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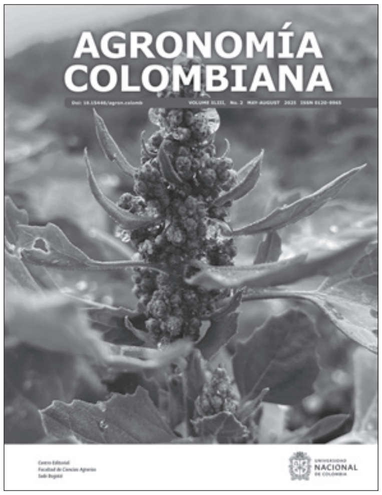

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INVITED EDITORIAL

Understanding microbiomes in agroecosystems: A challenge to address in the era of sustainable development

Comprender los microbiomas de agroecosistemas: un reto a enfrentar en la era del desarrollo sostenible

Among the 17 Sustainable Development Goals (SDGs) defined by the United Nations General Assembly in the 2030 Agenda, with the purpose of meeting current needs without affecting future generations, eradicating hunger is included as a priority (Tremblay *et al*., 2020; United Nations Environment Programme, 2015). It is for this reason that there is widespread recognition of the need for available, accessible, innocuous, nutritionally and organoleptically acceptable food, with a reduced environmental impact on its production. It has been estimated that resource-intensive agricultural practices occupy 43% of non-desert and non-ice-covered land, of which 87% is devoted to the production of crops of agronomic interest (Poore & Nemecek, 2018). Within the environmentally safe strategies to produce food, the exploitation of biological functions of microorganisms is identified, including nitrogen fixation, phosphate solubilization, production of indole acetic acid and siderophores, transformation of complex carbon sources, in addition to protection against pathogens and tolerance to abiotic stress in plants, among others. These capabilities have awakened a growing interest in knowing and understanding the microbial ecology of agricultural soils, especially through the analysis of microbiomes.

The microbiome, understood as a characteristic microbial community occupying a defined habitat with distinctive physicochemical properties (Berg *et al*., 2020), such as the soil of a given location or a plant, is a valuable opportunity to search for potential exploitable traits for the maintenance of agroecosystems. Within these, there is a remarkable variability of habitats that host microorganisms in the soil-plant continuum, including bulk soil, rhizosphere and rhizoplane, as well as endophytic and epiphytic microorganisms on stems and leaves (Xiong & Lu, 2022).

In the case of Colombia, a primary analysis of published studies on microbiomes in agricultural soils in the Scopus database, using the general search equation "microbiome" AND "soil" AND "Colombia" and excluding articles related to the field of medicine, revealed 15 articles published between 2017 and 2024. These studies focused primarily on the taxonomic diversity of rhizospheric fungi and bacteria associated with crops such as coffee, avocado, and tomato. In a megadiverse country like Colombia, it is essential to face the challenge of knowing not only the composition of microbial communities in multiple agroecosystems but also their functional diversity, to explore their possible biotechnological applications, providing new options for the sustainable management of agricultural production.

Given the current availability of metagenomics, metatranscriptomics, and metabolomics techniques, it might seem that understanding the microbial ecology of soils in agroecosystems is a simple task, however, there are limitations that must be recognized. At the most basic level, the definition of a prokaryotic species as an ecological unit is an ongoing topic of discussion. Likewise, the analysis of core microbiomes, understood as those microbial taxa shared between more than two samples from the same particular host or habitat (Neu *et al*., 2021), represents *per se* an essential challenge in an ecosystem as heterogeneous as the surface soil of any agroecosystem. This difficulty becomes especially relevant when recognizing that native species have specific niches that contribute to the cycling of elements and the development of their hosts.

It is widely recognized that microbial communities in an ecosystem as complex as agricultural soil have a direct or indirect impact on its functioning and stability. This is mainly due to the wide metabolic capacity of taxa that can carry out, in a unique way, processes of hydrolysis of complex polymers and oxidation/reduction of chemical elements, enabling the development of biogeochemical cycles. Despite recognizing this role, knowledge of the functional attributes of the soil microbiome remains restricted, especially due to technical limitations, such as the impossibility of culturing taxa with unique metabolic characteristics (Fierer, 2017). This makes the development of new techniques for cultivation and isolation of microorganisms, based on the use of ecological and genomic information, a valuable strategy for obtaining cost-effective and efficient bioinoculants. However, this type of biotechnological products is also a challenge, especially due to the cost of their production, their ability to colonize and express functions in the application habitat, as well as their effect on native microbial communities.

The interdisciplinary study of the taxonomic and functional composition of native microorganisms in the rhizosphere, rhizoplane, and phyllosphere, as well as the analysis of the topology and structure of microorganism-microorganism and microorganism-plant interaction networks, represents a potential opportunity to propose viable alternatives for the development of products such as biofertilizers or biocontrol agents. The use of cutting-edge techniques to address these investigations can generate a large amount of information, but it is the comprehensive and interrelated analysis of these data that can generate answers to contribute to establishing successful strategies for integrated management of agronomic practices that ensure the preservation of biodiversity and the well-being of our and future generations.

Literature cited

Berg, G., Rybakova, D., Fischer, D., Cernava, T., Vergès, M. C. C., Charles, T., Chen, X., Cocolin, L., Eversole, K., Corral, G. H., Kazou, M., Kinkel, L., Lange, L., Lima, N., Loy, A., Macklin, J. A., Maguin, E., Mauchline, T., McClure, R., … & Schloter, M. (2020). Microbiome definition re-visited: old concepts and new challenges. *Microbiome, 8*, Article 103. https://doi.org/10.1186/s40168-020-00875-0

Fierer, N. (2017). Embracing the unknown: disentangling the complexities of the soil microbiome. *Nature Reviews Microbiology*, 15(10), 579–590. Nature Publishing Group. https://doi.org/10.1038/nrmicro.2017.87

Neu, A. T., Allen, E. E., & Roy, K. (2021). Defining and quantifying the core microbiome: Challenges and prospects. *Proceedings of the National Academy of Sciences*, *118*(51), Article e2104429118. https://doi.org/10.1073/pnas.2104429118

Poore, J., & Nemecek, T. (2018). Reducing food's environmental impacts through producers and consumers. *Science*, *360*(6392), 987–992. https://doi.org/10.1126/science.aaq0216

Tremblay, D., Fortier, F., Boucher, J.-F., Riffon, O., & Villeneuve, C. (2020). Sustainable development goal interactions: An analysis based on the five pillars of the 2030 agenda. *Sustainable Development*, *28*(6), 1584–1596. https://doi.org/10.1002/sd.2107

United Nations Environment Programme. (2015). Policy coherence of the sustainable development goals: An International Resource Panel report. https://digitallibrary.un.org/record/1324550?ln=es&v=pdf

Xiong, C., & Lu, Y. (2022). Microbiomes in agroecosystem: Diversity, function and assembly mechanisms. *Environmental Microbiology Reports*, *14*(6), 833–849. John Wiley and Sons, Inc. https://doi.org/10.1111/1758-2229.13126

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Morpho-agronomic characterization and yield performance of quinoa (*Chenopodium quinoa* Willd.) genotypes

Caracterización morfoagronómica y desempeño de rendimiento de genotipos de quinua (*Chenopodium quinoa* Willd.)

Miguel Adolfo Pizo-Ossa[1*], Jennifer Lorena López-Hoyos[2], Diana Carolina Vásquez-Castro[3], Germán Andrés Aguilera-Arango[4], Ana Cruz Morillo-Coronado[5], and Yacenia Morillo-Coronado[1]

ABSTRACT

Quinoa (*Chenopodium quinoa* Willd.) stands out for its high nutritional value and agroecological adaptability, positioning it as a strategic crop in specialized markets. In Colombia, establishing crops in high Andean regions requires identifying improved genotypes with high yield potential and grain quality. The objective of this study was to agronomically characterize 14 improved sweet quinoa families, including the San Jorge family, and three commercial checks (Aurora, Blanca de Jericó, and Tunkahuan) under the agroecological conditions of Silvia (Cauca, Colombia), using a randomized complete block design with four replicates. Eleven morpho-agronomic variables were evaluated, and statistical analyses of ANOVA, Duncan's test ($P<0.05$), principal component analysis (PCA), hierarchical cluster analysis, and selection index were applied. The results revealed high variability among the genotypes. The highest yield per hectare and per plant was recorded for the genotype F56 (6623.1 kg ha^{-1} and 30.33 g), followed by the genotype FSJ. F25 stood out for the highest thousand-seed weight (3.13 g), and F38 for the largest grain diameter (2.38 mm). Aurora exhibited the most significant plant height (132.4 cm) and panicle length (53.9 cm), while F56 and F37 had the highest panicle diameter (68.79 mm) and stem diameter (16.5 mm). PCA explained 65.21% of the total variability, highlighting F56, FSJ, Aurora, and Blanca de Jericó as the genotypes with the most significant contribution. The most influential variables were plant height, yield, and thousand-seed weight. Cluster analysis grouped the 17 genotypes into three clusters: Group 1 (Aurora and Blanca de Jericó) characterized by robust architecture; Group 2 (Tunkahuan), with intermediate traits; and Group 3 (the remaining improved genotypes), which exhibited high productive efficiency and grain quality. Finally, the selection index effectively identified superior genotypes, positioning F56 and FSJ as promising candidates for future breeding and agronomic validation programs in high-altitude Andean zones.

Keywords: Andean crop, genetic variability, genetic diversity, genetic improvement, selection index.

RESUMEN

La quinua (*Chenopodium quinoa* Willd.) destaca por su valor nutricional y adaptabilidad agroecológica y la posiciona como un cultivo estratégico en mercados especializados. En Colombia, su consolidación en zonas altoandinas exige la identificación de genotipos mejorados con alto rendimiento y calidad de grano. El objetivo de esta investigación fue caracterizar agronómicamente 14 familias mejoradas de quinua dulce, incluida la familia San Jorge y tres testigos comerciales (Aurora, Blanca de Jericó y Tunkahuan) en condiciones del municipio de Silvia (Cauca, Colombia), mediante un diseño de bloques completamente aleatorizado con cuatro repeticiones. Se evaluaron 11 variables morfoagronómicas y se aplicaron análisis estadísticos de ANOVA, prueba de Duncan ($P<0.05$), análisis de componentes principales (ACP), análisis de conglomerados jerárquicos e índice de selección. Los resultados evidenciaron una alta variabilidad entre genotipos. El mayor rendimiento por hectárea y por planta fue registrado por el genotipo F56 (6623,1 kg ha^{-1} y 30,33 g), seguido del genotipo FSJ. F25 destacó por el mayor peso de mil semillas (3,13 g) y F38 presentó el mayor diámetro de grano (2,38 mm). Aurora, testigo comercial, exhibió la mayor altura de planta (132,4 cm) y longitud de panoja (53,9 cm), mientras que F56 y F37 registraron los valores más altos en diámetro de panoja (68,79 mm) y de tallo (16,5 mm), respectivamente. El ACP explicó el 65,21% de la variabilidad total, resaltando a F56, FSJ, Aurora y Blanca de Jericó como los genotipos de mayor contribución. Las variables más influyentes fueron altura de planta, rendimiento y peso de mil semillas. El análisis de agrupamiento clasificó los 17 genotipos en tres grupos: el grupo uno (Aurora y Blanca de Jericó), caracterizado por arquitectura robusta; el grupo dos (Tunkahuan), con características intermedias; y el grupo tres (resto de los genotipos mejorados), con alta eficiencia productiva y atributos de calidad. Finalmente, el índice de selección permitió identificar genotipos con alto potencial productivo y calidad física del grano, proponiendo a F56 y FSJ como candidatos clave para futuros programas de mejoramiento y validación en zonas altoandinas.

Palabras clave: cultivo andino, variabilidad genética, diversidad genética, mejoramiento genético, índice de selección.

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Introduction

Quinoa (*Chenopodium quinoa* Willd.) is a species native to the Andes, recognized for its high nutritional value, due to its high content of proteins, essential amino acids, vitamins, isoflavones, minerals, carbohydrates, and unsaturated fats (Sampaio *et al*., 2020). Quinoa is one of the few species with a remarkable capacity to adapt to extreme climatic and edaphic conditions without being significantly affected. Its cultivation is fundamental, as it represents a key strategy to confront climate change (Reguera *et al*., 2018). It exhibits efficient water use, tolerance to low soil moisture, and excellent adaptation to different climates, including deserts (Dehghanian *et al*., 2024). Therefore, quinoa contributes to food security in various regions of the world (Castro *et al*., 2023).

Quinoa yields have been boosted by genetic improvement, due to the nutritional properties of the seeds, especially the content of amino acids, macrocomponents (proteins, carbohydrates, and lipids), and some microcomponents such as vitamins, minerals, and polyphenols, which contribute to the manufacture of foods with health benefits (Argüello *et al*., 2024). However, the nutrients found can be significantly different among ecotypes, which has prompted an increase in quinoa breeding programs in recent years to study genetic variability in the expression of phenological, morphological, agronomic, and grain compositional traits (Delgado *et al*., 2024).

The central quinoa-producing departments in Colombia are Boyaca, Cundinamarca, Nariño, and Cauca, the latter of which has annual yields ranging from 2.5 to 3 t ha^{-1}. Meanwhile, in the Andean countries, annual yields of 1 to 1.5 t ha^{-1} have been reported, although some yields of 4 t ha^{-1} per year have also been reported (Montes-Rojas *et al*., 2020). These quinoa-producing regions have focused on developing a national strategy to increase yields through agroecological management, utilizing both ancestral and modern practices. Crop nutrition is carried out with organic matter collected and manufactured with crop residues predominant in each region (García *et al*., 2018). These productions are targeted at local markets to increase the country's per capita consumption of quinoa. In addition, exports have been boosted because quinoa has gained wide acceptance in North America, Africa, Asia, and Europe, mainly in countries seeking organic crops, to change the eating habits of consumers of products such as rice, potatoes, and other foods traditionally used (Pizo Ossa *et al*., 2024).

In this context, the Aurora and Tunkahuan genotypes have been widely used in the Nariño and Cauca regions due to their adaptability to mid- and high-altitude environments (2,500–3,000 m a.s.l.), their stable vegetative cycle, grain quality accepted by the market, and reliable performance under variable climatic conditions (Montes *et al*., 2018; Morillo-Coronado *et al.,* 2023). The Aurora ecotype has demonstrated exemplary performance in temperate environments, with a phenological cycle ranging from 160 to 170 d to harvest, average grain yields between 2,000 and 2,800 kg ha^{-1}, and a thousand-seed weight of approximately 2.7 g. Tunkahuan, on the other hand, is characterized by its short stature (average height of 95 cm), resistance to lodging, early maturity (125 to 140 d to harvest), low saponin content (below 0.05%), and an average yield of around 2,500 kg ha^{-1} (Pizo Ossa *et al.,* 2024; Urdanegui *et al.,* 2021). Their use as parental lines in breeding programs enables the development of advanced lines with high agronomic potential, as in the present study, which aims to identify promising genotypes adapted to the agroecological conditions of eastern Cauca.

In the department of Cauca, Colombia, this crop represents an opportunity and a key economic line for indigenous and peasant communities, as it contributes to food sovereignty and security in the region (Anchico-Jojoa *et al*., 2023). Quinoa has also contributed to combating malnutrition thanks to its nutritional properties and has boosted economic development. This crop has strengthened the ties between producers and their communities, fostering a solid and sustainable relationship with them, who encourage planting through entrepreneurship to develop sustainable alternatives, given the uses of this promising crop in agribusiness (Flórez-Martínez *et al*., 2024).

Given the increasing need to identify genotypes adapted to specific agroecological conditions, this study focuses on the morpho-agronomic characterization of 17 quinoa genotypes, including 14 families derived from the Aurora × Tunkahuan cross and three commercial checks. The aim of this evaluation was to select genotypes exhibiting desirable agronomic traits such as shorter growth cycles, superior grain physical quality, yield stability, and adaptability to adverse edaphic and climatic conditions. The outcomes of this research are expected to support breeding programs, enhance the diversification of the regional genetic base, and promote more resilient cropping systems in mid- to high-altitude environments.

Materials and methods

Plant material and experimental design

Fourteen sweet quinoa families, derived from the cross between the Aurora and Tunkahuan ecotypes including one identified as the "San Jorge family" were evaluated alongside three commercial checks: Aurora, Blanca de Jericó, and Tunkahuan (Tab. 1). The 17 genotypes were established in the field on deep, well-drained soils with loamy sand to sandy clay loam texture, rich in organic matter, classified as Andisols according to the USDA Soil Taxonomy and as Humic Andosols under the WRB-FAO classification system, using a completely randomized block design with four replicates. The experimental unit consisted of a plot measuring 14 m in length and 3.0 m in width, where the 17 genotypes were randomly assigned within each plot. Row spacing was set at 0.70 m, with 0.10 m between plants, resulting in a total of 68 rows. For the evaluation, plants located within the central 2 m were harvested, and border rows were excluded.

The quinoa genotypes evaluated in this study were cultivated under homogeneous agronomic conditions, thereby eliminating the influence of management variability on the observed responses. Organic fertilization was carried out using compost, produced from the co-composting of sugarcane filter cake (a byproduct of the sugar industry) and high-quality poultry manure, which was incorporated into the soil 20 d before sowing to enhance its physical and chemical structure and ensure optimal decomposition. Additionally, the organic fertilizer Agrofertil (produced by Agrofertil S.A.S., Colombia), commonly known as A.L.O.F.A. (Aerobically Fermented and Mineralized Liquid Organic Fertilizer), was applied both foliarly and via the root system throughout the crop cycle. This product is water-soluble and produced in Colombia through aerobic fermentation of macerated plant material, complemented with trace elements, humic and fulvic acids, Huila phosphorite, silicon oxide, and organic additives. The formulation is stabilized at a pH of 5.7, ensuring a balanced organic-mineral nutrient supply to the crop. This standardized management approach enabled the differences observed among genotypes to be primarily attributed to genetic expression and their interaction with the climatic and edaphic conditions of the study area.

Harvesting was conducted once the plants reached physiological maturity, determined by observing seed color, texture, and the fingernail test. The central 2 m of each plot was used for sampling, excluding the borders, with the aid of a 3-m handmade ruler. Plants were cut using pruning shears and labeled according to genotype and number of panicles. The samples were transported to a greenhouse, where panicles were separated and left to dry for 15 to 20 d. Subsequently, manual threshing was performed. Seeds were cleaned using sieves and mesh screens, then stored in

TABLE 1. List of evaluated quinoa genotypes, their type, and origin used in the field trial.

Code	Name / Family	Type of material	Origin
F1	Family 1	Aurora × Tunkahuan progeny	Experimental cross
F2	Family 2	Aurora × Tunkahuan progeny	Experimental cross
F3	Family 3	Aurora × Tunkahuan progeny	Experimental cross
F4	Family 4	Aurora × Tunkahuan progeny	Experimental cross
F5	Family 5	Aurora × Tunkahuan progeny	Experimental cross
F6	Family 6	Aurora × Tunkahuan progeny	Experimental cross
F7	Family 7	Aurora × Tunkahuan progeny	Experimental cross
F8	Family 8	Aurora × Tunkahuan progeny	Experimental cross
F9	Family 9	Aurora × Tunkahuan progeny	Experimental cross
F10	Family 10	Aurora × Tunkahuan progeny	Experimental cross
F11	Family 11	Aurora × Tunkahuan progeny	Experimental cross
F12	Family 12	Aurora × Tunkahuan progeny	Experimental cross
F13	Family 13	Aurora × Tunkahuan progeny	Experimental cross
F14	Family San Jorge	Aurora × Tunkahuan progeny	Experimental cross
T1	Aurora	Commercial check	National Institute of Agrarian Innovation (INIA), Peru
T2	Blanca de Jericó	Commercial check	Native germplasm collected and improved by farmers and Colombian universities, Antioquia, Colombia
T3	Tunkahuan	Commercial check	National Institute of Agricultural Research (INIAP), Ecuador

sealed bags and transported to the AGROSAVIA laboratory in Popayán for agronomic evaluation.

Location

The genotypes were evaluated during the second semester of 2023 in the San Fernando district, located in the municipality of Silvia, at an altitude of 2,660 m a.s.l., with an average annual temperature of 12°C, a relative air humidity of 80% and rainfall of 1,800 mm. The plants were fertilized organically, using cachaza (a byproduct of sugar cane and chicken manure production), complemented by manual weed control.

Harvesting was done manually when the genotypes reached harvest maturity between 120.31 and 168.48 d. For agronomic characterization, 11 agronomic variables were evaluated (Tab. 2), using the descriptors of quinoa and its wild relatives (Bioversity International *et al*., 2013).

TABLE 2. Agronomic variables for the characterization of 17 *Chenopodium quinoa* genotypes.

Variable	Abbreviation	Unit of measurement
Plant height	PH	cm
Panicle length	PL	cm
Panicle diameter	PD	mm
Stem diameter	SD	mm
Yield per plant	YPP	g
Yield (kg ha^{-1})	YPH	kg
Weight of one thousand seeds	WTS	g
Grain diameter	GD	mm
Grain thickness	GT	mm
Harvest index	HI	%
Grain moisture content	MC	%

Statistical analysis

The coefficient of variation and mean were calculated; an ANOVA analysis was used with a value ($P<0.05$) with its respective Duncan mean test ($P<0.05$). A Principal Component Analysis (PCA) was performed using the correlation matrix between the variables, which were plotted on a two-dimensional plane to group the quinoa genotypes evaluated. Subsequently, a hierarchical cluster analysis was performed using hierarchical grouping (UPGMA) where Euclidean distance was applied. Finally, for the selection of quinoa genotypes, the selection index (SI) was used using Equation 1:

$$SI = Y \frac{kg}{ha} (0.50) - WTS(0.20) - GD(0.30) \qquad (1)$$

where

Y: yield, WTS: thousand-seed weight, GD: grain diameter.

The software R version 4.4 was used to process the information.

Results and discussion

Table 3 presents the results of the analysis of variance ($P\leq0.05$), revealing statistically significant differences for most evaluated traits, except for harvest index ($P\leq0.0054$) and moisture content ($P\leq0.6641$). Highly significant differences ($P\leq0.0001$) were found for plant height, panicle length, panicle diameter, stem diameter, and thousand-seed weight, indicating a high level of genetic variability among the genotypes evaluated. In addition, significant differences were also detected for yield per plant ($P = 0.0009$), yield per hectare ($P\leq .0009$), grain diameter ($P\leq0.0007$), grain thickness ($P\leq0.0040$), and harvest index ($P\leq0.0054$). Conversely, no significant differences were observed for moisture content ($P\leq0.6641$), indicating a relatively uniform behavior for this trait across the genotypes.

Descriptive analysis for agronomic variables

For the statistical comparison among genotypes, Duncan's multiple range test ($P\leq0.05$) was used.

Regarding the plant height variable, the average was 99.35 cm. The maximum values were obtained by the Aurora, Blanca de Jericó, and Tunkahuan controls with 132.4, 121.2, and 114.2 cm, respectively. The minimum values were obtained by F27 with a value of 81.8 cm and F86 with a value of 82.6 cm. For the panicle length variable, the average was 43.49 cm. The commercial controls Aurora and Blanca obtained the maximum values in Jericó with 53.9 cm and 50.5 cm, respectively.

In comparison, the minimum value was obtained by FSJ with 33.6 cm. For the panicle diameter variable, the average was 61.28 mm. The maximum results were observed in F103 and F56 with 74.09 and 68.79 mm, respectively, while the minimum value was shown in F27 with 54.84 mm. For its part, the stem diameter variable presented an average of 15.62 mm, with the maximum value shown by

TABLE 3. Analysis of variance for morpho-agronomic variables evaluated in 17 quinoa genotypes.

Variables	Source of variation	SS	MS	F	*P-value*
Plant height (cm)	Genotype	126414.46	7900.9	96.28	<0.0001
	Replicate	23377.39	7792.46	94.96	<0.0001
	Total	203954.4			
Panicle length (cm)	Genotype	17454.21	1090.89	21.51	<0.0001
	Replicate	1573.13	524.38	10.34	<0.0001
	Total	52496.98			
Panicle diameter (mm)	Genotype	1221.21	76.33	12.37	<0.0001
	Replicate	35.27	11.76	1.91	0.1273
	Total	5321.89			
Stem diameter (mm)	Genotype	209.79	13.11	6.67	<0.0001
	Replicate	279.81	93.27	47.43	<0.0001
	Total	1787.41			
Yield per plant (g)	Genotype	810.3	50.6	3.2	0.0009
	Replicate	39.6	13.2	0.8	0.4784
	Total	1603.9			
Yield (kg ha^{-1})	Genotype	52688969.6	3293060.6	3.2	0.0009
	Replicate	2576885	858961.7	0.8	0.4783
	Total	104303939			
Weight of one thousand seeds (g)	Genotype	5.77	0.36	16.98	<0.0001
	Replicate	0.1	0.03	1.58	0.2075
	Total	6.89			
Grain diameter (mm)	Genotype	0.75	0.05	2.72	0.0007
	Replicate	0.11	0.04	2.08	0.1045
	Total	4.05			
Grain thickness (mm)	Genotype	0.2	0.01	1.05	0.0040
	Replicate	0.02	0.01	0.61	0.6122
	Total	2.44			
Harvest index (%)	Genotype	225.7	14.1	1.1	0.0054
	Replicate	65.1	21.7	1.7	0.1863
	Total	915.7			
Grain moisture content (%)	Genotype	71.3	4.5	0.8	0.6641
	Replicate	3.3	1.1	0.2	0.8957
	Total	337.7			

SS: Sum of squares, MS: mean square.

F37 at 16.5 mm, and the minimum value corresponds to F25 with 14.5 mm (Tab. 4).

The results obtained for plant height are like those reported by Chino Nicolas *et al*. (2019), who report heights between 100.4 to 136.8 cm, and suggest that plants within this range are easy to manage in intensive and mechanized agriculture, in addition to the fact that this characteristic is related to tolerance to grain lodging and sometimes with plants with late cycles. On the other hand, El-Harty *et al*. (2021) in studies conducted in Riyadh (Saudi Arabia) have categorized the height in three classifications, short (50-70 cm), medium (70-130 cm) and long (130-190 cm), and report that individuals with medium category have higher production of quinoa, while, in the long category presented lower yields; therefore, the classification obtained in the study, allowed to identify the families as medium and the controls as long.

For the panicle length variable, the values found in the study coincided with those reported by Morillo *et al*. (2020), who evaluated 19 quinoa materials in the department of Boyacá, with ranges that fluctuated between 12 cm and 45 cm. This variable is related to plant height and panicle diameter, especially at the point of development of the apical meristem, which contributes to its growth, as well as the joint interaction of genotypes with other factors such as the type of panicle, climatic conditions, planting density,

TABLE 4. Duncan's test of means ($P>=0.05$) for plant height, panicle length, panicle diameter, stem diameter, and harvest index in 17 genotypes of *Chenopodium quinoa*. Different letters in the row indicate significant differences according to Duncan's test ($P\leq0.05$).

Genotype	PH	PL	PD	SD	HI
F27	81.8 a	36.7 ab	54.84 a	15.2 bcd	40 a
F86	82.6 a	40.4 cd	63.83 cd	14.6 ab	45 a
F105	88.6 b	38.5 bc	58.45 abc	15.5 cde	44 a
F103	88.8 b	39.5 bc	74.09 f	15.6 cdef	43.2 a
F110	89.4 b	40.7 cd	59.35 abc	15.1 abc	43.2 a
Familia San Jorge	89.8 b	33.6 a	58.63 abc	15.6 cdef	41.1 a
F63	90 b	39.5 bc	57.96 ab	15.8 cdef	40.4 a
F23	93.1 bc	42 cde	61.62 bc	15.6 cdef	40 a
F38	95.4 c	46.2 fg	60.81 abc	15.2 bcd	44.4 a
F25	96 c	45.3 ef	58.15 ab	14.5 a	45.1 a
F34	104.7 d	43.7 def	60.13 abc	16.3 fg	38.4 a
F56	104.8 d	46.4 fg	68.79 e	16.3 fg	47.5 a
F60	107.7 d	47.2 fg	59.45 abc	16.2 fg	39.3 a
F37	108.4 d	49.2 gh	60.84 abc	16.5 g	40.4 a
Tunkahuan	114.2 e	46.1 fg	67.61 de	15.6 cdef	33.8 a
Blanca de Jericó	121.2 f	50.5 h	56.14 ab	16.1 efg	36.5 a
Aurora	132.4 g	53.9 i	61.04 bc	15.9 defg	34.9 a
Mean	99.35	43.49	61.28	15.62	41.01
C.V.(%)	10.71	16.28	16.76	9.60	13.08

PH: plant height; PL: panicle length; PD: panicle diameter; SD: stem diameter; HI: harvest index; CV(%): coefficient of variation. Means followed by the same letter within each variable do not differ significantly according to Duncan's multiple range test ($P\leq0.05$).

and fertility provided to the crop. Regarding the panicle diameter variable, the results align with those cited by Estrada-Zúniga *et al.* (2022) in agronomic studies, which reported values ranging from 47.5 to 72 mm, likewise. Manjarres *et al.* (2021) report a strong correlation between panicle length, diameter, and height.

Stem diameter values are similar to those reported by Hussain *et al.* (2020), where they recorded diameters ranging from 8.65 to 14.99 mm; furthermore, this variable contributes to the selection of genotypes with thick stems, which could improve yield, increase the number of inflorescence and other aspects such as the prevention of lodging caused by adverse weather conditions (prolonged winds, excessive rainfall), by conferring resistance to panicle weight, as well as tolerance to bird damage.

Descriptive analysis for performance variables

In the yield variable, the results are similar to those reported by Montes-Rojas *et al.* (2018), with yields ranging from 20 g to 32 g per plant. According to Hussain *et al.* (2020), this variable is directly related to genetic, environmental components, and genotype-by-environment interaction. Quinoa is very sensitive to abrupt changes in temperature, precipitation, and relative air humidity, which could have influenced the behavior of the controls, presenting the lowest values. At the same time, F56 and FSJ showed a better response to yield, with an average yield gain of 13.03 g (44.41%), which could be shown by exceeding the response obtained by the commercial genotypes. For yield (kg ha^{-1}), the results agree with those reported by Montes-Rojas *et al.* (2020), with values ranging between 2424.5 and 4193 kg ha^{-1}. Likewise, a yield gain of 2826.39 kg ha^{-1} (44.06%) was observed for FSJ and F56 compared to the commercial genotypes (Tab. 5).

Regarding the variable thousand-seed weight, the values are similar to those found by Morillo *et al.* (2020) in agromorphological evaluation, with an average weight of 2.40 g. In addition, an average gain of 0.99 g (31.93%) corresponding to Fl25 was observed, surpassing the response obtained by the controls. According to Madrid *et al.* (2018), they present a classification scale for this variable, where > 3 g is considered higher weight, integrated by 2 of 17 genotypes, from 2.5 to 3 g as medium weight, standing out (11 of 17 genotypes) and < 2.5 g as lower weights represented by the commercial controls. They also indicate that this variable is the primary component of yield, allowing for the measurement of the weight and number of seeds studied in each genotype, and is essential for selection in genetic improvement programs (García-Parra *et al.*, 2020).

TABLE 5. Duncan's test of averages for yield per plant, yield (kg ha^{-1}), thousand-seed weight, grain moisture content, grain diameter, and grain thickness in 17 *Chenopodium quinoa* accessions. Different letters in the row indicate significant differences according to Duncan's test ($P \leq 0.05$).

Genotype	YPP	YPH	WTS	MC	GD	GT
Blanca de Jericó	14.23 a	3150.7 a	2.12 a	13.3 a	2.24 abcd	1.09 a
F60	15.77 ab	3459.5 ab	2.44 bc	14 a	2.27 abcd	1.18 ab
Tunkahuan	16.99 abc	3704.8 abc	2.19 ab	14.5 a	2.1 abc	1.15 ab
Aurora	17.71 abc	3909.9 abc	2.03 a	13.4 a	2.09 a	1.1 ab
F27	20.19 abcd	4398.9 abcd	2.83 de	14.5 a	2.13 abc	1.21 b
F34	20.58 abcd	4491.3 abcd	2.66 cd	14.5 a	2.33 cd	1.18 ab
F103	21.33 abcd	4614.3 abcd	2.74 de	15.2 a	2.3 bcd	1.18 ab
F25	21.34 abcd	4654.1 abcd	3.13 f	14.4 a	2.12 abc	1.15 ab
F105	22.23 abcde	4852 abcde	2.85 de	14.4 a	2.11 abc	1.15 ab
F23	23.12 bcde	5043.2 bcde	2.7 cd	14.4 a	2.31 bcd	1.2 b
F63	23.12 bcde	5056 bcde	2.75 de	14.3 a	2.08 a	1.19 ab
F110	23.61 bcde	5135.4 bcde	2.65 cd	14.7 a	2.1 ab	1.2 b
F86	23.9 bcde	5224.9 bcde	3.02 ef	14.2 a	2.18 abc	1.16 ab
F38	24.31 bcde	5252.7 bcde	2.81 de	14.9 a	2.38 d	1.17 ab
F37	25.63 cde	5495.4 cde	3 def	16 a	2.15 abc	1.15 ab
Familia San Jorge	28.45 de	6206.6 de	2.8 de	14.5 a	2.16 abc	1.14 ab
F56	30.33 e	6623.1e	2.67 cd	14.4 a	2.25 abcd	1.19 ab
Mean	21.93	4780.75	2.67	14.45	2.19	1.16
C.V (%)	19.54	22.28	5.81	5.29	8.70	6.73

YPP: yield per plant; YPH: yield (kg ha^{-1}); WTS: thousand-seed weight; MC: moisture content; GD: grain diameter; GT: grain thickness; C.V. (%): coefficient of variation. Means followed by the same letter within each variable do not differ significantly according to Duncan's multiple range test ($P \leq 0.05$).

Regarding the grain diameter variable, the results are similar to those cited by Emrani *et al.* (2020), indicating an average of 2.09 mm, which suggests that quinoa is one of the crops where its ecotypes present greater variability and genetic diversity. However, this diversity has been progressively lost, due to the pressure of national and international markets, who demand large and white grains as a preference, being the markets the main demanders to propose the objectives of genetic improvement programs; therefore, this characteristic is one of the most suggested to be taken into account in breeding programs, because producers prefer larger grains desired by the agroindustry. Regarding the grain thickness variable, the results coincide with those reported by Chura Yupanqui *et al.* (2021), with an average of 1.06 mm. The F27 family shows a potential for thickness, surpassing the response obtained by the commercial genotypes.

Principal component analysis

With 11 agronomic variables evaluated, 14 families, and three commercial controls, two principal components were obtained, explaining 44.59% of the variance. The second component explained 21.66%, totaling 66.25% of the variance (Tab. 6).

TABLE 6. Eigenvalue for each of the dimensions in the principal component analysis using 11 agronomic variables for 17 genotypes of *Chenopodium quinoa.*

Components	Eigenvalue	Total variance %	Accumulated variance %
1	5.211	44.59	44.59
2	2.5319	21.66	66.25
3	1.2985	11.11	77.36
4	1.1552	9.88	87.24
5	0.5703	4.88	92.12
6	0.4016	3.44	95.56
7	0.3037	2.6	98.16
8	0.1464	1.25	99.41
9	0.0579	0.5	99.91
10	0.0101	0.09	99.99
11	0.0009	0.01	100.0

The Biplot graphical interaction analysis projected the parallel results between the variables (vectors) and the genotypes contained in the components (Fig. 1).

In Figure 2A, the genotypes Aurora (AU) and Blanca de Jericó (BDJ) showed the highest contributions to the formation of the first two principal components, followed by FSJ, F60, and F23. In Figure 2B, the variables with the most significant contributions were yield per plant (YPP) and yield per hectare (YPH), followed by grain thickness (GT), plant height (PH), and thousand-seed weight (WTS).

In the first quadrant, the variables PH, PD, SD, and PL are positively associated. They are represented by the genotypes Tunkahuan, Aurora, F34, and F37, which showed high values for these agronomic traits. These results align with those reported by Morillo *et al.* (2020), who find that tall plants tend to have thicker stems and longer panicles, traits that provide mechanical resistance to the size and weight of the panicle and help avoid lodging under adverse environmental conditions such as wind and rain.

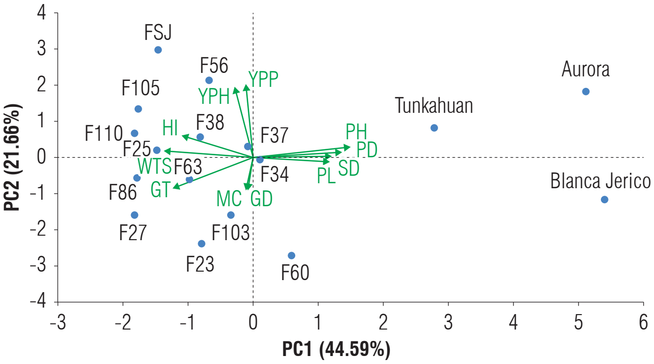

FIGURE 1. Representation of genotypes and variables in the first and second principal components using a composite Biplot graph for agronomic variables in 17 genotypes of *Chenopodium quinoa*. Abbreviations: PH: plant height; PL: panicle length; SD: stem diameter; PD: panicle diameter; GD: grain diameter; GT: grain thickness; MC: moisture content; WTS: thousand-seed weight; YPP: yield per plant; YPH: yield per hectare; HI: harvest index.

In the second quadrant, the variables YPP and YPH are grouped, with genotypes such as F56 and FSJ contributing most strongly in this direction. These variables, associated with productivity, showed a negative correlation with plant height and stem diameter, indicating that high-yielding genotypes tend to be shorter with thinner stems. This finding aligns with the findings of Foronda Limachi (2022), who report a positive correlation between YPP, YPH, and harvest index (HI).

The third quadrant includes genotypes such as F60, F23, and MC, along with variables GD, GT, and MC, indicating that these genotypes are associated with greater grain size, thickness, and moisture content. Although less productive, these materials could be relevant for breeding programs focused on grain quality traits.

In the fourth quadrant, the variable WTS is located along with the genotypes F27, F105, F110, F25, F86, and F63, which displayed higher values for thousand-seed weight. However, a negative correlation is observed with the yield and height-related variables (YPP, YPH, PH), suggesting that genotypes with heavier seeds may have lower overall plant productivity and shorter stature.

Finally, Blanca de Jericó, although positioned in the lower right quadrant, shows a contrasting pattern. It is far from most variables, indicating a low contribution to both principal components. However, it remains close to Aurora, suggesting possible similarities in phenotypic performance.

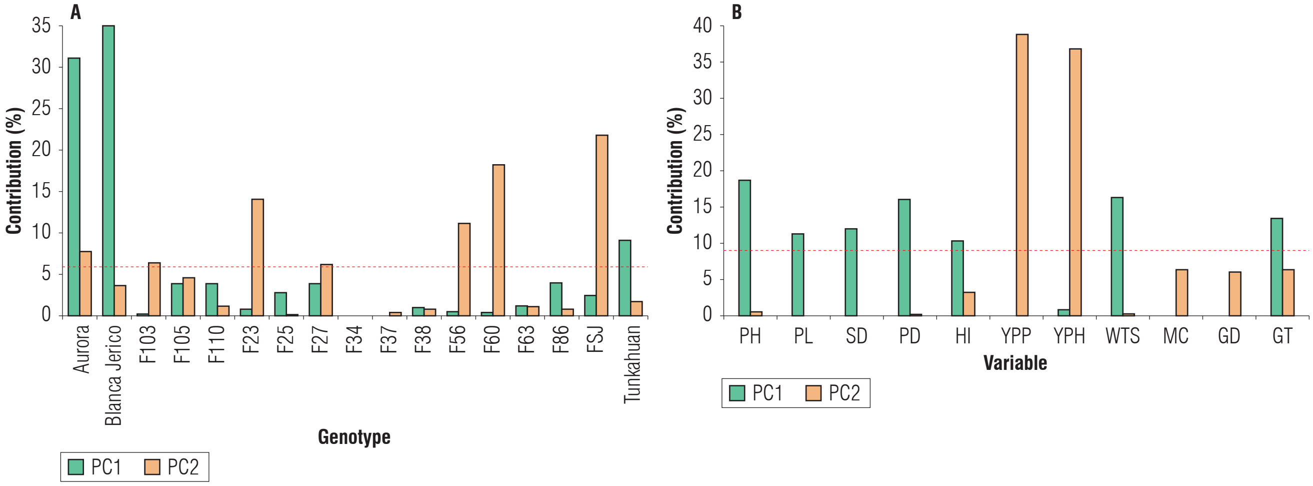

FIGURE 2. A) Contribution to the variance of quinoa genotypes in components 1 and 2 of the principal component analysis; B) Contribution to variance of yield variables where PL: plant height, MC: grain moisture content, PD: panicle diameter, SD: stem diameter, GT: grain thickness, HI: harvest index, PL: panicle length, WTS: thousand-seed weight, GD: grain diameter, YPH: yield per ha, YPP: yield per plant in components 1 and 2 of principal components analysis.

Cluster analysis

The optimal number of clusters was determined using the K-means algorithm, which identified the inflection point at K = 3, indicating that three clusters group the data more efficiently (Sinaga & Yang, 2020). The cluster analysis (Fig. 3) enabled the formation of three groups. The first group (A) consisted of four genotypes, Aurora and Blanca de Jericó. The second group (B) is composed of Tunkahuan. Finally, the third group (C) consisted of 14 materials: F60, FSJ, F105, F37, F38, F86, F110, F23, F63, F27, F34, F105, F103, and F25.

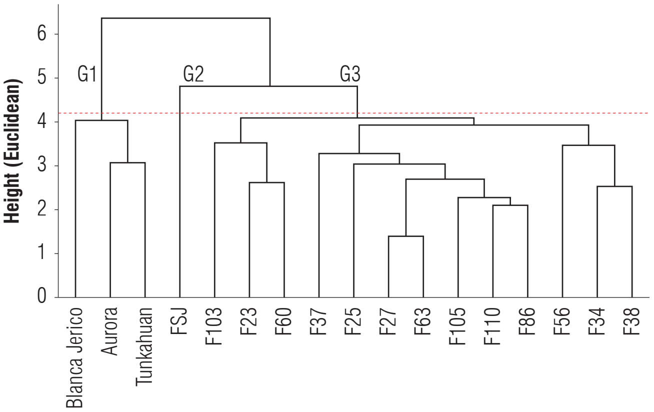

FIGURE 3. Dendrogram obtained by Principal Component Analysis for 17 genotypes of *Chenopodium quinoa* from 11 quantitative variables.

The grouping analysis allowed the identification of three clusters. The first group, consisting of the genotypes Aurora and Blanca de Jericó, was characterized by vigorous plant architecture, with heights ranging from 93.1 to 120.96 cm, stem diameters between 13.65 and 13.97 mm, and well-developed panicles in both diameter (50.12-53.73 mm) and length (37.74-38.15 cm). These structural features suggest a growth strategy focused on biomass accumulation and robustness, which could confer advantages in environments with heavy rainfall or strong winds. The second group, exclusively composed of Tunkahuan, displayed an intermediate profile. This ecotype exhibited relatively tall plants (114.2 cm) and long panicles (46.1 cm), characterized by a solid architecture. Its yield per plant (16.99 g) and per hectare (3704.8 kg ha^{-1}) were also intermediate. Although it did not outperform in any single trait, it maintained a balanced profile in terms of structure, yield, and grain quality, explaining its widespread use as a reference genotype and its value in breeding programs.

The third group, comprising most of the improved genotypes, stood out for its agronomic efficiency and grain quality. Genotypes F56 and FSJ recorded the highest yields per plant (30.33 and 28.45 g) and per ha (6623.1 and 6206.6 kg ha^{-1}), along with elevated harvest indices (47.5% and 41.1%, respectively). These findings suggest an efficient allocation of biomass toward reproductive structures. Regarding grain quality, genotypes such as F25, F86, and F27 exhibited the highest thousand-seed weights (3.13, 3.02, and 2.83 g, respectively), as well as greater grain thickness and diameter, which are desirable traits from an agro-industrial perspective. This group also displayed intermediate ranges in plant height (81.8 to 108.4 cm) and panicle length (33.6 to 49.2 cm), indicating that a tall stature is not necessarily required for achieving high yields, provided there is adequate physiological efficiency. This is exemplified by genotype F27, which, despite having the shortest plant height (81.8 cm), achieved outstanding performance in both yield and grain quality.

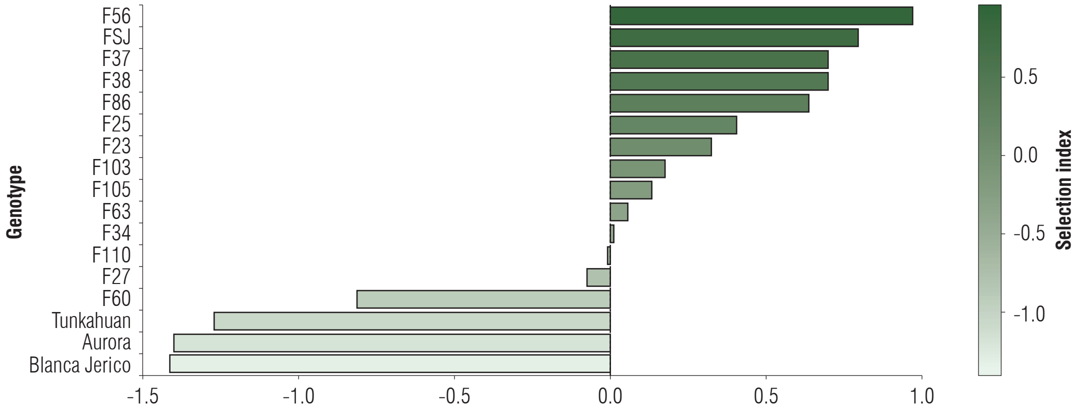

FIGURE 4. The selection index of promising quinoa genotypes in the locality of Silvia (Cauca), where families F56 stand out, followed by FSJ, F37, and F38.

Selection index

The selection index allowed for the clear differentiation among the evaluated quinoa genotypes. Genotype F56 exhibited the highest index value (0.96), with a yield of 6623.1 kg ha^{-1}, a thousand-seed weight of 2.7 g, and a grain diameter of 2.23 mm. It was followed by FSJ (0.79), F37 (0.69), F38 (0.69), and F86 (0.63), all of which showed yields exceeding 5,200 kg ha^{-1} and outstanding values in key quality traits. Notably, F86 recorded the highest thousand-seed weight (3.02 g), while F38 exhibited the largest grain diameter (2.34 mm). In contrast, the commercial checks Blanca de Jericó, Tunkahuan, and Aurora recorded the lowest selection index values (-1.41, -1.27, and -1.40, respectively), with yields ranging from 3150.7 to 3909.9 kg ha^{-1} and thousand-seed weights below the overall mean (2.68 g).

The selection index results highlighted the discriminative power of this tool in identifying quinoa genotypes with high productivity and superior grain quality. Genotype F56 stood out for combining high yield with desirable physical grain attributes, positioning it as a priority candidate for breeding programs adapted to the conditions of eastern Cauca. Likewise, genotypes FSJ, F37, F38, and F86 excelled in their overall agronomic performance, particularly in quality parameters such as thousand seed weight and grain diameter, traits highly valued by the agri-food industry. In contrast, the commercial checks exhibited lower performance, which may be associated with reduced adaptation to local conditions or limitations in biomass allocation to reproductive organs. This performance gap between improved and traditional genotypes reinforces the need to incorporate selection criteria based on multiple traits beyond yield alone.

These findings are consistent with those reported by Delgado *et al.* (2009), who demonstrate that the selection index enables the integration of multiple agronomic and quality traits to support informed decision-making in selecting outstanding materials. Although the index values observed in this study were lower than those reported by said authors (ranging from 1.78 to 0.96), the consistent performance in yield and grain quality of genotypes such as F56, FSJ, and F38 supports their inclusion in agronomic validation and technology transfer processes.

Conclusions

This study enabled the identification of sweet quinoa genotypes with superior agronomic performance under the agroecological conditions of Silvia, Cauca (Colombia). Genotype F56 stood out by exhibiting the highest selection index, with a yield exceeding 6600 kg ha^{-1}, and remarkable morpho-agronomic stability. These characteristics may be attributed to greater physiological efficiency in biomass allocation to reproductive organs, as evidenced by its high harvest index and the balance between compact plant architecture and high productivity, supported by the principal component analysis. These traits position F56 as a promising candidate for inclusion in genetic improvement programs. Similarly, FSJ proved to be an outstanding genotype, showing a high selection index, excellent yield, and favorable grain quality traits.

Both genotypes outperformed the commercial checks, which displayed more vigorous vegetative structures but lower productive efficiency. This behavior suggests that, under the agroecological conditions of the Cauca region, particularly at altitudes above 2600 m a.s.l. It is more advantageous to select genotypes with reduced plant height and enhanced resource allocation to grain production. Rather than prioritizing vigorous plant architecture, selection should focus on genotypes that optimize the production of grains with desirable physical attributes such as weight, diameter, and thickness, as observed in genotypes F25, F27, and F38.

This multi-trait selection strategy, which extends beyond yield alone, enabled the identification of agronomically efficient and highly competitive genotypes that align with the demands of the agro-industrial market, which values large, uniform, and heavy grains.

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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: MAPZ, YMC; Research: MAPZ, GAAA, YMC; Methodology: MAPZ, YMC; Data curation:

MAPZ, JLLH; Formal analysis: MAPZ, JLLH; Writing-original draft: MAPZ, JLLH; Supervision: YMC, DCVC, ACMC; Writing-review & editing: All authors. Visualization: MAPZ. All authors reviewed the final version of the manuscript.

Literature cited

Anchico-Jojoa, W., Peixoto, J. R., & Oliveira Júnior, A. A. (2023). Agronomic characterization and interaction of genotype by environment of quinoa under conditions of Brazil and Colombia. *Horticultura Brasileira, 41*, Article e2629. https://doi.org/10.1590/S0102-0536-2023-E2629

Argüello-Hernández, P., Samaniego, I., Leguizamo, A., Bernalte-García, M. J., & Ayuso-Yuste, M. C. (2024). Nutritional and functional properties of quinoa (*Chenopodium quinoa* Willd.) Chimborazo ecotype: Insights into chemical composition. *Agriculture, 14*(3), Article 396. https://doi.org/10.3390/agriculture14030396

Bioversity International, FAO, PROINPA, INIAF, & IFAD. (2013). *Descriptores para quinua (Chenopodium quinoa Willd.) y sus parientes silvestres*. https://hdl.handle.net/10568/72610

Castro-Albán, H. A., Castro-Gómez, R. D. P., & Alvarado-Capó, Y. (2023). Variabilidad morfoagronómica de quinua (*Chenopodium quinoa* Willd.) nativa tipo Chimborazo en Ecuador. *Agronomía Mesoamericana*, *34*(3), Article 53229. http://doi.org/10.15517/am.2023.53229

Chino Nicolas, E., Miranda Casas, R., & Del Castillo Gutiérrez, C. R. (2019). Comportamiento agronómico del cultivo de quinua (*Chenopodium quinoa* Willd.) con la aplicación de niveles de estiércol camélido. *Revista de Investigación e Innovación Agropecuaria y de Recursos Naturales, 6*(1), 41–49. https://riiarn.umsa.bo/index.php/RIIARn/article/view/22/6

Chura Yupanqui, E., Mujica Sánchez, A., Pinto Vargas, J., León Tacca, B., & Flores Ortega, A. L. (2021). Agromorphological characterization of F1 progenies of simple crosses of quinoa (*Chenopodium quinoa* Willd.) under greenhouse conditions. *Annals of the Romanian Society for Cell Biology, 25*(5), 2460–2481. http://annalsofrscb.ro/index.php/journal/article/view/4786/3846

Dehghanian, Z., Ahmadabadi, M., Asgari Lajayer, B., Gougerdchi, V., Hamedpour-Darabi, M., Bagheri, N., Sharma, R., Vetukuri, R. R., Astatkie, T., & Dell, B. (2024). Quinoa: A promising crop for resolving the bottleneck of cultivation in soils affected by multiple environmental abiotic stresses. *Plants, 13*(15), Article 2117. https://doi.org/10.3390/plants13152117

Delgado, A. I., Palacios, J. H., & Betancourt, C. (2009). Evaluación de 16 genotipos de quinua dulce (*Chenopodium quinoa* Willd.) en el municipio de Iles, Nariño (Colombia). *Agronomía Colombiana, 27*(2), 159–167. https://revistas.unal.edu.co/index.php/agrocol/article/view/11125

Delgado, H., Tapia, C., Manjarres-Hernández, E. H., Borja, E., Naranjo, E., & Martín, J. P. (2024). Phenotypic diversity of quinoa landraces cultivated in the Ecuadorian Andean region: In situ conservation and detection of promising accessions for breeding programs. *Agriculture, 14*(3), Article 336. https://doi.org/10.3390/agriculture14030336

EL-Harty, E. H., Ghazy, A., Alateeq, T. K., Al-Faifi, S. A., Khan, M. A., Afzal, M., Alghamdi, S. S., & Migdadi, H. M. (2021). Morphological and molecular characterization of quinoa genotypes. *Agriculture, 11*(4), Article 286. https://doi.org/10.3390/agriculture11040286

Emrani, N., Hasler, M., Patiranage, D. S. R., Maldonado-Taipe, N., Rey, E., & Jung, C. (2020). An efficient method to produce segregating populations in quinoa (*Chenopodium quinoa*). *Plant Breeding, 139*(6), 1190–1200. https://doi.org/10.1111/pbr.12873

Estrada-Zúñiga, R., Apaza-Mamani, V., Pérez-Ávila, A. A., Altamirano-Pérez, A. M., Neyra-Valdez, E., & Bobadilla, L. G. (2022). Comportamiento agronómico de 81 genotipos de quinua (*Chenopodium quinoa* Willd.) en el Perú. *Ecosistemas y Recursos Agropecuarios, 9*(1), Article e3134. https://doi.org/10.19136/era.a9n1.3134

Flórez-Martínez, D. H., Rodríguez-Cortina, J., Chávez-Oliveros, L. F., Aguilera-Arango, G. A., & Morales-Castañeda, A. (2024). Current trends and prospects in quinoa research: An approach for strategic knowledge areas. *Food Science & Nutrition, 12*(3), 1479–1501. https://doi.org/10.1002/fsn3.3891

Foronda Limachi, E. M. (2022). *Evaluación fenológica, morfológica y nutricional de cuatro progenies de quinua (Chenopodium quinoa* Willd.*) generación M3 bajo condiciones semicontroladas* [Doctoral dissertation, Universidad Mayor de San Andrés, Bolivia]. https://repositorio.umsa.bo/xmlui/handle/123456789/29078

García-Parra, M. A., García-Molano, J. F., & Carvajal-Rodríguez, D. C. (2018). Evaluación del efecto de la fertilización química y orgánica en la composición bromatológica de semillas de quinua (*Chenopodium quinoa* Willd.) en Boyacá–Colombia. *Revista de Investigación Agraria y Ambiental, 9*(2), 99–108. https://hemeroteca.unad.edu.co/index.php/riaa/article/view/2282

García-Parra, M. A., Roa-Acosta, D. F., Stechauner-Rohringer, R., García-Molano, F., Bazile, D., & Plazas-Leguizamón, N. (2020). Effect of temperature on the growth and development of quinoa plants (*Chenopodium quinoa* Willd.): A review on a global scale. *Sylwan, 164*(5), 411–433. https://agritrop.cirad.fr/595816/

Hussain, M. I., Muscolo, A., Ahmed, M., Asghar, M. A., & Al-Dakheel, A. J. (2020). Agro-morphological, yield and quality traits and interrelationship with yield stability in quinoa (*Chenopodium quinoa* Willd.) genotypes under saline marginal environment. *Plants, 9*(12), Article 1763. https://doi.org/10.3390/plants9121763

Madrid, D., Salgado, E., Verdugo, G., Olguín, P., Bilalis, D., & Fuentes, F. (2018). Morphological traits defining breeding criteria for coastal quinoa in Chile. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *46*(1), 190–196. https://doi.org/10.15835/nbha46110788

Manjarres-Hernández, E. H., Morillo-Coronado, A. C., Ojeda-Pérez, Z. Z., Cárdenas-Chaparro, A., & Arias-Moreno, D. M. (2021). Characterization of the yield components and selection of materials for breeding programs of quinoa (*Chenopodium quinoa* Willd.). *Euphytica, 217*(6), Article 101. https://doi.org/10.1007/s10681-021-02837-5

Montes-Rojas, C., Burbano-Catuche, G. A., & Muñoz-Certuche, E. F. (2020). Evaluación del rendimiento de quinua bajo diferentes densidades, fertilización y métodos de siembra en Cauca. *Biotecnología en el Sector Agropecuario y Agroindustrial, 18*(1), 46–55. https://revistas.unicauca.edu.co/index.php/biotecnologia/article/view/1410/1105

Montes-Rojas, C., Burbano-Catuche, G. A., Muñoz-Certuche, E. F., & Calderón-Yonda, Y. (2018). Descripción del ciclo fenológico de cuatro ecotipos de quinua (*Chenopodium quinoa* Willd.) en Puracé-Cauca, Colombia. *Biotecnología en el Sector Agropecuario y Agroindustrial, 16*(2), 26–37. https://revistas.unicauca.edu.co/index.php/biotecnologia/article/view/1163

Morillo, A. C., Manjarres, E. H., Reyes, W. L., & Morillo, Y. (2020). Intrapopulation phenotypic variation in Piartal (*Chenopodium quinoa* Willd.) from the Department of Boyacá, Colombia. *African Journal of Agricultural Research, 16*(8), 1195–1203. https://doi.org/10.5897/AJAR2020.14916

Morillo-Coronado, A. C., Manjarres-Hernández, E. H., & Pedreros-Benavides, M. C. (2023). Genetic diversity of quinoa (*Chenopodium quinoa* Willd.) from Cundinamarca, Colombia. *Plant Genetic Resources: Characterization and Utilization, 20*(3), 223–229. https://doi.org/10.1017/S1479262123000060

Pizo-Ossa, M. A., Montes-Rojas, C., Aguilera-Arango, G. A., Ramos-Villafañe, Y. P., López-Hoyos, J. L., & Morillo-Coronado, Y. (2024). Evaluation of the phenological cycle and morphological characterization of quinoa genotypes (*Chenopodium quinoa* Willd.). *Tropical and Subtropical Agroecosystems, 27*(2), Article 075. https://doi.org/10.56369/tsaes.5397

Reguera, M., Conesa, C. M., Gil-Gómez, A., Haros, C. M., Pérez-Casas, M. Á., Briones-Labarca, V., Bolaños, L., Bonilla, I., Álvarez, R., Pinto, K., Mujica, Á., & Bascuñán-Godoy, L. (2018). The impact of different agroecological conditions on the nutritional composition of quinoa seeds. *PeerJ Life* & *Enviroment, 6*, Article e4442. https://doi.org/10.7717/peerj.4442

Sampaio, S. L., Fernandes, Â., Pereira, C., Calhelha, R. C., Soković, M., Santos-Buelga, C., Barros, L., & Ferreira, I. C. F. R. (2020). Nutritional value, physicochemical characterization, and bioactive properties of the Brazilian quinoa BRS Piabiru. *Food* & *Function, 11*(4), 2969–2977. https://doi.org/10.1039/D0FO00055H

Sinaga, K. P., & Yang, M. S. (2020). Unsupervised K-means clustering algorithm. *IEEE Access, 8*, 80716–80727. https://doi.org/10.1109/ACCESS.2020.2988796

Urdanegui, P., Pérez-Ávila, Á., Estrada-Zuñiga, R., Neyra, E., Mujica, Á., & Corredor, F.-A. (2021). Rendimiento y evaluación agromorfológica de genotipos de quinua (*Chenopodium quinoa* Willd.) en Huancayo, Perú. *Agroindustrial Science, 11*(1), 63–71. https://doi.org/10.17268/agroind.sci.2021.01.08

Fruit productivity of “Pera” sweet orange grafted on different rootstocks in the mesoregion of Northeast Pará (Brazil)

Productividad de frutos en naranja dulce “Pera” injertada sobre diferentes portainjertos en la mesorregión del Nordeste de Pará (Brasil)

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ABSTRACT

Citrus is among the most important cultivated species in the world. However, the Northern region of Brazil, despite its available cultivation, still presents incipient production and faces numerous environmental factors that require further study to mitigate the impact of genotype-by-environment interactions. To address this issue, an experiment was set up in the municipality of Capitão Poço, Pará, using a completely randomized block design to evaluate six graft/rootstock combinations with AMMI and GGE Biplot analyses. The variable assessed was total fruit weight (FW), that is the total of fruits produced by the plant, measured in kg, in the 2018, 2019, 2020, and 2021a (first half of the year) and 2021b (second half of the year) harvests. Superior rootstocks were ‘Santa Cruz’ Rangpur lime (*C.* x *limonia* Osbeck) (T1) and ‘San Diego’ citrandarin (TSK x TRENG-314) (T10). Although T1 and T10 had low stability in certain years, for ideotype aspect T1 was superior in relation to the other rootstocks and, despite the search for more promising materials, which here were the least stable, it must be accepted that there are risks, as there is no way to predict production in later years. Future research should identify which environmental factors favor fruit productivity and which generate instability in the Capitão Poço region.

Keywords: *Citrus sinensis* (L.) Osbeck, graphical analysis, stability, adaptability, genetic improvement, perennial plants, Amazon region.

RESUMEN

Los cítricos están entre las especies cultivadas más importantes del mundo, pero la región norte de Brasil, a pesar de su área disponible para el cultivo, aún tiene una producción incipiente y, por presentar infinitas combinaciones de efectos ambientales, se requieren estudios que puedan evaluar el efecto de la interacción genotipo x ambiente. Para abordar esta problemática, se estableció un experimento en el municipio de Capitão Poço del Estado de Pará siguiendo un diseño en bloques completamente al azar con el fin de evaluar seis combinaciones de injerto/portainjerto mediante un análisis gráfico AMMI y GGE Biplot. La variable evaluada fue peso total de los frutos (FW) que corresponde a la suma del total de frutos producidos por la planta, medido en kg, en las cosechas de 2018, 2019, 2020 y 2021a (primera mitad del año) y 2021b (segunda mitad del año). Los portainjertos más destacados fueron los de limón Rangpur “Santa Cruz” (*C.* x *limonia* Osbeck) (T1) y citrandarin “San Diego” (TSK x TRENG-314) (T10). Aunque T1 y T10 tuvieron baja estabilidad para ciertos años, para el aspecto ideotipo T1 fue superior con relación a los demás portainjertos y a pesar de la búsqueda de materiales más prometedores, que en este caso fueron los menos estables, es necesario aceptar que existen riesgos, ya que no es posible predecir la producción en años posteriores. Investigaciones futuras deberían identificar cuáles factores ambientales favorecen la productividad frutícola y cuáles generan inestabilidad en la región de Capitão Poço.

Palabras clave: *Citrus sinensis* (L.) Osbeck, análisis gráfico, estabilidad, adaptabilidad, mejoramiento genético, plantas perennes, región Amazónica.

Introduction

Citrus fruits have become among the most important cultivated species in the world, consumed fresh or processed into juices, sweets, and other by-products (Passos *et al*., 2016; Silva *et al*., 2016). In the North region of Brazil, orange production stands out in the State of Pará, which in 2021 harvested 14,200 ha, yielding 233,051 t, associated with an economic value of US\$22 million and reaching an average yield of 16,412 kg ha^{-1}. Compared with other

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regions, Pará represents almost the entire northern region of Brazil (IBGE, 2021).

Given the importance of citrus farming, several studies have examined the potential of and minimized the challenges of the national citrus chain, seeking to provide security and enable socioeconomic development (Borges & Costa, 2005; Pimentel *et al.*, 2014). Among these studies, some evaluate scion/rootstock combinations resistant or tolerant to the principal biotic stresses (*e.g.*, pests or diseases) and abiotic stresses (*e.g.*, drought and high temperatures) (Bastos, Sombra, Andrade *et al.*, 2017; Bastos, Sombra, Loureiro *et al.*, 2017; Rodrigues *et al.*, 2016; Silva & Vieira, 2015).

The enormous potential for expanding fruit cultivation areas in Brazil generates a phenomenon known as genotype-by-environment interaction (GxE). This interaction results in significant differences in plant performance across heterogeneous locations (Cruz *et al.*, 2014). It is one of the main obstacles to plant selection.

In the manifestation of GxE interaction, the genotype's behavior becomes uncertain when changing from one location to another. This reaction reflects different responses of the same genes set to environmental changes (Muthoni *et al.*, 2015). There is no way to eliminate GxE interaction, as it is associated with a physiological reaction existing in plants (Adewale *et al.*, 2010). This event certainly disrupts the breeder's selection processes (Carvalho *et al.*, 2016). One possible solution to mitigate the effects of this interaction is to identify plants with broad adaptability and good stability in different environments (Cruz *et al.*, 2014).

Based on these particularities, plant genetic improvement programs usually aim to select several individuals with broad adaptation and stability, which can then be recommended for various environments (Malosetti *et al.*, 2013). There are three ways for GxE interaction to occur: detecting cultivar(s) with specificity for each environment; detecting cultivar(s) with high phenotypic stability; and obtaining environmental stratifications (Ramalho *et al.,* 2024). However, to get these results, it is necessary to perform statistical or graphical analyses on the genotypes and environments under study.

A graphical methodology for visualizing and interpreting GxE interaction is called AMMI ("Additive Main Effects and Multiplicative Interaction Model"), where the generated graphs make it easy to discern different interaction patterns, allowing better prediction of results across different genotypes and environments used in the analyses (Silva, 2016). Additionally, it provides information to understand how different genetic materials behave in terms of stability and adaptability (Karimizadeh *et al.*, 2016; Ramalho *et al.*, 2024).

A second and essential methodological procedure for estimating the effects of the existing GxE interaction is the so-called GGE biplot, which graphically expresses in biplot format an overview of the grouping of environments or mega environments and the superior or inferior genotypic performance in specific environments; it also allows the selection of genotypes based on the average relationship vs. stability, and discrimination vs. representativeness; and also finding the genotype that is perfect and desired by researchers (ideotype) (Yan, 2001; Yan, 2011; Yan & Holland, 2010; Yan & Kang, 2003; Yan & Tinker, 2006).

In the northeastern region of Pará, the municipality of Capitão Poço and other adjacent municipalities in the Guamá microregion are home to the largest citrus-growing region in Pará (SECOM, 2021). However, there are no studies on the stability and adaptability of rootstocks relevant to genetic improvement programs used by local producers. This justifies the need to understand the behavior of different genotypes under local conditions. Based on this information and using AMMI and GGE biplot analyses, the objective of this study was to evaluate the effects of genotype-by-environment (GxE) interactions on early productivity of orange clones, aiming to identify superior genotypes in environmental variations.

Materials and methods

We conducted our research in the rural part of the municipality of Capitão Poço, located in the northeast region of the state of Pará, Brazil, 73 m a.s.l., 47º03'34'' W, 01º44'47'' S. This municipality has a temperature range that varies from 25.7°C to 26.9°C, with an annual average of 26.2ºC (Silva *et al.*, 2011). According to the Köppen classification, the climate of the region is of the Am type (tropical monsoon climate), with annual precipitation around 2,500 mm, with a short dry season between September and November (monthly precipitation around 60 mm), and relative humidity of the air between 75% and 89% in the months with the least and most precipitation (Schwartz, 2007). The soil type is Dystrophic Yellow Latosol (Ribeiro *et al.*, 2006). The experimental design used was randomized blocks with six rootstocks (Tab. 1), four replicates, and 5 plants per plot, spaced 4.0 m between plants and 5.5 m between rows, with irrigation only during the dry season (August - November).

TABLE 1. Rootstocks used for the scions of 'Pera' sweet orange tree [*Citrus sinensis* (L.) Osbeck]. Lima Farm, Capitão Poço, PA, Brazil.

Number	Rootstock description
T1	"Santa Cruz" Rangpur lime (*C.* × *limonia* Osbeck)
T7	Hybrid LVK (Volkamer lemon [*C.* × *volkameriana* (Risso) V. Ten. & Pasq.]) x LCR-010 (Rangpur lime)
T10	"San Diego" citrandarin (TSK [Sunki mandarin] × TRENG-314 [*P. trifoliata* cv. Swingle])
T12	BRS Pompeu (TSKC [Sunki mandarin] x CTSW-028 Citrumelo Swingle [*Citrus paradisi* × *Poncirus trifoliata*])
T13	TSKC (Sunki mandarin [C. Sunki (Hayata) hort. x Tanaka] x CTSW-033 (Citrumelo Swingle [*Citrus paradisi* × *Poncirus trifoliata*])
T16	'Riverside' citrandarin (TSKC x TRENG-264)

The rootstock seedlings were produced in a greenhouse with 50% shade; the seeds to produce six rootstocks were obtained from the active germplasm bank of Embrapa Cassava and Fruits (Cruz das Almas, Bahia, Brazil). When the rootstocks reached the appropriate diameter (about 1 cm), inverted T-type budding was performed using buds of ´Pera' sweet orange [*Citrus sinensis* (L.) Osbeck] and the planting was carried out when the seedlings were about 11 months old after grafting and 0.90 cm tall in a nursery located in Santa Luzia, 15 km from the municipality of Capitão Poço, PA. Specific cultural practices for citrus cultivation were carried out in accordance with farm practices, including monitoring and removing unwanted plants, crowning plants, creating basins around plants to allow for water accumulation during rainfall and for irrigation, and using mulch. Fertilization was performed according to Lima Farm's nutritional program, with 1 kg of thermophosphate (20% P_2O_5) and 1 kg of 9-9-19 NPK formulation per year.

The characteristic evaluated was the total weight of fruits (FW), that is the sum of the fruits produced by each plant measured in kilograms in 2018, 2019, 2020, and 2021a (first half of the year) and 2021b (second half of the year) when the plants were three years old. This characteristic was assessed since it results from all the others that involve productivity aspects (number of fruits, fruit weight, and fruit size); therefore, for the purposes of this research, the total fruit weight was considered sufficient to describe the behavior of the rootstocks.

AMMI

The statistical treatment of the data, including analysis of variance and analysis of stability and adaptability via the AMMI model, was performed using the R program version 3.4.1 (R Core Team, 2020).

The AMMI analysis, described by Duarte and Vencovsky (1999), was based on the model:

$$Y_{ij} = \mu + g_i + a_j + \sum_{k=1}^{n} \lambda_k \gamma_{ik} \alpha_{jk} + \rho_{ij} + \bar{\varepsilon}_{ij} \qquad (1)$$

where Y_{ij} is the average response of the repetitions of the i-th progeny (i = 1, 2, 3, ..., g) in the j-th year (j = 1, 2, 3, ..., a); μ is the average of all progenies in all years (general average); g_i is the main effect of progeny "i"; a_j is the main effect of year "j"; λ_k, γ_{ik} and α_{jk} refer to the terms of the singular decomposition (SVD), also called principal component analysis (PCA), of the matrix $GE_{gxe}=\{(ge)_{ij}\}$, which expresses and captures the "pattern" regarding the interaction of progeny "i" with year "j"; ρ_{ij} represents the additional noise to be eliminated in the analysis, relative to the term routinely accepted as the interaction itself; and $\bar{\varepsilon}_{ij}$ is the experimental error, assuming i.i.d.~N(0, σ^2).

The AMMI analysis involves two steps: first, the main effects; then the additive part of the model (general mean, progeny, and year effects), adjusted by analysis of variance (ANOVA), generating a non-additivity residual $(\hat{ge})_{ij} = \bar{Y}_{ij} - \bar{Y}_{i.} - \bar{Y}_{.j} + Y_{..}$. That is, the ordinary least squares estimates of $(ge)_{ij}$; in the second step, the interaction (multiplicative part of the model) is adjusted using DVS or PCA applied to the matrix $GE_{gxe}=\{(\hat{ge})_{ij}\}$, resulting in the part named as "standard" (being the interaction – AMMI interaction), and the other denominated as "noise", which should be included in the residual in the analysis of variance.

To determine how many main axes should be considered to explain and graphically represent the interaction pattern, the criteria presented by Gauch Jr. and Zobel (1988) were adopted, where the proportion of the sum of squares SQ_{GxE} of the original interaction accumulated up to the n_{th} axis is considered:

$$\sum_{k=1}^{n} \lambda_k^2 / SQ_{GxE} \qquad (2)$$

GGE Biplot

The GGE Biplot method, according to Yan *et al*. (2000), considers the main effect of progeny and its interaction with years, which are essential and considered concomitantly. The GGE Biplot model maintains G of GxE together in the format of two multiplicative terms, employing Equation 3:

$$Y_{ij} - \bar{y}_j = y_1\varepsilon_{i1}\rho_{j1} + y_2\varepsilon_{i2}\rho_{j2} + \varepsilon_{ij} \quad (3)$$

where Y_{ij} symbolizes the average performance of the i-th progeny in the j-th year; $\bar{y}_j$ symbolizes the overall average of the progenies for year j; $y_1\varepsilon_{i1}\rho_{j1}$ is equivalent to the first principal component (IPCA1) associated with the eigenvalue from IPCA1; $y_2\varepsilon_{i2}\rho_{j2}$ is comparable to the second principal component (IPCA2) associated with the eigenvalue from IPCA2; ε_{i1} and ε_{i2} symbolize the scores of the first and second principal component, respectively, of the i-th progeny; ρ_{j1} and ρ_{j2} symbolize the scores of the first and second principal component, respectively, for the j-th year; ε_{ij} is equivalent to the model error associated with the i-th progeny in the j-th year (Yan & Kang, 2003).

Additionally, the information ratio (IR) proposed by Yan and Tinker (2006) was estimated to assess whether the biplot is suitable for displaying the patterns in a double-entry table. This relationship is interpreted based on each PC axis (interaction axis of the principal components analysis): IR ≥ 1 or close to 1 indicates patterns (associations between years), and a PC with IR < 1 indicates the absence of any pattern or information. Therefore, a biplot of dimension 2 can adequately represent the patterns in the data only if the first two PCs have IR ≥ 1 or close to 1.

Results and discussion

In the summary of the analysis of variance (Tab. 2), in all scion/rootstock combinations (G), significance was observed by the F test, that is, there are distinct performances that are indicative of the presence of superior materials and differences also occurring for the years of evaluation, as well as in the GxE interaction. This allows the continuity of the statistical analyses through the AMMI and GGE Biplot methodologies.

The experimental coefficients of variation as classified by Gomes (2022) were much higher than those reported by Costa *et al.* (2021). It is important to note that this scale does not represent the reality of perennial species or many fruit trees, including the orange tree. Therefore, although this scale is helpful for general discussions of agricultural research results, it is inappropriate because the precision assessment depends on the response variable in the study.

An appropriate CV classification for citrus should consider this species' peculiarities, the characteristics being evaluated, the number of replicates, and the experimental design, among other essential aspects. This may be because some variables are naturally more variable among fruits from the same plant or because the measurements themselves tend to be less homogeneous (Silva *et al.*, 2011). Therefore, it is evident that the productivity component (FW) is a trait under quantitative genetic control, with phenotypic performance strongly influenced by environmental conditions and shaped by distinct gene complexes (Cruz, 2012; Maia *et al.*, 2010).

TABLE 2. Summary of the analysis of variance for scion/rootstock combinations in 'Pera' sweet orange trees, being years (E); repetitions within years R(E); genotypes (G), effect of the interaction between genotypes and years (GxE), acumulative percentage (PA), mean square (QM) of the first four component axes (PC1, PC2, PC3, and PC4) on fruit weight (FW, kg/plant). Capitão Poço, PA, Brazil.

	E	R(E)	G	GxE
GL	4	15	5	20
	37599**	652**	4409**	754**
CV	103.48			
Mean	12.20			
	PA	QM		
PC1	69.7	144.236[b]		
PC2	98.8	80.282[c]		
PC3	99.4	2.845[a]		
PC4	100.0	4.657[a]		

ns: not significant; **: significant at 1%; *: significant at 5% by F test.

[a]: not significant; [b]: significant at 1%; [c]: significant at 5% by the Fisher-Ford test.

Table 2 also provides a summary of the principal component analysis, where the first two axes (PC1 and PC2) accounted for more than 82%, which is higher than the value reported by Costa (2019) at 66%, Costa *et al.* (2021) at 69%, and Carvalho *et al.* (2020) at 66.71%. Therefore, the first axis captured a greater portion of the main effects.

The data accumulated here by the first two component axes can be considered sufficient, since Yang *et al.* (2009) state that the first two principal components should account for at least 60% of the total variance. Since the F_{Gollob} test indicates the significance of only the first two axes, it allows us to conclude that the AMMI analysis with these axes captures all variations attributed to genetic and environmental effects directly related to the interaction, while discarding the effects of noise or stochastic effects, which can significantly hinder the analysis interpretation (Maia *et al.*, 2009). Therefore, the AMMI2 model used here aligns with the standard for studying GxE interaction within a data set.

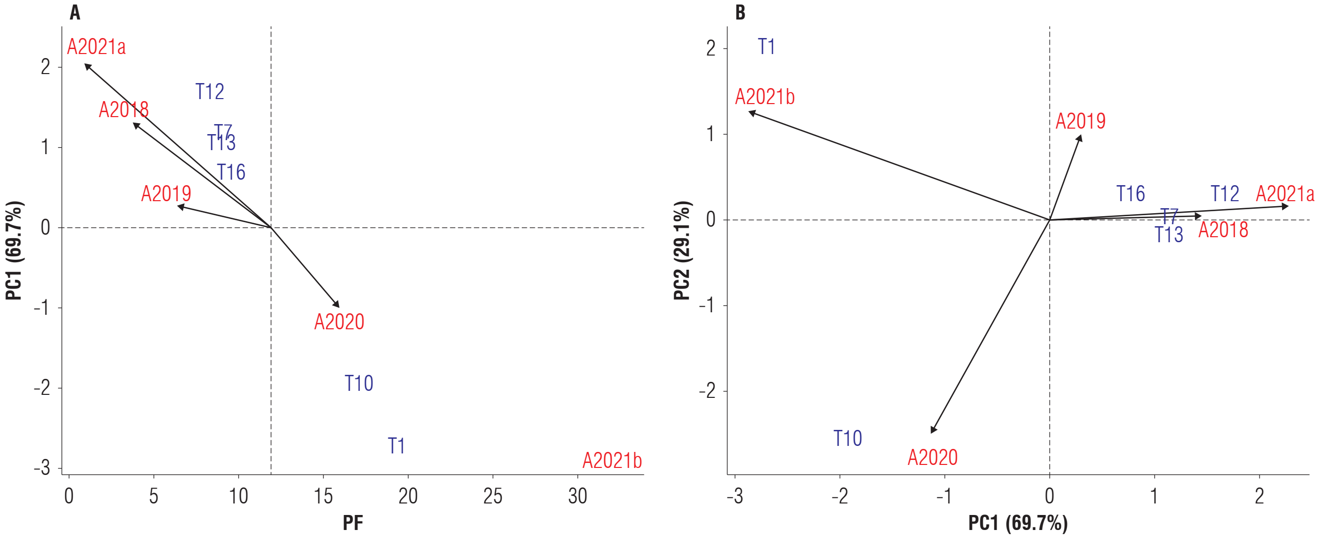

FIGURE 1. AMMI analysis for fruit weight (FW) trait. (A) Biplot AMMI1, means (x) vs. PC1 (y) and (B) Biplot AMMI2, PC1 (x) vs. PC2 (y), with environments corresponding to years for scion/rootstock combinations in 'Pera' sweet orange trees. Years are identified numerically, and combinations are recognized as T followed by a number. Capitão Poço, PA, Brazil.

In the AMMI1 analysis for FW (Fig. 1), T16 was the most stable material. Still, it contributed to the average, which is undesirable because it falls below the comparative average of all scion/rootstock combinations involved in the study. The materials with the most significant positive contributions (T1 and T10), unfortunately, exhibited instability, as evidenced by their distance from the horizontal axis and confirmed by the AMMI2 graph, which plots them far from the origin.

Based on graphic dispersion from scion/rootstock combinations, with different performances or contributions along the axes of the evaluated characteristics, it is possible to infer that materials with stability, specific adaptability, and better averages are valuable in genetic improvement programs. These results are consistent with those obtained by Carvalho *et al.* (2020), Ferrer *et al.* (2022), Huang *et al.* (2020), and Singh *et al.* (2023) in AMMI analysis.

In the GGE Biplot graphical analysis, summarized in the analysis of variance presented in Table 3, to verify the representativeness (PCs) of the treatment behavior, the first two axes account for a sum above 90%, exceeding the 60% minimum suggested by Yang *et al.* (2009). This confirms the reliability of the treatment performance distribution, which results from the G+GxE interaction and helps explain the total variation. The choice of a two-axis model, therefore, is satisfactory for portraying the behavior of the genetic material and the contribution of years, while disregarding potential noise or stochastic effects, which can cause distortions and make graph interpretation extremely difficult (Maia *et al.*, 2009). This model also exceeds the values reported by Costa (2019) (66%) and Costa *et al.* (2021) (69%).

Information ratios (IR) were also considered, with the first component axis showing values above 4 for all three characteristics, capturing almost all the contributions of genetic or non-environmental effects. In contrast, the second component reveals no clear pattern. Thus, a two-dimensional biplot portrays the data pattern, consistent with the approach defined by Yan and Tinker (2006). Consequently, the other component axes (PC3 and PC4) can be regarded as having only noise (IR<1), with no contribution to the interaction effect.

TABLE 3. Eigenvalues, explained variance (Ve%), cumulative explained variance (Va%), and information ratio (IR), considering the first four principal components (PCs), in fruit weight (FW, kg/plant) for scion/rootstock combinations in 'Pera' sweet orange trees. Capitão Poço, PA, Brazil.

Character	Parameters	PC1	PC2	PC3	PC4	PC5
FW	Eigenvalues	29.607	10.975	2.250	1.532	0.024
	Ve %	87.27	11.99	0.50	0.23	0.01
	Va %	87.27	99.26	99.76	99.99	100.00
	IR	4.36	0.60	0.03	0.01	0.00

In the graphical analysis of mega-environment identification and treatment specificity, we observed that for FW, no year was associated with T7, T13, T12, or T16, indicating no specificity in this aspect. In contrast, T10 was associated with 2020 and T1 with 2021b (Fig. 2).

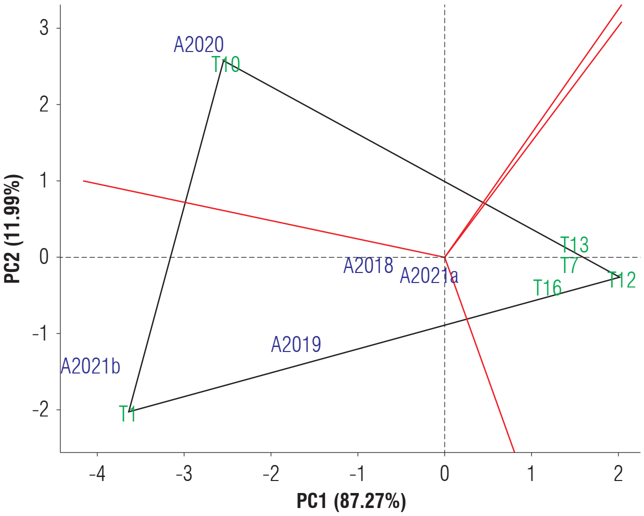

FIGURE 2. Indicative of which rootstocks performed best and in which years by GGE biplot ("Which-won-where") in fruit weight (FW) for scion/rootstock combinations in "Pera" sweet orange trees. Capitão Poço, PA, Brazil. The solid red lines delimit sectors that define the mega-environments.

Ideal test environments for identification and superior genotypes selection must possess both discriminative capacity and representativeness (Pereira *et al*., 2017). Environments with long vectors are the most discriminative, providing the most information about genotypes (Yan, 2016). The representative one is the one that forms the smallest angle with the average environment axis (EAM, the line that passes through the average environment and the biplot origin) and represents an average behavior across all environments. Therefore, in terms of the discriminative capacity, which aims to identify which sources of variation related to treatments and years have the most significant contribution to the variations, for FW (Fig. 3), the years 2019, 2020, and 2021b were the most discriminative, with treatments T1 and T10 also having an essential contribution in the total.

A second interpretation from the graphical analysis in Figure 3 is the identification of the year that represents the average of all others, effectively representing the average effect of all years. Thus, the year with the smallest angle relative to the EAM axis for FW was 2018, which was the average of all other years.

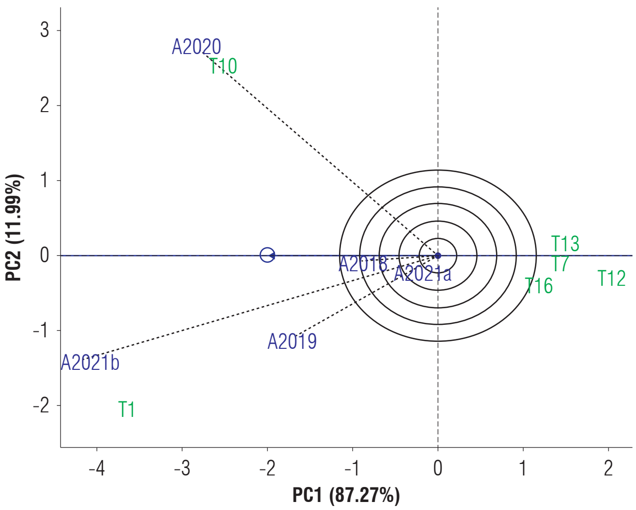

FIGURE 3. GGE biplot "discrimination and representativeness" to show the discrimination ability and representativeness of the test environments compared to the genetic materials in fruit weight (FW) for scion/rootstock combinations in 'Pera' sweet orange trees. Capitão Poço, PA, Brazil.

In Figure 4, the straight line (EAM) with an arrow indicates the scion/rootstock combinations that lie beyond the tip of the arrow are those that showed the highest average contribution among the materials evaluated (Yan, 2002; Yan, 2011). However, it is also noted that materials with longer vectors moving away from the EAM axis exhibit the least stability. Thus, in Figure 4 for FW, the treatments T7, T12, T13, and T16 were highly stable as indicated by their

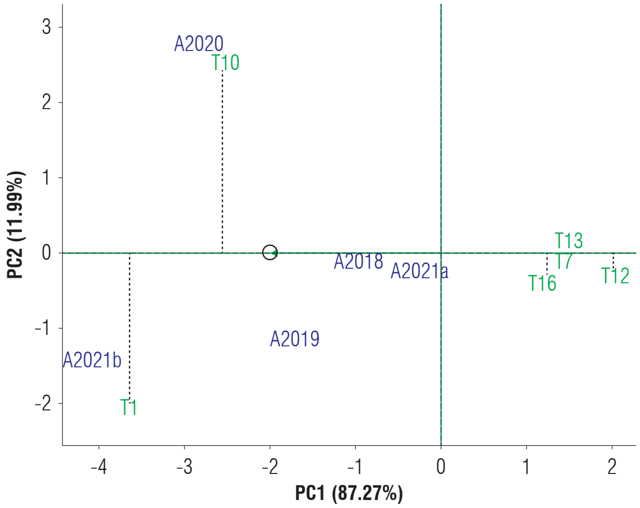

FIGURE 4. Average performance and stability of treatments by GGE biplot ("Average versus Stability") with environment mean axis (EAM) in fruit weight (FW) for scion/rootstock combinations in 'Pera' sweet orange trees. Capitão Poço, PA, Brazil.

proximity to the horizontal axis. Among the circles at the tip of the axis, those with the least stability were the ones with a positive contribution to the averages. This indicates that the environmental conditions generated different responses, with T1 and T10 exhibiting low stability and being more similar to genotypes with specific adaptability, due to their proximity to certain years.

A plant with above-average performance and high stability is the desired plant in genetic improvement programs and is called an "ideotype", or simply the perfect plant, associating the better relations between productivity, disease resistance and stability (Trethowan, 2014) in each specific situation. Although it is only a representative model, its identification serves as a reference in comparison with other materials. The graphical analysis of the GGE Biplot, titled "Average versus Stability," is an effective tool for identifying these ideotypes (Yan, 2011; Yan *et al*., 2007; Yan & Tinker, 2006).

The ideotype for the here-evaluated characteristic in the GGE Biplot (Fig. 5) is located at the center of the concentric circles; those plotted near this center or in the first circumcircles were considered promising. Thus, for FW, although T1 and T10 showed specificity and the greatest contributions to variation and were located in the circumcircles closest to the center, they were considered the best-performing ideotype among the materials evaluated.

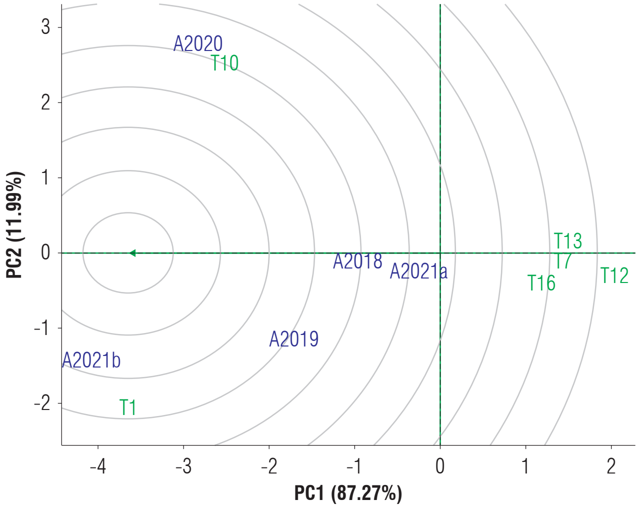

FIGURE 5. Classification of treatments in relation to ideotype (in the center of the concentric circles) by GGE biplot with environment-mean axis (EAM) in fruit weight (FW) for scion/rootstock combinations in 'Pera' sweet orange trees. Capitão Poço, PA, Brazil.

Citrus cultivation requires a variety of rootstocks for specific conditions, leading to differentiated performances in several characteristics, such as fruit quality and quantity, plant vigor and size, tolerance to abiotic factors, and resistance/tolerance to biotic factors (Medina *et al*., 2005; Santana *et al*., 2018). The results obtained here, selecting materials with greater stability, productivity, and a closer approach to the ideotype due to the presence of the GxE interaction, enabled the identification of promising clone/rootstock combinations. This study focused solely on fruit productivity, including components such as the number, weight, size, and quantity of fruits, again justifying its use here; however, in future research, evaluations of fruit quality and fruit harvest time should be added.

Conclusions

Comparing the results of AMMI and GGE Biplot analyses, the best rootstocks are "Santa Cruz" Rangpur lime (*C.* x *limonia* Osbeck) (T1) and "San Diego" citrandarin (TSK x TRENG-314) (T10).

The ideotype "Santa Cruz" Rangpur lime is shown to be closer to the target sought by genetic improvement.

"Santa Cruz" Rangpur lime presents low stability, which is a hindrance. Still, on the other hand, it appears to be specific to certain years.

In this situation, the breeder must choose the most promising materials in terms of positive contribution to the averages, even if it means sacrificing the desired stability.

Despite the search for more promising materials, which here were the least stable, it must be accepted that there are risks, as there is no way to predict production in later years.

Future research should identify which environmental factors favor fruit productivity and which generate instability in the Capitão Poço region of Brazil.

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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: EAG, OSP, WSSF. Data curation and experimental evaluation: FLG. Formal analysis: KH. Funding acquisition: FLG, EAG, OSP, WSSF. Research: FLG.

Writing – original draft: GKIY. Writing – review & editing: FLG, KH, EAG, OSP, WSSF. All authors have read and approved the final version of the manuscript.

Literature cited

Adewale, B. D., OIkonj, C., Oyekanmi, A. A., Akintobi, D. A. C., & Aremu, C. O. O. (2010). Genotypic variability and stability of some grain yield components of cowpea. *African Journal of Agricultural Research*, *5*(9), 874–880. https://www.cabidigitallibrary.org/doi/full/10.5555/20103197359

Bastos, D. C., Sombra, K. E. S., Andrade, H. M., Santos Filho, L. G., & Passos, O. S. (2017). Biometric evaluation of orange cultivars using different rootstocks in the semiarid region of Ceará, Brazil. *Citrus Research & Technology*, *38*(1), 71–76. https://www.researchgate.net/publication/323858350_Biometric_evaluation_of_orange_cultivars_using_different_rootstocks_in_the_semiarid_region_of_Ceara_Brazil

Bastos, D. C., Sombra, K. E. S., Loureiro, F. L. C., Costa e Silva, A. C., & Passos, O. S. (2017). Initial development of Tahiti acid lime trees on different rootstocks in the semiarid region of Ceará, Brazil. *Citrus Research & Technology*, *38*(1), 77–82. https://www.citrusrt.ccsm.br/article/10.4322/crt.ICC045/pdf/citrusrt-38-1-77.pdf

Borges, A. C. G., & Costa, V. M. H. M. (2005). A evolução do agronegócio citrícola paulista e o perfil da intervenção do estado. *Revista Brasileira Multidisciplinar -ReBraM*, *9*(2), 101–124. https://doi.org/10.25061/2527-2675/ReBraM/2006.v9i2.270

Carvalho, H. W. L., Teodoro, A. V., Barros, I., Carvalho, L. M., Soares Filho, W. S., Girardi, E. A., Passos, O. S., & Pinto-Zevallos, D. M. (2020). Rootstock-related improved performance of ´Pera´ sweet orange under rainfed conditions of Northeast Brazil. *Scientia Horticulturae*, *263*, Article 109148. https://doi.org/10.1016/j.scienta.2019.109148

Carvalho, L. M., Carvalho, H. W. L., Soares Filho, W. S., Martins, C. R., & Passos, O. S. (2016). Porta-enxertos promissores, alternativos ao limoeiro "Cravo", nos Tabuleiros Costeiros de Sergipe. *Pesquisa Agropecuária Brasileira*, *51*(2), 132–141. https://doi.org/10.1590/S0100-204X2016000200005

Costa, D. P. (2019). *Desempenho horticultural e tolerância à seca de laranjeira "Valência" sobre porta-enxertos híbridos de citros na região norte do estado de São Paulo* [Doctoral dissertation, Universidade Estadual de Santa Cruz]. https://www.biblioteca.uesc.br/pergamumweb/vinculos/201511084T.pdf

Costa, D. P., Stuchi, E. S., Girardi, E. A., Moreira, A. S., Gesteira, A. S., Coelho Filho, M. A., Ledo, C. A. S., Silva, A. L. V., Leão, H. C., Passos, O. S., & Soares Filho, W. S. (2021). Less is more: a hard way to get potential dwarfing hybrid rootstocks for Valencia sweet orange. *Agriculture*, *11*(4), Article 354. https://doi.org/10.3390/agriculture11040354

Cruz, C. D. (2012). *Princípios de genética quantitativa*. Viçosa, Editora UFV. https://livraria.funep.org.br/product/principios-de-genetica-quantitativa

Cruz, C. D., Carneiro, P. C. S., & Regazzi, A. J. (2014). *Modelos biométricos aplicados ao melhoramento genético* (3rd ed.). Viçosa, Editora UFV. https://www.editoraufv.com.br/produto/modelos-biometricos-aplicados-ao-melhoramento-genetico/1109038?srsltid=AfmBOopRnWNtPB3gP51YfsqIP1pSY2Bxz3NUTMiceYleDLktCj6qB3ru

Duarte, J. B., & Vencovsky, R. (1999). *Interação genótipos x ambientes: uma introdução à análise AMMI*. Sociedade Brasileira de Genética. Ribeirão Preto, SP, Brazil. https://www.researchgate.net/publication/235941257_Interacao_genotipos_x_ambientes_uma_introducao_a_analise_AMMI

Ferrer, V., Paymal, N., Quinton, C., Costantino, G., Paoli, M., Froelicher, Y., Ollitrault, P., Tomi, F., & Luro, F. (2022). Influence of the rootstock and the ploidy level of the scion and the rootstock on sweet orange (*Citrus sinensis*) peel essential oil yield, composition and aromatic properties. *Agriculture*, *12*(2), Article 214. https://doi.org/10.3390/agriculture12020214

Gauch Jr., H. G., & Zobel, R. W. (1988). Predictive and postdictive success of statistical analysis of yield trials. *Theoretical and Applied Genetics*, *76*(1), 1–10. https://doi.org/10.1007/BF00288824

Gomes, F. P. (2022). *Curso de estatística experimental* (15th ed.). FEALQ, São Paulo. https://loja.fealq.org.br/produtos/curso-de-estatistica-experimental-15-edicao/

Huang, L., Grosser, J., Gmitter Jr., F. G., Sim, C. A., & Wang, Y. (2020). Effects of scion/rootstock combination on flavor quality of orange juice from Huanglongbing (HLB)-affected trees: a two-year study of the targeted metabolomics. *Journal of Agricultural and Food Chemistry*, *68*(10), 3286–3296. https://doi.org/10.1021/acs.jafc.9b07934

IBGE – Instituto Brasileiro de Geografia e Estatística (2025, April). *PAM – Produção Agrícola Municipal 2021 IBGE*. https://www.ibge.gov.br/estatisticas/economicas/agricultura-e-pecuaria/9117-producao-agricola-municipal-culturas-temporarias-e-permanentes.html?=&t=resultados

Karimizadeh, R., Asghari, A., Chinipardaz, R., Sofalian, O., & Ghaffarii, A. (2016). Determining yield stability and model selection by AMMI method in rain-fed durum wheat genotypes. *Turkish Journal of Field Crops*, *21*(2), 174–183. https://dergipark.org.tr/en/pub/tjfc/issue/37058/425744

Maia, M. C. C., Oliveira, L. C., Alvares, V. S., Maciel, V. T., Lessa, L. S., & Roncatto, G. (2010). Caracterização agroindustrial de clones experimentais de cupuaçuzeiro. Anais do Congresso Brasileiro de Fruticultura 21. Frutas: saúde, inovação e responsabilidade. Natal, October 12-22. https://www.alice.cnptia.embrapa.br/alice/bitstream/doc/866128/1/23705.pdf

Maia, M. C. C., Resende, M. D. V., Paiva, J. R., Cavalcanti, J. J. V., & Barros, L. M. (2009). Seleção simultânea para produção, adaptabilidade e estabilidade genotípicas em clones de cajueiro, via modelos mistos. *Pesquisa Agropecuária Tropical*, *39*(1), 43–50. https://revistas.ufg.br/pat/article/view/5704

Malosetti, M., Ribaut, J.-M., & van Eeuwijk, F. A. (2013). The statistical analysis of multi-environment data: modeling genotype-by-environment interaction and its genetic basis. *Frontiers in Physiology*, *4*, Article 44. https://doi.org/10.3389/fphys.2013.00044

Medina, C. L., Rena, A. B., Siqueira, D. L., & Machado, E. C. (2005). Fisiologia dos citros. In D. Mattos Junior, J. D. De Negri, R. M. Pio, & J. Pompeu Junior (Eds.), *Citros* (pp. 148–195). Fundag. https://pt.scribd.com/doc/257581876/Mattos-Junior-et-al-2005-Citros-Livro-Digital

Muthoni, J., Shimelis, H., & Melis, R. (2015). Genotype x environment interaction and stability of potato tuber yield and bacterial wilt resistance in Kenya. *American Journal of Potato Research*, *92*, 367–378. https://doi.org/10.1007/s12230-015-9442-z

Passos, O. S., Machado, C. F., Souza, A. S., Soares Filho, W. S., Souza, F. V. D., Cunha Sobrinho, A. P., Gesteira, A. S., Girardi, E. A., Barbosa, C. J., Santos Filho, H. P., Nascimento, A. S., & Rosa, R. C. C. (2016). *Banco ativo de germoplasma de citros da Embrapa Mandioca e Fruticultura*. Embrapa. https://www.infoteca.cnptia.embrapa.br/infoteca/bitstream/doc/1038877/3/FolderCitrosBAG2016portugues.pdf

Pereira, H. S., Alvares, R. C., Silva, F. C., Faria, L. C, & Melo, L. C. (2017). Genetic, environmental and genotype x environment interaction effects on the common bean grain yield and commercial quality. *Semina: Ciencias Agrárias*, *38*(3), 1241–1250. https://doi.org/10.5433/1679-0359.2017v38n3p1241

Pimentel, U. V., Martins, A. B. G., Barbosa, J. C., & Cavallari, L. L. (2014). Nutrição do porta-enxerto 'Flying Dragon'. *Revista Brasileira de Fruticultura*, *36*(2), 495–502. https://doi.org/10.1590/0100-2945-193/13

Ramalho, M. A. P, Abreu, A. F. B., Santos, J. B., & Nunes, J. A. R. (2024). *Aplicações da genética quantitativa no melhoramento de plantas autógamas*. UFLA. https://www.editoraufv.com.br/produto/aplicacoes-da-genetica-quantitativa-no-melhoramento-de-plantas-autogamas/1110838?srsltid=AfmBOopIuGDEwzhyfbXrFAQgl9Ev44eoOtSuZibziNXwaDTQKwJGdMWB

Ribeiro, S. I., Silva, E. G., & Ribeiro, N. S. V. (2006). Desempenho de laranjeiras em Capitão Poço, PA. Belém, PA: Embrapa Amazônia Oriental. 16 p. il (Embrapa Amazônia Oriental. Boletim de pesquisas e desenvolvimento, 57).

Rodrigues, M. J. S., Oliveira, E. R. M., Girardi, E. A., Ledo, C. A. S., & Soares Filho, W. S. (2016). Produção de mudas de citros com diferentes combinações copa e porta-enxerto em viveiro protegido. *Revista Brasileira de Fruticultura*, *38*(10), 187–201. https://doi.org/10.1590/0100-2945-284/14

Santana, L. G. L., Souza, E. S., Passos, O. S., Gesteira, A. S., Ledo, C. A. S., & Soares Filho, W. S. (2018). Vigor and mortality of citrus progênies with potential use as rootstocks. *Scientia Agricola*, *75*(4), 339–345. https://doi.org/10.1590/1678-992X-2016-0455

Schwartz, G. (2007). Manejo sustentável de florestas secundárias: espécies potenciais no nordeste do Pará, Brasil. *Amazônia: Ciência & Desenvolvimento*, *3*(5), 125–147. https://www.alice.cnptia.embrapa.br/alice/bitstream/doc/578357/1/ManejoSustentavelFloresta.pdf

SECOM – Secretaria de Comunicação do Estado do Pará (2021, June 8). *Pará se destaca cada vez mais no cenário nacional de citrus, devido à inexistência de pragas*. https://agenciapara.com.br/noticia/28860/para-se-destaca-cada-vez-mais-no-cenario--nacional-de-citrus-devido-a-inexistencia-de-pragas de citrus, devido à inexistência de pragas. Agência Pará.

Silva, A. R., Cecon, P. R., Rêgo, E. R., & Nascimento, M. (2011). Avaliação do coeficiente de variação experimental para caracteres de frutos de pimenteiras. *Revista Ceres*, *58*(2), 168–171. https://doi.org/10.1590/S0034-737X2011000200006

Silva, A. V. S., & Vieira, T. A. (2015). Agricultura e relações de gênero em uma comunidade rural de Santarém, Pará. *Cadernos de Agroecologia*, *10*(3). https://revista.aba-agroecologia.org.br/cad/article/view/17654/13283

Silva, C. E. F., Gama, B. M. V., Oliveira, L. M. T. M., Araujo, L. T., Araujo, M. L., Oliveira Junior, A. M., & Abud, A. K. S. (2016). Uso da laranja lima e seus resíduos no desenvolvimento de novos produtos. *Revista Brasileira de Engenharia de Biossistemas*, *10*(1), 69–96. https://doi.org/10.18011/bioeng2016v10n1p69-96

Singh, N., Sharma, R. M., Dubey, A. K., Awashi, O. P., Saha, S., Bharadwaj, C., Sharma, V. K., Sevanthi, A. M., Kumar, A., & Deepak. (2023). Citrus improvement for enhanced mineral nutrients in fruit juice through interspecific hybridization. *Journal of Food Composition and Analysis*, *119*, Article 105259. https://doi.org/10.1016/j.jfca.2023.105259

Trethowan, R. M. (2014). Defining a genetic ideotype for crop improvement. In D. Fleury, D., & R. Whitford (Eds.), *Crop breeding* (pp. 1–20). Humana New York, NY. https://link.springer.com/protocol/10.1007/978-1-4939-0446-4_1

Yan, W. (2001). GGEbiplot – A windows application for graphical analysis of multienvironment trial data and other types of two-way data. *Agronomy Journal, 93*(5), 1111–1118. https://doi.org/10.2134/agronj2001.9351111x

Yan, W. (2002). Singular-value partitioning in biplot analysis of multi-environment trial data. *Agronomy Journal, 94*(5), 990–996. https://doi.org/10.2134/agronj2002.9900

Yan, W. (2011). GGE biplot vs. AMMI graphs for genotype-by-environment data analysis. *Journal of the India Society of Agricultural Statistics, 65*(2), 181–193. https://www.researchgate.net/publication/304347399_GGE_biplot_vs_AMMI_graphs_for_genotype-by-environment_data_analysis

Yan, W. (2016). Analysis and handling of G x E in a practical breeding program. *Crop Science, 56*(5), 2106–2118. https://doi.org/10.2135/cropsci2015.06.0336

Yan, W., & Holland, J. B. (2010). A heritability-adjusted GGE biplot test environment evaluation. *Euphytica, 171*, 355–369. https://doi.org/10.1007/s10681-009-0030-5

Yan, W., Hunt, L. A., Sheng, Q., & Szlavnics, Z. (2000). Cultivar evaluation and mega-environment investigation based on GGE biplot. *Crop Science, 40*(3), 597–605. https://doi.org/10.2135/cropsci2000.403597x

Yan, W., & Kang, M. S. (2003). *GGE biplot analysis: a graphical tool for breeders, geneticists, and agronomists.* CRC Press. https://www.routledge.com/GGE-Biplot-Analysis-A-Graphical-Tool-for-Breeders-Geneticists-and-Agronomists/Yan-Kang/p/book/9780367454791?srsltid=AfmBOorI1NAD2K3YoHnY43du8eqIpDgZYjbALny2F7BqWlAeSPFrkIVu

Yan, W., Kang, M. S., Ma, B., Woods, S., & Cornelius, P. L. (2007). GGE biplots vs. AMMI analysis of genotype-by-environment data. *Crop Science, 47*(2), 643–653. https://doi.org/10.2135/cropsci2006.06.0374

Yan, W., & Tinker, N. A. (2006). Biplot analysis of multi-environment trial data: principles and applications. *Canadian Journal of Plant Science, 86*(3), 623–645. https://doi.org/10.4141/P05-169

Yang, R.-C., Crossa, J., Cornelius, P. L., & Burgueño, J. (2009). Biplot analysis of genotype x environment interaction: proceed with caution. *Crop Science, 49*(5), 1564–1576. https://doi.org/10.2135/cropsci2008.11.0665

Evaluation of an automatic drainage recirculation system in rose crop in terms of S, Na^+, and Cl^-

Evaluación de un sistema de recirculación automática de drenajes en el cultivo de rosa, en términos de S, Na^+ y Cl^-

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ABSTRACT

In Colombia, mixtures of substrates such as burned rice husk (BRH) and coconut fiber (CF) are used for the cultivation of cut flowers. Fertigation is applied to ensure 30% drainage, which can contaminate water and soil. In a drainage recycling system, there is a tendency for ions that are poorly absorbed by plants, such as sodium and chloride, to accumulate, which can create an ionic imbalance in the solution or salinize the substrate. An automatic drainage recycling system was built in rose cv. 'Charlotte' established in mixtures 100BRH, 65BRH:35CF, and 35BRH:65CF with 0%, 50%, and 100% drainage recycling. The contents of S, Na^+, and Cl^- were evaluated in drainages, substrates, and plant tissue at 0, 5, and 8 weeks after pruning (WAP). The SO_4^{2-} and Na^+ ions presented a similar behavior over time, with contents significantly higher in the substrates with higher CF contents at 0 WAP and the opposite at 8 WAP. In addition, the higher the BRH content and the recycling percentage, the higher the SO_4^{2-} in the drains. There was no significant difference in the S contents in substrates and plant tissue. Drainage recycling (50% and 100%) significantly increased Na^+ contents in the substrate. The Cl^- concentrations were significantly different at 8 WAP, being higher in treatments with recycling (50% and 100%), regardless of the type of substrate mixture.

Keywords: intensive horticulture, cut flowers, organic substrates.

RESUMEN

En Colombia se utilizan mezclas de sustratos como la cascarilla de arroz quemada (CAQ) y la fibra de coco (FC) para el cultivo de flores de corte. Se aplican volúmenes de fertirriego que aseguran drenajes de cerca del 30% que pueden contaminar aguas y suelos. En un sistema de reciclaje de drenajes, hay tendencia a la acumulación de iones que son poco absorbidos por las plantas como el sodio y el cloruro, los cuales pueden crear un desbalance iónico en la solución o salinizar el sustrato. Se construyó un sistema automático para el reciclaje de drenajes en un cultivo de rosa cv. 'Charlotte' establecido en los sustratos 100CAQ, 65CAQ:35FC y 35CAQ:65FC con 0, 50 y 100% de reciclaje de drenaje y se evaluaron los contenidos de S, Na^+ y Cl^- en drenaje, sustrato y tejido vegetal a las 0, 5 y 8 semanas después de poda (SDP). Los iones SO_4^{2-} y Na^+ presentaron un comportamiento similar en el tiempo, con contenidos significativamente mayores en los sustratos con mayor porcentaje de FC en la 0 SDP y lo contrario en la 8 SDP. Además, a mayores porcentajes de CAQ y de reciclaje mayor contenido de SO_4^{2-} en la solución drenada; entre tanto, no se constató diferencia significativa en los contenidos de S en sustrato y tejido. El reciclaje del drenaje (50 y 100%) incrementó significativamente el contenido de Na^+ en el sustrato. Las concentraciones de Cl^- fueron significativamente diferentes en la 8 SDP, con mayores concentraciones en los tratamientos con reciclaje (50 y 100%), independiente del tipo de mezcla de sustratos.

Palabras clave: horticultura intensiva, flores de corte, sustratos orgánicos.

Introduction

In intensive soilless horticulture, drainage recirculation systems are required for the proper collection, evaluation, recompositing, and recycling of the generated effluents. This type of system requires an approximate programming model to recycle the nutrient solution according to the most influential variables affecting quality of fertigation. Under the conditions of cultivation in the Bogotá plateau, the development of technologies that are within the reach of farmers and that allow the management and control of procedures such as fertigation, solution recycling, and climate control in greenhouse crops is needed. These technologies must be characterized by low cost and by a level

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of complexity that allows their use and management by personnel with basic knowledge of systems and cultivation conditions. In some cut flower crops in the Bogotá plateau, drainage is directed towards a reservoir to later use the irrigation water to prepare a new fertigation solution. This is not considered recycling, since the chemical composition of the reservoir water is altered by ions that are poorly absorbed by plants (Cuervo *et al.*, 2011).

In soilless culture systems, ions that are not absorbed by plants are drained and tend to accumulate in the recirculating nutrient solution, therefore, they must be discharged from the system. Frequently, in high-temperature environments, ion accumulation can lead to an increase in electric conductivity (EC) of the substrate up to 2.0 dS m^{-1} (Carmassi *et al.*, 2013). Roy *et al.* (2014) and Sonneveld *et al.* (1999) concluded that depending on the species, the absorption of sodium and chloride by the plant increases with the increases in their concentrations in the root environment, which is advantageous in counteracting their accumulation in the rhizosphere (Dotaniya & Meena, 2015). However, excess salts in the irrigation solution can negatively affect the vase life of harvested flowers (Atta-Aly *et al.*, 1998; Fujimoto *et al.*, 2000; Metwally *et al.*, 2018; Riley, 1987).

The composition of a nutrient solution is defined by total salt concentration, pH, micronutrient concentrations, macronutrient ratios, and irrigation water composition (Savvas & Adamidis, 1999). When using a drainage recycling system, the physiological response of the cultivated species must be considered. It has been established that nutrient uptake by the plant is specific to each solute and follows Michaelis-Menten dynamics (Claassen & Barber, 1974). Bugbee (2004) categorizes essential nutrients based on their absorption rate in the solution as: i) active absorption and rapid removal (NO_3^-, NH_4^+, P, K, Mn); ii) intermediate absorption (Mg, S, Fe, Zn, Cu, Mo, Cl); and iii) passive absorption and slow removal (Ca, B), as is the case of the efficient absorption of monovalent ions (Schippers, 1980). The mechanism of ion absorption differs for each ion and for each plant species; therefore, the terms "passive" and "active" absorption are general concepts, since plant-specific characteristics play an important role (Coskun & White, 2023), including the stoichiometry of some elements (Ågren & Weih, 2020).

The total nutrient content in the solution determines the EC. However, because of the differential removal rates from nutrient uptake, EC mainly reflects the concentrations of calcium, magnesium, and sulfate remaining in the solution, while micronutrients contribute less than 0.1% to this variable (Bugbee, 2004).

In species such as chrysanthemum, slightly saline waters (1 g L^{-1} NaCl) can be used (Lee & Van Iersel, 2008); meanwhile, when low-quality irrigation water is used, EC normally increases rapidly due to the accumulation of ions such as sodium, chloride, and sulfate and, in the case of hard water, calcium and magnesium. Bicarbonate ions commonly present in groundwater are neutralized by the application of acid. In contrast, micronutrients such as boron or metals could accumulate to toxic levels (Metwally *et al.*, 2018); however, their concentrations are on the order of µmol L^{-1}, and unlike other ions that can be determined indirectly by measuring EC, these ions must be monitored by expensive laboratory analysis (Carmassi *et al.*, 2013; Olympios, 1999).

Savvas (2003) reports that, especially in the case of actively absorbed macronutrients, such as N, P, and K, keeping their concentrations low could result in good yields. The best results are obtained when the concentrations of elements in the solution correspond approximately to the nutrient: water uptake ratio (Domingues *et al.*, 2012; Trejo-Téllez & Gómez-Merino, 2012). Under these conditions, plants do not consume energy to actively take up or exclude ions (Alaoui *et al.*, 2022); however, nutrient:water uptake ratios fluctuate in response to climatic conditions, making it difficult to prepare a solution that is consistent with that ratio (Savvas, 2003).

In practice, in systems based on organic substrates, it is difficult to track variations in the concentrations of micronutrients (Cu, Fe, Mn, Zn, and B), possibly due to changes in the physical, chemical, and microbiological characteristics of the substrates. In systems based on coconut fiber, excesses of Na^+ and Cl^- can occur due to the high contents of these ions in the substrate (Abad *et al.*, 2002). In addition to the essential ions, it is necessary to consider the absorption of other ions, such as sodium and chloride, to avoid accumulations in the root environment. The ratios and amounts of nutrients absorbed change with the stage and development conditions of the plants. Unexpected changes in the composition of the nutrient solution occur frequently in commercial systems, making it necessary to perform analyses in the root environment (Sonneveld, 2000). Similarly, the composition and volume of the drained nutrient solution vary over time and influence the number of reuse cycles.

Sulfur is an essential element for plants, playing roles in the formation of amino acids and the synthesis of proteins and chlorophyll. In soil, sulfate is the form accessible to plants, but it is susceptible to leaching. Sulfur contents in soils are related to the contents of organic matter, and its chemical transformations are mostly catalyzed by microorganisms (Kertesz & Mirleau, 2004); a similar process occurs in organic substrates, where microorganisms oxidize sulfur into sulfuric acid (Handreck & Black, 2010); in addition, this acid contributes to a high EC (Kämpf *et al.*, 2009).

Chloride is recognized as a component of the photolysis system in photosystem II, as a stomatal regulator in several species, and as a counter anion. Chloride originates from soil, water irrigation, rain, traces of fertilizers, or atmospheric pollution, so no physiological problems are expected due to its deficiency, and the concentration of chloride at toxic levels is the main concern (Wen *et al.*, 2017; Zhang *et al.*, 2021). While average Cl^- content in plants ranges from 2.0 to 20.0 mg g^{-1} dry weight (DW), the critical tissue Cl^- content for toxicity is about 4-7 and 15-35 mg g^{-1} DW for Cl^--sensitive glycophytes and Cl^--tolerant glycophytes, respectively (Colmenero-Flores *et al.*, 2019). One of the reasons for the rapid accumulation of chloride is its weak retention in soils and subsequent leaching due to its high mobility (Cakmak *et al.*, 2023). Although chloride is an essential element for plants, its contents in the plant dry mass is relatively low and the addition of this ion to the solution can be a strategy to decrease nitrate contents in crops, due to the antagonistic effects between these two ions (Chapagain *et al.*, 2003). On average, chloride concentrations in the external solution greater than 20 mM can produce toxic effects in sensitive species, while for tolerant species, concentrations can be up to five times higher without affecting growth (Cakmak *et al.*, 2023).

The concentration of Na^+ in plant tissue is generally high, typical of a macroelement. As a charged ion, the lipid bilayer exhibits very low permeability for Na^+, but it can be transported across the plasma membrane by both low- and high-affinity transport systems, many of which normally transport K^+ into root cells (Taiz *et al.*, 2015). This is possibly related to the role of Na^+ as a partial replacement for K^+ in functions such as osmoregulation (Cakmak *et al.*, 2023). Salt-sensitive plants depend mainly on the exclusion of Na^+ through plasma membrane (Blumwald *et al.*, 2000) and have the ability to compartmentalize and accumulate Na^+ in root cells (Cakmak *et al.*, 2023), thereby reducing its transport to the aerial parts. However, in some ornamental species continuously exposed to high concentrations of Na^+, this capacity can be lost (Cabrera *et al.*, 2009; Cabrera & Perdomo, 2003; Farnham *et al.*, 1985).

In general, roses are classified as sensitive to salinity, tolerating NaCl concentrations between 15 and 30 mM with proper management of irrigation and fertilization. Tolerance depends on the type and concentration of salts, the cultivation system, the type of substrate, the irrigation system and the cultivar and the rootstock (Cabrera, 2003; Cabrera *et al.*, 2009; Cabrera & Perdomo, 2003; Lorenzo *et al.*, 2000; Niu & Rodriguez, 2008).

The objective of this research was to determine the effect of the drainage recycling percentage at three levels (0, 50, and 100%) and the type of mixture of the substrates: 100% burned rice husk (100BRH); 65% burned rice husk, 35% coconut fiber (65BRH); and 35% burned rice husk, 65% coconut fiber (35BRH) on the concentrations of sulfur (S in plant tissue and substrates, and SO_4^{2-} in drainage water), Na^+, and Cl^- during the stages of flowering stem development corresponding to 0, 5 and 8 weeks after pruning (WAP) in rose plants cv. 'Charlotte'.

Materials and methods

Plant material and growth conditions

The research was carried out at the Center for Agricultural Biotechnology of SENA, located in the municipality of Mosquera (4°41' N, 74°13' W; 2,516 m a.s.l.), with annual average temperature and precipitation of 12.6°C and 670 mm, respectively, and with characteristics of the lower montane dry forest (bs-MB) life zone (Guzmán González, 1996). A traditional wooden greenhouse covered with Agroclear® plastic (Andean Chemical Products, Colombia) was used, with five spans of 65 x 6.8 m each, planted with rose cv. 'Charlotte' grafted onto 'Natal Briar'. The crop consisted of 33 raised beds of 15 x 0.8 m, in which 8 L pots were placed, for a planting density of 7 plants m^{-2}.

The fertilizer formula, in mg L^{-1}, was 170 total N (15% NH_4^+), 35 P, 150 K, 110 Ca, 60 Mg, 82 S, 1 Mn, 0.5 Zn, 0.5 Cu, 3 Fe, 0.5 B, and 0.1 Mo. The formula was developed based on commercial formulas commonly used in the region and was further adjusted to reflect the specific characteristics of the available irrigation water. Standard phytosanitary management protocols for this crop were consistently applied throughout the study.

Treatment application and experimental design

For the establishment of the plants, mixtures of the substrates burned rice husk (BRH), with a degree of burning between 70% and 100%, and coconut fiber (CF) (Tab. 1) were used, as follows: 100% burned rice husk (100BRH); 65% burned rice husk, 35% coconut fiber (65BRH); and 35% burned rice husk, 65% coconut fiber (35BRH). The mixing ratios corresponded to the levels of the substrate experimental factor. An automatic drainage recycling system (ADRS) was used, which recycled the drainage at three levels —0, 50, and 100%— and served as the recycling percentage factor. The ADRS methodology is described in Cuervo *et al.* (2011) and Cuervo *et al.* (2012).

The treatments were established as a combination of the levels of the recycling percentage factor and types of substrates, for a total of nine treatments with three replicates each (Tab. 2). The experimental unit was a culture bed. This bifactorial arrangement was carried out under a design of plots divided into completely randomized blocks, where the main plot corresponded to the recycling percentage factor and the subplot to the substrate factor.

$$Y_{ijk} = \mu + \alpha_i + \delta_k + \eta_{ik} + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk} \qquad (1)$$

with $i = 1, 2, 3$; $j = 1, 2, 3$; $k = 1, 2, 3$;

where:

μ is the effect of the overall mean;

α_i is the effect of the *i-th* level of the recycling percentage factor;

δ_k is the effect of the *k-th* block;

η_{ik} is the effect of random error on the parent plot (recycle percentage per block);

β_j is the effect of the *j-th* level of the substrate factor;

$(\alpha\beta)_{ij}$ is the effect of the *ij-th* interaction between the two factors (recycling percentage per substrate);

ε_{ijk} is the effect of random error in the subplot;

Y_{ijk} is the observation in the *k-th* block of the *i-th* level of the percentage recycling factor and the *j-th* level of the substrate factor.

TABLE 2. Treatments evaluated in rose cv. 'Charlotte' grown in substrates with automatic drainage recycling system.

Treatment code	Treatments	Substrates	Recycling (%)
T1	100BRH-0R*	100BRH	0
T7	65BRH-0R*	65BRH	
T4	35BRH-0R*	35BRH	
T2	100BRH-50R	100BRH	50
T8	65BRH-50R	65BRH	
T5	35BRH-50R	35BRH	
T3	100BRH-100R	100BRH	100
T9	65BRH-100R	65BRH	
T6	35BRH-100R	35BRH	

100BRH = 100% burned rice husk; 65BRH = 65% burned rice husk, 35% coconut fiber; 35BRH = 35% burned rice husk, 65% coconut fiber.

*Treatments that do not enter the ADRS.

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Response variables and statistical analysis

In each replicate (bed), sampling of drainage water, substrates, and complete plants was carried out at the stages of development of the flowering stem corresponding to 0, 5, and 8 weeks after pruning (WAP). The samples were sent to the Soil and Water Laboratory at the Faculty of Agricultural Sciences, Universidad Nacional de Colombia, Bogotá campus, to determine the contents of S, Na^+, and Cl^-. The protocols used for the chemical analyses were the following: the chemical characterization of the plant tissue and substrates for Na, S and Cl^- was carried by calcination at 475°C and measured by atomic absorption spectrophotometry. The drainages were chemically characterized by the following methods. Sulfate (SO_4^{2-}): valuation by turbidimetry (precipitation with barium chloride); sodium: atomic absorption spectrophotometry; and chloride (Cl^-): titration with $AgNO_3$ 0.0141 N (Vélez Carvajal, 2012).

TABLE 1. Chemical properties of the substrates used in the experiment.

Substrate	pH	EC	OC	N	P	Ca	K	Mg	Na	Cu	Fe	Mn	Zn	B	S
		(dS m^{-1})				(%)						(mg kg^{-1})			
100BRH	5.53	6.82	27.2	0.51	0.06	0.11	0.01	0.04	0.03	4.4	225	136	54	28	481
65BRH	5.31	6.52	23.6	0.39	0.08	0.4	0.01	0.06	0.08	13.4	433	87	50	34	470
35BRH	5.18	5.18	6.04	26.6	0.5	0.06	0.16	0.01	0.17	19.1	704	66	47	-	548

EC – electric conductivity, OC – organic carbon.

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Statistical analysis

The statistical software R was used to perform the analysis of variance (ANOVA) and the subsequent Tukey comparison test ($P<0.05$) for each evaluation week (Mendiburu, 2023). Scientific visualizations were created using ggplot2 package v3.4.2. (Wickham, 2016).

Results and discussion

Sulfur concentrations in drainage, substrates, and plant tissue

Coconut fiber is a material with high contents of lignin and cellulose (Abad *et al.*, 2002), carbon sources required for the metabolism of S-oxidizing microorganisms, such as bacteria of the genus *Thiobacillus* (Lucheta & Lambais, 2012; Tourna *et al.*, 2014), which can increase the temporary immobilization of S in organo-sulfur compounds (Kertesz & Mirleau, 2004). The oxidation of S to H_2SO_4 or to SO_4^{2-} in organic substrates requires the activity of microorganisms in the presence of a medium with low water saturation, which makes substrates with a higher content of BRH an appropriate medium for this reaction. In addition, the acidity of the medium and the EC are increased (Cabrera *et al.*, 2017), which was also described by Roig *et al.* (2004) for mixtures of CF and expanded clays.

The behavior of the concentrations of S and Na^+ (Tabs. 3-4) shows a positive relationship, as mentioned by Cakmak *et al.* (2023), who noted that when analyzing the ions in the nutrient solution, counter-ions must be considered: if an anion is absorbed at a low rate, the same occurs with its counter-ion.

For Na^+, Cl^-, and SO_4^{2-} contents, a positive relationship between them and the EC is observed; these results are consistent with those referenced by Cabrera *et al.* (2017).

Sodium concentrations in drainage, substrates, and plant tissue

At 0 WAP, significantly higher concentrations of Na^+ were obtained for CF substrates, independent of the recycling percentage of the solution (Tab. 4). At 5 WAP, there was no difference in the concentrations of the ion in the drainage. At 8 WAP, significantly higher Na^+ contents were recorded for the substrates with the highest BRH content and in treatments where drainage recycling was carried out; the opposite was observed in 0 WAP. At the substrate level, for 0 and 5 WAP stages, there was no significant difference between the Na^+ contents; while at 8 WAP, at higher CF contents, significantly higher ion contents were observed, regardless of the recycling percentage. In plant tissue, no statistical differences were found for Na^+.

In general, a growing trend in Na^+ concentrations in the drainage can be observed over time, which may be related to the fact that CF materials, which contain high concentrations of Na^+, underwent washing and leaching of this ion, which accumulated as the solution was recycled. Additionally, the CF-based substrates may have adsorbed Na^+, due to their high capacity for cation retention, which increased over time, possibly due to the activation by acids, such as sulfuric, from microbial activity (Hettiarachchi *et al.*, 2016). With respect to the concentration of the contents within the tissue, there was no evidence of increased uptake of Na^+ into plant tissue associated with the concentrations present in the drainage solution, which contrasts with the findings reported by Solís-Pérez and Cabrera (2007).

Chloride concentrations in drainage water

Chloride concentrations were only significantly different at 8 WAP. At this stage, ion concentrations were significantly higher in recycling treatments independent of the substrate mixture, a typical occurrence in recycling systems (Tab. 5). This behavior may result from Cl^- competing with NO_3^- for absorption (Sambo *et al.*, 2019). During 8 WAP, plants can enhance NO_3^- uptake (Rodríguez & Flórez, 2012), which promotes Cl^- exclusion and reduces inhibition of NO_3^- absorption (Griffiths & York, 2020; Massa *et al.*, 2009). In addition, the concentrations of NO_3^- were approximately twice those of Cl^- (data not shown).

TABLE 3. Mean values ± SD of sulfur contents in drainage water (expressed as SO_4^{2-} mg L^{-1}); substrate (expressed as S mg kg^{-1}); and plant tissue (expressed as S mg kg^{-1}), in rose cv. 'Charlotte' grown in organic substrates with an automatic drainage recycling system (ADRS). Measurements were taken at 0, 5, and 8 weeks after pruning (WAP). Means followed by different letters indicate significant differences at $P<0.05$ according to Tukey's multiple comparison test.

Week	Treatment																	
	100BRH0R		100BRH50R		100BRH100R		35BRH0R		35BRH50R		35BRH100R		65BRH0R		65BRH50R		65BRH100R	
SO_4^{2-} in drainage (mg L^{-1})																		
0	341.3±12.1	c	458±90.4	abc	339.3±55.8	c	433.3±51.3	bc	584.3±146.9	ab	560±138.4	abc	457±13.1	abc	483.3±196.9	b	671±51.4	a
5	413.2±122.9	a	520.2±151.7	a	485.8±252.1	a	510.1±108.5	a	425.4±63.4	a	586.9±237.1	a	431.3±75.3	a	671.8±62.9	a	450.8±79.9	a
8	451.33±84.33	d	1203.57±296.07	ab	1528.32±270.26	a	528.79±123.19	d	806.82±192.44	cd	1125.02±59.19	bc	560.86±57.31	d	1059.71±284.1	bc	1238.41±65.17	ab
S in substrates (mg kg^{-1})																		
0	1626.5±666.3	a	2500.1±799.4	a	4058.8±4317.9	a	8065.2±1841.2	a	5133.2±672.6	a	6843.2±598.5	a	5133.4±1588.6	a	4821.9±4055.5	a	3540.8±1205.2	a
5	4781.75±1737.15	a	8061.26±6597.34	a	5354.27±2832.08	a	6234.9±2964.4	a	8393.9±2882.7	a	6651.99±1850.1	a	7918.8±508.8	a	8062.6±5786.3	a	6970.8±2474.1	a
8	2661.22±422.66	a	5600.04±1519.26	a	7304.4±1676.15	a	7277.3±1286.6	a	11765.7±8911.0	a	15471.99±11314.4	a	4794.5±1073.1	a	10611.2±6806.8	a	10558.6±8019.3	a
S in plant tissue (mg kg^{-1})																		
0	2484.3±212.2	a	2646.7±225.9	a	2520.3±616.7	a	2653.7±31.9	a	2393.7±327.9	a	2704±175.1	a	2472.7±124.0	a	2561.7±111.3	a	2456.7±461.4	a
5	1801.7±96.8	a	1892.3±164.2	a	2027.3±81.7	a	1857.3±98.1	a	2043.3±137	a	2058.67±367.5	a	1845.3±40.1	a	1828.7±223.5	a	2075±92.6	a
8	1948.7±384.5	a	1893.7±129.5	a	1845±51.0	a	1952.3±28.5	a	1845±84.9	a	1882±355.8	a	1879±153.5	a	1723.3±103.5	a	1803±165	a

TABLE 4. Mean values ± SD of sodium contents in drainage water, expressed as Na^+ (mg L^{-1}); substrate, expressed as a percentage; and plant tissue, expressed as a percentage, in crop rose cv. 'Charlotte' grown in organic substrates with an automatic drainage recycling system (ADRS) at 0, 5, and 8 weeks after pruning (WAP). Means followed by different letters indicate significant differences at $P<0.05$ according to Tukey's multiple comparison test.

Week	Treatment																	
	100BRH0R		100BRH50R		100BRH100R		35BRH0R		35BRH50R		35BRH100R		65BRH0R		65BRH50R		65BRH100R	
Na^+ in drainage (mg L^{-1})																		
0	150±6.93	c	230.33±42.12	ab	191.67±30.01	bc	186.33±18.01	bc	273±61.02	a	289.33±51.5	a	186.33±4,16	bc	247±78	ab	303.67±15.04	a
5	298.98±147.65	a	358.58±91.84	a	373.5±112.44	a	319.36±114.37	a	317.65±106.78	a	418.97±173.58	a	265.52±51.19	a	391.88±136.95	a	312.63±42.16	a
8	187.76±21.38	e	405.98±100.78	abc	551.8±120.07	a	233.39±63.05	de	267.82±68.6	cde	388.06±26.72	bcd	223.72±19.42	e	385.65±100.39	bcd	438.8±38.54	ab
Na^+ in substrates (%)																		
0	0.12±0.05	a	0.15±0.01	a	0.25±0.15	a	0.48±0.18	a	0.28±0.02	a	0.36±0.11	a	0.3±0.09	a	0.32±0.26	a	0.23±0.09	a
5	0.19±0.09	a	0.23±0.06	a	0.23±0.08	a	0.56±0.54	a	0.32±0.14	a	0.25±0.04	a	0.28±0.04	a	0.38±0.37	a	0.28±0.04	a
8	0.08±0.06	b	0.24±0.08	ab	0.25±0.11	ab	0.21±0.08	ab	0.36±0.2	ab	0.4±0.11	a	0.1±0	b	0.25±0.05	ab	0.26±0.14	ab
Na^+ in plant tissue (%)																		
5	0.05±0.03	a	0.09±0.07	a	0.04±0.02	a	0.05±0.01	a	0.07±0.04	a	0,06±0,04	a	0.06±0.05	a	0.06±0.03	a	0.06±0.04	a
8	0.05±0.01	a	0.06±0	a	0.05±0.02	a	0.06±0.03	a	0.05±0.01	a	0.04±0.01	a	0.05±0.01	a	0.05±0.03	a	0.04±0	a

TABLE 5. Mean values ± SD of chloride contents in drainage water (expressed as Cl^- mg L^{-1}) in crop rose cv. 'Charlotte' grown in organic substrates with an automatic drainage recycling system (ADRS). Measurements were taken at 0, 5, and 8 weeks after pruning (WAP). Means followed by different letters indicate significant differences at $P<0.05$ according to Tukey's multiple comparison test.

Week	Treatment								
	100 BRH0R	100BRH50R	100BRH100R	35BRH0R	35BRH50R	35BRH100R	65BRH0R	65BRH50R	65BRH100R
	Cl^- in drainage (mg L^{-1})								
0	159.3±6 a	162±11.3 a	172.7±28.7 a	154.3±11.0 a	244.3±58.7 a	214.7±59.7 a	167±25.2 a	207.3±57.7 a	264±17.4 a
5	258.9±135.2 a	309.7±88.3 a	328.8±115.7 a	271.4±93.9 a	273.9±109.0 a	362.1±156.2 a	218.1±36.4 a	329.7±134.1 a	258.1±42.7 a
8	179.0±33.7 b	303.0±80.7 ab	417.9±108.4 a	215.6±53.9 b	204.0±40.6 b	295.5±25.0 ab	209.8±22.2 b	271.4±63.9 b	319.7±38.8 ab

Conclusions

The implementation of automatic drainage recirculation systems in rose crops can reduce effluent discharge and minimize environmental impact. However, it is essential to monitor and regulate the accumulation of ions such as Na^+ and Cl^-, since their increase during advanced growth stages may affect stem quality and vase life. This implies that growers must adjust recirculation frequency and substrate composition according to the phenological stage to maintain ionic balance and prevent salinization issues. In addition, the proportion between burned rice husk and coconut fiber directly influences nutrient and salt absorption dynamics and accumulation. Mixtures with higher coconut fiber content promote retention of Na^+ and SO_4^{2-} as time progresses toward the flowering stage of the floral stem under 100% recirculation, while those with higher burned rice husk content tend to accumulate more sulfates under prolonged recirculation. At 8 WAP, the Cl^- concentrations were significantly higher in the treatments with recycling (50 and 100%), regardless of the type of mixture of substrates. For the floriculture sector, a strategy based on substrate mixture rotation or adjustment could optimize nutrient uptake, extend substrate lifespan, and maintain rose quality in intensive production systems.

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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

WJCB and VJFR: conceptualization, funding acquisition, research, writing – original draft, visualization, and editing. WJCB, YAGC, and VJFR: writing the final version of the article. YAGC contributed to the translation of the article. All authors have read and approved the final version of the manuscript.

Literature cited

Abad, M., Noguera, P., Puchades, R., Maquieira, A., & Noguera, V. (2002). Physico-chemical and chemical properties of some coconut coir dusts for use as a peat substitute for containerised ornamental plants. *Bioresource Technology*, *82*(3), 241–245. https://doi.org/10.1016/S0960-8524(01)00189-4

Ågren, G. I., & Weih, M. (2020). Corrigendum: Multi-dimensional plant element stoichiometry – looking beyond carbon, nitrogen, and phosphorus. *Frontiers in Plant Science*, *11*, Article 915. https://doi.org/10.3389/fpls.2020.00915

Alaoui, I., El Ghadraoui, O., Serbouti, S., Ahmed, H., Mansouri, I., El Kamari, F., Taroq, A., Ousaaid, D., Squalli, W., & Farah, A. (2022). The mechanisms of absorption and nutrients transport in plants: A review. *Tropical Journal of Natural Product Research*, *6*(1), 8–14. https://scispace.com/papers/the-mechanisms-of-absorption-and-nutrients-transport-in-2x628x8q

Atta-Aly, M. A., Saltveit, M. E., & El-Beltagy, A. S. (1998). Saline growing conditions induce ripening of the non-ripening mutants *nor* and *rin* tomato fruits but not of *Nr* fruit. *Postharvest Biology and Technology*, *13*(3), 225–234. https://doi.org/10.1016/S0925-5214(98)00010-6

Blumwald, E., Aharon, G. S., & Apse, M. P. (2000). Sodium transport in plant cells. *Biochimica et Biophysica Acta (BBA) – Biomembranes, 1465*(1–2), 140–151. https://doi.org/10.1016/S0005-2736(00)00135-8

Bugbee, B. (2004). Nutrient management in recirculating hydroponic culture. *Acta Horticulturae*, *648*, 99–112. https://doi.org/10.17660/ActaHortic.2004.648.12

Cabrera, R. I. (2003). Nitrogen balance for two container-grown woody ornamental plants. *Scientia Horticulturae*, *97*(3–4), 297–308. https://doi.org/10.1016/S0304-4238(02)00151-6

Cabrera, R. I., & Perdomo, P. (2003). Reassessing the salinity tolerance of greenhouse roses under soilless production conditions. *HortScience*, *38*(4), 533–536. https://doi.org/10.21273/hortsci.38.4.533

Cabrera, R. I., Solís-Pérez, A. R., & Cuervo-Bejarano, W. J. (2017). Tolerancia y manejo de salinidad, pH y alcalinidad en cultivos de flores. In V. J. Flórez (Ed.), *Consideraciones sobre producción, manejo y poscosecha de flores de corte con énfasis en rosa* *y clavel* (pp. 63–73). Editorial Universidad Nacional de Colombia. https://academia.ceniflores.org/CentroDocumental/consideraciones-sobre-produccion-manejo-y-poscosecha-de-flores-de-corte-con-enfasis-en-rosa-y-clavel/

Cabrera, R. I., Solís-Pérez, A. R., & Sloan, J. J. (2009). Greenhouse rose yield and ion accumulation responses to salt stress as modulated by rootstock selection. *HortScience*, *44*(7), 2000–2008. https://doi.org/10.21273/hortsci.44.7.2000

Cakmak, I., Brown, P., Colmenero-Flores, J., Husted, S., Kutman, B. Y., Nikolic, M., Rengel, Z., Schmidt, S., & Zhao, F.-J. (2023). Micronutrients. In Z. Rengel, I. Cakmak, & P. J. White (Eds.), *Marschner's mineral nutrition of plants* (4th ed., pp. 283–385). Academic Press. https://doi.org/10.1016/B978-0-12-819773-8.00017-4

Carmassi, G., Romani, M., Diara, C., Massa, D., Maggini, R., Incrocci, L., & Pardossi, A. (2013). Response to sodium chloride salinity and excess boron in greenhouse tomato grown in semi-closed substrate culture in a Mediterranean climate. *Journal of Plant Nutrition*, *36*(7), 1025–1042. https://doi.org/10.1080/01904167.2013.766209

Chapagain, B. P., Wiesman, Z., Zaccai, M., Imas, P., & Magen, H. (2003). Potassium chloride enhances fruit appearance and improves quality of fertigated greenhouse tomato as compared to potassium nitrate. *Journal of Plant Nutrition*, *26*(3), 243–658. https://doi.org/10.1081/PLN-120017671

Claassen, N., & Barber, S. A. (1974). A method for characterizing the relation between nutrient concentration and flux into roots of intact plants. *Plant Physiology*, *54*(4), 564–568. https://doi.org/10.1104/pp.54.4.564

Colmenero-Flores, J. M., Franco-Navarro, J. D., Cubero-Font, P., Peinado-Torrubia, P., & Rosales, M. A. (2019). Chloride as a beneficial macronutrient in higher plants: new roles and regulation. *International Journal of Molecular Sciences*, *20*(19), Article 4686. https://doi.org/10.3390/ijms20194686

Coskun, D., & White, P. J. (2023). Ion-uptake mechanisms of individual cells and roots: short-distance transport. In Z. Rengel, I. Cakmak, & P. J. White (Eds.), *Marschner's mineral nutrition of plants* (4th ed., pp. 11–71). Academic Press. https://doi.org/10.1016/B978-0-12-819773-8.00018-6

Cuervo-Bejarano, W. J., Flórez-Roncancio, V. J. (2024). Evaluation of electrical conductivity and pH in a nutrient solution with recirculating system in rose crop. *Agronomía Colombiana, 42*(2), Article e115607. https://doi.org/10.15446/agron.colomb.v42n2.115607

Cuervo, W. J., Flórez, V. J., & González, C. A. (2012). Aspectos a tener en cuenta para optimizar un sistema de cultivo en sustrato con reciclaje de drenajes. *Agronomía Colombiana*, *30*(3), 379–387. https://revistas.unal.edu.co/index.php/agrocol/article/view/29029

Cuervo, W. J., Flórez, V. J., & González, C. A. (2011). Generalidades de la automatización y control para el reciclaje de drenajes en cultivos bajo cubierta. In V. J. Florez (Ed.), *Sustratos, manejo del clima, automatización y control en sistemas de cultivo sin suelo* (pp. 247–275). Editorial Universidad Nacional de Colombia. https://academia.ceniflores.org/CentroDocumental/sustratos-manejo-del-clima-automatizacion-y-control-en-sistemas-de-cultivo-sin-suelo/

Domingues, D. S., Takahashi, H. W., Camara, C. A. P., & Nixdorf, S. L. (2012). Automated system developed to control pH and concentration of nutrient solution evaluated in hydroponic lettuce production. *Computers and Electronics in Agriculture*, *84*, 53–61. https://doi.org/10.1016/j.compag.2012.02.006

Dotaniya, M. L., & Meena, V. D. (2015). Rhizosphere effect on nutrient availability in soil and its uptake by plants: A review. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, *85*, 1–12. https://doi.org/10.1007/s40011-013-0297-0

Farnham, D. S., Hasek, R. F., & Paul, J. L. (1985). *Water quality: Its effects on ornamental plants.* Cooperative Extension University of California. Division of Agriculture and Natural Resources. Leaflet 2995. https://ucanr.edu/sites/cetest/files/55088.pdf

Fujimoto, S. Y., Ohta, M., Usui, A., Shinshi, H., & Ohme-Takagi, M. (2000). Arabidopsis ethylene-responsive element binding factors act as transcriptional activators or repressors of GCC box-mediated gene expression. *Plant Cell*, *12*(3), 393–404. https://doi.org/10.1105/tpc.12.3.393

Griffiths, M., & York, L. M. (2020). Targeting root ion uptake kinetics to increase plant productivity and nutrient use efficiency. *Plant Physiology*, *182*(4), 1854–1868. https://doi.org/10.1104/PP.19.01496

Guzmán González, D. A. (1996). *Zonas de vida o formaciones vegetales. Área jurisdiccional CAR*. Corporación Autónoma Regional. https://sie.car.gov.co/server/api/core/bitstreams/4d8734ed-15b4-4992-955b-f696d6a4417c/content

Handreck, K., & Black, N. (2010). *Growing media for ornamental plants and turf* (4th ed.). University of New South Wales Press. https://archive.org/details/growingmediaforo0000hand

Hettiarachchi, E., Perera, R., Chandani Perera, A. D. L., & Kottegoda, N. (2016). Activated coconut coir for removal of sodium and magnesium ions from saline water. *Desalination and Water Treatment*, *57*(47), 22341–22352. https://doi.org/10.1080/19443994.2015.1129649

Kämpf, A. N., Fior, C. S., & Leonhardt, C. (2009). Lowering pH value with elemental sulfur in the substrate for *ex vitro* acclimatization. *Acta Horticulturae*, *812*, 415–420. https://doi.org/10.17660/ActaHortic.2009.812.58

Kertesz, M. A., & Mirleau, P. (2004). The role of soil microbes in plant sulphur nutrition. *Journal of Experimental Botany*, *55*(404), 1939–1945. https://doi.org/10.1093/jxb/erh176

Lee, M. K., & Van Iersel, M. W. (2008). Sodium chloride effects on growth, morphology, and physiology of chrysanthemum (*Chrysanthemum x morifolium*). *HortScience*, *43*(6), 1888–1891. https://doi.org/10.21273/hortsci.43.6.1888

Lorenzo, H., Cid, M. C., Siverio, J. M., & Ruano, M. C. (2000). Effects of sodium on mineral nutrition in rose plants. *Annals of Applied Biology*, *137*(1), 65–72. https://doi.org/10.1111/j.1744-7348.2000.tb00058.x

Lucheta, A. R., & Lambais, M. R. (2012). Sulfur in agriculture. *Revista Brasileira de Ciência do Solo*, *36*(5), 1369–1379. https://doi.org/10.1590/s0100-06832012000500001

Massa, D., Mattson, N. S., & Lieth, H. J. (2009). Effects of saline root environment (NaCl) on nitrate and potassium uptake kinetics for rose plants: A Michaelis-Menten modelling approach. *Plant and Soil*, *318*, 101–115. https://doi.org/10.1007/s11104-008-9821-z

Mendiburu, F. (2023). *R package version 1.3-7 Statistical procedures for agricultural research* (Version 1.3-1). https://CRAN.R-project.org/package=agricolae

Metwally, A. M., Radi, A. A., El-Shazoly, R. M., & Hamada, A. M. (2018). The role of calcium, silicon and salicylic acid treatment in protection of canola plants against boron toxicity stress. *Journal of Plant Research*, *131*(6), 1015–1028. https://doi.org/10.1007/s10265-018-1008-y

Niu, G., & Rodriguez, D. S. (2008). Responses of growth and ion uptake of four rose rootstocks to chloride- or sulfate-dominated salinity. *Journal of the American Society for Horticultural Science*, *133*(5), 663–669. https://doi.org/10.21273/jashs.133.5.663

Olympios, C. M. (1999). Overview of soilless culture: advantages, constraints, and perspectives. In R. Choukr-Allah (Ed.), *Protected cultivation in the Mediterranean region* (pp. 307–324).

Riley, M. M. (1987). Micronutrients: Boron toxicity in barley. *Journal of Plant Nutrition*, *10*(9–16), 2109–2115. https://doi.org/10.1080/01904168709363761

Rodríguez, M., & Flórez, V. (2012). Changes in EC, pH and in the concentrations of nitrate, ammonium, sodium and chlorine in the drainage solution of a crop of roses on substrates with drainage recycling. *Agronomía Colombiana*, *30*(2), 266–273. https://revistas.unal.edu.co/index.php/agrocol/article/view/15773

Roig, A., Cayuela, M. L., & Sánchez-Monedero, M. A. (2004). The use of elemental sulphur as organic alternative to control pH during composting of olive mill wastes. *Chemosphere*, *57*(9), 1099–1105. https://doi.org/10.1016/j.chemosphere.2004.08.024

Roy, S. J., Negrão, S., & Tester, M. (2014). Salt resistant crop plants. *Current Opinion in Biotechnology, 26*, 115–124. https://doi.org/10.1016/j.copbio.2013.12.004

Sambo, P., Nicoletto, C., Giro, A., Pii, Y., Valentinuzzi, F., Mimmo, T., Lugli, P., Orzes, G., Mazzetto, F., Astolfi, S., Terzano, R., & Cesco, S. (2019). Hydroponic solutions for soilless production systems: issues and opportunities in a smart agriculture perspective. *Frontiers in Plant Science*, *10*(923), 1–17. https://doi.org/10.3389/fpls.2019.00923

Savvas, D. (2003). Nutritional management of vegetables and ornamental plants in hydroponics. In R. Dris, R. Niskanen, & S. M. Jain (Eds.), *Crop management and postharvest handling of horticultural products fruits and vegetables* (Vol. 1, pp. 37–87). Science Publishers.

Savvas, D., & Adamidis, K. (1999). Automated management of nutrient solutions based on target electrical conductivity, pH, and nutrient concentration ratios. *Journal of Plant Nutrition*, *22*(9), 1415–1432. https://doi.org/10.1080/01904169909365723

Schippers, P. A. (1980). Composition changes in the nutrient solution during the growth of plants in recirculating nutrient culture. *Acta Horticulturae*, *98*, 103–118. https://doi.org/10.17660/actahortic.1980.98.9

Solís-Pérez, A. R., & Cabrera, R. I. (2007). Evaluating counter-ion effects on greenhouse roses subjected to moderately-high salinity. *Acta Horticulturae*, *751*, 375–380. https://doi.org/10.17660/ActaHortic.2007.751.47

Sonneveld, C. (2000). *Effects of salinity on substrate grown vegetables and ornamentals in greenhouse horticulture* [Doctoral dissertation, Wageningen University]. https://doi.org/10.18174/121235

Sonneveld, C., Baas, R., Nijssen, H. M. C., & De Hoog, J. (1999). Salt tolerance of flower crops grown in soilless culture. *Journal of Plant Nutrition*, *22*(6), 1033–1048. https://doi.org/10.1080/01904169909365692

Taiz, L., Zeiger, E., Møller, I. M., & Murphy, A. (2015). *Plant physiology and development* (6th. ed.). Sinauer Associates, Inc., Publishers.

Tourna, M., Maclean, P., Condron, L., O'Callaghan, M., & Wakelin, S. A. (2014). Links between sulphur oxidation and sulphur-oxidising bacteria abundance and diversity in soil microcosms based on *soxB* functional gene analysis. *FEMS Microbiology Ecology*, *88*(3), 538–549. https://doi.org/10.1111/1574-6941.12323

Trejo-Téllez, L. I., & Gómez-Merino F. C. (2012). Nutrient solutions for hydroponic systems. In T. Asao (Ed.), *Hydroponics - A standard methodology for plant biological researches* (pp. 1-22). InTech. https://doi.org/10.5772/2215.

Vélez Carvajal, N. A. (2012). *Comportamiento de macronutrientes en un sistema de cultivo sin suelo para clavel estándar cv. Delphi con recirculación de drenajes en la Sabana de Bogotá* [Master thesis, Universidad Nacional de Colombia]. https://repositorio.unal.edu.co/handle/unal/11550

Wen, Z., Tyerman, S. D., Dechorgnat, J., Ovchinnikova, E., Dhugga, K. S., & Kaiser, B. N. (2017). Maize NPF6 proteins are homologs of Arabidopsis CHL1 that are selective for both nitrate and chloride. *The Plant Cell*, *29*(10), 2581–2596. https://doi.org/10.1105/tpc.16.00724

Wickham, H. (2016). *Ggplot2: Elegant graphics for data analysis* (2nd ed.). Springer. https://link.springer.com/book/10.1007/978-3-319-24277-4

Zhang, X., Franzisky, B. L., Eigner, L., Geilfus, C. M., & Zörb, C. (2021). Antagonism of chloride and nitrate inhibits nitrate reductase activity in chloride-stressed maize. *Plant Growth Regulation*, *93*(3), 279–289. https://doi.org/10.1007/s10725-020-00685-2

Effect of water deficit on photosynthesis and yield in pea plants (*Pisum sativum* L.): A systematic review

Efecto del déficit hídrico en la fotosíntesis y el rendimiento de arveja (*Pisum sativum* L.): una revisión sistemática

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ABSTRACT

Water deficit caused by drought is common and extreme because of climate change. Pea (*Pisum sativum* L.) crops undergo water deficits depending on the sowing season and location. Some mechanisms of these plants in response to drought include stomatal closure and a reduction in photosynthetic capacity. However, as a consequence, such mechanisms disrupt reproductive processes such as flowering and pod filling, which diminishes the yield of plants. Although these mechanisms have been studied, there is no systematic review of the effect of water deficit on photosynthesis and yield in *P. sativum*. This review aimed to combine, summarize, and interpret the current knowledge on the impact of water deficiency on photosynthesis and yield in *P. sativum*. We carried out a systematic review using the databases Scopus, Web of Science, and ScienceDirect. Water deficit diminishes CO_2 assimilation by as much as 25%. This reduction is apparently due to stomatal closure (reduced by 28%) and, to a lesser degree, to variables such as the real efficiency of photosystem II (reduced by 15%). Water deficit reduces pea yield by 30% compared to control treatments (watered plants). The yield components most affected are the number of pods per plant, grains per plant, and pods per square meter. Few studies have assessed the relationship among yield, photosynthesis, and water status in *P. sativum* under water deficit conditions. Furthermore, significant knowledge gaps remain with respect to the combined effects of water deficit and interacting environmental factors (such as light intensity, temperature, and vapour pressure deficit) on the responses of field pea photosynthesis and yield. Additionally, there is a need to standardise methodologies for assessing water deficit status in both plant tissue and soil.

Keywords: drought, water stress, legumes, water potential, water use efficiency.

RESUMEN

El déficit hídrico causado por sequías es común y extremo debido al cambio climático. Los cultivos de arveja (*Pisum sativum* L.) sufren déficit hídrico, dependiendo de la época de siembra y la ubicación. Algunos mecanismos de estas plantas en respuesta a la sequía son el cierre de estomas y la reducción de la capacidad fotosintética. Sin embargo, como consecuencia, se alteran procesos reproductivos como la floración y el llenado de vainas, lo que disminuye el rendimiento de las plantas. Aunque estos mecanismos han sido estudiados, no existe una revisión sistemática del efecto del déficit hídrico en la fotosíntesis y el rendimiento en *P. sativum*. Esta revisión tuvo como objetivo combinar, resumir e interpretar el conocimiento actual sobre los efectos del déficit hídrico en la fotosíntesis y el rendimiento en *P. sativum*. Se realizó una revisión sistemática utilizando las bases de datos Scopus, Web of Science y ScienceDirect. El déficit hídrico disminuye la asimilación de CO_2 en 25%. Esta reducción se debe aparentemente al cierre estomático (reducido en un 28%) y, en menor medida, a variables como la eficiencia real del fotosistema II (reducida en un 15%). El déficit hídrico reduce el rendimiento de arveja en un 30% en comparación con los tratamientos de control (plantas regadas). Los componentes del rendimiento más afectados son número de vainas por planta, granos por planta y vainas por metro cuadrado. Pocos estudios han evaluado la relación entre el rendimiento, la fotosíntesis y el estado hídrico en *P. sativum* en condiciones de déficit hídrico. Adicionalmente, prevalecen varios vacíos del conocimiento acerca de los efectos combinados del déficit hídrico con otros factores ambientales (como intensidad lumínica, temperatura y déficit de presión de vapor) en la respuesta del rendimiento y la fotosíntesis de la arveja. También existe la necesidad de estandarizar metodologías para evaluar el déficit hídrico tanto en plantas como en el suelo.

Palabras clave: sequía, estrés hídrico, leguminosas, potencial hídrico, uso eficiente del agua.

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Introduction

Because of their nutritional attributes and capacity for nitrogen-fixing, legumes (such as *Pisum sativum* L.) have great potential to deal with the challenge of sustainably alimentation an increasing population (Sońta & Rekiel, 2020). The pea grain is rich in protein (200 g kg^{-1}) (Foyer *et al*., 2016) and has health benefits that include antihypertensive, antioxidant, and anti-inflammatory properties. For humans, peas reduce cholesterol levels and regulate intestinal bacterial activity (Ge *et al*., 2020). Additionally, pea crops have lower environmental impact because of lower nitrogen-fertilizer dependency (Sońta & Rekiel, 2020), reducing the carbon footprint. Despite health and environmental benefits, research on legumes such as peas has been less than on cereals (Sońta & Rekiel, 2020).

As a result of the increase in climate variability, the yield and quality of pea grains are affected by extreme climatic conditions (drought, flooding, and heat waves). Climate change in the tropics will affect agriculture more severely in developing countries (Lee *et al*., 2023), particularly the yield of legumes (Foyer *et al*., 2016; Soba *et al*., 2022). Extreme conditions include water stress caused by drought, which affects the physiology and yield of peas (Nadeem *et al*., 2019). This stress reduces photosynthetic rate, chlorophyll (chl) content, stomatal conductance, number of grains per pod, number of pods, and yield by up to 50% (Nadeem *et al*., 2019; Ney *et al*., 1994). These impacts are more severe when plants undergo water stress in the reproductive stage (Fougereux *et al*., 1997; Henriet *et al*., 2019).

Water stress effects on photosynthesis are well-studied in plants. These stress effects mainly are affected by stomatal closure that prevents CO_2 entry into the substomatal cavity and, thus, the entrance of CO_2 into mesophyll cells (Flexas *et al*., 2012). Depending on the intensity, water stress also affects the activity and synthesis of photosynthetic enzymes and chlorophyll (Analin *et al*., 2023; Chaves *et al*., 2009). Couchoud *et al*. (2020) demonstrate that photosynthesis in peas mainly decreases from stomatal closure, and the decrease is less severe in the Afila cultivar.

Water stress diminishes yield not only because of its effects on leaves but also because of its effects on the reproductive organs. Water stress triggers flower fall, stigma dehydration, and pollen infertility (Soba *et al*., 2022; Suzuki *et al*., 2014). Moreover, water stress decreases the number of reproductive nodes of the pea plants, the average grain weight, and the number of pods per plant, and increases the number of aborted seeds (Henriet *et al*., 2019).

Gas exchange variables such as CO_2 assimilation and stomatal conductance could be used to predict yield and, directly or indirectly, to evaluate the water status of plants and crops (Furbank *et al*., 2019). Although the relationship between yield and leaf photosynthesis is not straightforward due to factors such as canopy, genotype, transpiration, and environmental conditions, under specific circumstances, leaf photosynthesis has been used to predict mass accumulation in crops (Wu *et al*., 2019). Knowing the photosynthesis-yield relationship with a water deficit would help select management alternatives, detect deficits earlier, and predict pea yields.

This study aimed to summarize and interpret current knowledge about water deficit effects on yield and photosynthesis in *Pisum sativum*. Specifically, we addressed the following questions: Which factors have been evaluated in water deficits? Which methodological approaches have been used? Which yield component or photosynthesis-related process is most affected by water deficits? And what is the effect of water deficits on yield and photosynthesis in pea plants? We hypothesize the following: Enough information exists addressing the impact of drought stress on photosynthesis and yield. Furthermore, we expect that canopy CO_2 assimilation is positively correlated with yield but not with leaf CO_2 assimilation. Finally, yield components such as grains per area or pods per area are positively related to yield.

Materials and methods

Web of Science, Scopus, and ScienceDirect databases were searched to identify original studies on drought, irrigation, or water stress effects on yield and photosynthesis in peas (*Pisum sativum* L.). The search terms were as follows: drought, water stress, aridity, and irrigation. For yield and photosynthesis, the terms were these: yield, productivity, photosynthesis, and gas exchange (Tab. 1). The search was broad in order to obtain a significant number of articles related to the subject.

Literature such as reviews, abstracts, theses, or meta-analyses was excluded. During screening, studies on drought, irrigation, or water stress in *P. sativum* organs, plants, or crops were selected. Studies whose experimental unit was organelles or studies that were carried out on different species were omitted. Finally, studies where drought, irrigation, or water stress treatment were not clearly defined were excluded (Fig. 1).

TABLE 1. Databases, searching terms, search date, and number of publications.

Database	Search string	Search terms	N°	Search date
Scopus	Title-Abstract-Key words	(*Pisum sativum* OR pea OR *P. sativum*) AND (drought OR water stress OR aridity OR water scarcity OR irrigation) AND (yield OR productivity OR photosynthesis OR chlorophyll OR gas exchange OR stomata OR carbon dioxide fixation OR photosystem OR photochemistry OR radiation use efficiency)	805	09/01/2024
Web of Science	Topic (TS)	(*Pisum sativum* OR pea OR *P. sativum*) AND TS= (drought OR water stress OR aridity OR water scarcity OR irrigation) AND TS= (yield OR productivity OR photosynthesis OR chlorophyll OR gas exchange OR stomata OR carbon dioxide fixation OR photosystem OR photochemistry OR radiation use efficiency)	880	09/01/2024
ScienceDirect	Title, Abstract, or Author-specified keywords	(*Pisum sativum*) AND (drought OR water stress OR irrigation) AND (yield OR photosynthesis OR chlorophyll OR radiation use efficiency)	50	09/01/2024
Total			**1735**	

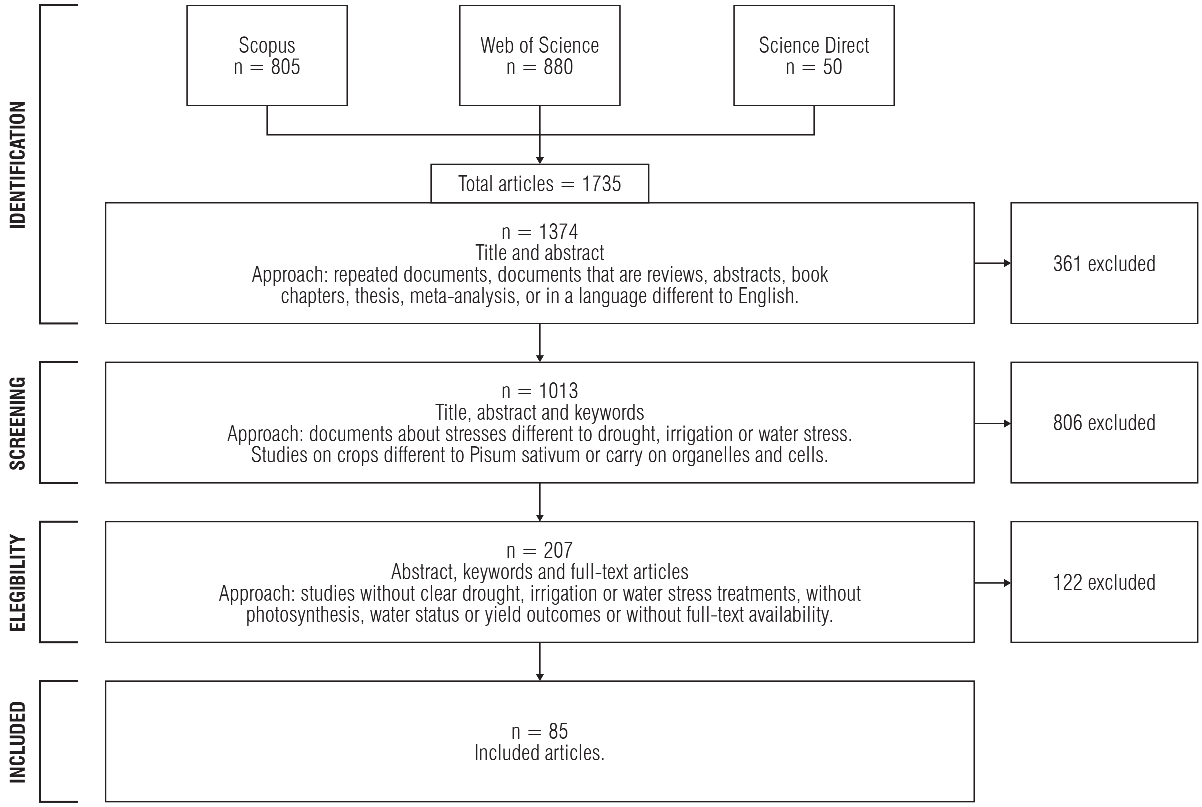

FIGURE 1. Flow diagram of the identification, screening, and selection of studies related to water stress on the yield and photosynthesis of *Pisum sativum* L.

Variables related to yield, yield components, growth, water status, and gas exchange were extracted from the selected studies. From each selected article, both the response variables and other treatment variables (*e.g.*, genotype, temperature, phenological stage, etc.) were entered into an Excel file. The absolute values of each variable reported in the literature were extracted. Finally, the relative increase or decrease from water or irrigation deficit treatments compared to the control treatments (in which the plants were well irrigated) were calculated. When the authors reported the results on bar or point plots, the plugin figure calibration of ImageJ was used (available at http://www.astro.physik.uni-goettingen.de/~hessman/ImageJ/Figure_Calibration/) to obtain numeric data.

To summarize, the effect of water or irrigation deficit on photosynthesis and yield, boxplots of each treatment combination were plotted. The boxplots represented the relative change of the treatments in comparison to the control treatment. In each boxplot, minimally the data from two studies were plotted. Plots were made using the ggplot2 and ComplexHeatmap packages in R software V. 4.0.5.

Results and discussion

Common factors and variables

Most of the studies assessed the interaction between water deficit and sowing date, followed by water deficit versus genotype, water deficit versus duration, and water deficit versus phenology (Fig. 2). The remaining factors were evaluated by fewer than five studies. They were related to environmental conditions (temperature, light, CO_2), chemical substances, fertilizers (organic matter, phosphorus, potassium, nitrogen, and sulfur), timing of the measurements, nanoparticles, and osmolytes (such as proline and glycine betaine).

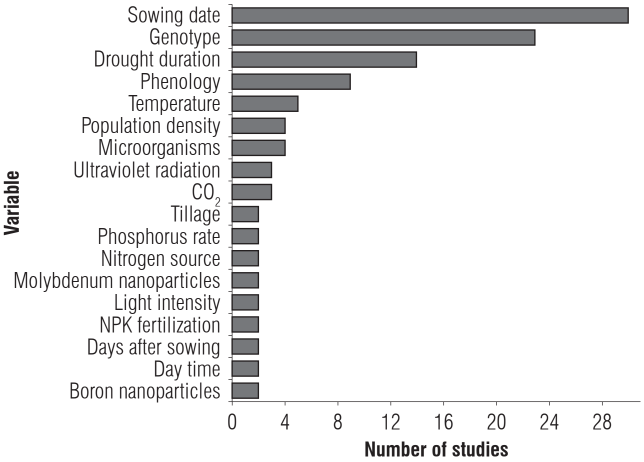

FIGURE 2. Frequency of evaluated factors (more than one study). Evaluated factors were evaluated along with water deficit in studies of water or irrigation deficit on *Pisum sativum*.

The interaction of water deficit with other environmental variables, represented by sowing dates and water-deficit duration, is typical in the field and significantly impacts pea crop yield. For instance, Bueckert *et al.* (2015) find that the sowing date substantially affects the yield and duration of the reproductive phase of pea plants. They also show a positive correlation between yield and precipitation and a negative correlation between the duration of the reproductive phase and the mean daily maximum temperature.

Breeding to obtain genotypes tolerant to water stress is a common strategy, but it demands time and resources. However, this strategy has remarkable benefits: Rodríguez-Maribona *et al.* (1992) report that, for the 'Desso' cultivar undergoing a water deficit, yield decreases around 40%, while, for the 'Fride' cultivar, the decrease is only 10%. 'Fride' cultivar presents a mechanism of greater efficiency in water use or its conservation in the face of drought events. Another example is reported by Nemeskéri *et al.* (2015), who demonstrate that mild and early-ripening cultivars are less susceptible to water deficit than late-ripening cultivars.

The duration of water withholding determines the intensity of water stress in plants. Still, without soil and plant measurements, it is difficult to know the intensity of the water stress. Some authors (Embiale *et al.*, 2016) only report the withholding duration but not the water potential of plants or soils. However, others report the water potential of both plants and soil, while some report it only for soil (Al-Quraan *et al.*, 2021; Frechilla *et al.*, 2000; Leport *et al.*, 1998).

Some factors are rarely measured yet are essential due to their practicality or their interaction with water stress. For example, fertilizers are practical and frequently used. Still, only one study evaluates both phosphorus and potassium fertilization on pea plants undergoing irrigation deficit (Carter & Stoker, 1988), yielding no benefit. However, Jin *et al.* (2015) find that fertilization with phosphorus increases the WUE (water use efficiency) and growth of pea plants under drought conditions, and Abd El–Mageed *et al.* (2017) observe the same pattern with potassium fertilization in soybeans.

Other factors such as temperature, light or UV radiation, and water deficit might coincide and be more severe and common under a climate change scenario (Lee *et al.*, 2023; Zandalinas *et al.*, 2021). For instance, Zandalinas *et al.* (2021) show that the response of *Arabidopsis thaliana* to high light, heat stress, osmotic stress, and oxidative stress is different when plants are subjected to each stress individually compared to when such stresses are combined. The same authors find that the combination of stresses reduces the survival and growth of plants than do each stress individually. This underscores the necessity of researching the effect of stress combinations on the physiology and yield of pea crops.

Having selected the most common variables related to yield, photosynthesis, and water status, the majority of the studies evaluated yield (41) and total dry mass (24) (Fig. 3). On the other hand, 24 studies evaluated stomatal conductance, 21 looked at CO_2 assimilation and only 23 measured relative water content (RWC) or leaf water potential (Fig. 3).

It is difficult to establish the relationship between yield, water status, and photosynthesis in pea plants undergoing drought because few studies have simultaneously assessed these processes. Only five of the selected studies evaluated yield and stomatal conductance simultaneously, and only

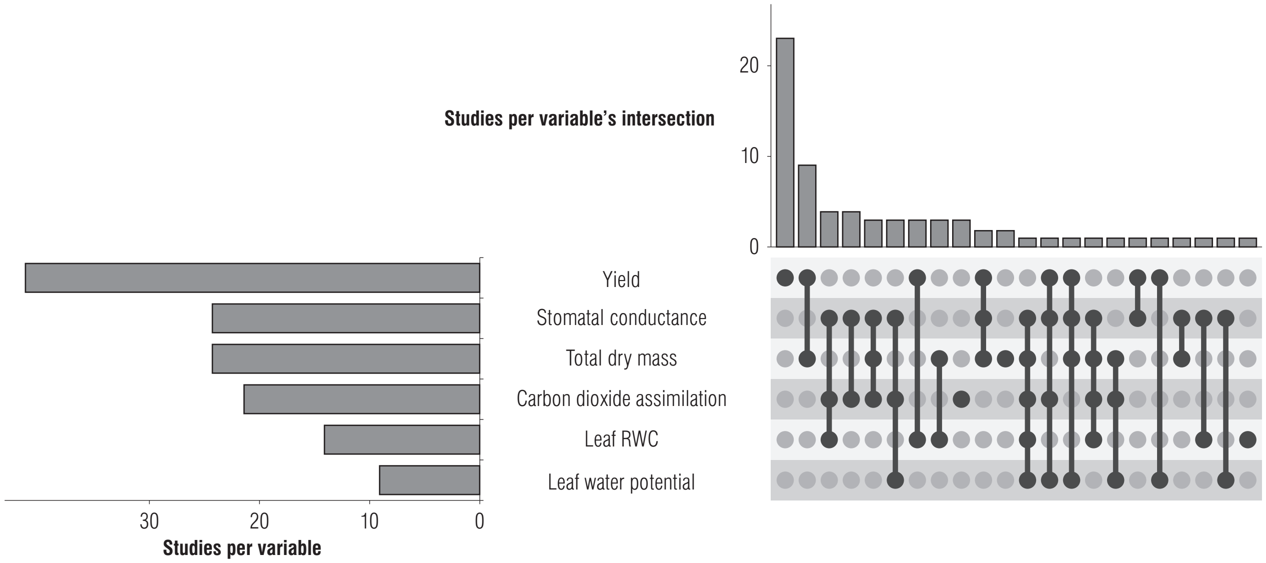

FIGURE 3. UpSet plot of the most common variables related to yield, photosynthesis, and water status of pea plants undergoing water deficit. Studies per variable indicate the number of articles in which the variables were measured. Each point indicates in which studies the variable was measured; when two variables (two points) are linked by a line, both variables were measured in the same study. RWC – relative water content.

one assessed yield and CO_2 assimilation (Fig. 3). Additionally, few studies evaluated both yield and water status (only six measured leaf water potential or RWC and yield) (Fig. 3). Currently, the diagnosis of water stress is based on the spectral response of plants and crops, which is related to their photosynthetic response and water status (Beebe *et al*., 2013; Furbank *et al*., 2019). The limited knowledge of the relationship between yield, photosynthesis, and water status makes it difficult to use technologies to recognize water stress earlier.

Because photosynthesis is the primary source of dry mass and yield of crops, determining the relationship between yield and photosynthesis would benefit the prediction of yields beforehand (Honda *et al*., 2021; Wu *et al*., 2019). The relationship between leaf photosynthesis and crop yield is not straightforward because other factors, such as canopy architecture, nitrogen dynamics, phenology, and crop management, also play a role (Wu *et al*., 2019). However, considering these factors allows us to find the best match between leaf photosynthesis and yield. For example, Honda *et al*. (2021) reveal a strong relationship between the photosynthesis of the flag leaf and the yield of rice plants during the heading stage.

Effect of water deficit on water status

All studies assessed irrigation or water deficit in pea plants. Still, only 23 of the 85 studies measured a variable related to water status in plants (Fig. 3). The most common variables were leaf RWC (13 studies) and leaf water potential (7 studies) (Fig. 3). Meanwhile, other water status variables were measured in five articles each. In general, leaf water potential was more affected (50%) by water deficit than other variables (Fig. 4).

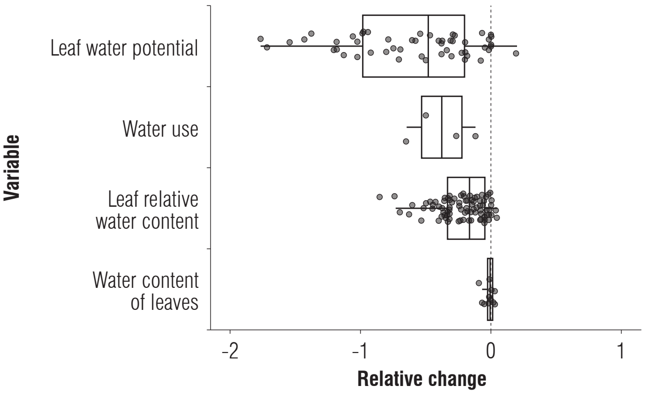

FIGURE 4. Relative changes from water deficit on water status variables in *P. sativum* L. Each boxplot represents the relative change of 34 cases (represented by each point) in 27 studies. Boxes represent 50% of the observations (relative change of each treatment different from control), and whiskers represent the minimum and maximum value or 1.5 times the interquartile range.

The main variables used as indicators of water stress were leaf water potential and leaf RWC. Generally, leaf water potential is more variable and sensitive to water deficit than leaf RWC (Fig. 4). Both variables indicate the water status of plants. Higher RWC in plants undergoing water deficit is related to the osmotic adjustment mechanism, where the accumulation of solutes maintains the water content.

The fact that leaf water potential has been more sensitive than leaf RWC probably indicates an osmotic adjustment in pea plants (Turner *et al.*, 2007; Turner, 2018). However, only Nogués *et al.* (1998) measure both variables. They did not discuss whether there was an osmotic adjustment, indicating a gap in knowledge on osmotic adjustment in peas.

Water deficit effect on photosynthesis

Although stomatal limitation is a mechanism to conserve water in plants, it is also the leading cause of a reduction in CO_2 assimilation in plants undergoing drought or irrigation deficit. Only three studies report simultaneously the internal CO_2 concentration and stomatal limitation. This stomatal limitation should have been reported by the authors because devices used to measure CO_2 assimilation and stomatal conductance (measured in 19 and 17 studies, respectively) also measure ambient and internal CO_2 concentration.

In our study, chlorophyll showed contrasting behavior: despite the minor changes in the total chlorophyll of plants undergoing drought, chlorophyll a and b decreased by approximately 30% when plants suffered a water deficit (Fig. 5). CO_2 assimilation was reduced by 25% due to the water deficit. Gas diffusion variables such as stomatal conductance, internal CO_2, and transpiration were similarly affected (~28%), while some photochemistry variables such as *Fv/Fm* (Maximum quantum efficiency of PSII photochemistry), ϕPSII (PSII operating efficiency), qP (Photochemical quenching), and NPQ (Non-photochemical quenching) were affected to a lesser extent (15%) (Fig. 5). Generally, water deficits slightly increased the water use efficiency (WUE) when measured at the leaf level (μmol CO_2 $mmol^{-1}$ H_2O) but not at the crop level (Fig. 5).

Gas exchange and chlorophyll content variables are more frequently measured than variables related to photochemistry (*e.g*, ϕPSII, Jmax, OJIP analysis variables). Regarding the variability of photosynthetic variables between studies, *Fv/Fm* was much less variable than CO_2 assimilation, transpiration, and stomatal conductance (Fig. 5). The low variability of *Fv/Fm* indicated that the plant uses biochemical mechanisms to protect the photosynthetic apparatus. Depending on the intensity of the stress, if it is moderate, dynamic photoinhibition occurs, and PSII recovery happens through the turnover of the D1 protein (Pandey *et al.*, 2023). However, if the stress is severe, there would be damage at the PSII level, leading to a decrease in the maximum potential photochemical efficiency of photosystem II and consequently a decrease in the operational performance of the photosystem (Pandey *et al.*, 2023).

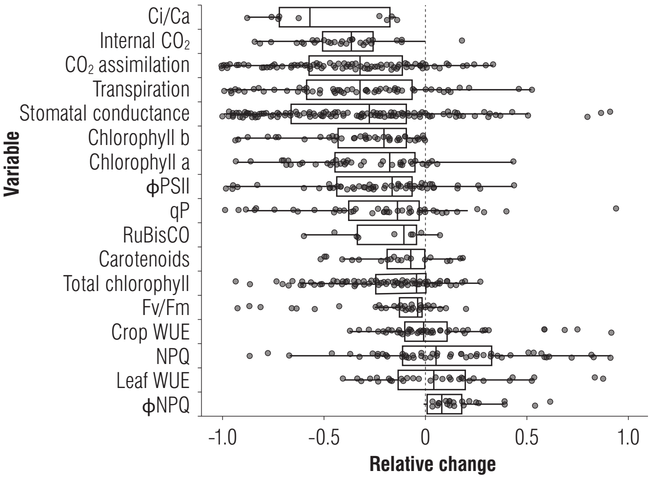

FIGURE 5. Relative changes from water deficits in photosynthesis-related variables in *P. sativum* L. Each boxplot represented the relative change of 173 cases (represented by each point) from 35 studies. Boxes represent 50% of the observations (relative change of each treatment different from the control), and whiskers represent the minimum and maximum value or 1.5 times the interquartile range. Ci – internal CO_2 concentration, Ca – ambient CO_2 concentration, ϕPSII – actual quantum efficiency of PSII, qP – photochemical quenching, *Fv/Fm* – maximum quantum efficiency of PSII, NPQ – non-photochemical quenching, WUE – water use efficiency.

The photosynthetic response of pea plants follows a similar pattern to other crops: Initially, there is stomatal limitation caused by a considerable reduction in stomatal conductance, followed by a decrease in internal CO_2 concentration and CO_2 assimilation, and finally a slight reduction in ϕPSII, *Fv/Fm* and chlorophyll content (Fig. 5) (Flexas *et al.*, 2012; Sun *et al.*, 2020). According to Flexas *et al.* (2012), the first response of plants to water deficits is stomatal closure, which occurs under mild and moderate water deficits. On the other hand, under severe water stress, variables such as chlorophyll content or integrity of photosynthetic enzymes are disturbed (non-stomatal or biochemical limitation). Most studies have assessed the effect of mild or moderate stress on pea plants, but few have assessed severe stress.

Although the first response of plants to water stress is closing their stomata, when the stress is severe or long-lasting, biochemical damage occurs (Flexas *et al.*, 2012; Sun *et al.*, 2020). During water stress, reactive oxygen species are accumulated, leading to oxidative damage that causes membrane damage and turnover of the D1 protein (Wang *et al.*, 2018). This protein is part of PSII; thus, its turnover decreases the activity of PSII (Wang *et al.*, 2018). Additionally, chlorophyll biosynthesis declines because of the decrease of enzymes responsible for the synthesis of chlorophyll intermediates such as protoporphyrinogen IX oxidase, Mg-chelatase, and protochlorophyllide oxidoreductase (Dalal & Tripathy, 2012).

Despite the reduction of the contents of chlorophyll a and b, there was no such reduction for total chlorophyll content (Fig. 5). The reason might be the scale used to measure each parameter: the contents of chlorophyll a and b were measured in the laboratory, in most cases per unit mass; whereas, total chlorophyll content was calculated per unit area and, in half of the cases, with a SPAD chlorophyll meter. Although the chlorophyll meter is a non-destructive method and is helpful in the field, it is less sensitive to fluctuations in chlorophyll content than destructive measurements (Parry *et al.*, 2014).

Water deficit effect on yield

In general, yield was reduced by 30% under water deficit compared to the control treatments. However, the yield ranged from 20% to 80% less than the control treatment. The yield components most affected by water deficit were pods and grains per plant; meanwhile, variables such as pod dry mass, harvest index, 1,000 seed weight, and the average mass of one grain were slightly affected by water deficit (Fig. 6).

Variables such as seed yield, grains per plant, pods per plant, and grains per pod were frequently measured. In contrast, others, such as aborted grains per pod, flowers per plant, and grains per square meter, were rarely measured. The variability of the more common variables, seed yield, and grains per plant was higher among studies than grains per pod, harvest index, and 1,000-seed mass (Fig. 6).

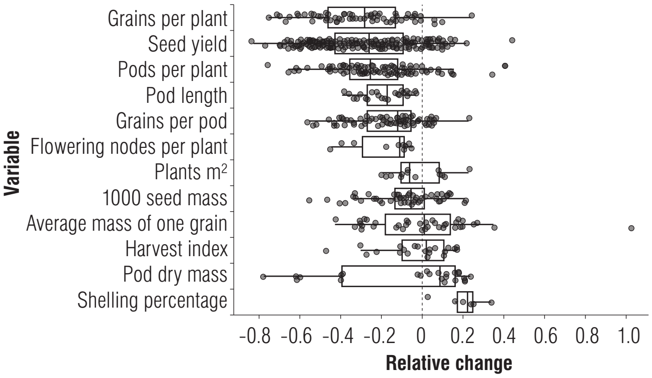

FIGURE 6. Relative changes of yield-related variables in *P. sativum* L. from water deficit. Each boxplot represents the relative change of 758 cases (represented by each point) from 82 studies. Boxes represent 50% of the observations (relative change of each treatment different from control), and whiskers represent the minimum and maximum value or 1.5 times the interquartile range.

Yield, represented as the grain mass produced per unit area or per plant, was reduced by water stress, with a reduction of 30% compared to control treatments (Fig. 6). Grains per plant showed the most similar pattern to yield, with comparable reductions and variability. Several studies report the relationship between water deficit and the reduction of reproductive structures such as flowers and pods, mainly when the water deficit occurs during flowering or pod-filling stages (Fougereux *et al.*, 1997; Martin & Jamieson, 1996). This explanation aligns with the fact that most studies measuring yield assessed plants undergoing water deficit during their entire cycle or critical stages such as flowering and pod filling.

Variables related to the number of grains per plant or area were more affected by water deficits than variables related to grain size. Variables related to grain size, such as the mass of 1,000 seeds or the average mass of one grain, are conservative compared to variables associated with the number of grains, such as grains per pod or pods per plant (Fougereux *et al.*, 1997; Jeuffroy *et al.*, 2010). Grain size is usually affected if the water stress occurs only during the pod-filling stage (Jeuffroy *et al.*, 2010; Munier-Jolain, 2010).

Although they were rarely measured, variables such as flowers per plant, reproductive nodes per plant, or number of nodes with pods can indicate if the water deficit occurred during flowering (Farooq *et al.*, 2017). This is because the number of flowers, pollen viability, and pistil function are severely affected by terminal droughts, which reduce the number of pods (Fang *et al.*, 2010). If the drought event occurred between flowering and the final stage of seed abortion, the primary affected variable would be the number of grains per pod. Therefore, if a study aims to elucidate the timing of the stress and the process concerned, it is essential to consider variables related to both reproductive structures and yield, such as the number of grains (Farooq *et al.*, 2017; Lecoeur, 2010).

In some studies, seed yield was not measured, but biomass yield was. Water deficit sharply decreased total and shoot dry mass, while leaf area and root dry mass were less affected. Even the relative growth rate (RGR) of roots was higher in some plants that suffered a water deficit compared to the control treatments (Fig. 7).

Growth is the first to be affected and one of the most susceptible processes to water deficit (Fig. 7). The shoot growth rate was more affected than root growth because roots can grow at a lower water potential than leaves. Their osmotic adjustment occurs faster than in leaves (Hsiao & Xu, 2000). In both cases, the cell expansion process involves the absorption of water and the weakening of the cell wall, and both methods are reduced by water deficits (Farooq *et al.*, 2009). Additionally, the dry mass of a plant depends mainly on photosynthesis; thus, any decline in the photosynthetic process diminishes CO_2 assimilation and carbohydrate synthesis.

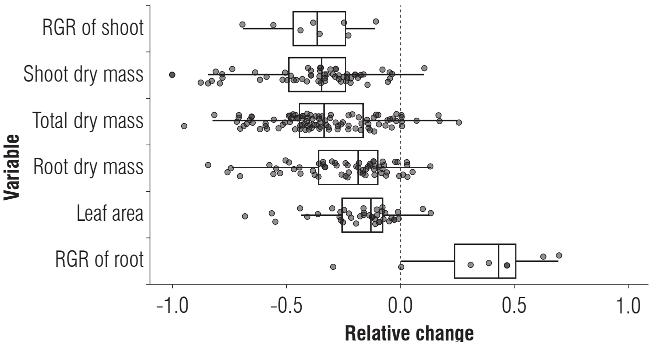

FIGURE 7. Relative changes from water or irrigation deficit on growth-related variables in *P. sativum* L. Each boxplot represents the relative change of 94 cases (represented by each point) from 33 studies. Boxes represent 50% of the observations (relative change of each treatment different from control), and whiskers represent the minimum and maximum value or 1.5 times the interquartile range. RGR – relative growth rate.

The reduction of growth points and sinks (such as young shoots and pods as in Figures 6 and 7 diminishes the sink strength of the plants. This decrease may induce feedback inhibition, thereby reducing the photosynthetic rate. In other grain crops, such as barley, for instance, the reduction of grain number through spike trimming results in sugar accumulation in stems and leaves, which in turn suppresses leaf photosynthesis (Serrago *et al*., 2013). These findings highlight the importance of assessing source–sink dynamics and photosynthetic performance under drought stress conditions.

Limits and challenges

Some methodological problems prevent the standardization or complete analysis of some variables. Several authors did not report the stage or moisture content at which grains were harvested; this could affect the estimation of water deficit effects on yield. Many authors reported the days on which the measurements were taken, but not the phenological stage of the plants, which hinders the relationship between phenology and water deficit.

Variables used as indicators of drought or deficit irrigation should be unified to facilitate comparisons between studies. Some studies used only the days of withholding water as a drought indicator. In contrast, other studies used the soil and leaf water potential. Authors should at least indicate water stress with a measure of soil moisture, but ideally with a measure of soil and plant water potential, as well as the depth at which the soil potential or moisture was measured.

Because this study evaluated articles whose drought and control treatments were well established, articles whose objective was to assess sowing dates with different weather conditions were not considered. Additionally, due to differences in levels of water deficits and units, the difference between water deficit levels was not established. Still, they were categorized between extremes: with or without a water deficit.

During the article screening, several studies examining the salt effect on pea plants or crops were identified. These studies were not considered in this review. Still, a future review of this topic could expand knowledge on osmotic stress in peas. Osmotic stress affects plants undergoing both a water deficit and salinity because both stresses reduce the water potential of the soil solution (Zhao *et al*., 2020). Salinity reduces the water potential due to the accumulation of salts. In contrast, water deficits decrease the water available in the soil, thus lowering the water potential of the soil solution. Additionally, both stresses share several genetic and enzymatic responses (Forni *et al*., 2017).

There is a lack of information on the relationship between yield, water status, and photosynthesis in pea plants. Although the interaction between water deficits and other factors has been evaluated, other essential factors such as light, fertilization, soil type, chemical primers, microorganisms, and population density have rarely been assessed. Environmental variables such as soil type, vapor pressure deficit, or light commonly interact with water deficits under field conditions. At the same time, fertilization, microorganisms, and chemical primers might alleviate water deficits.

Conclusions

Water deficit significantly reduces CO_2 assimilation of pea plants due to substantial stomatal closure and slight photochemical impairment. Contrarily, non-photochemical quenching, and water use efficiency rise moderately as strategies to quench energy excess and avoid water loss (Fig. 8). Pea yield appears to diminish because of reductions in grains per square meter, resulting from a lower number of pods per plant, grains per pod, or flowers per plant during the reproductive stage. Similarly, most growth processes decrease, except for the relative growth of the root.

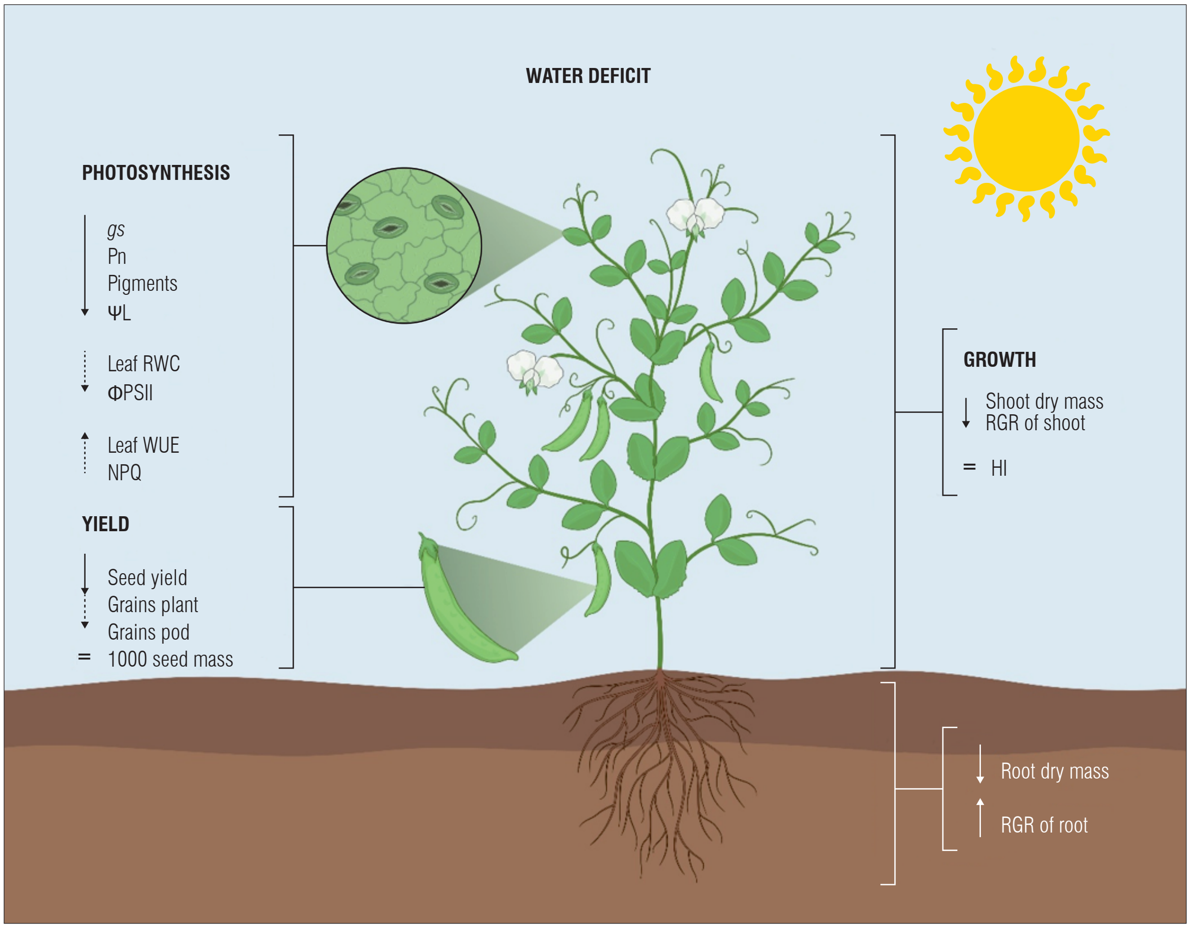

FIGURE 8. The main photosynthetic and yield processes are affected by water deficit. *gs* –Stomatal conductance; Pn – CO_2 assimilation; Ψ_L – leaf water potential; RWC –relative water content; ϕPSII –photosystem II maximum real efficiency; WUE –water use efficiency; HI –harvest index; RGR –relative growth rate. Solid and dashed arrows mean a strong and a moderate impact on each process. Created in BioRender. https://BioRender.com/x1ioacv.

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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

OHAS, DMAA, JAH, and LMM contributed to the study’s conceptualization and design. Data curation, formal analysis, research, and writing of the original draft were performed by OHAS. DMAA, JAH, and LMM carried out the critical review, commentary, and revision of the manuscript. All authors have read and approved the final version of the manuscript.

Literature cited

Abd El–Mageed, T. A., El-Sherif, A. M. A., Ali, M. M., & Abd El-Wahed, M. H. (2017). Combined effect of deficit irrigation and potassium fertilizer on physiological response, plant water status, and yield of soybean in calcareous soil. *Archives of Agronomy and Soil Science, 63*(6), 827–840. https://doi.org/10.1080/03650340.2016.1240363

Al-Quraan, N. A., Al-Ajlouni, Z. I., & Qawasma, N. F. (2021). Physiological and biochemical characterization of the GABA shunt pathway in pea (*Pisum sativum* L.) seedlings under drought stress. *Horticulturae, 7*(6), Article 125. https://doi.org/10.3390/horticulturae7060125

Analin, B., Bakka, K., & Challabathula, D. (2023). Exacerbation of drought-induced physiological and biochemical changes in leaves of *Pisum sativum* upon restriction of COX and AOX pathways of mitochondrial oxidative electron transport. *Journal of Biosciences, 49*(1), Article 5. https://doi.org/10.1007/s12038-023-00380-0

Beebe, S. E., Rao, I. M., Blair, M. W., & Acosta-Gallegos, J. A. (2013). Phenotyping common beans for adaptation to drought. *Frontiers in Physiology, 4*, Article 35. https://doi.org/10.3389/fphys.2013.00035

Bueckert, R. A., Wagenhoffer, S., Hnatowich, G., & Warkentin, T. D. (2015). Effect of heat and precipitation on pea yield and reproductive performance in the field. *Canadian Journal of Plant Science*, *95*(4), 629–639. https://doi.org/10.4141/cjps-2014-342

Carter, K. E., & Stoker, R. (1988). Responses of non-irrigated and irrigated garden peas to phosphorus and potassium on Lismore stony silt loam. *New Zealand Journal of Experimental Agriculture, 16*(1), 11–15. https://doi.org/10.1080/03015521.1988.10425608

Chaves, M. M., Flexas, J., & Pinheiro, C. (2009). Photosynthesis under drought and salt stress: Regulation mechanisms from whole plant to cell. *Annals of Botany, 103*(4), 551–560. https://doi.org/10.1093/aob/mcn125

Couchoud, M., Salon, C., Girodet, S., Jeudy, C., Vernoud, V., & Prudent, M. (2020). Pea efficiency of post-drought recovery relies on the strategy to fine-tune nitrogen nutrition. *Frontiers in Plant Science, 11*, Article 204. https://doi.org/10.3389/fpls.2020.00204

Dalal, V. K., & Tripathy, B. C. (2012). Modulation of chlorophyll biosynthesis by water stress in rice seedlings during chloroplast biogenesis. *Plant, Cell and Environment, 35*(9), 1685–1703. https://doi.org/10.1111/j.1365-3040.2012.02520.x

Embiale, A., Hussein, M., Husen, A., Sahile, S., & Mohammed, K. (2016). Differential sensitivity of *Pisum sativum* L. cultivars to water-deficit stress: Changes in growth, water status, chlorophyll fluorescence, and gas exchange attributes. *Journal of Agronomy, 15*(2), 45–57. https://doi.org/10.3923/ja.2016.45.57

Fang, X., Turner, N. C., Yan, G., Li, F., & Siddique, K. H. M. (2010). Flower numbers, pod production, pollen viability, and pistil function are reduced, and flower and pod abortion increased in chickpea (*Cicer arietinum* L.) under terminal drought. *Journal of Experimental Botany, 61*(2), 335–345. https://doi.org/10.1093/jxb/erp307

Farooq, M., Gogoi, N., Barthakur, S., Baroowa, B., Bharadwaj, N., Alghamdi, S. S., & Siddique, K. H. M. (2017). Drought stress in grain legumes during reproduction and grain filling. *Journal of Agronomy and Crop Science, 203*(2), 81–102. https://doi.org/10.1111/jac.12169

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., & Basra, S. M. A. (2009). Plant drought stress: effects, mechanisms and management. *Agronomy for Sustainable Development, 29*(1), 185–212. https://doi.org/10.1051/agro:2008021

Flexas, J., Gallé, A., Galmés, J., Ribas-Carbo, M., & Medrano, H. (2012). The response of photosynthesis to soil water stress. In R. Aroca (Ed.), *Plant responses to drought stress: From morphological to molecular features* (pp. 129–144). Springer Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-32653-0_5

Fougereux, J.-A., Doré, T., Ladonne, F., & Fleury, A. (1997). Water stress during reproductive stages affects seed quality and yield of pea (*Pisum sativum* L.). *Crop Science, 37*(4), 1247–1252. https://doi.org/10.2135/cropsci1997.0011183X003700040036x

Foyer, C. H., Lam, H. M., Nguyen, H. T., Siddique, K. H. M., Varshney, R. K., Colmer, T. D., Cowling, W., Bramley, H., Mori, T. A., Hodgson, J. M., Cooper, J. W., Miller, A. J., Kunert, K., Vorster, J., Cullis, C., Ozga, J. A., Wahlqvist, M. L., Liang, Y., Shou, H., ..., & Considine, M. J. (2016). Neglecting legumes has compromised human health and sustainable food production. *Nature Plants, 2*(8), Article 16112. https://doi.org/10.1038/NPLANTS.2016.112

Frechilla, S., González, E. M., Royuela, M., Minchin, F. R., Aparicio-Tejo, P. M., & Arrese-Igor, C. (2000). Source of nitrogen nutrition (nitrogen fixation or nitrate assimilation) is a major factor involved in pea response to moderate water stress. *Journal of Plant Physiology, 157*(6), 609–617. https://doi.org/10.1016/S0176-1617(00)80003-6

Furbank, R. T., Jimenez-Berni, J. A., George-Jaeggli, B., Potgieter, A. B., & Deery, D. M. (2019). Field crop phenomics: enabling breeding for radiation use efficiency and biomass in cereal crops. *New Phytologist, 223*(4), 1714–1727. https://doi.org/10.1111/nph.15817

Ge, J., Sun, C. X., Corke, H., Gul, K., Gan, R. Y., & Fang, Y. (2020). The health benefits, functional properties, modifications, and applications of pea (*Pisum sativum* L.) protein: Current status, challenges, and perspectives. *Comprehensive Reviews in Food Science and Food Safety, 19*(4), 1835–1876. https://doi.org/10.1111/1541-4337.12573

Henriet, C., Aimé, D., Térézol, M., Kilandamoko, A., Rossin, N., Combes-Soia, L., Labas, V., Serre, R.-F., Prudent, M., Kreplak, J., Vernoud, V., & Gallardo, K. (2019). Water stress combined with sulfur deficiency in pea affects yield components but mitigates the effect of deficiency on seed globulin composition. *Journal of Experimental Botany, 70*(16), 4287–4303. https://doi.org/10.1093/jxb/erz114

Honda, S., Ohkubo, S., San, N. S., Nakkasame, A., Tomisawa, K., Katsura, K., Ookawa, T., Nagano, A. J., & Adachi, S. (2021). Maintaining higher leaf photosynthesis after the heading stage could promote biomass accumulation in rice. *Scientific Reports, 11*(1), Article 7579. https://doi.org/10.1038/s41598-021-86983-9

Hsiao, T. C., & Xu, L. K. (2000). Sensitivity of growth of roots versus leaves to water stress: biophysical analysis and relation to water transport. *Journal of Experimental Botany, 51*(350), 1595–1616. https://doi.org/10.1093/jexbot/51.350.1595

Jeuffroy, M.-H., Lecoeur, J., & Roche, R. (2010). The seed number. In N. Munier-Jolain, V. Biarnes, I. Chaillet, J. Lecoeur, & M.-H. Jeuffroy (Eds.), *Physiology of the pea crop* (pp. 104–109). CRC Press. https://www.taylorfrancis.com/chapters/edit/10.1201/b10504-6/seed-number-marie-h%C3%A9l%C3%A8ne-jeuffroy-j%C3%A9r%C3%A9mie-lecoeur-romain-roche?context=ubx&refId=932a093a-1c4e-463d-b6c0-1d22111fad9f

Jin, J., Lauricella, D., Armstrong, R., Sale, P., & Tang, C. (2015). Phosphorus application and elevated CO_2 enhance drought tolerance in field pea grown in a phosphorus-deficient vertisol. *Annals of Botany, 116*(6), 975–985. https://doi.org/10.1093/aob/mcu209

Lecoeur, J. (2010). Vegetative development: The morphogenesis of plant organs. In N. Munier-Jolain, V. Biarnès, I. Chaillet, J. Lecoeur, & M.-H. Jeuffroy (Eds.), *Physiology of the pea crop* (pp. 3–43). CRC Press. https://www.taylorfrancis.com/chapters/edit/10.1201/b10504-2/vegetative-development-morphogenesis-plant-organs-lecoeur?context=ubx&refId=fb9bfba7-40ad-414a-9c9c-e2cc4ed6286f

Lee, H., Calvin, K., Dasgupta, D., Krinner, G., Mukherji, A., Thorne, P., Trisos, C., Romero, J., Aldunce, P., & Barret, K. (2023). IPCC, 2023: Climate Change 2023: Synthesis Report, Summary for Policymakers. Contribution of Working Groups I, II, and III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, H. Lee and J. Romero (Eds.)]. IPCC, Geneva, Switzerland. https://www.ipcc.ch/report/ar6/syr/downloads/report/IPCC_AR6_SYR_LongerReport.pdf

Leport, L., Turner, N. C., French, R. J., Tennant, D., Thomson, B. D., & Siddique, K. H. M. (1998). Water relations, gas exchange, and growth of cool-season grain legumes in a Mediterranean-type environment. *European Journal of Agronomy, 9*(4), 295–303. https://doi.org/10.1016/S1161-0301(98)00042-2

Martin, R. J., & Jamieson, P. D. (1996). Effect of timing and intensity of drought on the growth and yield of field peas (*Pisum sativum* L.). *New Zealand Journal of Crop and Horticultural Science, 24*(2), 167–174. https://doi.org/10.1080/01140671.1996.9513949

Munier-Jolain, N. (2010). Individual seed weight. In N. Munier-Jolain, V. Biarnes, I. Chaillet, J. Lecoeur, & M.-H. Jeuffroy (Eds.), *Physiology of the pea crop* (pp. 109–117). CRC Press.

Nadeem, M., Li, J., Yahya, M., Sher, A., Ma, C., Wang, X., & Qiu, L. (2019). Research progress and perspective on drought stress in legumes: a review. *International Journal of Molecular Sciences, 20*(10), Article 2541. https://doi.org/10.3390/ijms20102541

Nemeskéri, E., Molnár, K., Vígh, R., Nagy, J. J., & Dobos, A. (2015). Relationships between stomatal behaviour, spectral traits, and water use and productivity of green peas (*Pisum sativum* L.) in dry seasons. *Acta Physiologiae Plantarum, 37*(2), Article 34. https://doi.org/10.1007/s11738-015-1776-0

Ney, B., Duthion, C., & Turc, O. (1994). Phenological response of pea to water stress during reproductive development. *Crop Science, 34*(1), 141–146. https://doi.org/10.2135/cropsci1994.0011183x003400010025x

Nogués, S., Allen, D. J., Morison, J. I. L., & Baker, N. R. (1998). Ultraviolet-B radiation effects on water relations, leaf development, and photosynthesis in droughted pea plants. *Plant Physiology, 117*(1), 173–181. https://doi.org/10.1104/pp.117.1.173

Pandey, J., Devadasu, E., Saini, D., Dhokne, K., Marriboina, S., Raghavendra, A. S. S., & Subramanyam, R. (2023). Reversible changes in the structure and function of the photosynthetic apparatus of pea (*Pisum sativum*) leaves under drought stress. *Plant Journal, 113*(1), 60–74. https://doi.org/10.1111/tpj.16034

Parry, C., Blonquist Jr., J. M., & Bugbee, B. (2014). *In situ* measurement of leaf chlorophyll concentration: Analysis of the optical/absolute relationship. *Plant, Cell and Environment, 37*(11), 2508–2520. https://doi.org/10.1111/pce.12324

Rodríguez-Maribona, B., Tenorio, J. L., Conde, J. R., & Ayerbe, L. (1992). Correlation between yield and osmotic adjustment of peas (*Pisum sativum* L.) under drought stress. *Field Crops Research, 29*(1), 15–22. https://doi.org/10.1016/0378-4290(92)90072-H

Serrago, R. A., Alzueta, I. I., Savin, R., & Slafer, G. A. (2013). Understanding grain yield responses to source-sink ratios during grain filling in wheat and barley under contrasting environments. *Field Crops Research, 150*, 42–51. https://doi.org/10.1016/j.fcr.2013.05.016

Soba, D., Arrese-Igor, C., & Aranjuelo, I. (2022). Additive effects of heatwave and water stresses on soybean seed yield is caused by impaired carbon assimilation at pod formation but not at flowering. *Plant Science, 321*, Article 111320. https://doi.org/10.1016/j.plantsci.2022.111320

Sońta, M., & Rekiel, A. (2020). Legumes – use for nutritional and feeding purposes. *Journal of Elementology, 25*(3), 835–849. https://doi.org/10.5601/jelem.2020.25.1.1953

Sun, Y., Wang, C., Chen, H. Y. H., & Ruan, H. (2020). Response of plants to water stress: a meta-analysis. *Frontiers in Plant Science, 11*, Article 978. https://doi.org/10.3389/fpls.2020.00978

Suzuki, N., Rivero, R. M., Shulaev, V., Blumwald, E., & Mittler, R. (2014). Abiotic and biotic stress combinations. *New Phytologist, 203*(1), 32–43. https://doi.org/10.1111/nph.12797

Turner, N. C. (2018). Turgor maintenance by osmotic adjustment: 40 years of progress. *Journal of Experimental Botany, 69*(13), 3223–3233. https://doi.org/10.1093/jxb/ery181

Turner, N. C., Abbo, S., Berger, J. D., Chaturvedi, S. K., French, R. J., Ludwig, C., Mannur, D. M., Singh, S. J., & Yadava, H. S. (2007). Osmotic adjustment in chickpea (*Cicer arietinum* L.) results in no yield benefit under terminal drought. *Journal of Experimental Botany, 58*(2), 187–194. https://doi.org/10.1093/jxb/erl192

Wang, Z., Li, G., Sun, H., Ma, L., Guo, Y., Zhao, Z., Gao, H., & Mei, L. (2018). Effects of drought stress on photosynthesis and photosynthetic electron transport chain in young apple tree leaves. *Biology Open, 7*(11), Article 035279. https://doi.org/10.1242/bio.035279

Wu, A., Hammer, G. L., Doherty, A., von Caemmerer, S., & Farquhar, G. D. (2019). Quantifying impacts of enhancing photosynthesis on crop yield. *Nature Plants, 5*(4), 380–388. https://doi.org/10.1038/s41477-019-0398-8

Zandalinas, S. I., Fritschi, F. B., & Mittler, R. (2021). Global warming, climate change, and environmental pollution: recipe for a multifactorial stress combination disaster. *Trends in Plant Science, 26*(6), 588–599. https://doi.org/10.1016/j.tplants.2021.02.011

Influence of naphthaleneacetic acid on the yield and bunch composition of the interspecific O × G hybrid of oil palm

Influencia del ácido naftalenacético en el rendimiento y la composición del racimo del híbrido interespecífico O × G de palma de aceite

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ABSTRACT

The interspecific hybrid O × G of oil palm is a promising genotype whose cultivated area in Colombia has increased in recent years due to its high productivity attributed to the formation of parthenocarpic fruits and its outstanding diseases tolerance. This study aimed to evaluate the effect of two forms of naphthaleneacetic acid, applied at different concentrations, on bunch composition variables that explain the yield of oil palm hybrids. A split-plot experimental design was employed, where the main factor was the type of auxins and the subfactor was dosage. A total of 31 bunch composition variables were evaluated through correlation analysis, principal component analysis, and a predictive yield model. Oil production was primarily associated with fruit set, bunch weight, and the weight of parthenocarpic fruits. There was no significant correlation with normal fruit traits such as seed weight or the kernel and seed-to-fruit ratio. The proposed model successfully explains oil yield based on the evaluated variables, which were influenced by the type and dose of auxin. The optimal response was obtained with 450 mg L^{-1} of sodium salt naphthaleneacetic acid (NAAS) and 1200 mg L^{-1} of naphthaleneacetic acid (NAA). These treatments promoted an ideal fruit set, increased parthenocarpic fruit weight, bunch weight, and oil-to-bunch ratio, as reflected in yield. These findings confirm the potential of auxins to enhance productivity and offer valuable insights for genetic improvement and the sustainability of oil palm cultivation.

Keywords: auxins, *Elaeis oleifera* (Kunth) Cortés × *Elaeis guineensis* Jacq., oil palm hybrid, artificial pollination, parthenocarpic fruits, oil production.

RESUMEN

El híbrido interespecífico O × G de palma de aceite es un genotipo promisorio cuya área de siembra en Colombia ha aumentado en los últimos años debido a su elevada productividad atribuida a la formación de frutos partenocárpicos y a su sobresaliente tolerancia a enfermedades. Esta investigación tuvo como objetivo determinar el efecto de dos moléculas de ácido naftalenacético, aplicadas en diferentes concentraciones, sobre las variables de composición del racimo que explican el rendimiento del híbrido de palma de aceite. Se empleó un diseño experimental de parcelas divididas, donde el factor principal fue el tipo de auxinas y el subfactor fue la dosis. Se evaluaron un total de 31 variables de composición del racimo mediante análisis de correlación, análisis de componentes principales y un modelo predictivo de rendimiento. La producción de aceite se asoció principalmente con el cuajado, el peso del racimo y el peso de los frutos partenocárpicos. No hubo correlación significativa con los rasgos normales de los frutos, como el peso de las semillas o la relación entre el grano y la semilla y el fruto. El modelo propuesto explica con éxito el rendimiento de aceite basándose en las variables evaluadas, que se vieron influidas por el tipo y la dosis de auxina. La mejor respuesta se obtuvo con 450 mg L^{-1} de sal sódica de ácido naftalenacético (SANA) y con 1200 mg L^{-1} de ácido naftalenacético (ANA), tratamientos que promovieron el llenado de los frutos, incrementaron el peso de los frutos partenocárpicos y del racimo, así como el porcentaje de aceite en racimo, reflejándose en un mayor rendimiento. Estos resultados confirman el potencial de las auxinas para mejorar la productividad y aportan información clave para el mejoramiento genético y la sostenibilidad del cultivo.

Palabras clave: auxinas, *Elaeis oleifera* (Kunth) Cortés × *Elaeis guineensis* Jacq., híbrido de palma de aceite, polinización artificial, frutos partenocárpicos, producción de aceite.

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Introduction

Oil palm is one of the world's most significant oil crops due to its high productivity, its applications in industry, as human food, animal feed, nutraceutical products, and biofuel production. Global demand for palm oil is projected to reach 156 million t by 2050 (Murphy *et al*., 2021; Pirker *et al*., 2016). Increased oil production can be achieved without requiring more land or additional water, energy and fertilizers for crop cultivation, considering environmental protection and climate change (Chew *et al*., 2021). Colombia stands as the largest oil palm producer in Latin America and the fourth worldwide, playing a key role in meeting the growing global demand through sustainable intensification practices (Corley & Tiker, 2015; Fedepalma, 2015; Fedepalma, 2021). The primary source of commercial palm oil production comes from the species *Elaeis guineensis* Jacq., the African oil palm (Corley & Tiker, 2015). However, in recent years in Colombia, the O × G hybrid has emerged as a productive alternative to achieve higher yields without expanding the area of cultivated oil palm through the induction of parthenocarpic fruits (PF) (Daza *et al*., 2020; Nieto Mogollon *et al*., 2024; Romero *et al*., 2021).

O x G interspecific hybrids are derived from a cross between the two most notable species, the American oil palm, *Elaeis oleifera* (Kunth) Cortés, and the African oil palm *Elaeis guineensis* Jacq. (Arias *et al*., 2015). These show intermediate characteristics between their parental stock in certain agronomically interesting aspects, such as their high-quality oil rich in oleic acid and exhibiting high productivity potential (Avila *et al*., 2016; Montoya *et al*., 2023). These hybrids are partially resistant to bud rot disease, the most significant epidemiological threat to oil palm worldwide, particularly in Colombia, where it severely affects plantations of the African oil palm. This impact led to a marked decline in productivity and triggered a severe social and economic crisis, necessitating the implementation of an emergency plan to replace African oil palm plantations with the hybrid, particularly in municipalities such as Tumaco (Avila *et al*., 2016).

Nevertheless, under natural pollination conditions, hybrids exhibit low fruit set, poor bunch formation, and a reduced oil extraction rate. These limitations in bunch development may be linked to factors such as limited production of male inflorescences, low pollen viability, and germination capacity, as well as the presence of indehiscent peduncular bracts in female inflorescences, which complicate pollen entry, making the oil potential lower than that of the African oil palm. Assisted pollination emerged as a technique that, while improving oil potential through enhancements in bunch size, weight, and fruit set, is a labor-intensive and a costly activity with a very short application window, reducing its effectiveness (Daza *et al*., 2020; Hormaza *et al*., 2012; Mosquera-Montoya *et al*., 2023; Rincón *et al*., 2013).

Parthenocarpy is the mechanism of seedless fruit generation, which can occur with or without pollination or flower fertilization. During the ovary's development, this process can be artificially stimulated for agricultural purposes by applying natural or synthetic phytohormones, such as gibberellins, cytokinins, and auxins (Azzeme *et al*., 2020). The increase in hormonal levels and their interactions induce ovary growth, resulting in the formation of seedless fruits, which translates into more outstanding flesh content as the seeds and seed cavities are replaced by an expanded mesocarp (Azzeme *et al*., 2020; Liu *et al*., 2018; Mandal *et al*., 2022; Zhang *et al*., 2021). Developing parthenocarpic fruits is a desirable strategy for improving palm oil production. In the O x G hybrid, the accumulation of oil relies on the development of both normal, fertile fruits and parthenocarpic fruits, achieved by removing the kernel and expanding the mesocarp, leading to more significant oil accumulation within the fruits (Montoya *et al*., 2023; Rincón *et al*., 2013).

Recent studies have shown that inducing parthenocarpy is effective in increasing yield without affecting oil quality, fruit development, or bunch components using the auxin naphthaleneacetic acid (NAA) (Cayón Salinas *et al*., 2022; Daza *et al*., 2020; Romero *et al*., 2021). However, there are no studies explaining the effect of NAA on the behavior of bunch composition variables that increase yield in the hybrid, which would help to explain yield through the most representative variables, considering that these phenotypic traits related to bunch composition are of great interest in oil palm breeding programs (Rios *et al*., 2018; Van Hintum *et al*., 2000).

Taking into account the above, in recent years, the hybrid has emerged as an alternative for oil palm production, replacing much of African oil palm cultivation, especially in Latin America (Avila *et al*., 2016; Rincón *et al*., 2013), due to its tolerance to pests and diseases, as well as its high yield per hectare per year with the use of NAA, and a reduction of production costs without increasing the planting area, thanks to the rise in oil extraction rate (Romero *et al*., 2021; Ruiz *et al*., 2021). This study aimed to determine the effect of two NAA molecules on the bunch composition variables that explain the yield of the O × G oil palm hybrid. The results precisely characterize the key variables determining the performance of the O × G hybrid, offering strategic insights that strengthen selection and agronomic improvement programs. The application of auxins, particularly naphthaleneacetic acid in its sodium salt form, positively affects bunch composition, enhancing oil accumulation and significantly contributing to the productivity and competitiveness of the oil palm agroindustry.

Materials and methods

Location and plant material

This experiment was conducted from October 2023 to May 2024 in the Palmeiras commercial plantation in Tumaco, Nariño, Colombia (1°26'50.75" N, 78°42'12.83" W). Climatic conditions during the experiment corresponded to the typical rainy season of the region, characterized by a bimodal rainfall pattern, with a cumulative rainfall of 840.5 mm during this period, an average relative air humidity of 82.7%, mean temperature of 27.0°C, and a mean daily light integral of 32.6 mol m^{-2} d^{-1}. A nine-year-old hybrid interspecific *Elaeis oleifera* (Coari-Brazil) x *Elaeis guineensis* (La Mé-Ivory Coast) was used. The palms were planted at a density of 115 trees per ha in an experimental field with clay soil characterized by pH 4.6, EC 0.14 dS m^{-1}, and 2.67% organic matter.

Experiment design and treatments

The experiment followed a split-plot structure with a 2 × 3 factorial arrangement. The main factor consisted of two types of auxin molecules: (1) 1-Naphthaleneacetic acid (NAA) and (2) 1-Naphthaleneacetic acid, sodium salt (NAAS). The secondary factor corresponds to the auxin dose, with three treatments: (1) application at 450 mg L^{-1}, (2) application at 1200 mg L^{-1}, and (3) a control treatment consisting of pollen (15 g talc + 1 g pollen per inflorescence). Female inflorescences were treated through liquid application, using 200 ml of the corresponding solution per inflorescence. NAA (product number N0640, Sigma) and NAAS (product number S572896, Sigma) were used as the auxin sources. For each treatment (subplot), three replicates were established, each consisting of 10 palms (each one considered as an experimental unit), from which three bunches per palm were analyzed.

Isolation of inflorescences and treatment application

Female inflorescences were isolated beforehand at phenological stage PS601, or pre-anthesis 1, as described by Rosero *et al.* (2017); they were isolated with polyester bags to prevent the entry of external pollen, ensuring that fruit development resulted exclusively from the applied treatments, while allowing gas exchange and supporting inflorescence development under similar natural conditions. The pollen used in this experiment was collected from selected pisifera palms (*Elaeis guineensis* (La Mé)). The treatments were applied when the inflorescences reached phenological stage PS607 of anthesis, when the flowers had receptive stigmas (Hormaza *et al.*, 2012). The application was made through a sprinkler, uniformly spraying each inflorescence with the corresponding dose. Subsequently, the inflorescence was sealed again. Three applications were made every eight days with the above-mentioned solution, and the isolation bags were removed 10 d after the last application.

TABLE 1. Analyzed variables from the bunch components of the (O x G) hybrid.

Abbreviation	Description	Abbreviation	Description
BW	Bunch weight	E/F	Endocarp-to-fruit ratio
SW	Stalk weight	K/F	Kernel-to-fruit ratio
S/B	Stalk-to-bunch ratio	ASW	Average seed weight
RN	Rachillae number	O/Fnf	Oil-to-fruit ratio in normal fruits
NAF	Number of aborted fruits	O/DMpf	Oil-to-dry-mesocarp ratio in parthenocarpic fruits
NNF	Number of normal fruits	M/Mnpf	Moisture-to-mesocarp ratio in parthenocarpic fruits
NFW	Normal fruit weight	NFS	Normal fruit set
NPF	Number of parthenocarpic fruits	PFS	Parthenocarpic fruit set
WPF	Weight of parthenocarpic fruits	FS	Fruit set (PFS + NFS)
WPNF	Weight of parthenocarpic and normal fruits	NFBO	Oil contribution of normal fruits to the bunch
AWpf	Average weight of parthenocarpic fruit	PFBO	Oil contribution of parthenocarpic fruits to the bunch
WFS	Weight of seeds per fruit	O/B	Oil-to-bunch ratio (NFBO + PFBO) or bunch oil percentage
WE	Weight of endocarp	LF	Loose fruits
O/DMnf	Oil-to-dry-mesocarp ratio in normal fruits	OC	Oil content or yield (kg oil/bunch)
M/Mnf	Moisture-to-mesocarp ratio in normal fruits	DTM	Days to maturity
M/nf	Mesocarp-to-normal fruit ratio		

Response variables

The harvest and analysis of bunches were carried out following Prada and Romero (2012), who established parameters for accurately quantifying all variables involved in bunch analysis of the (O × G) hybrid, including those related to bunch composition, structure, and oil productivity (Tab. 1). The harvest was performed when the optimum harvest point was reached, approximately 175 d after anthesis, between phenological stages PS807 and PS809 (Romero *et al*., 2025).

Data analysis

To determine the correlation between variables, we performed a Pearson correlation analysis ($P<0.05$) (Pearson, 1900), presented as a colored heatmap using the 'PROC CORR' procedure and the 'PROC TEMPLATE' function. We performed a principal component analysis (PCA) using 'PROC PRINCOMP' to reduce the dimensionality of the variables and explore the relationships and grouping patterns among them (Jolliffe, 2002), helping to explain yield. We performed a cluster analysis using the 'CLUSTER' procedure, applying the average linkage method based on squared Euclidean distances: the analysis aimed to group related variables (Jain, 1988). We generated a dendrogram to visualize relationships, providing insights into potential patterns within the dataset.

We proposed a model to explain the total oil yield through the PROC REG procedure with the Oil Content (OC) as a function of the bunch composition variables using a multiple linear regression equation as follows:

$$Y = \beta_0 + \sum_{i=1}^{n} \beta_{\mathrm{i}} X_{\mathrm{i}} + \varepsilon \qquad \text{(Eq. 1)}$$

where:

Y represents the yield through the oil content (OC) in the bunch; β_0 is the model intercept; β_{i} are the regression coefficients associated with each predictor variable; and X_{i} corresponds to the explanatory variables included in the model, each representing an analyzed bunch composition characteristic. The term ε accounted for the random error, capturing the variability not explained by the predictor variables. The variables ultimately retained in the model were selected based on statistical significance and their explanatory contribution to model fit.

Finally, we analyzed the most essential variables in the study using analysis of variance (ANOVA) with the 'PROC MIXED' procedure. We applied the residual maximum likelihood (REML) estimation method. We used the following linear mixed model, considering hormone and dose as fixed effects, and the replicate number as random effects:

$$Y_{ijk} = \mu + \alpha_{\mathrm{i}} + \beta_j + (\alpha\beta)_{ij} + u_k + \varepsilon_{ijk} \qquad \text{(Eq. 2)}$$

where $Yijk$ is the response variable observed for the k - th experimental unit receiving the $l - th$ hormone and the $j - th$ dose, μ is the overall mean, α_i represents the fixed effect of the $l - th$ hormone, and βj represents the fixed effect of the $j - th$ dose. The term $(\alpha\beta)_{ij}$ corresponds to the fixed effect of the hormone × dose interaction. The random effect u_k represents the $k - th$ subject, which is assumed to follow a normal distribution with mean zero and variance σ_u^2. Finally, ε_{ijk} is the random error term, supposed to follow a normal distribution with zero mean and variance σ_ε^2. All analyses were performed using SAS® statistical software version 9.4 (SAS, Inc., 2013).

Results

We harvested the bunches when they ripened, identified by an intense orange color, the presence of stretch marks arranged in a circular pattern around the apical part of the fruit, and the presence of fruit detachment. In most bunches, fruit set (FS) exceeded 80%. Subsequently, each of the 31 variables included in the bunch analysis was quantified immediately after harvest.

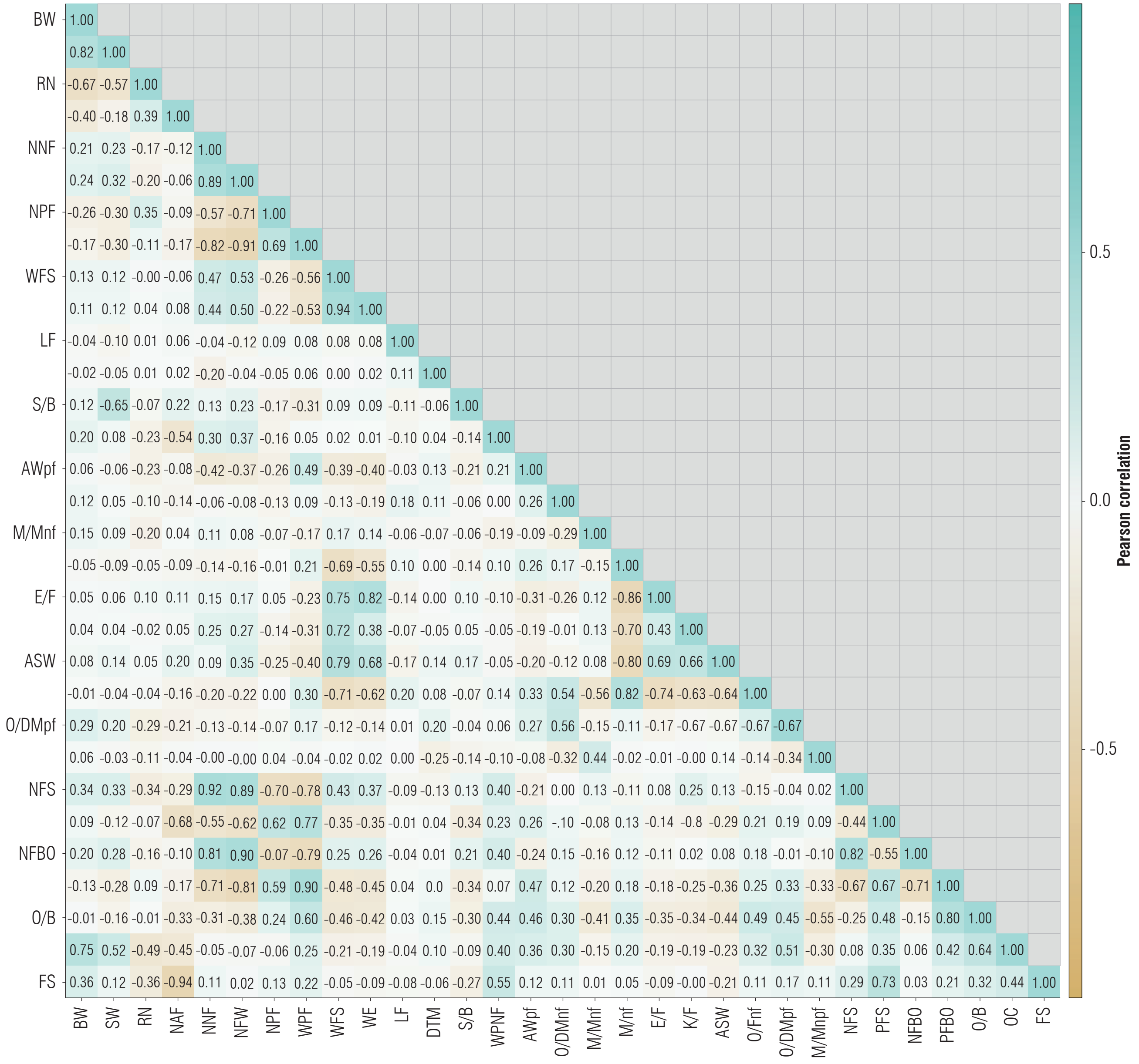
To identify associations, we performed a Pearson correlation analysis among the 31 variables evaluated in this study, represented through a heatmap (Fig. 1). Variables that showed a statistically significant positive correlation ($P<0.05$) included bunch weight (BW) and stalk weight (SW) (r = 0.82). We also observed associations among variables related to normal fruits, such as normal fruit set (NFS) and the oil contribution of normal fruits to bunch (NFBO) (r = 0.82), as well as between the number of normal fruits (NNF) and their weight (NFW) (r = 0.89). Similarly, we found a positive correlation between moisture and the oil-to-fruit ratio in normal fruits (M/nf and O/Fnf) (r = 0.82).

We also detected significant associations between seed-related variables, such as the weight of seeds per fruit (WFS) and the weight of endocarp (WE) (r = 0.90), as well as with the endocarp-to-fruit ratio (E/F) (r = 0.82). Additionally, we identified positive correlations among parthenocarpic variables, such as weight of parthenocarpic fruits (WPF) and the contribution of these fruits to the bunch oil percentage (PFBO) (r = 0.90). The latter was also highly correlated with the oil-to-bunch ratio (O/B) (r = 0.80).

Most of the variables showing negative correlations correspond to relationships between characteristics of normal and parthenocarpic fruits. For instance, the number of normal fruits (NNF) was negatively correlated with the weight of parthenocarpic fruits (WPF) (r = -0.82), which, in turn, was negatively correlated with the weight of normal fruits (NFW) (r = -0.91). The latter also negatively correlated with the oil contribution of parthenocarpic fruits to the bunch (PFBO) (r = -0.81).

Similarly, a negative correlation was observed between fruit set (FS) and the number of aborted fruits (NAF) (r = -0.94). Finally, the mesocarp-to-normal fruit ratio (M/nf) was negatively correlated with seed-related variables, such as average seed weight (ASW) (r = -0.80) and the endocarp-to-fruit ratio (E/F) (r = -0.86). The above suggests that oil production in the O × G hybrid is favored by a higher fruit set, which is reflected in increased bunch weight. In this context, parthenocarpic fruits, lacking the structural components typical of normal fruits, may allocate more resources to oil content, thereby increasing the bunch oil percentage (O/B).

The PCA revealed two principal components that together explained 47.24% of the total variability in the dataset (Fig. 2). The first principal component (PC1) accounted



	BW	SW	RN	NAF	NNF	NFW	NPF	WPF	WFS	WE	LF	DTM	S/B	WPNF	AWpf	O/DMnf	M/Mnf	M/nf	E/F	K/F	ASW	O/Fnf	O/DMpf	M/Mnpf	NFS	PFS	NFBO	PFBO	O/B	OC	FS
BW	1.00																														
	0.82	1.00																													
RN	-0.67	-0.57	1.00																												
	-0.40	-0.18	0.39	1.00																											
NNF	0.21	0.23	-0.17	-0.12	1.00																										
	0.24	0.32	-0.20	-0.06	0.89	1.00																									
NPF	-0.26	-0.30	0.35	-0.09	-0.57	-0.71	1.00																								
	-0.17	-0.30	-0.11	-0.17	-0.82	-0.91	0.69	1.00																							
WFS	0.13	0.12	-0.00	-0.06	0.47	0.53	-0.26	-0.56	1.00																						
	0.11	0.12	0.04	0.08	0.44	0.50	-0.22	-0.53	0.94	1.00																					
LF	-0.04	-0.10	0.01	0.06	-0.04	-0.12	0.09	0.08	0.08	0.08	1.00																				
	-0.02	-0.05	0.01	0.02	-0.20	-0.04	-0.05	0.06	0.00	0.02	0.11	1.00																			
S/B	0.12	-0.65	-0.07	0.22	0.13	0.23	-0.17	-0.31	0.09	0.09	-0.11	-0.06	1.00																		
	0.20	0.08	-0.23	-0.54	0.30	0.37	-0.16	0.05	0.02	0.01	-0.10	0.04	-0.14	1.00																	
AWpf	0.06	-0.06	-0.23	-0.08	-0.42	-0.37	-0.26	0.49	-0.39	-0.40	-0.03	0.13	-0.21	0.21	1.00																
	0.12	0.05	-0.10	-0.14	-0.06	-0.08	-0.13	0.09	-0.13	-0.19	0.18	0.11	-0.06	0.00	0.26	1.00															
M/Mnf	0.15	0.09	-0.20	0.04	0.11	0.08	-0.07	-0.17	0.17	0.14	-0.06	-0.07	-0.06	-0.19	-0.09	-0.29	1.00														
	-0.05	-0.09	-0.05	-0.09	-0.14	-0.16	-0.01	0.21	-0.69	-0.55	0.10	0.00	-0.14	0.10	0.26	0.17	-0.15	1.00													
E/F	0.05	0.06	0.10	0.11	0.15	0.17	0.05	-0.23	0.75	0.82	-0.14	0.00	0.10	-0.10	-0.31	-0.26	0.12	-0.86	1.00												
	0.04	0.04	-0.02	0.05	0.25	0.27	-0.14	-0.31	0.72	0.38	-0.07	-0.05	0.05	-0.05	-0.19	-0.01	0.13	-0.70	0.43	1.00											
ASW	0.08	0.14	0.05	0.20	0.09	0.35	-0.25	-0.40	0.79	0.68	-0.17	0.14	0.17	-0.05	-0.20	-0.12	0.08	-0.80	0.69	0.66	1.00										
	-0.01	-0.04	-0.04	-0.16	-0.20	-0.22	0.00	0.30	-0.71	-0.62	0.20	0.08	-0.07	0.14	0.33	0.54	-0.56	0.82	-0.74	-0.63	-0.64	1.00									
O/DMpf	0.29	0.20	-0.29	-0.21	-0.13	-0.14	-0.07	0.17	-0.12	-0.14	0.01	0.20	-0.04	0.06	0.27	0.56	-0.15	-0.11	-0.17	-0.67	-0.67	-0.67	-0.67								
	0.06	-0.03	-0.11	-0.04	-0.00	-0.00	0.04	-0.04	-0.02	0.02	0.00	-0.25	-0.14	-0.10	-0.08	-0.32	0.44	-0.02	-0.01	-0.00	0.14	-0.14	-0.34	1.00							
NFS	0.34	0.33	-0.34	-0.29	0.92	0.89	-0.70	-0.78	0.43	0.37	-0.09	-0.13	0.13	0.40	-0.21	0.00	0.13	-0.11	0.08	0.25	0.13	-0.15	-0.04	0.02	1.00						
	0.09	-0.12	-0.07	-0.68	-0.55	-0.62	0.62	0.77	-0.35	-0.35	-0.01	0.04	-0.34	0.23	0.26	-.10	-0.08	0.13	-0.14	-0.8	-0.29	0.21	0.19	0.09	-0.44	1.00					
NFBO	0.20	0.28	-0.16	-0.10	0.81	0.90	-0.07	-0.79	0.25	0.26	-0.04	0.01	0.21	0.40	-0.24	0.15	-0.16	0.12	-0.11	0.02	0.08	0.18	-0.01	-0.10	0.82	-0.55	1.00				
	-0.13	-0.28	0.09	-0.17	-0.71	-0.81	0.59	0.90	-0.48	-0.45	0.04	0.0	-0.34	0.07	0.47	0.12	-0.20	0.18	-0.18	-0.25	-0.36	0.25	0.33	-0.33	-0.67	0.67	-0.71	1.00			
O/B	-0.01	-0.16	-0.01	-0.33	-0.31	-0.38	0.24	0.60	-0.46	-0.42	0.03	0.15	-0.30	0.44	0.46	0.30	-0.41	0.35	-0.35	-0.34	-0.44	0.49	0.45	-0.55	-0.25	0.48	-0.15	0.80	1.00		
	0.75	0.52	-0.49	-0.45	-0.05	-0.07	-0.06	0.25	-0.21	-0.19	-0.04	0.10	-0.09	0.40	0.36	0.30	-0.15	0.20	-0.19	-0.19	-0.23	0.32	0.51	-0.30	0.08	0.35	0.06	0.42	0.64	1.00	
FS	0.36	0.12	-0.36	-0.94	0.11	0.02	0.13	0.22	-0.05	-0.09	-0.08	-0.06	-0.27	0.55	0.12	0.11	0.01	0.05	-0.09	-0.00	-0.21	0.11	0.17	0.11	0.29	0.73	0.03	0.21	0.32	0.44	1.00

FIGURE 1. Heatmap of Pearson correlations among the composition variables of oil palm bunches in the (O x G) hybrid. The description of the variables is provided in Table 1.

for 29.55%, and the second principal component (PC2) contributed 16.73% to the total variance (Fig. 2A). The results, represented in the biplot, show a clear separation and differential clustering of the evaluated variables. In the upper left quadrant, variables related to fruit set (FS), mesocarp weight, and oil content—both in normal fruits (O/Fnf, M/nf, O/DMnf) and parthenocarpic fruits (AWpf, O/DMpf)—are grouped. These variables exhibit strong associations with one another and contribute positively to the bunch oil percentage (O/B). This indicates that mesocarp development and accumulation efficiency in both fruit types are key to overall bunch oil yield.

In contrast, the lower right quadrant contains variables with an opposite correlation pattern, notably the number of aborted fruits (NAF), moisture content in the mesocarp of normal and parthenocarpic fruits (M/Mnf, M/Mnpf), and seed-related traits in normal fruits: weight of seeds per fruit (WFS), average seed weight (ASW), weight of endocarp (WE), endocarp-to-fruit ratio (E/F), and kernel-to-fruit ratio (K/F). These variables negatively correlate with oil percentage, suggesting that increased development of reproductive structures (such as seeds and endocarp) or higher mesocarp moisture content may be associated with reduced oil yield per bunch.

The hierarchical clustering analysis (Fig. 2B) enabled the classification of variables into four main groups with distinct characteristics, facilitating a clearer understanding of the factors influencing bunch formation in the O × G hybrid. Group I includes variables related to structural growth and reproductive biology, such as fruit set, parthenocarpic fruit set, number of aborted fruits, and loose fruits, which directly reflect reproductive performance. Group II comprises variables associated with bunch architecture, such as bunch weight, peduncle weight, and the proportion of normal fruits, including the number of normal fruits, which directly reflect structural development and productive potential. Group III brings together key variables for oil production efficiency, such as the bunch oil percentage (O/B), oil content in the mesocarp of both normal and parthenocarpic fruits (O/DMnf and O/DMpf), and the average weight of parthenocarpic fruits (AWpf). This group is identified as the most relevant for yield optimization, given its strong contribution to final oil content.

Finally, Group IV includes variables that do not directly contribute to oil yield, such as those related to seed structure (average seed weight, endocarp weight, kernel and endocarp-to-fruit ratio) and fruit moisture content. These variables negatively or marginally influence the hybrid's

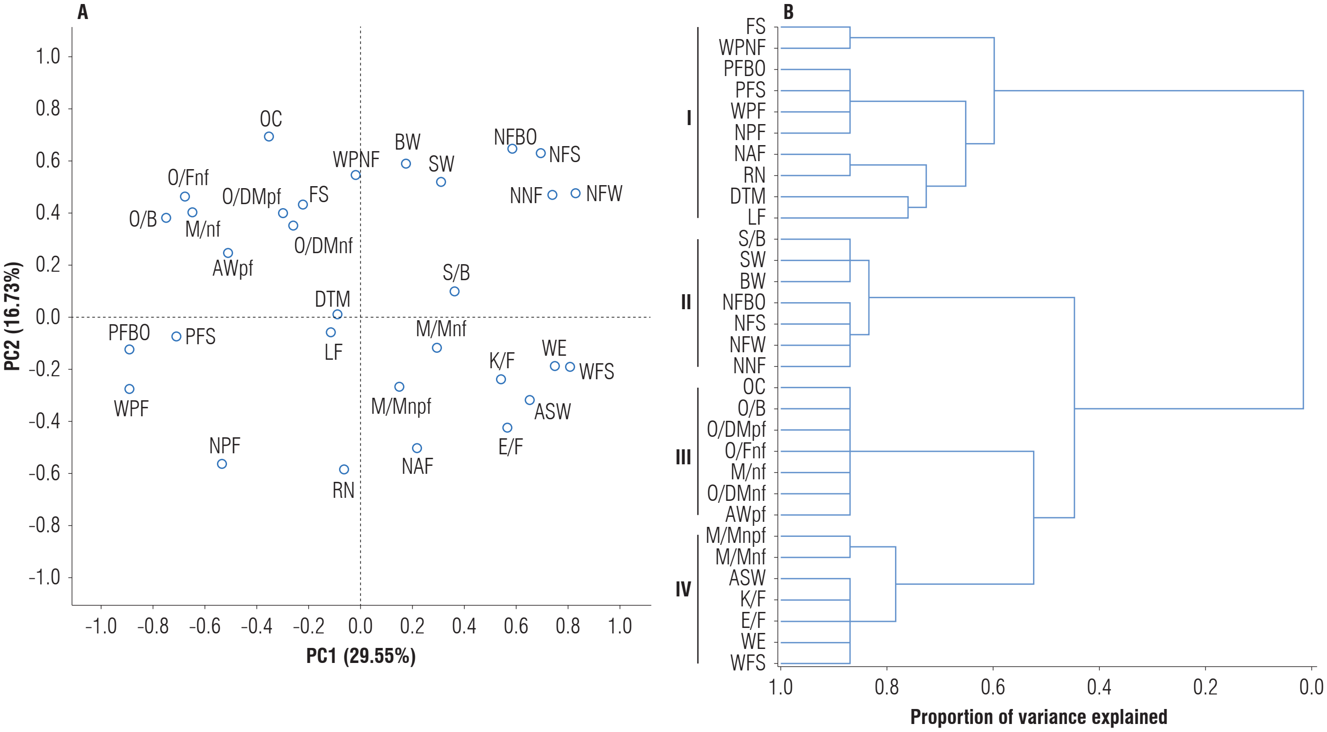


FIGURE 2. Principal component analysis of highly correlated bunch composition variables of the O × G hybrid (A) and dendrogram of their cluster grouping (B). The description of the variables is provided in Table 1.

productive efficiency. This analysis reveals that bunch formation in the O × G hybrid is primarily influenced by fruit set and mesocarp oil content in normal and parthenocarpic fruits.

A regression model was developed to determine which variables analyzed in this study significantly influenced the oil content in the O × G hybrid. The resulting regression model showed an excellent fit to the data, with a coefficient of determination (R^2) of 0.9635 and an adjusted R^2 of 0.9599, indicating that approximately 96% of the variability in oil content (OC) can be explained by the independent variables included in the model. The root mean square error (RMSE) was 328.29, reflecting high prediction accuracy. The mean oil content value was 5.29 kg of oil/bunch, and the coefficient of variation (CV) was 6.19%, indicating low relative dispersion of the data around the mean and suggesting consistency in the measurements. Furthermore, the model yielded an F-statistic of 266.93 with a $P < 0.0001$, confirming that the model is statistically significant and that at least one of the predictor variables had a statistically significant effect on oil content.

All variables included in the model showed a significant effect ($P<0.05$) on oil content, with bunch weight (BW) identified as the most influential predictor (Tab. 2). The analysis revealed that increases in oil content in parthenocarpic fruits and the number of rachillae (RN) are positively associated with oil yield. Overall, the developed regression model proved to be a robust and accurate tool for explaining and predicting oil yield in the *Elaeis oleifera* × *E. guineensis* (O × G) hybrid, providing key information for decision-making in breeding and genetic selection programs.

TABLE 2. Regression analysis of variables affecting yield (OC): model estimates and fits statistics.

Variable	Parameter estimate	Standard error	*t*-value	*P*-value	VIF
Intercept	-10043.0	929.12	-10.81	<0.0001	-
AWpf	123.73	46.36	2.67	0.0090	1.67
O/Fnf	34.97	4.17	8.38	<0.0001	1.26
O/DMpf	54.75	9.0	6.09	<0.0001	1.44
M/Mnpf	-51.55	5.05	-10.22	<0.0001	1.24
BW	0.31	0.01	29.43	<0.0001	1.86
RN	31.95	9.05	3.53	0.0007	2.05
NNF	4.21	1.68	2.51	0.0137	5.69
NFW	1.68	0.26	6.45	<0.0001	11.17
WPF	3.23	0.23	14.0	<0.0001	7.63

VIF: Variance inflation factor.

We performed an ANOVA to evaluate the individual and combined effects of auxin (hormone) application and its different doses on key variables related to oil content. This analysis included the variables previously identified in the regression model and others relevant to oil palm productivity, as presented in Table 3. The ANOVA results revealed significant effects of hormone, doses, and interaction on several analyzed variables. Auxin application had a significant impact on increasing bunch oil percentage (O/B) and the average weight of parthenocarpic fruits (AWpf) (Figs. 3A and 3B).

On the other hand, the dose factor significantly affected most of the evaluated variables, except M/Mnpf and RN, indicating that the level of auxin application directly influenced bunch formation and composition. Moreover, the hormone × dose interaction (H × D) was significant for multiple key variables, including BW, S/B, O/B, OC, AWpf, and O/Fnf. This effect was evident in variables such as oil content (OC), oil-to-fruit ratio in normal fruits (O/Fnf), and the weight of parthenocarpic fruits (WPF). Notably, although the highest dose of NAA (1200 mg L^{-1}) showed a prominent effect, the intermediate dose of NAAS (450 mg L^{-1}) presented very similar values, since it increased both BW and O/B (Figs. 3A and 3C), clearly reflected in the oil content (OC) (Fig. 3D). Additionally, fruit set (Fig. 3E) was equally effective with auxin application (artificial pollination) and the control treatment (assisted pollination). These results indicated that the hormone effect on these variables depended on the applied dose, reflecting a differential response among the evaluated variables.

TABLE 3. ANOVA Type III F-values for the effects of hormone, dose, and their interaction.

Effects	df	BW	S/B	O/B	OC	AWpf	O/Fnf	O/DMpf	M/Mnpf	RN	NNF	NFW	WPF
Hormone	1	1.88	2.16	4.11*	0.04	5.87*	0.04	0.03	2.88	0.83	3.47	0.25	1.94
Dose	2	0.78*	2.56*	15.80*	5.35*	12.19*	3.48*	3.14*	2.65	0.17	15.54*	41.54*	34.10*
H x D	2	1.27*	1.60*	4.16*	4.25*	0.37*	3.49*	0.65	1.69	2.68	0.21	0.17	0.74

df (degrees of freedom), BW (bunch weight), S/B (stalk-to-bunch ratio), O/B (oil-to-bunch ratio), OC (oil content), AWpf (average weight of parthenocarpic fruit), O/Fnf (oil-to-fruit ratio in normal fruits), O/DMpf (oil-to-dry-mesocarp ratio in parthenocarpic fruits), M/Mnpf (moisture-to-mesocarp ratio in parthenocarpic fruits), RN (rachillae number), NNF (number of normal fruits), NFW (normal fruit weight), WPF (weight of parthenocarpic fruits). *$P<0.05$.

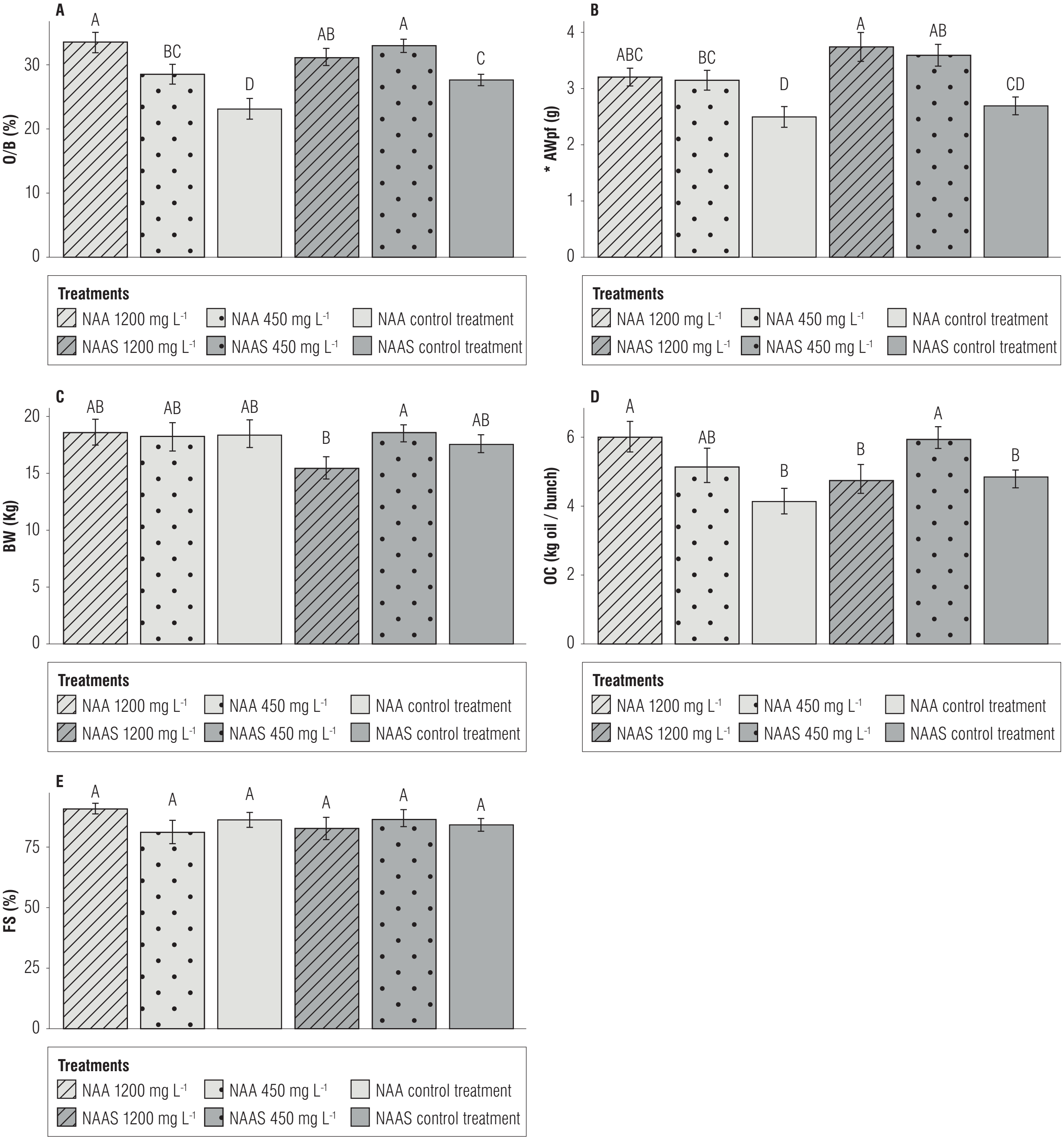


FIGURE 3. Effect of different treatments on the composition variables of oil palm bunches in the (O x G) hybrid: Naphthaleneacetic acid (NAA), sodium naphthaleneacetic acid salt (NAAS), and control treatment (assisted pollination). Vertical bars indicate the standard error. Different letters denote significant differences between treatments according to the LSD test ($P<0.05$) for the least significant difference. *The AWpf parameter considers the weight of normal fruits in the assisted pollination (control treatment).

Discussion

The agronomic management of the interspecific hybrid (O x G) presents significant challenges for oil palm growers, particularly due to its low natural pollination rate (Corley & Tiker, 2015). This limitation has led to the widespread adoption of artificial pollination using NAA as a key strategy to ensure commercial production (Mosquera-Montoya *et al*., 2023). In this context, understanding the factors that influence the hybrid's agronomic performance is essential for optimizing its management and productivity.

Results of this study identified the key variables that determine the hybrid's agronomic performance, providing strategic information useful for strengthening selection and breeding programs. In particular, the traits that showed a significant and positive correlation with oil yield were especially relevant, as their variation in the same direction represented a strategic factor for yield optimization (Krualee *et al*., 2013; Rios *et al*., 2018; Singh *et al*., 2014). These variables included FS, BW, WPNF, and O/B, particularly when associated with parthenocarpic fruits (O/DMpf, PFBO). Altogether, these traits explained the productive behavior of the hybrid, since adequate fruit set enables subsequent fruit development (Socha *et al*., 2019), increasing fruit weight and, consequently, bunch weight. This enhances the fruit-to-bunch and oil-to-bunch ratios (Henson & Dolmat, 2004; Socha *et al*., 2019; Swaray *et al*., 2021).

Similarly, the PCA and cluster analysis enabled the identification of the variables with the most significant influence on oil yield (OC), namely the oil content in the mesocarp of normal fruits (O/DMnf) and parthenocarpic fruits (O/DMpf), as well as the average weight of parthenocarpic fruits (AWpf). In contrast to these yield-promoting variables, mesocarp moisture content and seed-related traits negatively impacted performance. An increase in moisture content is typically associated with a lower oil-to-mesocarp ratio, which also does not favor yield and is often associated with a decline in oil quality during storage (Basyuni *et al*., 2017; Romero *et al*., 2025). Likewise, the presence of seeds and endocarp in normal fruits is also considered disadvantageous, as noted by several authors (Rios *et al*., 2018; Tanya *et al*., 2021), since their absence allows for a greater proportion of the fruit to be composed of fleshy mesocarp, thereby enhancing the actual production of marketable oil (Corley & Tiker, 2015; Htwe *et al*., 2022) in spite of constraints on palm kernel oil production.

The results described above were consistent with the developed regression model (Tab. 2), in which the most statistically significant variables were bunch weight and those primarily associated with parthenocarpic fruits. Thus, parthenocarpic fruits and related variables emerge as the most critical factors for optimizing oil production in the hybrid. Several studies have demonstrated that the induction of parthenocarpic fruits through auxin application is a practice that not only ensures fruit formation but also significantly enhances crop profitability by increasing yield (Hormaza Martínez *et al*., 2010; Mosquera-Montoya *et al*., 2023; Romero *et al*., 2021).

Our results revealed significant effects of the hormone, dose, and interaction on several analyzed variables. In particular, auxin application had a significant effect on bunch weight (BW), oil-to-bunch ratio (O/B), and the average weight of parthenocarpic fruits (AWpf). Similar results with auxin application are reported by Cayón Salinas *et al*. (2022), Romero *et al*. (2021), and Daza *et al*. (2020), who demonstrate that auxin use enhances the oil-to-dry mesocarp ratio, oil-to-bunch ratio, bunch weight, and fruit set (FS). The latter (FS) showed values exceeding the ideal fruit set threshold (> 80%) as reported by Mohd Haniff and Mohd Roslan (2002). It is essential to highlight that, although the highest dose of NAA (1200 mg L^{-1}) exhibited outstanding effects on OB and OC—also reported by Romero *et al*. (2021)—the sodium salt form of 1-naphthaleneacetic acid (NAAS) at an intermediate dose (450 mg L^{-1}) proved to be similarly effective in this study, which may be attributed to their solubility and transport properties. NAAS, being more soluble, might be absorbed more efficiently, leading to distinct physiological responses. These treatments not only increased fruit and bunch biomass but also enhanced oil production efficiency, with a clear effect on oil yield (OC) (Fig. 3). Thomas *et al*. (1973) report that in oil palms, auxins are the group of hormones primarily responsible for inducing parthenocarpic fruit formation. More recent evaluations, in which other hormones such as gibberellins and an ethylene precursor were tested, confirm that auxins were the most effective in inducing parthenocarpy, with a direct impact on yield (Cayón *et al*., 2022; Daza *et al*., 2020). Auxins promote optimal fruit development through hormonal signaling that triggers the transformation of the ovary into fruit, enhances fruit set, and stimulates cell division (Montoya *et al*., 2023; Pandolfini *et al*., 2007; Socha *et al*., 2019; Somyong *et al*., 2018). This hormone regulates key cell cycle transitions and facilitates cell elongation by acidifying the extracellular space, activating proteins such as expansins and xyloglucan endotransglucosylase/hydrolases. These proteins relax the cell wall and enable cell expansion by allowing water influx into the cell (Campanoni & Nick, 2005; Perrot-Rechenmann, 2010), and auxins can also increase sink activity. Collectively, these processes support growth and biomass accumulation in the mesocarp (Bennett & Leyser, 2014; Romero *et al*., 2021; Zhang *et al*., 2021). These findings open opportunities for further research involving the use of this molecule as a potential sustainable intensification strategy, understood as productivity improvement through efficient input use (Lerner *et al*., 2017; Sekaran *et al*., 2021).

Altogether, the results reaffirm the ability of auxins to enhance yield and profitability, considering the cost-benefit trade-off when compared to assisted pollination, due to the increase in oil output, making it a highly beneficial strategy for oil palm growers (Mosquera-Montoya *et al*., 2023).

An interesting response was observed with the highest dose of NAAS (1200 mg L^{-1}), which led to a decrease in bunch weight and oil content. This effect was likely due to the disruption caused by supra-optimal doses on hormonal homeostasis and growth regulation. At high concentrations, auxins can become toxic, and when applied exogenously, synthetic auxins are less susceptible to homeostatic control mechanisms, such as degradation, conjugation, transport, or sequestration, compared to natural auxins (Mellor *et al*., 2016; Taiz *et al*., 2017). This underscores the importance of applying appropriate doses when inducing parthenocarpic fruit formation.

These findings help clarify the behavior of the variables that explain oil yield in the hybrid in response to auxin application, highlighting their role in bunch formation and architecture, biomass accumulation, and improving oil production efficiency. This supports the progressive replacement of oil palm plantations in recent years in Colombia (Avila *et al*., 2016; Rincón *et al*., 2013; Romero *et al*., 2021), due to the hybrid's direct impact on productivity, as well as its high profitability and sustainability.

Conclusions

The variables derived from the analysis of bunch composition effectively explained the productive performance of the interspecific hybrid O × G. The developed model identified key variables that, collectively, provided valuable insights for genetic improvement programs targeting this hybrid. Applying auxins significantly enhanced crop productivity, primarily by promoting fruit set, in a similar manner to assisted pollination, and by increasing bunch biomass, especially through the induction of parthenocarpic fruits. These fruits, lacking seeds and endocarp, have a higher mesocarp proportion, resulting in increased oil content per bunch. The optimal doses were 450 mg L^{-1} of NAAS and 1200 mg L^{-1} of NAA, highlighting the importance of using appropriate concentrations to induce parthenocarpy effectively. These findings confirmed the potential of auxins as a strategy to boost oil production, with significant economic implications that enhance crop profitability and strengthen the sustainability of the oil palm sector.

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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

KAML and ALBO designed and conducted field experiments. JEPD, KAML, and DMAM contributed to data analysis. KAML, DMAM, and HEBL prepared the draft of the manuscript. All authors reviewed and approved the final version.

Literature cited

Arias, D., González, M., Prada, F., Ayala-Diaz, I., Montoya, C., Daza, E., & Romero, H. M. (2015). Genetic and phenotypic diversity of natural American oil palm (*Elaeis oleifera* (H.B.K.) Cortés) accessions. *Tree Genetics & Genomes*, *11*(6), Article 122. https://doi.org/10.1007/s11295-015-0946-y

Avila-Diazgranados, R. A., Daza, E. S., Navia, E., & Romero, H. M. (2016). Response of various oil palm materials (*Elaeis guineensis* and *Elaeis oleifera* × *Elaeis guineensis* interspecific hybrids) to bud rot disease in the southwestern oil palm-growing area of Colombia. *Agronomía Colombiana*, *34*(1), Article 53760. https://doi.org/10.15446/agron.colomb.v34n1.53760

Azzeme, A. M., Abdullah, S. N. A., Aziz, M. A., & Megat Wahab, P. E. (2020). Overexpression of oil palm *EgDREB1* in tomato decreased fruit size and produced parthenocarpic fruits. *Biologia Plantarum*, *64*(1), 58–67. https://doi.org/10.32615/bp.2019.084

Basyuni, M., Amri, N., Putri, L. A. P., Syahputra, I., & Arifiyanto, D. (2017). Characteristics of fresh fruit bunch yield and the physicochemical qualities of palm oil during storage in North Sumatra, Indonesia. *Indonesian Journal of Chemistry*, *17*(2), 182–190. https://doi.org/10.22146/ijc.24910

Bennett, T., & Leyser, O. (2014). The auxin question: A philosophical overview. In E. Zažímalová, J. Petrášek, & E. Benková (Eds.), *Auxin and its role in plant development* (pp. 3–19). Springer. https://doi.org/10.1007/978-3-7091-1526-8_1

Campanoni, P., & Nick, P. (2005). Auxin-dependent cell division and cell elongation. 1-naphthaleneacetic acid and 2,4-dichlorophenoxyacetic acid activate different pathways. *Plant Physiology*, *137*(3), 939–948. https://doi.org/10.1104/pp.104.053843

Cayón Salinas, D. G., Ligarreto Moreno, G. A., Magnitskiy, S., Rosero, G., & Leguizamón, O. (2022). Application of naphthalene acetic acid and gibberellic acid favours fruit induction and development in oil palm hybrid (*Elaeis oleifera* x *Elaeis guineensis*). *Experimental Agriculture*, *58*, Article e35. https://doi.org/10.1017/s001447972200031x

Chew, C. L., Ng, C. Y., Hong, W. O., Wu, T. Y., Lee, Y.-Y., Low, L. E., Kong, P. S., & Chan, E. S. (2021). Improving sustainability of palm oil production by increasing oil extraction rate: a review. *Food and Bioprocess Technology*, *14*(4), 573–586. https://doi.org/10.1007/s11947-020-02555-1

Corley, R. H. V., & Tiker, P. B. (2015). The origin and development of the oil palm industry. In R. H. V. Corley, & P. Tinker (Eds.), *The oil palm* (pp. 1–29). John Wiley & Sons Ltd. https://doi.org/10.1002/9781118953297.ch1

Daza, E., Ayala-Díaz, I., Ruiz-Romero, R., & Romero, H. M. (2020). Effect of the application of plant hormones on the formation of parthenocarpic fruits and oil production in oil palm interspecific hybrids (*Elaeis oleifera* Cortes x *Elaeis guineensis* Jacq.). *Plant Production Science*, *24*(3), 354–362. https://doi.org/10.1080/1343943X.2020.1862681

Fedepalma. (2015). *Anuario estadístico 2015: la agroindustria de la palma de aceite en Colombia y en el mundo 2010–2014*. https://publicaciones.fedepalma.org/index.php/anuario/article/view/11721

Fedepalma. (2021). *Anuario estadístico 2021: principales cifras de la agroindustria de la palma de aceite en Colombia y en el mundo 2016–2020*. https://publicaciones.fedepalma.org/index.php/anuario/issue/view/1556

Henson, I. E., & Dolmat, M. T. (2004). Seasonal variation in yield and developmental processes in an oil palm density trial on a peat soil: 2. Bunch weight components. *Journal of Oil Palm Research*, *16*(2), 106–120. https://jopr.mpob.gov.my/seasonal-variation-in-yield-and-developmental-processes-in-an-oil-palm-density-trial-on-a-peat-soil-2-bunch-weight-components

Hormaza Martínez, P. A., Forero Hernández, D. C., Ruiz Romero, R., & Romero Angulo, H. M. (2010). *Fenología de la palma de aceite africana (Elaeis guineensis Jacq.) y del híbrido interespecífico (Elaeis oleifera [Kunt] Cortes x Elaeis guineensis Jacq.).* Cenipalma. https://repositorio.fedepalma.org/handle/123456789/80999

Hormaza, P., Mesa Fuquen, E., & Romero, H. M. (2012). Phenology of the oil palm interspecific hybrid *Elaeis oleifera* × *Elaeis guineensis*. *Scientia Agricola*, *69*(4), 275–280. https://doi.org/10.1590/S0103-90162012000400007

Htwe, Y. M., Shi, P., Zhang, D., Li, Z., Xiao, Y., Yang, Y., Lei, X., & Wang, Y. (2022). Programmed cell death may be involved in the seedless phenotype formation of oil palm. *Frontiers in Plant Science*, *13,* Article 832017. https://doi.org/10.3389/fpls.2022.832017

Jain, A. K. (1988). *Algorithms for clustering data*. Prentice Hall.

Jolliffe, I. T. (2002). Principal component analysis for special types of data. In I. T. Jolliffe (Ed.), *Principal component analysis* (2nd ed., pp. 338–372). Springer. https://doi.org/10.1007/0-387-22440-8_13

Krualee, S., Sdoodee, S., Eksomtramage, T., & Sereeprasert, V. (2013). Correlation and path analysis of palm oil yield components in oil palm (*Elaeis guineensis* Jacq.). *Agriculture and Natural Resources*, *47*(4), 528–533. https://li01.tci-thaijo.org/index.php/anres/article/view/243092

Lerner, A. M., Zuluaga, A. F., Chará, J., Etter, A., & Searchinger, T. (2017). Sustainable cattle ranching in practice: moving from theory to planning in Colombia's livestock sector. *Environmental Management*, *60*(2), 176–184. https://doi.org/10.1007/s00267-017-0902-8

Liu, J., Zhai, R., Liu, F., Zhao, Y., Wang, H., Liu, L., Yang, C., Wang, Z., Ma, F., & Xu, L. (2018). Melatonin induces parthenocarpy by regulating genes in gibberellin pathways of 'Starkrimson' pear (*Pyrus communis* L.). *Frontiers in Plant Science*, *9*, Article 946. https://doi.org/10.3389/fpls.2018.00946

Mandal, N. K., Kumari, K., Kundu, A., Arora, A., Bhowmick, P. K., Iquebal, M. A., Jaiswal, S., Behera, T. K., Munshi, A. D., & Dey, S. S. (2022). Cross-talk between the cytokinin, auxin, and gibberellin regulatory networks in determining parthenocarpy in cucumber. *Frontiers in Genetics*, *13*, Article 957360. https://doi.org/10.3389/fgene.2022.957360

Mellor, N., Band, L. R., Pěnčík, A., Novák, O., Rashed, A., Holman, T., Wilson, M. H., Voß, U., Bishopp, A., King, J. R., Ljung, K., Bennett, M. J., & Owen, M. R. (2016). Dynamic regulation of auxin oxidase and conjugating enzymes *AtDAO1* and *GH3* modulates auxin homeostasis. *Proceedings of the National Academy of Sciences*, *113*(39), 11022–11027. https://doi.org/10.1073/pnas.1604458113

Mohd Haniff, H., & Mohd Roslan, N. (2002). Fruit set and oil palm bunch components. *Journal of Oil Palm Research*, *14*(2), 24–33. https://jopr.mpob.gov.my/fruit-set-and-oil-palm-bunch-components

Montoya, C., Mejia-Alvarado, F. S., Botero-Rozo, D., Ayala-Diaz, I. M., & Romero, H. M. (2023). Parthenocarpy-related genes induced by naphthalene acetic acid in oil palm interspecific O × G [*Elaeis oleifera* (Kunth) Cortés × *Elaeis guineensis* Jacq.] hybrids. *Frontiers in Genetics*, *14*, Article 1099489. https://doi.org/10.3389/fgene.2023.1099489

Mosquera-Montoya, M., Camperos, J. E., Ruiz, E., Hernández, D., García, A., Vargas, L. E., Mesa, E., Munévar, D., & Sinisterra, K. (2023). Evidence of sustainable intensification in the production of palm oil from crops planted with *Elaeis oleifera* x *Elaeis guineensis* in Colombia. *Frontiers in Sustainable Food Systems*, *7*, Article 1217653. https://doi.org/10.3389/fsufs.2023.1217653

Murphy, D. J., Goggin, K., & Paterson, R. R. M. (2021). Oil palm in the 2020s and beyond: challenges and solutions. *CABI Agriculture and Bioscience*, *2*(1), Article 39. https://doi.org/10.1186/s43170-021-00058-3

Nieto Mogollon, D. I., Venturini, O. J., Ocampo Batlle, E. A., Martinez González, A., Munar-Florez, D. A., Ramirez-Contreras, N. E., García-Nuñez, J. A., Borges, P. T., & Silva Lora, E. E. (2024). Environmental and energy issues in biodiesel production using palm oil from the interspecific hybrid OxG and *Elaeis guineensis*: a case study in Colombia. *Oilseeds and fats, Crops and Lipids-OCL*, *31*, Article 25. https://doi.org/10.1051/ocl/2024020

Pandolfini, T., Molesini, B., & Spena, A. (2007). Molecular dissection of the role of auxin in fruit initiation. *Trends in Plant Science*, *12*(8), 327–329. https://doi.org/10.1016/j.tplants.2007.06.011

Pearson, K. (1900). I. Mathematical contributions to the theory of evolution. – VII. On the correlation of characters not quantitatively measurable. *Philosophical Transactions of the Royal Society of London. Series A, Containing Papers of a Mathematical or Physical Character*, *195*(262-273), 1–47. https://doi.org/10.1098/rsta.1900.0022

Perrot-Rechenmann, C. (2010). Cellular responses to auxin: Division versus expansion. *Cold Spring Harbor Perspectives in Biology*, *2*(5), Article a001446. https://doi.org/10.1101/cshperspect.a001446

Pirker, J., Mosnier, A., Kraxner, F., Havlík, P., & Obersteiner, M. (2016). What are the limits to oil palm expansion? *Global Environmental Change*, *40*, 73–81. https://doi.org/10.1016/j.gloenvcha.2016.06.007

Prada, C., F., & Romero, H. M. (2012). *Muestreo y análisis de racimos en el cultivo de la palma de aceite*. Tecnologías para la agroindustria de la palma de aceite: guía para facilitadores. Javegraf, Bogotá, Colombia. https://repositorio.fedepalma.org/handle/123456789/107697

Rincón, S. M., Hormaza, P. A., Moreno, L. P., Prada, F., Portillo, D. J., García, J. A., & Romero, H. M. (2013). Use of phenological stages of the fruits and physicochemical characteristics of the oil to determine the optimal harvest time of oil palm interspecific OxG hybrid fruits. *Industrial Crops and Products*, *49*, 204–210. https://doi.org/10.1016/j.indcrop.2013.04.035

Rios, S. A., Cunha, R. N. V., Lopes, R., Barcelos, E., Rocha, R. N. C., & Lima, W. A. A. (2018). Correlation and Path analysis for yield components in Dura oil palm germplasm. *Industrial Crops and Products*, *112*, 724–733. https://doi.org/10.1016/j.indcrop.2017.12.054

Romero, H. M., Daza, E., Ayala-Díaz, I., & Ruiz-Romero, R. (2021). High-oleic palm oil (hopo) production from parthenocarpic fruits in oil palm interspecific hybrids using naphthalene acetic acid. *Agronomy*, *11*(2), Article 290. https://doi.org/10.3390/agronomy11020290

Romero, H. M., Ruiz-Romero, R., Caicedo-Zambrano, A. F., Ayala-Diaz, I., & Rodríguez, J. L. (2025). Determining the optimum harvest point in oil palm interspecific hybrids (O×G) to maximize oil content. *Agronomy*, *15*(4), Article 887. https://doi.org/10.3390/agronomy15040887

Rosero, G., Santacruz, L., & Carvajal, S. (2017). Influencia del destape de la inflorescencia en la polinización asistida del híbrido OxG. *Palmas, 38*(1), 49–62. https://publicaciones.fedepalma.org/index.php/palmas/article/view/12044

Ruiz-Alvarez, E., Daza, E. S., Caballero-Blanco, K., & Mosquera-Montoya, M. (2021). Complementing assisted pollination with artificial pollination in oil palm crops planted with interspecific hybrids O × G (*Elaeis guineensis* × *Elaeis oleifera*): Is it profitable? *Oilseeds and fats, Crops and Lipids – OCL*, *28*, Article 27. https://doi.org/10.1051/ocl/2021014

SAS – Statistical Analysis Software Institute Inc. (2013). *The statistical analysis software (SAS®) version 9.04*. SAS Institute Inc., Cary.

Sekaran, U., Lai, L., Ussiri, D. A. N., Kumar, S., & Clay, S. (2021). Role of integrated crop-livestock systems in improving agriculture production and addressing food security – A review. *Journal of Agriculture and Food Research*, *5*, Article 100190. https://doi.org/10.1016/j.jafr.2021.100190

Singh, R., Low, E.-T. L., Ooi, L. C.-L., Ong-Abdullah, M., Nookiah, R., Ting, N.-C., Marjuni, M., Chan, P.-L., Ithnin, M., Manaf, M. A. A., Nagappan, J., Chan, K.-L., Rosli, R., Halim, M. A., Azizi, N., Budiman, M. A., Lakey, N., Bacher, B., Van Brunt, A., …, & Sambanthamurthi, R. (2014). The oil palm *VIRESCENS* gene controls fruit colour and encodes a R2R3-MYB. *Nature Communications*, *5*(1), Article 4106. https://doi.org/10.1038/ncomms5106

Socha, J., Cayón, D., Ligarreto, G., & Chaves, G. (2019). Effect of pollen doses on fruit formation and oil production in two hybrid palm genotypes (*Elaeis oleifera* H.B.K. Cortes x *Elaeis guineensis* Jacq.). *Agronomía Colombiana*, *37*(1), 12–17. https://doi.org/10.15446/agron.colomb.v37n1.75313

Somyong, S., Walayaporn, K., Jomchai, N., Naktang, C., Yodyingyong, T., Phumichai, C., Pootakham, W., & Tangphatsornruang, S. (2018). Transcriptome analysis of oil palm inflorescences revealed candidate genes for an auxin signaling pathway involved in parthenocarpy. *PeerJ*, *6*, Article e5975. https://doi.org/10.7717/peerj.5975

Swaray, S., Amiruddin, M. D., Rafii, M. Y., Jamian, S., Ismail, M. F., Jalloh, M., Eswa, M., Marjuni, M., Akos, I. S., & Yusuff, O. (2021). Oil palm inflorescence sex ratio and fruit set assessment in *dura* × *pisifera* biparental progenies on fibric peat soil. *Agronomy*, *11*(7), Article 1380. https://doi.org/10.3390/agronomy11071380

Taiz, L., Zeiger, E., Møller, I. M., & Murphy, A. S. (2017). *Fisiologia e desenvolvimento vegetal* (6th ed.). Artmed.

Tanya, P., Hadkam, Y., Taeprayoon, P., & Srinives, P. (2013). Estimates of repeatability and path coefficient of bunch and fruit traits in Bang Boet *Dur*a oil palm. *Journal of Oil Palm Research*, *25*(1), 108–115. https://jopr.mpob.gov.my/wp-content/uploads/2013/10/joprv25april2013-Patcharin1.pdf

Thomas, R. L., Seth, A. K., Chan, K. W., & Ooi, S. C. (1973). Induced parthenocarpy in the oil-palm. *Annals of Botany*, *37*(3), 447–452. https://doi.org/10.1093/oxfordjournals.aob.a084711

Van Hintum, Th. J. L., Brown, A. H. D., Spillane, C., & Hodgkin, T. (2000). *Core collections of plant genetic resources (Technical Bulletin No. 3).* International Plant Genetic Resources Institute IPGRI. Rome, Italy. https://research.wur.nl/en/publications/core-collections-of-plant-genetic-resources

Zhang, S., Gu, X., Shao, J., Hu, Z., Yang, W., Wang, L., Su, H., & Zhu, L. (2021). Auxin metabolism is involved in fruit set and early fruit development in the parthenocarpic tomato "R35-P". *Frontiers in Plant Science*, *12*, Article 671713. https://doi.org/10.3389/fpls.2021.671713

Influence of propagules and inoculation method on the development of potato early dying caused by *Verticillium* spp.

Influencia de los propágulos y el método de inoculación en el desarrollo de la marchitez temprana de la papa causada por *Verticillium* spp.

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ABSTRACT

Potato early dying is a significant disease in potato-producing countries. However, the development of the disease as a result of infection by the different propagules of the pathogen and at different times of inoculation is unclear. In this study, the infection of potato plants var. Diacol Capiro by two types of *Verticillium* spp. propagules, conidia, and microsclerotia (MS), was assessed. Conidia were evaluated based on inoculation of the soil at the sowing time of the tuber, or in soil drench and root immersion 49 days after sowing (das). Microsclerotia were evaluated for their incorporation into soil at different densities at the time of sowing. The tests were carried out under a complete factorial design in a completely randomized arrangement with five repetitions per treatment; the experimental unit was a plant. Non-inoculated plants grown in sterile soil were used as controls in both cases. Variables measured included disease severity, area under the disease progress curve (AUDPC), incubation period, phenological development of the plants, and yield components. Disease incubation periods ranging from 65 to 70 d were recorded when *Verticillium* spp. was inoculated at the time of sowing. With inoculation at 49 das, the incubation period was reduced to 23 days after inoculation (dai), with lower disease severity than at the time of sowing. The results showed that all inoculation methods, times, and strains of *Verticillium* spp. caused the typical symptoms of the disease and led to the development of early death. Infection of potato plants was possible with the inoculation of conidia or MS of the pathogen; however, in the latter case, it depended on the inoculum density. While the disease induced by the different inoculation methods led to a reduction in tuber weight, it did not impact the number of tubers produced per plant.

Keywords: conidia, microsclerotia, potato diseases, soil-borne pathogens, vascular wilt.

RESUMEN

La marchitez temprana es una enfermedad importante en los países productores de papa. Sin embargo, el desarrollo de la enfermedad bajo diferentes propágulos del patógeno y momentos de la inoculación no es claro. En este estudio, se evaluó la infección de plantas de papa variedad Diacol Capiro con dos tipos de propágulos de *Verticillium* spp., conidias y microesclerocios (MS). Las conidias se evaluaron en inoculación al suelo al momento de la siembra de los tubérculos, o en drench e inmersión de raíces a los 49 días después de la siembra (dds). La infección por MS se evaluó mediante incorporación al suelo en diferentes densidades al momento de la siembra. Los experimentos se realizaron bajo un diseño factorial completamente al azar con cinco repeticiones por tratamiento; la unidad experimental fue una planta. En ambos casos como controles se utilizaron plantas no inoculadas sembradas en suelo estéril. Como variables se evaluaron la severidad de la enfermedad, el área bajo la curva de progreso de la enfermedad (AUDPC), el período de incubación, el desarrollo fenológico de las plantas y componentes de rendimiento. Los períodos de incubación de la enfermedad variaron entre 65 y 70 d cuando *Verticillium* spp. se inoculó al momento de la siembra. Con la inoculación a los 49 dds, el periodo de incubación se redujo a 23 días después de la inoculación (ddi) con menor severidad con respecto a la inoculación al momento de la siembra. Los resultados mostraron que todos los métodos, momentos de inoculación y aislamientos de *Verticillium* spp. causaron síntomas típicos y llevaron al desarrollo de la marchitez temprana. La infección de las plantas de papa fue posible con la inoculación de conidias o MS, siendo estos últimos dependientes de la densidad de inóculo. Con la inoculación por los diferentes métodos, la enfermedad redujo el peso de los tubérculos, pero no afectó el número de tubérculos por planta.

Palabras clave: conidios, microesclerocios, enfermedades de papa, patógenos del suelo, marchitamiento vascular.

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Introduction

The potato is the fourth most important crop in the world. It has a tremendous economic impact due to its nutritional contribution of carbohydrates, minerals, and vitamins (Lima *et al*., 2018). However, its performance is affected by various factors, including genotype, environmental conditions, agronomic practices, and limiting pests and diseases (Jakubowski *et al.,* 2024). One of the diseases affecting this crop is early dying caused by *Verticillium* spp., which may reduce plant performance by 50% or more (Nieto, 1988). For this reason, it has attracted interest in potato-producing areas worldwide. Potato early dying has become one of the most economically significant diseases affecting the crop, not only because of its negative impact on yield and tuber quality but also due to the accumulation of inoculum in the soil, which can render infested fields unsuitable for future potato production (Li *et al*., 2019; Simko & Haynes, 2017). Among the pathogenic *Verticillium* species in cultivated plants, the most noteworthy include *V. albo-atrum* Reinke & Berthold, *V. dahliae* Kleb (Daami-Remadi *et al*., 2011), and *V. tricorpus* Isaac, all of which infect stems, vascular tissue, and tubers (Nair *et al*., 2019). Of these, *V. albo-atrum* and *V. dahliae* are widely distributed and are the most common species (Klosterman *et al*., 2009; Nair *et al*., 2019; Powelson & Rowe, 1993). In Colombia, *V. albo-atrum* and *V. dahliae* have been reported to be associated with potato early dying in Antioquia, Boyacá, Nariño, Norte de Santander, and Cundinamarca (Gómez-Caro & Mendoza-Vargas, 2020; Nieto, 1988).

Verticillium dahliae has a wide host range, including more than 200 plant species. It can survive for long periods in the soil, even in the presence of plants, by forming resistance structures called microsclerotia (MS) (Johnson & Dung, 2010). Microesclerotia can be spread by crop residues, transportation of contaminated seed, air currents, irrigation, and other agricultural activities (Zhang *et al*., 2023) and can survive for up to 10 years in soil (Steere & Kirk, 2015). Meanwhile, *V. albo-atrum* has a more limited host range, encompassing 30-40 plant species. It survives for shorter periods through melanized hyphae that can remain viable for 3 to 5 years in soil (Gómez-Caro & Mendoza-Vargas, 2020; Johnson & Dung, 2010). Finally, *V. tricorpus* produces resistance structures, including chlamydospores, resting dark mycelium, and large, irregularly shaped MS. However, it is a less aggressive species than *V. dahliae* and *V. albo-atrum* (Nair *et al*., 2019).

Other species of the pathogen that until now have been of lesser importance but have attracted increasing interest are *V. nonalfalfae* and *V. alfalfae*. These refer to pathotypes of *V. albo-atrum sensu lato*, named based on a taxonomic revision of the genus (Inderbitzin *et al*., 2011; Inderbitzin & Subbarao, 2014). *Verticillium nonalfalfae* is morphologically indistinguishable from *V. alfalfae* but differs in host range and DNA characters (EPPO, 2020). The presence of *V. nonalfalfae* has been reported in Canada, Cuba, Germany, Japan, Slovenia, and the United Kingdom, and it infects several hosts, including hops, petunia, spinach, and potato (Inderbitzin *et al*., 2011). On the other hand, Li and Li (2021) report *V. alfalfae* causing disease on seven non-alfalfa plant species including bluish dogbane, common vetch, cotton, erect milkvetch, potato, sainfoin, and sunflower; it is reported in Canada, France, Germany, Iran, Japan, New Zealand, Russia, Sweden, and the USA (Pegg & Brady, 2002).

The MS or melanized hyphae formed by *V. dahliae* and *V. albo-atrum,* respectively, can colonize roots in response to plant exudates, which they use as signals to recognize their hosts and initiate infection (Klosterman *et al*., 2009). The fungus penetrates the plant's roots directly or through wounds (Gómez-Caro & Mendoza-Vargas, 2020). Once the fungus enters the root, it colonizes the bark, and the mycelium can enter the xylem vessels. Once there, the fungus travels to the upper third of the plant via the vascular system as conidia. As the fungus colonizes the vascular system, symptoms such as chlorosis, necrosis, and leaf wilting begin to appear (Steere & Kirk, 2015). The pathogen-induced early dying of potato plants can result in premature senescence, leading to decreased tuber production (Rowe & Powelson, 2002).

To date, research on this disease caused by *Verticillium* spp. has mainly focused on countries such as Australia, Canada, China, and the USA. These studies have helped to understand infection by this pathogen in various economically important plant species, such as alfalfa, cotton, eggplant, lettuce, maple, okra, olive, potato, rapeseed, sunflower, spinach, strawberry, tomato, and watermelon (Rowe & Powelson, 2002; Wu *et al*., 2022), and recently on avocado in Colombia (Ramírez-Gil & Peterson, 2019). Some studies on *Verticillium* spp. have been conducted under field conditions, using *in situ* inoculum in the plot's soil as the source of the pathogen. These include studies by Platt and Sanderson (1987), Uppal *et al.* (2008), Trapero, Serrano *et al*. (2013), and Mulero-Aparicio *et al*. (2020), who used different types of *Verticillium* propagules and inoculum densities varying between 4×10^6 and 1×10^7 conidia ml^{-1}, 5 and 21 MS g^{-1} soil, and 35 UCF g^{-1}, respectively. Although these studies have contributed significantly to knowledge of *Verticillium* disease in various crops, results may vary due to inoculum density, the type of propagule used, and abiotic and biotic factors that affect assay development under field conditions.

In the specific case of potatoes, research has been carried out using artificial inoculation methods in varieties 'Russet' and 'Kennebec' type in the USA and Canada (Bae *et al.*, 2007; Platt & Sanderson, 1987), 'Shepody' in China (Zhang *et al.*, 2023), and 'Victoria' and 'Tasmania' in Australia (Nair *et al.*, 2019). In Colombia, Nieto (1988), Guerrero *et al.* (1991), and Benavides *et al.* (1995) have studied the disease in varieties such as ICA Nariño, ICA Tequendama, and Parda Pastusa.

In studies using artificial inoculation, a concept known as pathogenicity testing is used, which is based on Koch's postulates. Despite their secular history, the postulates have stood the test of time because of their philosophical essence and conceptual stability (Volcy, 2008). The importance of pathogenicity tests in the study of *Verticillium* spp. is evident in the research carried out by Bae *et al.* (2007). Daami-Remadi *et al.* (2011) used concentrations of 1×10^7 and 8×10^6 conidia ml^{-1}, respectively, and successfully reproduced the disease in potato varieties.

Meanwhile, in Colombia, some work has been carried out with inoculations of the pathogen at the time of plant emergence using concentrations of $5x10^5$ and 5×10^7 conidia ml^{-1} (Guerrero *et al.*, 1991), and with 5 g of wheat previously inoculated with *Verticillium* (Benavides *et al.*, 1995). Regarding the use of MS of the pathogen as inoculum, Land *et al.* (2017) conducted a study in commercial cotton lots, using 250 ml of a suspension containing 1×10^7 MS ml^{-1} that was incorporated into the substrate within the first 12 cm pots. In all these trials, disease symptoms were observed, and the pathogen was confirmed.

As described, various studies have separately documented the use of pathogenicity tests with different inoculation methods and *Verticillium* spp. inoculum densities. Among the most frequently used methods are the application of a suspension of the pathogen to the soil (drench) (Leon-Ttacca *et al.*, 2018), immersion of roots in the suspension of the pathogen (Serrano *et al.*, 2023; Trapero, Díez *et al.*, 2013), incorporation of propagules into substrate (wheat) (Benavides *et al.*, 1995) or into the soil (Platt & Sanderson, 1987), and inoculation by puncture or wound (Leon-Ttacca *et al.*, 2018; Zhang *et al.*, 2023). Although the disease has been reproduced using different methods and inoculum densities, no studies have shown how the pathogenesis process varies across different infective structures, such as conidia, microsclerotia, or melanized mycelium formed by the different *Verticillium* species reported in potato.

Since no comparative studies on the timing of pathogen inoculation and its effects on potato early dying parameters exist, the possible outcomes of disease development under variations in inoculation time, propagule type, and inoculation method remain unresolved. This information would not only provide insight into the development of potato early dying under different inoculation methods, but also into the mechanisms underlying it. But it would also facilitate the interpretation and discussion of the results obtained. Additionally, it is necessary to clarify whether the disease can occur due to pathogen infection at more advanced stages of plant development, rather than only at the sowing time. Therefore, this work sought to evaluate different inoculation methods, using conidia and microsclerotia from *Verticillium* spp. obtained from infected plants in commercial fields in Cundinamarca (Colombia) on potato early dying development.

Materials and methods

The study was carried out in a greenhouse at the Facultad de Ciencias Agrarias of the Universidad Nacional de Colombia (UNAL), Bogotá campus, located at 4°38'17.3 N and 74°05'20.3" W, at an altitude of 2630 m a.s.l. The study took place over the second half of 2021 and the first half of 2022. The environmental conditions during the experiments were an average daily temperature of ± 23°C, 50% and 70% relative humidity, and a natural photoperiod of 12 h. The volume required for watering the plants was determined by daily quantification of their evapotranspiration requirements, as described by Hainaut *et al.* (2016).

Plant material and *Verticillium* strains

As plant material, certified potato seed tubers var. Diacol Capiro was used. The seed tubers were planted in 3 kg plastic pots containing soil from non-agricultural areas previously sterilized through autoclaving for 2 cycles of 30 min at 121°C and 21 psi, followed by 20 min of drying. Soil fertilization was carried out at the time of sowing by applying the commercial fertilizer Sir 24 (Precisagro SAS, Colombia) containing $12N\text{-}24P_2O_5\text{-}12K_2O+10CaO+1.5S$ at a dose of 40 g per plant.

In the study, two monosporic strains of *Verticillium* spp. obtained from infected potato plants in commercial fields in Cundinamarca (Colombia), and one reference strain of *V. albo-atrum* from potato was used. The *V. albo-atrum* strain was provided by the microbiology laboratory of the Colombian Agricultural Research Corporation (Agrosavia). The other two strains were obtained from plants of the Diacol Capiro variety with symptoms of potato early dying collected in commercial plots in Funza (4°44’56.0” N, 74°12’04.0” W) (Funza 104 strain) and Mosquera (4°39’48.0” N, 74°14’42.0” W) (Mosquera 98 strain). These two strains (Funza 104 and Mosquera 98) were selected based on the high disease severity of the commercial crop plants from which they were isolated and their contrasting *in vitro* characteristics, including colony appearance, growth rate, and the propagules formed by the pathogen. The strain Funza 104 was characterized by the formation of conidia and melanized mycelium, and the strain Mosquera 98 by the profuse production of microsclerotia (MS); *V. albo-atrum* (Agrosavia) was characterized by the production of conidia only. Previously, the strains Funza 104 and Mosquera 98 were morphologically and molecularly identified by Mendoza-Vargas *et al.* (2025) as belonging to the genus *Verticillium,* with greater phylogenetic proximity to the species *V. nonalfalfae* and *V. alfalfae*, which have not been previously reported in Colombia.

Multiplication of *Verticillium* strains

The inoculum of the three *Verticillium* strains [Funza 104, Mosquera 98, and *V. albo-atrum* (Agrosavia)] for the tests was produced on sterile rice, following the protocol proposed by Guerrero *et al.* (1992), and adjusted by the laboratory. For this purpose, flasks containing 30 g of rice and 20 ml of sterile distilled water (SDW) were autoclaved for 20 min in two cycles at 121°C, 21 psi, followed by 20 min of drying. The flasks, with rice as substrate, were inoculated with 1 ml of a conidial suspension ($1{\times}10^7$) of Funza 104 or Agrosavia strain, separately, and placed in an incubator (Incucell® V, Medcenter, Planegg, Germany) at 21°C for 11 d. To prepare each conidial suspension, a plug of Potato Dextrose Agar (PDA) medium (Oxoid®, Thermo Scientific, USA) with seven-day-old mycelial growth colonies of each strain was added to sterile tubes containing 12 ml of SDW, shaken in a Vortex® for 2 min, and filtered with sterile gauze. Conidia were counted in a Neubauer chamber (Neubauer, VWR, Darmstadt, Germany), and the suspensions of $1{\times}10^7$ conidia ml^{-1} were adjusted.

For the essays involving microsclerotia, the strain Mosquera 98 was multiplied using the same methodology described above for conidial production. Still, approximately 15 d were required to obtain microsclerotia (MS). After this period, the MS were separated following the methodology proposed by Hawke and Lazarovits (1994), which consisted of preparing sand (50 g) with 37% HCl on watch glasses and allowing this to act for 24 h. Subsequently, this mixture was thoroughly washed with abundant SDW to neutralize its acidity. The MS were recovered by sieving (100, 50, and 25 μm), collected on filter paper, and stored in sealed Falcon® tubes until inoculation.

Pathogenicity tests

Different methods and times of inoculation of the pathogen were evaluated. These varied according to the propagules formed by each *Verticillium* strain. Conidia as inoculum were assessed from the Funza 104 and Agrosavia strains, and MS from Mosquera 98. Two moments of inoculation of conidia were evaluated, at the sowing time and 49 days after sowing (das). In this case, plants were inoculated at 49 das, since the time period between 45 and 55 das in potato coincides with an active root growth, beginning of tuberization, and the maximum vegetative growth stage, when the pathogen may strongly impair water and nutrient transport (Klosterman *et al*., 2009; Steere & Kirk, 2015).

For conidia inoculation (Funza 104 and Agrosavia) the treatments were: (i) incorporation of propagules in the substrate (rice) into the soil at the time of sowing (IPS) and (ii) 49 das (IPS 49); (iii) incorporation of conidia into the soil at the time of sowing (CS); (iv) inoculation by immersion of roots in the suspension of conidia 49 das (150 ml plant^{-1}; 10 min) (IR); and (v) inoculation of the conidial suspension by soil drench 49 das (120 ml plant^{-1}) (DS). For these treatments, a concentration of $1{\times}10^7$ conidia ml^{-1} was used (Ashraf *et al*., 2012; Mendoza-Vargas *et al.,* 2025). In the case of propagules into the substrate (rice), the amount of rice needed to achieve the desired conidia density for inoculation was previously determined (data not shown). In each inoculation method evaluated for conidia, five potato plants var. Diacol Capiro were inoculated, and five non-inoculated plants were used as controls. The assessed treatments are explained in Table 1.

For MS (Mosquera 98 strain), test plants were inoculated with five different concentrations: 2, 5, 10, 30, and 60 MS g^{-1} of soil. The suspension of MS was prepared by counting the required number of MS in a Neubauer chamber and suspending them in 600 ml of SDW. The inoculation was carried out at sowing by incorporating the MS suspension directly into the soil and thoroughly mixing to ensure uniformity. In each MS concentration evaluated, five potato plants var. Diacol Capiro were inoculated, and five non-inoculated (treated only with SDW) were used as controls. Previously, the viability of MS, measured as the germination rate, was assessed in PDA medium following the method of Coley-Smith and Javed (1970). The

TABLE 1. Inoculation methods, strain of *Verticillium* spp., and amount of conidia used as inoculum in potato plants var. Diacol Capiro.

Inoculation method	Strain of the pathogen	Amount of substrate or suspension per plant	Final concentration
Incorporation of propagules in the substrate (rice) into the soil at the time of sowing (IPS)	Funza	4.35 g of rice	1×10^7 conidia ml^{-1}
	Agrosavia	0.8 g of rice	
Incorporation of propagules in the substrate (rice) into the soil 49 das (IPS 49)	Funza	4.35 g of rice	
	Agrosavia	0.8 g of rice	
Incorporation of conidia into the soil at the time of sowing (CS)	Funza	120 ml	
	Agrosavia		
Inoculation by immersion of roots in the suspension of conidia 49 das (IR)	Funza	150 ml	
	Agrosavia		
Inoculation of the conidial suspension by soil drench 49 das (DS)	Funza	120 ml	
	Agrosavia		

MS viability was determined to be 70%, which was used to adjust the amount of MS per gram of soil for each inoculum density assessed.

Monitoring of disease development and plant phenology

Disease monitoring was carried out using the severity scale for *Verticillium* in potatoes proposed by Hunter *et al.* (1968). This is a five-level scale, where 0: no symptoms of the disease; 1: slight wilting and discoloration of leaves; 2: moderate wilting affecting less than half of the leaves on the plants; 3: severe wilting affecting more than half of the leaves on the plants; and 4: plant death due to wilting. The results were used to calculate the disease severity index (DSI) according to Equation 1 (Chávez-Arias *et al.*, 2020).

$$DSI = \frac{\Sigma(nv)}{V} \tag{1}$$

where n is the level of infection according to the scale, v is the number of plants present in each level, and V is the total number of evaluated plants.

Evaluation was conducted twice a week for 17 weeks (120 d), by visually inspecting all plants in each pot across all treatments. Using the data obtained for each treatment, the incubation period (Simko & Haynes, 2017), disease incidence, and the area under the disease progress curve (AUDPC) were calculated according to Campbell and Madden (1990). The formula used for calculating the AUDPC is presented in Equation 2.

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

where n is the number of evaluations, y_i and y_{i+1} are the values of the severity scale that were obtained at every evaluation time, and $(t_{i+1} - t_i)$ is the time interval between evaluations.

To confirm the presence of the pathogen in the symptomatic plants at the end of the study, stem explants from each treatment were seeded on PDA media according to the protocol described by EPPO (2007). Pure culture isolations from each treatment were prepared and visually confirmed as *Verticillium* by microscopic observations (Olympus CX31) at 10X and 40X of 7-d cultures according to the morphology described by Barnett and Hunter (1998).

The phenological monitoring of the plants was carried out weekly using the BBCH scale (Meier, 2018)—the German acronym for Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie— selecting from the outset the most vigorous stem in each pot. With these data, the effect of the disease on the phenological development of the potato plants was determined. Once the plant cycle in each treatment was completed, the inoculated and control plants were harvested. The number of tubers per plant, tuber weight, and caliber were evaluated in accordance with Colombian technical standard 341 (ICONTEC, 2018). The experiments were conducted until harvest (120 das), and harvest time was carefully monitored to ensure consistency among treatments.

Experimental design

The test was carried out using a complete factorial design in a completely randomized arrangement with five replicates (plants) per treatment. Two trials were conducted based on the type of *Verticillium* propagules used as inoculum: conidia or MS. The first trial, which involved conidia, included two inoculation timings: (i) at sowing and (ii) at 49 das. The factor evaluated was the inoculation method, comprising five different methods, while the levels considered were the *Verticillium* strains (Funza 104 and Agrosavia). The second trial, focusing on MS, involved inoculating with varying MS densities. Here, the factor evaluated was the inoculum density, with five different densities tested, and the level was represented by the single Mosquera 98 strain.

Data analysis

The free software RStudio version 4.4.1 (Core R Team 2024) was used to analyze the data. The Shapiro-Wilk ($P>0.05$) and Bartlett ($P>0.05$) tests were used to assess normality and homogeneity of variances. ANOVA analysis of variance ($P<0.05$) was performed, along with Tukey's multiple comparison tests ($P<0.05$). A multivariate analysis of variance (MANOVA) ($P<0.05$) was utilized to analyze the harvest data, facilitating the examination of correlations among response variables originating from the same experimental units. This approach enabled a simultaneous assessment of the effects of various factors. In addition, cluster analysis was performed to further explore treatment relationships.

Results

Characteristic potato early dying symptoms were observed in plants inoculated with the Funza 104, Agrosavia, or Mosquera 98 strains, regardless of inoculation method, timing, or the propagule type used (MS or conidia). The symptoms resembled those typically reported, loss of turgor, unilateral chlorosis of the lower leaves extending from the edge to cover the entire leaf blade, subsequent necrosis starting from the borders of the leaflets that progress to the complete leaves advancing from the base of the stem towards the upper part of the plants, accompanied by wilting and subsequent necrosis of the stems (Ashraf *et al.*, 2012; Gómez-Caro & Mendoza-Vargas, 2020; Trapero, Serrano *et al.*, 2013). For all conidial inoculation methods and for most MS densities evaluated, the same pattern of symptom development was observed over time. The differences between the evaluated treatments were associated with the number of affected plants and the severity of the disease.

In the treatments inoculated with conidia (Funza 104 and Agrosavia), a 100% incidence of the disease was achieved, confirming that the evaluated inoculation methods successfully induced pathogen infection and reproduced the disease (Fig. 1). The inoculation with MS (Mosquera 98), a 20% incidence of the disease was obtained with the density of 5 and 30 MS g^{-1} of soil, and 40% incidence was obtained with the density of 10 and 60 MS g^{-1} of soil. For the density of 2 MS g^{-1} of soil, there was no development of symptoms associated with potato early dying caused by *Verticillium* (Fig. 2).

Development of the disease from the inoculation of *Verticillium* conidia

Regarding the incubation period (Fig. 3A), the results showed that all treatments inoculated at the time of sowing presented higher values [59-71 d after inoculation (dai)] than treatments inoculated at 49 das, where the symptoms appeared between 17-32 dai (Fig. 3B), this being the shortest incubation period observed. The inoculation with conidia in the soil (CS) presented significant differences ($P<0.05$) compared to the treatment with inoculation of propagules in substrate (rice) (IPS), for both Funza 104 and Agrosavia. The incubation period for the treatment with incorporation of conidia into the soil (CS) with the Agrosavia strain was the longest (71 dai) of all the treatments evaluated. On the other hand, plants inoculated at 49 das did not show significant differences in this variable (Fig. 3B). In the control treatment plants, disease symptoms did not develop during the study.

The AUDPC values in Figure 4 represent the amount of disease per treatment and *Verticillium* strain over 120 d (from the appearance of the first disease symptoms until the end of the evaluations). Significant differences ($P<0.05$) in AUDPC are observed between inoculation methods and the two inoculation timings for all treatments. The results show that the disease caused by the strains Funza 104 and Agrosavia in potato plants inoculated at sowing presents higher AUDPC values (110-160, respectively). Additionally, these plants showed shorter incubation periods and a faster increase in disease severity compared to plants inoculated at 49 das (65-110 d, respectively) (Fig. 6A-B). Inoculation of propagules in the substrate (rice) (IPS) at the time of sowing with the strain Funza 104 presented the highest AUDPC value (160.82). Additionally, this treatment showed significant differences ($P<0.05$) with respect to the treatments with incorporation of conidia into the soil (CS) with Funza 104 and Agrosavia strains at the time of sowing (Fig. 4A). In contrast, the treatment with inoculation of conidia in the substrate 49 das (IPS 49) of the Agrosavia strain presented the lowest AUDPC value (70.55) of all the treatments (Fig. 4B).

The results for treatments with inoculation of conidia into the substrate (rice) at 0 and 49 das showed significant differences ($P<0.05$) in AUDPC values (Fig. 4B), despite using the same inoculation method and differing only in the time of inoculation. In this case, potato early dying progressed more rapidly when the pathogen was inoculated

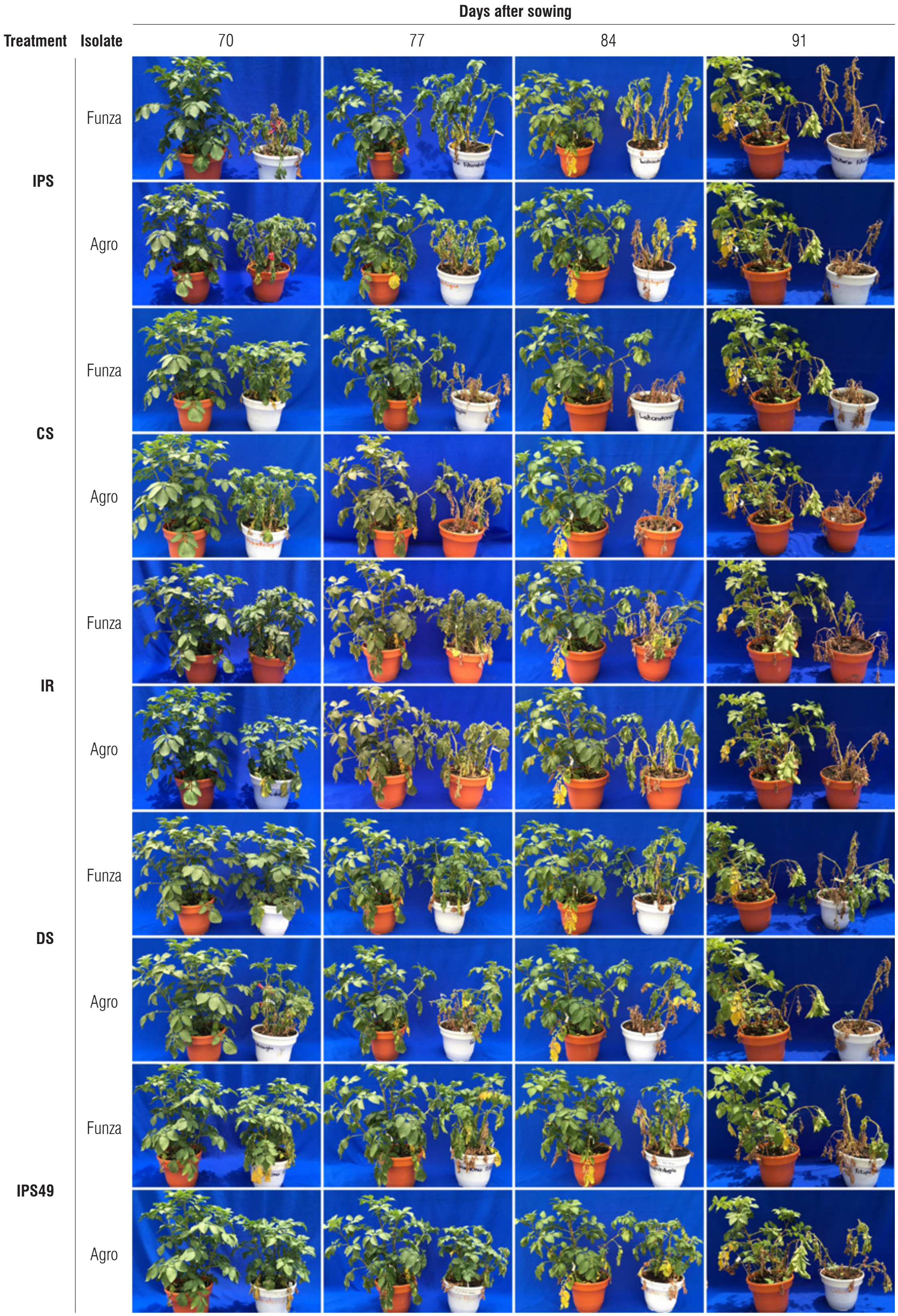

FIGURE 1. Development of potato early dying caused by *Verticillium* in plants var. Diacol Capiro under different inoculation methods at 70, 77, 84, and 91 d after sowing (das). IPS: incorporation of propagules in substrate (rice); CS: incorporation of conidia into the soil; IR: immersion of roots in a suspension of conidia; DS: applications of conidia in drench to the soil; IPS 49: incorporation of propagules in substrate (rice) at 49 das. Agro: Agrosavia strain; Funza: Funza 104 strain. The pots on the left of each image correspond to the control.

FIGURE 2. Development of potato early dying caused by *Verticillium* at 76, 84, 99, and 111 d after sowing in the plants var. Diacol Capiro inoculated at the sowing time with different densities of microsclerotia (2, 5, 10, 30, and 60 MS g^{-1} of soil) of the Mosquera 98 strain. The pots on the left of each image correspond to the control.

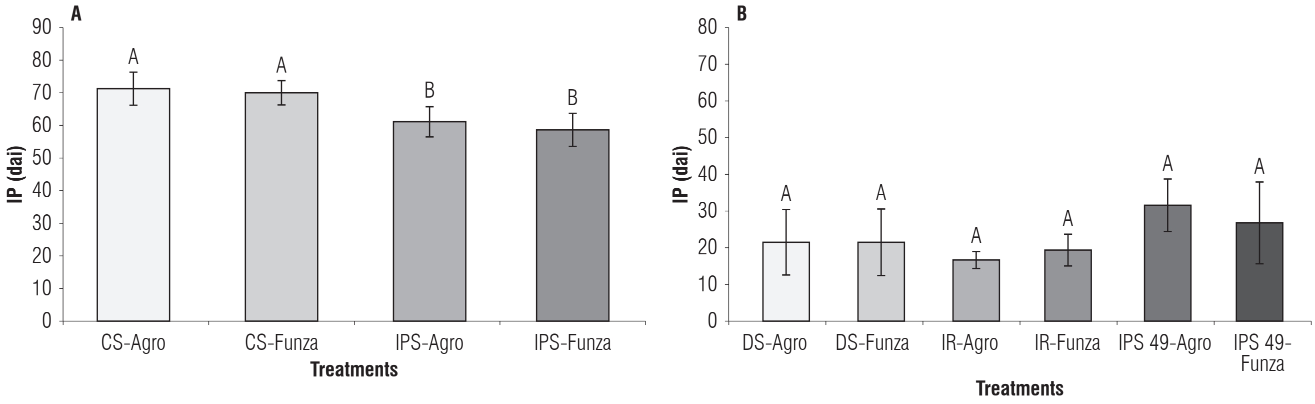

FIGURE 3. Incubation period (IP) of potato early dying in plants var. Diacol Capiro after the inoculation of *Verticillium* conidia under different methods and strains of the pathogen: A) at the time of sowing and B) 49 d after sowing (das). Treatments at the time of sowing: CS: incorporation of conidia into the soil; IPS: incorporation of propagules in substrate (rice). Treatments 49 das: DS: applications of conidia in drench to the soil; IR: immersion of roots in a suspension of conidia; IPS 49: incorporation of propagules to substrate (rice) at 49 das. Agro: Agrosavia strain; Funza: Funza 104 strain. Bars represent the mean of five values ± SE (n=5). Different letters indicate significant differences according to the Tukey test ($P \leq 0.05$).

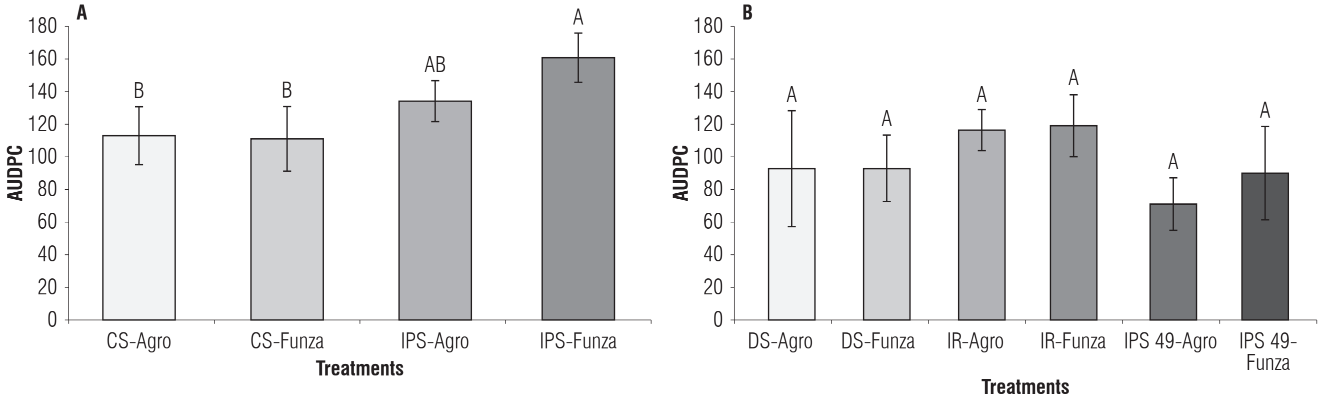

FIGURE 4. Area under the disease progress curve (AUDPC) of potato early dying in plants var. Diacol Capiro after the inoculation of *Verticillium* conidia under different methods and strains of the pathogen: A) at the time of sowing and B) 49 d after sowing (das). Treatments at the time of sowing: CS: incorporation of conidia into the soil; IPS: incorporation of propagules in substrate (rice); DS: applications of conidia in drench to the soil. Treatments 49 das: IR: immersion of roots in a suspension of conidia; IPS 49: incorporation of propagules in substrate (rice) at 49 das. Agro: Agrosavia strain; Funza: Funza 104 strain. Bars represent the mean of five values ± SE (n=5). Different letters indicate significant differences according to the Tukey´s test (*P≤0.05).*

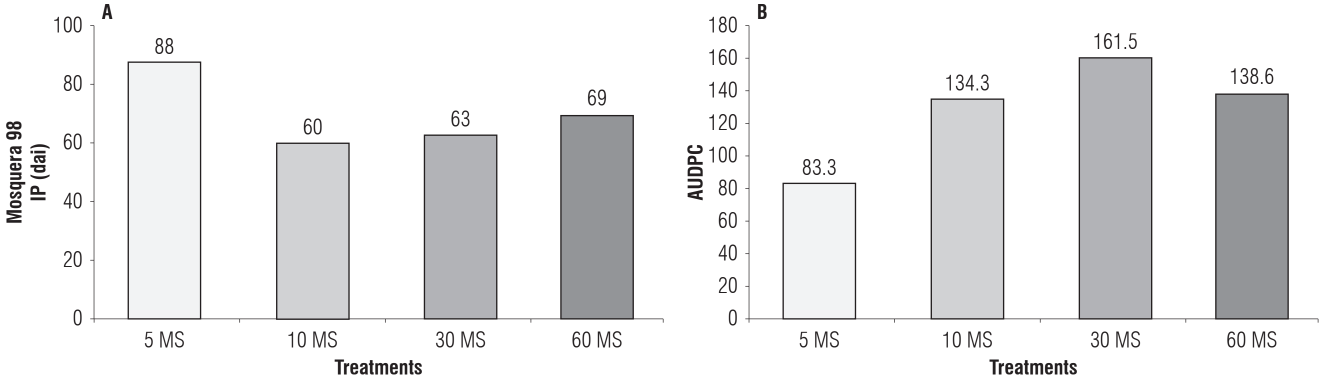

FIGURE 5. Development of potato early dying in plants var. Diacol Capiro from inoculation of *Verticillium* microsclerotia (Mosquera 98): A) Incubation periods (IP), B) Area under the disease progress curve (AUDPC) for each density of *Verticillium* microsclerotia (MS). The data presented only include information on diseased plants.

at sowing. The results showed that plants inoculated with *Verticillium* conidia by soil drench (DS) and incorporation of propagules into the substrate (rice) 49 das (IPS 49), with the Funza 104 and Agrosavia strains, generated the lowest severity values for potato early dying (Fig. 6A and 6B).

Development of the disease from inoculation of *Verticillium* microsclerotia

The incubation period of potato early dying in plants inoculated with MS of Mosquera 98 was in the range of 60 to 88 dai, the longest being the treatment with a density of 5 MS g^{-1} of soil (Fig. 5A). Regarding the AUDPC values, the highest was found with the density of 30 MS g^{-1} of soil (Fig. 5B). Due to the small number of plants affected by this inoculation method, it was not possible to perform a statistical analysis of the data obtained.

Development of the plants

Regarding the phenological development of potato plants, var. Diacol Capiro with the two types of propagules (conidia and MS), the first disease symptoms were observed after 45 das at phenological stage PS4 (tuberization) and the disease severity increased 60-90 das at phenological stages PS5 (tuber development) and PS6 (flowering), regardless the inoculation method, timing of inoculation, strain or type of inoculum used (Fig. 6). After 90 das, the potato plants reached values over 2.5 and the maximum disease severity of 4.0 at 120 das with conidia as inoculum (Fig. 6A and B). In the case of MS, a similar tendency was observed, but with lower disease severity values (Fig. 6C). Necrosis of leaves caused by *Verticillium* infection led to leaf fall and subsequently to the affected stems dying, altering the phenological development of the plants. Upon inoculation

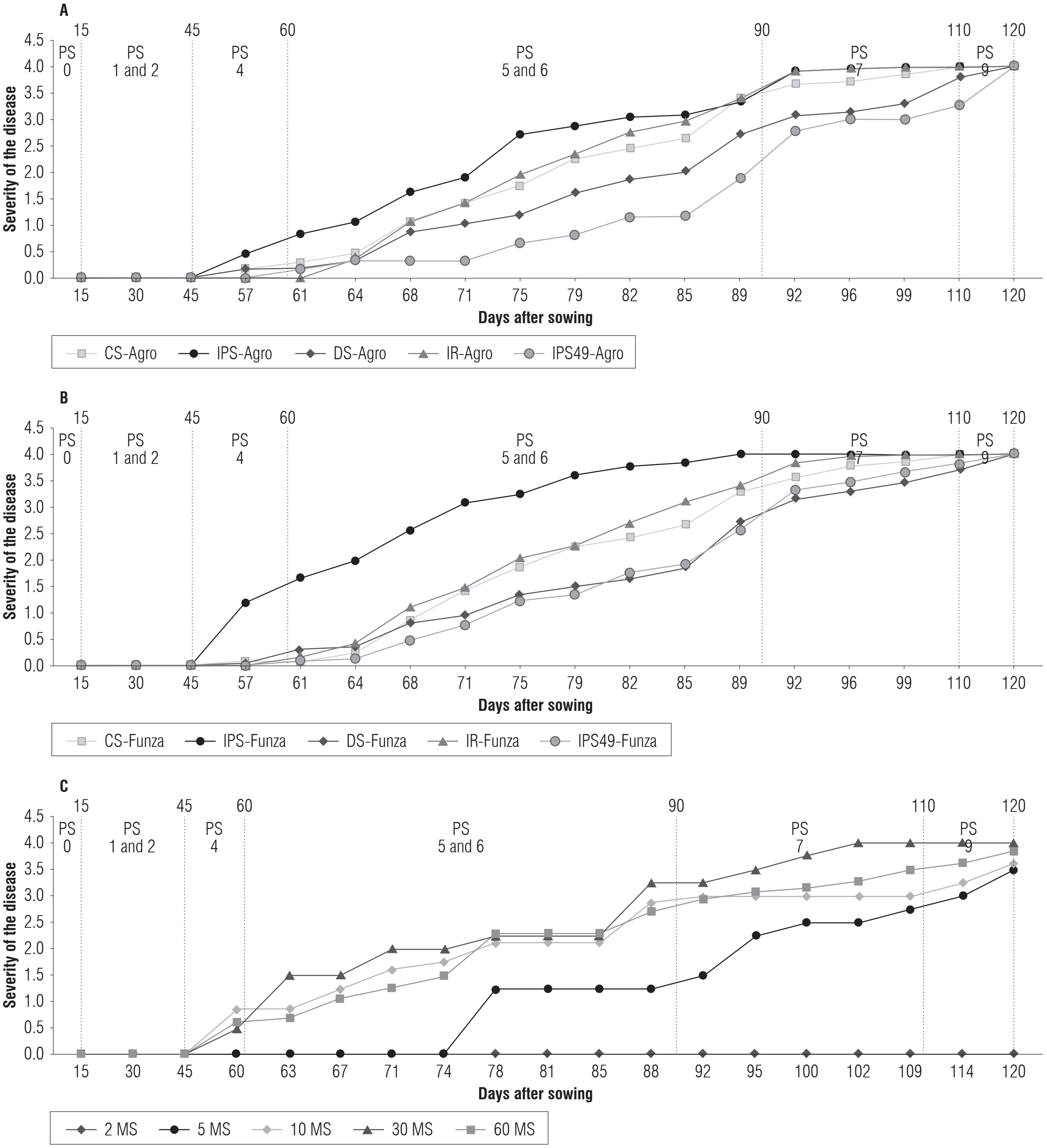

FIGURE 6. Severity of potato early dying and phenological stages of plants var. Diacol Capiro inoculated with three different sources of *Verticillium* inoculum. A) Inoculation of conidia Agrosavia strain (Agro) under different methods, B) Inoculation of conidia Funza 104 strain (Funza) under different methods, C) Inoculation of microsclerotia Mosquera 98 strain (Mosquera) under different densities of MS (2, 5, 10, 30, and 60 MS g^{-1} of soil). Treatments: CS: incorporation of conidia into the soil at the time of sowing (st); IPS: incorporation of propagules in substrate (rice) at st; DS: applications of conidia in drench to the soil 49 d after sowing (das); IR: immersion of roots in a suspension of conidia 49 das; IPS 49: incorporation of conidia in substrate (rice) at 49 das. Dashed vertical lines indicate the transition between main phenological stages according to the BBCH scale. Stage 0 (PS0): sprouting (0-15 das); stage 1 (PS1): leaf development (15-30 das), stage 2 (PS2): development of lateral stems (30-45 das), stage 4 (PS4): tuberization (45-60 das), stage 5 (PS5): tuber development (60-90 das), stage 6 (PS6): flowering (60-80), stage 7 (PS7): tuber filling/initial ripening (90-110 das) and stage 9 (PS9): senescence (110-120 das).

with conidia, the plants showed senescence at 60-67 das, and plant death was observed from 84 das. Premature senescence of potato plants inoculated with the pathogen was consistently observed across all inoculation methods and *Verticillium* strains evaluated. Plants inoculated with conidia reached phenological stage PS9 according to the BBC scale, as early as 67 das in most inoculation methods with the Agrosavia (Fig. 7) or the Funza 104 strain (Fig. 8).

These results show that the plants inoculated with Agrosavia and Funza 104 strains senesced 28-35 d earlier than the control plants (non-inoculated), which flowered (PS6)

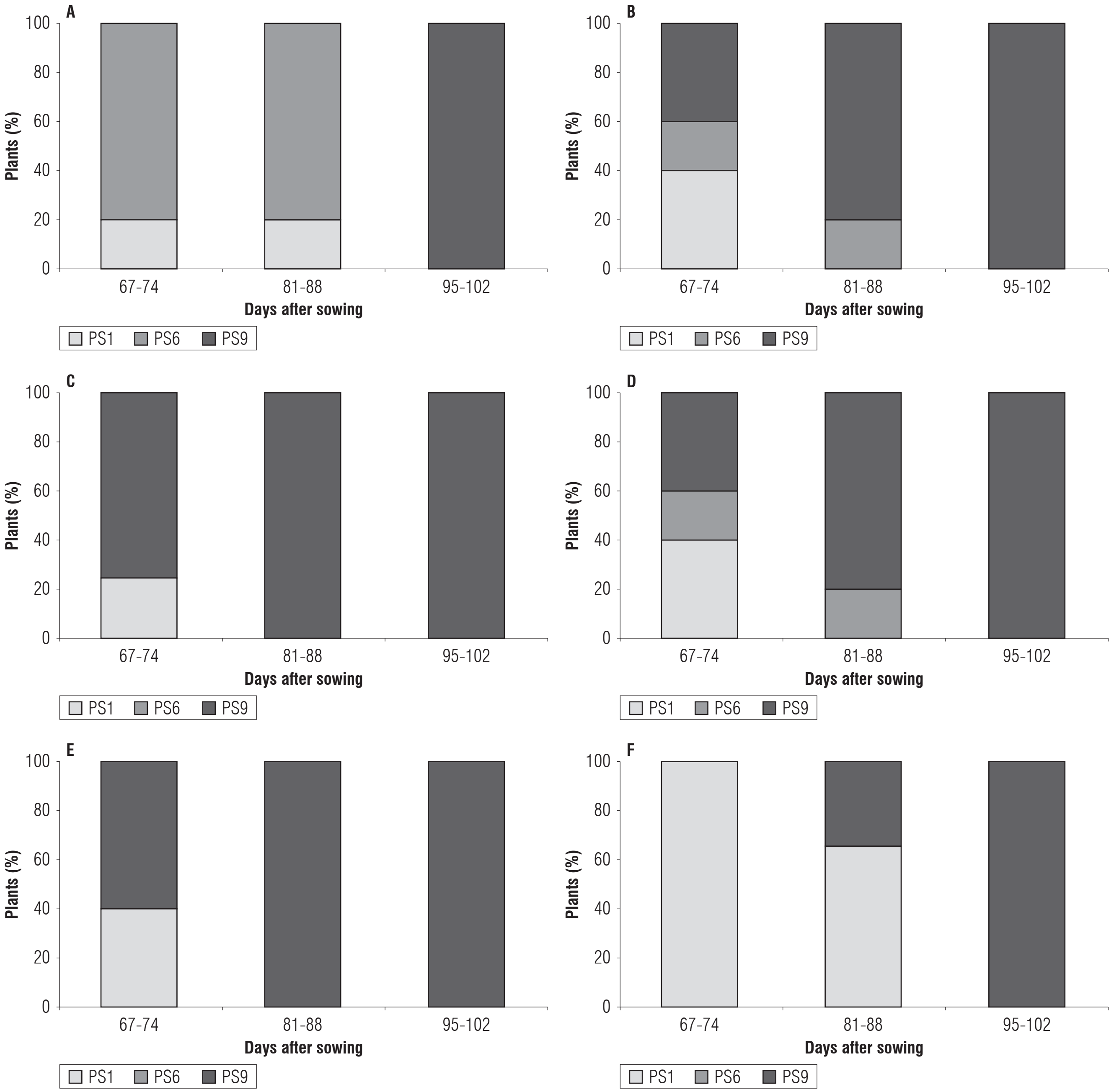

FIGURE 7. Developmental status of potato var. Diacol Capiro during the final phenological stages of plants inoculated with conidia of the strain Agrosavia (Agro). The figure illustrates the percentage of plants in each phenological stage (PS), for leaf development (PS1), flowering (PS6), and senescence (PS9) according to the BBCH scale. A) Control plants non-inoculated, B) Incorporation of propagules in substrate (rice), C) Incorporation of conidia into the soil, D) Immersion of roots in the suspension of conidia, E) Application of conidia by soil drench, F) Incorporation of propagules in substrate (rice) at 49 days after sowing (das). Note the early dying of plants under *Verticillium* inoculation (PS9) starting at 64-67 das, compared to control plants (A), which reached PS9 (senescence) at 95-102 das.

between 67 and 88 das and began to show senescence symptoms at 95 das (Figs. 7A and 8A). In the case of plants inoculated with MS (Mosquera 98 strain), the alteration of the phenological development of the plants was less remarkable; however, leaf fall associated with wilting was also recorded, which affected the phenology of the plants, causing them to enter the senescence stage around 20 d earlier than the control plants.

Isolation of *Verticillium* from plants

In the symptomatic potato plants, var. Diacol Capiro, inoculated with the two types of propagules of the pathogen

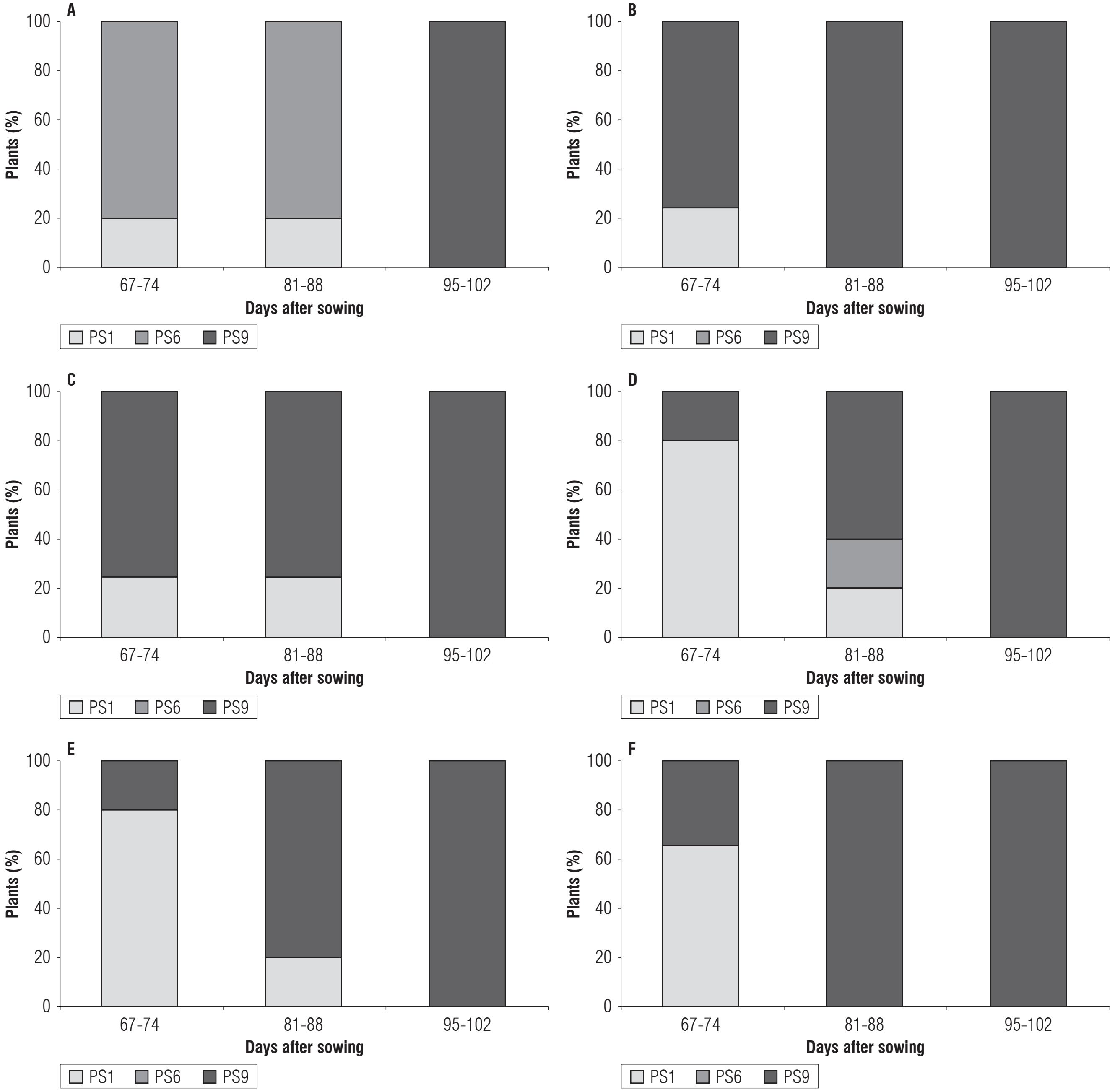

FIGURE 8. Developmental status of potato var. Diacol Capiro during the final phenological stages of plants inoculated with conidia of the strain Funza 104 (Funza). The figure illustrates the percentage of plants in the phenological stages (PS), leaf development (PS1), flowering (PS6), and senescence (PS9) according to the BBCH scale. A) Control plants non-inoculated, B) Incorporation of propagules in substrate (rice), C) Incorporation of conidia into the soil, D) Immersion of roots in a suspension of conidia, E) Application of conidia by soil drench, F) Incorporation of propagules in substrate (rice) at 49 days after sowing (das). Note the early dying of plants under *Verticillium* inoculation (PS9) starting at 64-67 days after sowing (das), compared to control plants (A), which reached PS9-senescence at 95-102 das.

(conidia and MS) and the three strains of *Verticillium* evaluated, the browning of the vascular bundles of the stems reported for the disease was observed in cross stem sections taken from the base of the diseased plants (Daami-Remadi *et al*., 2011; Powelson *et al*., 1993; Simko & Haynes, 2017). In isolations carried out in PDA medium from these stems, the growth of *Verticillium* colonies was confirmed, with typical mycelial growth of this genus as reported by Klosterman *et al.* (2009), Rubilar (2010), and Leon-Ttacca *et al.* (2018). In the colonies obtained, the characteristic whorled conidiophores were formed (Barnett & Hunter, 1998; Leon-Ttacca *et al*., 2018). In stems of control plants, the presence of vascular browning was not observed, and no *Verticillium* colonies were isolated. These results allowed us to confirm successful infection by the pathogen and that the symptoms presented in the potato plants var. Diacol Capiro, inoculated and maintained under greenhouse conditions, corresponded to potato early dying disease caused by *Verticillium* spp.

Harvest

From the MANOVA analysis of harvest data from plants inoculated with conidia, the inoculation method had a highly significant effect ($P>0.05$) on the set of response variables, and neither the strain nor the interaction had significant effects. From the clusters obtained, two possible groups were observed. The first group comprised the drench methods, application of conidia to the soil, and incorporation of propagules into the substrate for 49 das. The second group included root immersion and the incorporation of propagules into the substrate at sowing. Group 1 had the highest average tuber weights, and Group 2 had the lowest. Regarding the MS test, the analysis showed no significant differences in the evaluated MS densities.

Regarding the number of tubers per plant, for the two types of inoculated propagules (conidia and MS), no significant differences ($P>0.05$) were found between inoculation method, inoculum density, *Verticillium* strain, and the control treatments. Regarding tuber quality, in the conidia test, the most significant proportion of these (a range of 67% to 98% of the total tubers) corresponded to third-class quality, and no tubers classified as zero or first-class quality were obtained. In the case of MS, no zero-class quality tubers were recorded. Still, a small number of first-class quality tubers (maximum 2) were observed. In contrast, between 90% and 95% of the total tubers were classified as second or third-class quality, with similar proportions for the two classes. In both cases (conidia and MS), the control treatment presented the same behavior as the inoculated treatments for this variable.

Discussion

Under natural conditions, wilting of potato plants due to *Verticillium* begins during tuberization, as a result of infection by the inoculum present in the soil (Busch & Edgington, 1967), and it can also originate from inoculum present in seed tubers (Ayers, 1952; Robinson & Ayres, 1961). In this study, a comparative evaluation was carried out of the development of potato early dying under different methods, inoculation timing and types of *Verticillium* propagules (conidia and microsclerotia), in order to expose the host plants (seed tubers and newly emerged plants) to inoculum that can be found on the surface of the tuber, in the soil near the seed, and near the root system of developing plants. Our research revealed that almost all plants inoculated with the three *Verticillium* strains and treated with the different methods developed the characteristic symptoms of early wilting. The results also confirmed that *Verticillium* can infect established plants (49 das), and was not limited to early root from potato tuber seed at the time of sowing.

Characteristic symptoms of the disease developed under all the inoculation methods evaluated. These results are in accordance with previous studies, in which *Verticillium* inoculation methods such as soil drench (Leon-Ttacca *et al*., 2018), root immersion (Trapero, Díez *et al*., 2013), incorporation of propagules (conidia) in substrate (wheat) and application of propagules (conidia) to the soil (Platt & Sanderson, 1987) were applied to potatoes; the results find that it is possible to induce potato early dying by *Verticillium*. Based on the results obtained, the inoculation of a suspension of conidia into the soil is a recommended method, as it allows the exact inoculum density to be determined. Aditionally, the pathogen is similar to what is found in natural conditions. Due to its practicality, incorporating propagules into the substrate (rice) underlines the effectiveness of rice as a substrate for the multiplication and growth of *Verticillium* spp., but it also demonstrates the ease of adjustment to the required inoculum concentration. Regarding the timing of inoculation, it is suggested that it should be done at sowing, as this best represents the *Verticillium* infective process under natural commercial cultivation conditions.

In this study, a progressive increase in the severity of the disease was observed over time, ending with the wilting and death of the plants, which is in accordance with Guerrero *et al*. (1991), Bae *et al*. (2007), Johnson and Dung (2010), and Gómez-Caro and Mendoza-Vargas (2020). These symptoms appear once the pathogen has penetrated and invaded the xylem, where it generates numerous conidia that move systemically through the host's vascular system, causing chlorosis, necrosis, and, finally, leaf wilting (Johnson & Dung, 2010; Steere & Kirk, 2015).

Observing the development of the disease from the inoculation of *Verticillium* conidia demonstrated that the incubation period was shorter (23 dai) in the treatments where the pathogen was inoculated at 49 das. However, although symptom onset in these treatments was faster, disease progression was less severe than in treatments with conidia applied to the soil and propagules in substrate (rice), where the pathogen was inoculated at the time of sowing. Johnson and Dung (2010) state that, although *Verticillium* infection in potatoes can occur in the early stages of crop growth, wilting symptoms usually appear in the last phase of development, coinciding with the rapid tuber growth stage. Therefore, the treatments (*Verticillium* inoculation) applied at sowing exhibited a more extended incubation period and higher disease than those inoculated at 49 das. This difference is probably because, when the *Verticillium* inoculum reaches the crop after it has been established and its root system is developed, infection and the subsequent appearance of symptoms occur more rapidly. Moreover, although studies quantifying phenological susceptibility are limited, Johnson and Dung (2010) also found that *Verticillium* inoculation at sowing resulted in more severe disease than inoculation at a later stage. This implies heightened susceptibility during early plant development, which likely contributes to the more extended incubation period and greater disease levels observed in these treatments.

In the development of potato early dying through MS inoculation, we observed that the incubation periods coincided with the intervals reported by Trapero, Díez *et al.* (2013) in three of the five densities evaluated (10, 30, and 60 MS g^{-1} of soil), which range between 60 and 80 d. Furthermore, we observed an increase in disease (higher AUDPC) with increasing MS levels in the soil. However, at soil densities greater than 30 MS g^{-1}, the AUDPC decreased. Similar results are reported by Xiao and Subbarao (1998) in cauliflower, where the incidence of wilting due to *V. dahliae* is 16%, even at 4 MS g^{-1}, and inoculum densities greater than 20 MS g^{-1} of soil did not cause a further reduction in plant growth. Likewise, these authors point out that the minimum MS concentration in soil required to cause wilting, as well as the number of infected plants at increasing MS density, depends on the cultivated plant species. In the present work, we found that infection with the pathogen and development of the disease in potato var. Diacol Capiro was at a density of 5 MS g^{-1} of soil. The results obtained in this and other studies reflect the difficulty of working with *Verticillium* resistance propagules such as MS, due to the limited protocols available for them. Although numerous methods have been described for their quantification, in most cases, sample processing is complex and laborious, MS recovery rates are low, and isolates are often inconsistent. In summary, the low efficiency of these techniques has been attributed, among other factors, to the possible dormancy that MS may present in the semi-selective media used for their production (López-Escudero *et al.*, 2003). Moreover, MS have been reported to survive in soil for 10 to 15 years as melanized microsclerotia in the absence of a suitable host (Duressa *et al.*, 2013).

Pullman and DeVay (1982) report that cotton plants inoculated with *V. dahliae* mainly presented inhibition of growth and development. This finding is consistent with observations of potatoes in the current study, in which leaf fall and vascular wilting affected plant phenology. In addition, this would lead to a lower photosynthetic rate, directly due to wilting and leaf drop, and indirectly through vascular blockage by the pathogen, resulting in reduced tuber production. In this regard, in the 1960s, it was speculated that the pathogen's toxins are responsible for leaf necrosis in plants infected by *V. dahliae* (Stoddart & Carr, 1966). However, the proteins secreted by *V. dahliae* are mostly intrinsic toxins that cause leaf wilting, fulfilling two main functions: altering the host's physical barriers through their enzymatic activity, and manipulating its defense system through their synergistic contribution to virulence (Chen *et al.*, 2021). In *V. dahliae,* the best-known toxin is the necrosis and ethylene-inducing protein (Vd-NEP), which induces wilting in cotton leaves (Wang *et al.*, 2004) and cell death in tobacco (Santhanam *et al.*, 2013; Zhou *et al.*, 2012) and is a virulence factor in tomato and *Arabidopsis thaliana*. Potato plants var. Diacol Capiro inoculated with *Verticillium* sceneced approximately 20 d before non-inoculated plants coinciding with those results reported by Rowe and Powelson (2002), in which the death of potato plants affected by the pathogen was accelerated between 20 and 30 d. These findings underscore the altered phenological development of potato plants caused by *Verticillium* spp. infection.

Studies carried out by Steere and Kirk (2015), and Gómez-Caro and Mendoza-Vargas (2020) report that potato early dying by *Verticillium* causes that plants cannot develop tubers of significant size, resulting in a lower proportion of tubers in the zero and first-class categories, which negatively affects the performance of diseased plants and makes early dying a limiting disease for the crop. In this study, a greater number of tubers was found in the second and third-class categories, along with a reduction in tuber weight in the affected plants. According to Botseas and Rowe (1994), tuber size may have been affected by a decrease in photosynthetic rate resulting from the premature loss of foliage, a consequence of chlorosis that progresses to necrosis and ultimately causes the plant's death. Plants subjected to methods of inoculation of *Verticillium* conidia by soil drench (DS), incorporation of conidia into the soil (CS), and incorporation of propagules into the substrate at 49 das (IPS 49) had a greater tuber weight. In contrast, plants subjected to root immersion (IR) 49 das and those inoculated by incorporating propagules into the substrate at the time of sowing (IPS) had lower weights. According to the results, the pathogen inoculation methods that showed the highest AUDPC values (CS and IPS), which resulted in premature plant death around 30-35 d before the control plants, presented the lowest harvested tuber weights.

Given the increasing importance of potato early dying caused by *Verticillium* spp. in Colombia, the need to conduct studies that consider melanized hyphae as a source of inoculum is evident, as these resistance structures, which form some *Verticillium* species, have been little studied. Likewise, the results of the present study could be expanded by evaluating other potato varieties, infection processes with mixtures of different types of propagules in the development of the disease under different soil conditions, including pH, texture, and humidity. This would broaden our understanding of the development of potato early dying caused by *Verticillium* within the framework of a pathogen that produces different infective propagules, making this a complex disease to study and control.

Conclusions

This study revealed that potato early dying caused by *Verticillium* may occur due to plant infection at sowing time or during later growth stages, such as the onset of tuberization and the maximum vegetative growth stage, 45 to 55 days after sowing. However, the results suggest that the disease is more severe in plants inoculated at sowing. It was found that the first symptoms in potato plants var. Diacol Capiro can be observed between 65 and 70 d, regardless of the type of *Verticillium* propagule used as inoculum. Although either conidia or microsclerotia can infect potato plants, the density of microsclerotia plays an essential role in plant infection, with densities of minimum 5 MS g^{-1} of soil.

Additionally, it was found that plants affected by the pathogen senesce prematurely, 28 to 35 d earlier than pathogen-free plants. This finding highlights the altered phenological development of infected plants, leading to reduced tuber production and quality, with a more pronounced effect when infection occurs at sowing. To further understand the disease's development, evaluations using melanized hyphae as a source of inoculum are necessary, as it is a typical structure of the pathogen for persisting in soil. Finally, this study lays the groundwork for a systematic evaluation of potato varieties in Colombia and the Andean region. A similar approach could be employed to screen *Solanum* species related to *Solanum tuberosum* or *Solanum phureja* to identify resistant sources.

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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: SG; Formal data analysis: KSA and KSS; Funding acquisition: SGC; Research: KSA and KSS. Methodology: SGC; Validation: KSA and KSS and SGC; Writing – original draft: KSA and KSS; Writing – review & editing: KSA, KSS, and SGC; Supervision: SGC. All authors have read and approved the final version of the manuscript.

Literature cited

Ashraf, A., Rauf, A., Abbas, M. F., & Rehman, R. (2012). Isolation and identification of *Verticillium dahliae* causing wilt on potato in Pakistan. *Pakistan Journal of Phytopathology*, *24*(2), 112–116. https://www.academia.edu/37983367/ISOLATION_AND_IDENTIFICATION_OF_VERTICILLIUM_DAHLIAE_CAUSING_WILT_ON_POTATO_IN_PAKISTAN

Ayers, G. W. (1952). Studies on verticillium wilt of potatoes. *American Potato Journal*, *29*, 201–205. https://doi.org/10.1007/BF02881339

Bae, J., Atallah, Z. K., Jansky, S. H., Rouse, D. I., & Stevenson, W. R. (2007). Colonization dynamics and spatial progression of

Verticillium dahliae in individual stems of two potato cultivars with differing responses to potato early dying. *Plant Disease*, *91*(9), 1137–1141. https://doi.org/10.1094/PDIS-91-9-1137

Barnett, H. L., & Hunter, B. B. (1998). *Illustrated genera of imperfect fungi* (4th ed.). The American Phytopathological Society. https://es.scribd.com/document/415060565/Barnett-Hunter-Illustrated-genera-of-imperfect-fungi-APS-pdf

Benavides, J. A., Rodríguez, R. P., Arteaga Meneses, G., & Guerrero, O. (1995). Comportamiento de dos variedades de papa a la inoculación de *Verticillium albo-atrum* Reinke & Berth y *Globodera pallida* (Stone) Beherens. *Revista de Ciencias Agrícolas*, *13*(1-2), 103–22. https://revistas.udenar.edu.co/index.php/rfacia/article/view/1177

Botseas, D. D., & Rowe, R. C. (1994). Development of potato early dying in response to infection by two pathotypes of *Verticillium dahliae* and co-infection by *Pratylenchus penetrans*. *Phytopathology*, *84*(3), 275–282. https://doi.org/10.1094/Phyto-84-275

Busch, L. V., & Edgington, L. V. (1967). Correlation of photoperiod with tuberization and susceptibility of potato to *Verticillium albo-atrum*. *Canadian Journal of Botany*, *45*(5), 691–693. https://doi.org/10.1139/b67-076

Campbell, C. L., & Madden, L. V. (1990). *Introduction to plant disease epidemiology*. John Wiley & Sons.

Chávez-Arias, C. C., Gómez-Caro, S., & Restrepo-Díaz, H. (2020). Mitigation of the impact of vascular wilt and soil hypoxia on cape gooseberry plants by foliar application of synthetic elicitors. *HortScience*, *55*(1), 121–132. https://doi.org/10.21273/HORTSCI14550-19

Chen, J. Y., Klosterman, S. J., Hu, X. P., Dai, X. F., & Subbarao, K. V. (2021). Key insights and research prospects at the dawn of the population genomics era for *Verticillium dahliae*. *Annual Review of Phytopathology*, *59*, 31–51. https://doi.org/10.1146/annurev-phyto-020620-121925

Coley-Smith, J. R., & Javed, Z. U. R. (1970). Testing the viability of fungal sclerotia. *Annals of Applied Biology, 65*(1), 59–63.

Daami-Remadi, M., Jabnoun-Khiareddine, H., Ayed, F., & El Mahjoub, M. (2011). Comparative aggressiveness of *Verticillium dahliae*, *V. albo-atrum* and *V. tricorpus* on potato as measured by their effects on wilt severity, plant growth and subsequent yield loss. *Functional Plant Science and Biotechnology*, *5*(Special issue 1), 1–8. https://www.researchgate.net/publication/311651854_Functional_Plant_Science_and_Biotechnology_Comparative_Aggressiveness_of_Verticillium_dahliae_V_albo-_atrum_and_V_tricorpus_on_Potato_as_Measured_by_their_Effects_on_Wilt_Severity_Plant_Growth_and_Sub

Duressa, D., Anchieta, A., Chen, D., Klimes, A., Garcia Pedrajas, M. D., Dobinson, K. F., & Klosterman, S. J. (2013). RNA-seq analyses of gene expression in the microsclerotia of *Verticillium dahliae*. *BMC Genomics, 14*, Article 607. https://doi.org/10.1186/1471-2164-14-607

EPPO. (2020). PM 7/78 (2) *Verticillium nonalfalfae* and *V. dahliae*. *EPPO Bulletin, 50*(3), 462–476. https://doi.org/10.1111/epp.12701

EPPO. (2007). *Verticillium albo-atrum* and *Verticillium dahliae* on hop. *EPPO Bulletin*, *37*(3), 528–535. https://doi.org/10.1111/j.1365-2338.2007.01160.x

Gómez Caro, S., & Mendoza Vargas, L. A. (2020). Madurez temprana del cultivo de papa, una enfermedad limitante que todos debemos conocer. *Revista Papa*, *51*, 32–40. http://hdl.handle.net/20.500.12324/37792

Guerrero, O., Benavides, J., Rodríguez, R. (1992). Pérdidas ocasionadas por *Verticillium albo-atrum* Reinke y Berth., *Globodera pallida* (Stone) Beherens y sus interacciones en dos variedades de papa. *Revista ICA*, *27*(1), 13–23. http://hdl.handle.net/20.500.12324/16882

Guerrero Guerrero, O., Benavides Pazmiño, J., & Rodríguez Escobar, R. (1991). Efecto de la inoculación de *Verticillium albo-atrum* Reinke y Berth y *Globodera pallida* (Stone) Beherens en las variedades de papa ICA-Nariño y Parda pastusa. *Revista ICA*, *26(*1-2), 9–18. http://hdl.handle.net/20.500.12324/644

Hainaut, P., Remacle, T., Decamps, C., Lambert, & R., Sadok, W. (2016). Higher forage yields under temperate drought explained by lower transpiration rates under increasing evaporative demand. *European Journal of Agronomy, 72*, 91–98. https://doi.org/10.1016/j.eja.2015.10.002

Hawke, M. A., & Lazarovits, G. (1994). Production and manipulation of individual microsclerotia of *Verticillium dahliae* for use in studies of survival. *Phytopathology*, *84*(9), 883–890. https://doi.org/10.1094/Phyto-84-883

Hunter, D. E., Darling, H. M., Stevenson, F. J., & Cunningham, C. E. (1968). Inheritance of resistance to Verticillium wilt in Wisconsin. *American Potato Journal*, *45*, 72–78. https://doi.org/10.1007/BF02862864

Inderbitzin, P., Bostock, R. M., Davis, R. M., Usami, T., Platt, H. W., & Subbarao, K. V. (2011). Phylogenetics and taxonomy of the fungal vascular wilt pathogen *Verticillium*, with the descriptions of five new species. *PLoS ONE*, *6*(12), Article e28341. https://doi.org/10.1371/journal.pone.0028341

Inderbitzin, P., & Subbarao, K. V. (2014). *Verticillium* systematics and evolution: how confusion impedes Verticillium wilt management and how to resolve it. *Phytopathology*, *104*(6), 564–574. https://doi.org/10.1094/PHYTO-11-13-0315-IA

Jakubowski, T., Rolbiecki, S., Rolbiecki, R., Wichrowska, D., Figas, A., Jagosz, B., Atilgan, A., Pal-Fam, F., Keszthelyi, S., & Krakowiak-Bal, A. (2024). Response of *Solanum tuberosum* L. to drip irrigation and nitrogen application: productivity, nutrition composition, bioactive compounds, antioxidant activity. *Journal of Plant Protection Research*, *64*(3), 307–315. https://www.plantprotection.pl/pdf-187983-113252?filename=113252.pdf

Johnson, D. A., & Dung, J. K. S. (2010). Verticillium wilt of potato – the pathogen, disease and management. *Canadian Journal of Plant Pathology*, *32*(1), 58–67. https://doi.org/10.1080/07060661003621134

Klosterman, S. J., Atallah, Z. K., Vallad, G. E., & Subbarao, K. V. (2009). Diversity, pathogenicity, and management of Verticillium species. *Annual Review of Phytopathology*, *47*, 39–62. https://doi.org/10.1146/annurev-phyto-080508-081748

Land, C. J., Lawrence, K. S., Burmester, C. H., & Meyer, B. (2017). Cultivar, irrigation, and soil contribution to the enhancement of Verticillium wilt disease in cotton. *Crop Protection*, *96*, 1–6. https://doi.org/10.1016/j.cropro.2017.01.002

Leon-Ttacca, B., Arévalo-Gardini, E., & Bouchon, A. S. (2018). Muerte repentina de *Theobroma cacao* L. causado por *Verticillium dahliae* Kleb. en el Perú y su biocontrol *in vitro*. *Ciencia* *y Tecnología Agropecuaria*, *20*(1), 117–148. http://www.scielo.org.co/pdf/ccta/v20n1/en_0122-8706-ccta-20-01-00117.pdf

Li, F., & Li, Y. (2021). Evaluation of pathogenicity, systemic colonisation, and host range of *Verticillium alfalfae* in a greenhouse. *Crop and Pasture Science*, *72*(5), 383–392. https://doi.org/10.1071/CP20449

Li, H., Wang, Z., Hu, X., Shang, W., Shen, R., Guo, C., Guo, Q., & Subbarao, K. V. (2019). Assessment of resistance in potato cultivars to Verticillium wilt caused by *Verticillium dahliae* and *Verticillium nonalfalfae*. *Plant Disease*, *103*(6), 1357–1362. https://doi.org/10.1094/PDIS-10-18-1815-RE

Lima, F. S. O., Matos, V. S., Silva, E. S., Carvalho, M. A. S., Teixeira, R. A., Silva, J. C., & Correa, V. R. (2018). Nematodes affecting potato and sustainable practices for their management. In M. Yildiz (Ed.), *Potato – from Incas to all over the world* (Ch. 6, pp. 107–124). Intech Open. https://doi.org/10.5772/intechopen.73056

López Escudero, F. J., Núñez Santos, D., & Blanco López, M. A. (2003). Aislamiento de *Verticillium dahliae* del suelo y caracterización morfológica de sus microesclerocios. *Boletín de Sanidad Vegetal, Plagas*, *29*(4), 613–626. https://www.mapa.gob.es/ministerio/pags/Biblioteca/Revistas/pdf_plagas%2FBSVP-29-04-613-626.pdf

Meier, U. (Ed.) (2018). *Growth stages of mono- and dicotyledonous plants: BBCH monograph*. Blackwell. https://doi.org/10.5073/20180906-074619

Mendoza-Vargas, L. A, León-Rueda, W. A, Ramírez-Gil, J. G, & Gómez-Caro, S. (2025). Identifying the causal agent of potato early dying and key epidemiological parameters at meso-scale in the high tropics. *Tropical Plant Pathology*, *50*(1), Article 35. https://doi.org/10.1007/s40858-025-00692-6

Mulero-Aparicio, A., Varo, A., Agustí-Brisach, C., López-Escudero, F. J., & Trapero, A. (2020). Biological control of Verticillium wilt of olive in the field. *Crop Protection*, *128*, Article 104993. https://doi.org/10.1016/j.cropro.2019.104993

Nair, P. V. R., Wiechel, T. J., Crump, N. S., & Taylor, P. W. J. (2019). Seed tuber incidence, identification and pathogenicity of *Verticillium* species infecting potatoes in South East Australia. *Australasian Plant Pathology*, *48*, 637–650. https://doi.org/10.1007/s13313-019-00667-7

Nieto, L. E. (1988). La madurez prematura de la papa causada por *Verticillium* spp. en Colombia. *Revista ICA*, *23*(4), 334–340. http://hdl.handle.net/20.500.12324/532

Pegg, G. F., & Brady, B. L. (2002). *Verticillium wilts*. CABI Publishing. https://www.cabidigitallibrary.org/doi/book/10.1079/9780851995298.0000

Platt, H. W., & Sanderson, J. B. (1987). Comparison of inoculation methods for field studies on varietal response to Verticillium wilt of potatoes. *American Potato Journal*, *64*, 87–92. https://doi.org/10.1007/BF02853441

Powelson, M. L., & Rowe, R. C. (1993). Biology and management of early dying of potatoes. *Annual Review of Phytopathology*, *31*, 111–126. https://doi.org/10.1146/annurev.py.31.090193.000551

Pullman, G. S., & DeVay, J. E. (1982). Epidemiology of *Verticillium* wilt of cotton: effects of disease development on plant phenology and lint yield. *Phytopathology*, *72*(5), 554–559. https://doi.org/10.1094/Phyto-72-554

Ramírez-Gil, J. G., & Peterson, A. T. (2019). Current and potential distributions of the most important diseases affecting Hass avocado in Antioquia Colombia. *Journal of Plant Protection Research*, *59*(2), 214–228. https://doi.org/10.24425/jppr.2019.129288

Robinson, D. B., & Ayers, G. W. (1961). Verticillium wilt of potato in relation to vascular infection of the tuber. *Canadian Journal of Plant Science*, *41*(4), 703–708. https://doi.org/10.4141/cjps61-104

Rowe, R. C., & Powelson, M. L. (2002). Potato early dying: management challenges in a changing production environment. *Plant Disease*, *86*(11), 1184–1193. https://doi.org/10.1094/PDIS.2002.86.11.1184

Rubilar Rubilar, M. A. (2010). *Estudios de patogenicidad y detección mediante PCR de Verticillium albo-atrum Reinke* & *Berth., en Kiwi dorado (Actinidia chinensis planch) cultivar Hort16A* [Undergraduate thesis, Universidad de Chile]. https://repositorio.uchile.cl/handle/2250/112411

Santhanam, P., van Esse, H. P., Albert, I., Faino, L., Nürnberger, T., & Thomma, B. P. H. J. (2013). Evidence for functional diversification within a fungal NEP1-like protein family. *Molecular Plant-Microbe Interactions*, *26*(3), 278–286. https://doi.org/10.1094/MPMI-09-12-0222-R

Serrano, A., Rodríguez-Jurado, D., Ramírez-Tejero, J. A., Luque, F., López-Escudero, F. J., Belaj, A., Román, B., & León, L. (2023). Response to *Verticillium dahliae* infection in a genetically diverse set of olive cultivars. *Scientia Horticulturae*, *316*, Article 112008. https://doi.org/10.1016/j.scienta.2023.112008

Simko, I., & Haynes, K. G. (2017). Maturity-adjusted resistance of potato (*Solanum tuberosum* L.) cultivars to Verticillium wilt caused by *Verticillium dahliae*. *American Journal of Potato Research*, *94*, 173–177. https://doi.org/10.1007/s12230-016-9553-1

Steere, L., & Kirk, W. (2015). Potato early die complex (*Verticillium* wilt). *Extension Bulletin E-3207*. https://www.canr.msu.edu/resources/potato_early_die_complex_e3207

Stoddart, J. L., & Carr, A. J. H. (1966). Properties of wilt-toxins produced by *Verticillium albo-atrum* Reinke and Berth. *Annals of Applied Biology*, *58*(1), 81–92. https://doi.org/10.1111/j.1744-7348.1966.tb05073.x

Trapero, C., Díez, C. M., Rallo, L., Barranco, D., & López-Escudero, F. J. (2013). Effective inoculation methods to screen for resistance to Verticillium wilt in olive. *Scientia Horticulturae*, *162*, 252–259. https://doi.org/10.1016/j.scienta.2013.08.036

Trapero, C., Serrano, N., Arquero, O., Del Río, C., Trapero, A., & López-Escudero, F. J. (2013). Field resistance to *Verticillium* wilt in selected olive cultivars grown in two naturally infested soils. *Plant Disease*, *97*(5), 668–674. https://doi.org/10.1094/PDIS-07-12-0654-RE

Uppal, A. K., El Hadrami, A., Adam, L. R., Tenuta, M., & Daayf, F. (2008). Biological control of potato *Verticillium* wilt under controlled and field conditions using selected bacterial antagonists and plant extracts. *Biological Control*, *44*(1), 90–100. https://doi.org/10.1016/j.biocontrol.2007.10.020

Volcy, C. (2008). Génesis y evolución de los postulados de Koch y su relación con la fitopatología. Una revision. *Agronomía Colombiana*, *26*(1), 107–115. https://revistas.unal.edu.co/index.php/agrocol/article/view/13924

Wang, J. Y., Cai, Y., Gou, J. Y., Mao, Y. B., Xu, Y. H., Jiang, W. H., & Chen, X. Y. (2004). VdNEP, an elicitor from *Verticillium dahliae*, induces cotton plant wilting. *Applied and Environmental Microbiology*, *70*(8), 4989–4995. https://doi.org/10.1128/AEM.70.8.4989-4995.2004

Wu, M., Li, Q., Xia, G., Zhang, Y., & Wang, F. (2022). New insights into defense responses against *Verticillium dahliae* infection revealed by a quantitative proteomic analysis in *Arabidopsis thaliana*. *Functional Plant Biology*, *49*(11), 980–994. https://doi.org/10.1071/fp22006

Xiao, C. L., & Subbarao, K. V. (1998). Relationships between *Verticillium dahliae* inoculum density and wilt incidence, severity, and growth of cauliflower. *Phytopathology*, *88*(10), 1108–1115. https://doi.org/10.1094/PHYTO.1998.88.10.1108

Zhang, Y., Kang, L., Gao, J., Puri, K. D., Jia, R., Zhang, Z., Zhang, J., & Zhao, J. (2023). Systemic colonization of potato plants by *Verticillium dahliae* leads to infection of tubers and sprouting buds. *Plant Disease*, *107*(3), 750–757. https://doi.org/10.1094/PDIS-05-22-1029-RE

Zhou, B. J., Jia, P. S., Gao, F., & Guo, H. S. (2012). Molecular characterization and functional analysis of a necrosis- and ethylene-inducing, protein-encoding gene family from *Verticillium dahliae*. *Molecular Plant-Microbe Interactions*, *25*(7), 964–975. https://doi.org/10.1094/MPMI-12-11-0319

SUPPLEMENTARY TABLE S1. Analysis of variance (ANOVA) results for area under the disease progress curve (AUDPC) in potato plants inoculated with conidia of different *Verticillium* strains (Agrosavia and Funza 104) and inoculation methods.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
AUDPC	9	22465	2496.08	4.9353	0.0003155
Residuals	3	16690	505.76		

Df = degrees of freedom; Sum Sq = sum of squares; Mean Sq = mean square; F value = Fisher's statistic; Pr(>F) = *P*-value. Significance was considered at $P<0.05$.

SUPPLEMENTARY TABLE S2. Analysis of variance (ANOVA) results for incubation period (IP) in potato plants inoculated with conidia of different *Verticillium* strains (Agrosavia and Funza 104) and inoculation methods.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
AUDPC	9	19571.1	2174.57	53.589	$2.2e^{-16}$
Residuals	3	1339.1	40.58		

Df = degrees of freedom; Sum Sq = sum of squares; Mean Sq = mean square; F value = Fisher's statistic; Pr(>F) = *P*-value. Significance was considered at $P<0.05$.

SUPPLEMENTARY TABLE S3. Analysis of variance (ANOVA) results for average total weight of tubers harvested in potato plants inoculated with conidia of different *Verticillium* strains (Agrosavia and Funza 104) and inoculation methods.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Method	4	428.91	107.226	2.0963	0.0966
Strain	2	64.37	32.187	0.6293	0.5375
Residuals	46	2352.87	51.149		

Df = degrees of freedom; Sum Sq = sum of squares; Mean Sq = mean square; F value = Fisher's statistic; Pr(>F) = *P*-value. Significance was considered at $P<0.05$.

SUPPLEMENTARY TABLE S4. Multivariate analysis of variance (MANOVA) results for average total weight of tubers harvested in potato plants inoculated with conidia of different *Verticillium* strains (Agrosavia and Funza 104) and inoculation methods.

	Df	Pillai	approx F	num Df	Den Df	Pr(>F)
Method	4	0.63872	5.3959	8	92	$1.421e^{-05}$
Strain	2	0.06774	0.8064	4	92	0.5242
Residuals	46					

Df = degrees of freedom; Pillai = Pillai's trace statistic; approx F = approximate F value; num Df = numerator degrees of freedom; den Df = denominator degrees of freedom; Pr(>F) = *P*-value. Significance was considered at $P<0.05$.

SUPPLEMENTARY TABLE S5. Multivariate analysis of variance (MANOVA) results for the number of tubers harvested in potato plants inoculated with conidia of different *Verticillium* strains (Agrosavia and Funza 104) and inoculation methods.

	Df	Pillai	approx F	num Df	Den Df	Pr(>F)
Method	4	0.46860	2.1288	12	138	0.01870
Strain	2	0.22414	1.8933	6	90	0.09052
Residuals	46					

Df = degrees of freedom; Pillai = Pillai's trace statistic; approx F = approximate F value; num Df = numerator degrees of freedom; den Df = denominator degrees of freedom; Pr(>F) = *P*. Significance was considered at $P<0.05$.

SUPPLEMENTARY TABLE S6. Multivariate analysis of variance (MANOVA) results for average total weight of tubers harvested in potato plants inoculated with microesclerotia of different *Verticillium* strain (Mosquera 98).

Source	Df	Pillai	approx F	num Df	Den Df	Pr(>F)
Method	5	0.67189	1.61614	15	84	0.08659
Strain	1	0.04448	0.40348	3	26	0.75170
Residuals	28					

Df = degrees of freedom; Pillai = Pillai's trace statistic; approx F = approximate F value; num Df = numerator degrees of freedom; den Df = denominator degrees of freedom; Pr(>F) = *P*. Significance was considered at $P<0.05$.

Arbuscular mycorrhizae induce resistance against Fusarium wilt in onion in Boyacá, Colombia

Micorrizas arbusculares inducen la resistencia contra marchitamiento por *Fusarium* en cebolla en Boyacá, Colombia

Raúl Hernando Posada Almanza[1], Sud Sair Sierra Roncancio[1,2*], and Cindy Julieth Rodríguez Bernal[3]

ABSTRACT

Bulb onion (*Allium cepa* L.) is a globally consumed vegetable, and as the global population increases, demand for this crop is continuously rising. Unfortunately, production is significantly reduced—up to 40%—due to Fusarium wilt, a fungal disease caused by the *Fusarium* genus. In Boyacá, Colombia, one of the central onion-producing regions, chemical control is the primary method for controlling this disease despite the negative impact of chemicals on soil health and their decreasing efficacy. One alternative management strategy is resistance induction through microorganisms, which has been tested with the *Trichoderma* genus but not with native populations of arbuscular mycorrhizal fungi (AMF). This study aims to evaluate the resistance-inducing effect of a consortium of native AMF from Boyacá on the bulb onion. *Fusarium oxysporum* pathogens and native AMF were isolated from *A. cepa* L. crops in Boyacá and tested under greenhouse conditions for 18 weeks in a completely randomized design. The study evaluated the effects of the pathogen and AMF consortium on leaf number, average leaf area, and bulb growth. It found resistance-induction and growth promotion effects, as well as the adverse effects of the pathogen and the combined effects of both microorganisms. The findings suggest that native AMF consortia from Boyacá exerted a protective impact against Fusarium wilt, improving plant productivity under sterile soil conditions.

Keywords: infection prevention, inoculation time, mycorrhizal consortium, sanitary status.

RESUMEN

La cebolla de bulbo (*Allium cepa* L.) es una hortaliza consumida a nivel global y con el crecimiento poblacional mundial su demanda se hace cada vez más grande. Desafortunadamente su producción disminuye hasta un 40% debido al marchitamiento por *Fusarium*, enfermedad causada por hongos del género *Fusarium*. En Boyacá, Colombia, una de las principales regiones productoras de cebolla, el control químico es el principal método contra esta enfermedad, aunque presenta impactos negativos en la salud del suelo y su eficacia ha disminuido. Una de las alternativas de manejo es la resistencia inducida por microorganismos, como se ha probado con hongos del género *Trichoderma*, pero no con poblaciones nativas de hongos micorrízicos arbusculares (HMA). El objeto del presente trabajo fue evaluar el efecto inductor de resistencia al marchitamiento por *Fusarium* utilizando un consorcio de HMA nativos de Boyacá en cebolla de bulbo. Se realizaron aislamientos del patógeno *Fusarium oxysporum* y de HMA a partir de cultivos de *A. cepa* L. nativos de Boyacá, y se realizaron pruebas en condiciones de invernadero durante 18 semanas con un diseño completamente al azar. Se evaluaron los efectos del patógeno y del consorcio de HMA sobre el número de hojas, el área foliar promedio y el crecimiento del bulbo. Se encontraron efectos de inducción de resistencia y de promoción de crecimiento; también se observaron los efectos adversos del patógeno, así como efectos conjuntos de ambos tipos de microorganismos. Se concluyó que los consorcios de HMA de Boyacá promovieron un efecto protector contra el marchitamiento por *Fusarium*, mejorando la productividad de la planta en condiciones de esterilidad del suelo.

Palabras clave: prevención de infección, tiempo de inoculación, consorcio micorrízico, estado sanitario.

Introduction

The global human population reached 8,000 million in 2022 and is expected to increase to 9,000 million by 2050 (ONU, 2019; 2022), requiring more efficient food production. Intensive agriculture and chemical inputs have increased the prevalence of diseases and insect pests while negatively impacting soil microbiota (Rojas Rodríguez & Ortuño, 2007). This, along with acquired resistance to some pathogens, highlights the need to explore mechanisms

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aligned with natural dynamics (Islam *et al*., 2024; Yin *et al*., 2023).

Boyacá leads onion production in Colombia, accounting for 41% of the total output with 260,970 t in 2023 (MinAgricultura, 2024). Colombia's bulb onion production systems operate within diverse biophysical, socio-ecosystem, and socioeconomic environments. Despite this variability, they can sustain and strengthen their supply, ensuring a steady provision for local and national markets throughout the year. However, they are impacted by imports from neighboring countries.

Worldwide, *Fusarium* species are the primary fungal soil pathogens affecting onion crops (Delgado-Ortiz *et al*., 2016; Montes-Belmont *et al*., 2003), leading to Fusarium wilt. It is essential to note that the plant's phenological stage can increase susceptibility to pathogens; the susceptibility can be increased or reduced in mature or nursery plants (Frare *et al*., 2019). In onion bulbs, *Fusarium* spp. fungi can initially cause leaf yellowing, followed by wilting, leaf curling, rot, root breakage, and eventually plant death (Gardezi *et al*., 2001; Martínez-Salgado *et al*., 2021). If infection occurs late, symptoms may not appear until storage (Cramer, 2000).

Economically significant *Fusarium* species include *F. proliferatum, F. solani*, and *F. oxysporum*, which can reduce crop yields by up to 40% (Betancourth García *et al*., 2020; Martínez-Salgado *et al*., 2021). Fusarium wilt control in onion crops heavily relies on chemical inputs (Naeini *et al*., 2010; Navarro *et al*., 2021; Vergel *et al*., 2016), overlooking alternatives like biological control with *Trichoderma* spp. (Delgado-Oramas, 2020), endophytes (Abdelrahman *et al*., 2016), resistance induction by extracts or microorganisms (Fontana *et al*., 2021), and exposure to UV radiation (Winona *et al*., 2025).

Among the three plant defense mechanisms—physical, chemical, and induced (Couto & Zipfel, 2016)—plants activate induced responses when they detect pathogen-associated molecules, triggering pattern-induced resistance. In response to specific microbial effectors, this leads to effector-triggered immunity or acquired resistance, often resulting in the hypersensitive response characterized by localized cell death (Jones & Dangl, 2006).

In recent decades, interest in using arbuscular mycorrhizal fungi (AMF) for food production and plant disease control has increased (Whipps, 2004). AMF are microorganisms that play a crucial role in plant-associated biological processes, enhancing growth, yields, and biochemical components that strengthen defense capacities against pathogens such as fungi and bacteria (Amin & Ahmed, 2023; Błaszczyk *et al*., 2014). Plants respond favorably to AMF interactions, improving growth and increasing resistance to abiotic stressors such as drought, temperature fluctuations, salinity, heavy metal toxicity, and nutrient deficiency or excess (Datta & Kulkarni, 2012; Gardezi *et al*., 2001; Rivera Méndez *et al*., 2014; Wilches Ortiz *et al*., 2019).

The AMF also contribute to maintaining the structural stability of soils, whether in forest or crop conditions, by secreting glomalin, mucilages, and hydrophobins through their hyphae: these contribute to soil aggregation (Rashid *et al*., 2016) by generating hyphal networks that trap and bind soil particles, providing cohesion to the particles and stability to the aggregates (Leifheit *et al*., 2014; Schütz *et al*., 2022). The inductive defense effect of arbuscular mycorrhizal fungi is recognized in plants such as *Elymus nutans* (Zhang *et al*., 2022), *Solanum lycopersicum* (Badrbani *et al*., 2024; Saha *et al*., 2022), *Plantago lanceolata* (Qu *et al*., 2021), and other plant species such as *Poncirus trifoliata* (Liu *et al., 2024*) and *Zea mays* (Hao *et al*., 2012). This mechanism is used to control plant pathogens (Dey & Ghosh, 2022). The defensive effect of AMF extends beyond the root level. These fungi also mediate in the control of fungal foliar diseases (Kashyap *et al*., 2024). Plant defenses are induced not only by arbuscular mycorrhizal fungi but also by an adequate supply of nutrients (Stratton *et al*., 2022).

Regarding bulb onions, there are positive references. Agudelo Becerra and Casierra-Posada (2004) find that undefined AMF in field conditions increase resistance to *F. oxysporum*, inhibiting pathogen growth, reducing bulb damage, and mitigating salinity effects; after that, Jaime *et al*. (2008) report a reduction of about 50% in the incidence of white rot in field conditions using *Glomus intraradices*. Yağmur *et al*. (2024) found up to a 73% reduction in the severity of basal wilt expression using *Funneliformis mosseae* against *F. oxysporum* in greenhouse conditions. Studies on *Rhizophagus irregularis*-inoculated *A. cepa* crops demonstrate positive effects on growth, quality, and yields, driven by increased chlorophyll content and improved nutritional properties (El-Sherbeny *et al*., 2022; Rozpądek *et al*., 2016). However, Ghanbarzadeh *et al*. (2016) report that the simultaneous inoculation with *F. mosseae* and *T. harzianum* stimulated onion growth but partially inhibited *F. mosseae* colonization.

When evaluating antagonistic or suppressive effects against pathogens, an important aspect is the concentration at which the pathogen induces disease symptoms. Generally, the increase in *F. oxysporum* concentration is directly correlated to the severity of the symptoms. For example, in cotton (*Gossypium hirsutum*), wilt symptoms and reductions in plant growth occur at soil inoculum levels of 10^3 conidia and become more severe at 10^4 conidia/g and higher (Hao *et al.*, 2009). In Mexican lime (*Citrus* x *aurantifolia*), severity steadily increases as microconidia density rose from 500 to 8000 per g of soil (Morgan & Timmer, 1984). In chickpea (*Cicer arietinum*), maximum disease intensity is observed at chlamydospore densities as low as 6 to 50 per g of soil, depending on the race of *F. oxysporum* (Navas-Cortés *et al.*, 2007). Similarly, in watermelon (*Citrullus lanatus*), wilt incidence is strongly linked to inoculum densities ranging from 100 to 1200 CFU/g (Zhou & Everts, 2003).

Even in the indirect transmission of the pathogen, such as laurel wilt in avocado, caused by the fungus *Raffaelea lauricola* and transmitted by the exotic ambrosia beetle *Xyleborus glabratus*, symptom severity is lower at 10^2 than at higher concentrations, and both 10^2 and 10^3 conidia cause less disease than 10^4 and 10^5 (Hughes *et al.*, 2015).

Evaluating the interactions of native AMF consortia in *A. cepa* crops helps expand the field research on sustainable alternative onion production. This study posited: (1) that pathogen concentration does not influence the severity of Fusarium wilt in *A. cepa*, and (2) that AMF provided a protective effect against Fusarium wilt, enhancing plant productivity. The study tested for differences among treatments with varying pathogen concentrations. It established whether AMF-inoculated plants exhibited better growth and productivity.

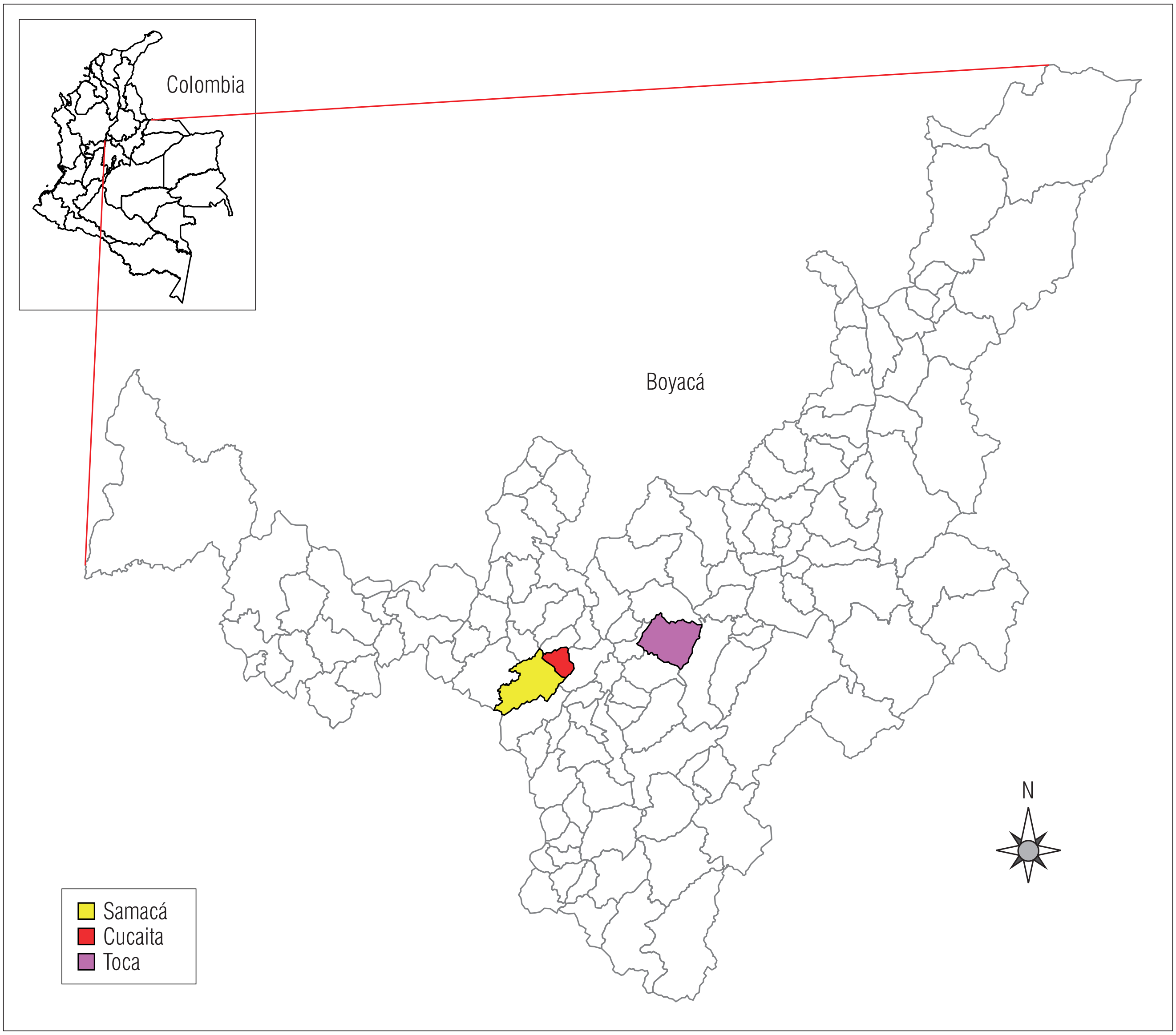

FIGURE 1. Sampling sites in the municipalities of Cucaita, Toca, and Samacá in Boyacá, Colombia.

Materials and methods

Sampling

Samples were collected in 2022 from three municipalities in Boyacá—Cucaita, Toca, and Samacá—at elevations ranging from 2,641 to 2,838 m a.s.l. Two farms growing onions were chosen in each municipality. Ten random top-soil subsamples were taken from each farm and combined to yield approximately 1,000 g of soil (Fig. 1). Additionally, onions in the bulb thickening phenological phase, showing Fusarium wilt symptoms were collected. All samples were stored in labeled plastic bags and kept refrigerated until processed at the Zenkinoko SAS laboratory in Cucaita.

Extraction of AMF spores

The AMF spores were extracted by wet sieving and decantation following Sieverding (1984). Ten grams of soil were processed through a series of mesh sieves with openings of 2000, 500, 250, 120, and 45 µm. The material retained in the smaller sieves was subjected to sucrose gradient centrifugation (70-80%). The spores were examined using a ZEISS Stemi 305 stereomicroscope. They were subsequently extracted with a syringe fitted with a yellow micropipette tip and stored in refrigeration at 2-4°C in 2 ml conical tubes containing 100 µl of distilled water, at a rate of 40 spores per tube. For experiments, only the three most abundant species were selected.

Only spores that appeared viable (based on visual assessment and the presence of cytoplasmic content), not parasitized, broken, or perforated, were selected. Morphospecies or higher taxonomic classification was determined based on morphological characteristics, including spore and hyphal coloration, presence or absence of a shield, number of walls and layers, shape and attachment of the subtending hypha, presence, absence, and position of the septum, presence of scars, presence of a sacculus, ornamentation, whether spores were solitary or clustered, the type of aggregation, and reaction to Melzer's reagent. Genus-level identification was performed according to the existing literature. Species-level identification was conducted where possible by comparing morphological traits with data from the International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi (INVAM) (https://invam.wvu.edu), Professor Sidney Stürmer's collection (https://sites.google.com/site/cicgfma/home), and Professor Janusz Blaszkowski's Glomeromycota collection (http://www.zor.zut.edu.pl) (Blaszkowski, 2012), as well as recently described species up to December 2023.

Isolation of *Fusarium* spp. strains

The isolation of *Fusarium* spp. followed the methodology described by Hernández *et al*. (2019). Onion root segments (~2 cm) from sick plants were cleaned with distilled water to remove soil residues, surface-sterilized with 2% sodium hypochlorite for three minutes, rinsed with distilled water, immersed in 70% ethanol for one minute, and subsequently rewashed with distilled water. The roots were dried on filter paper and plated (4 fragments per Petri dish) on potato dextrose agar (PDA) without antibiotics or antimycotics. A total of 40 plates were incubated at 28°C until visible colonies appeared, within 1 week.

Of 57 colonies obtained, the 20 that exhibited cotton-like growth and characteristic pink, red, or white *Fusarium* pigmentation (Duarte *et al*., 2016) were selected. Using a mycological loop, mycelial tip fragments were subcultured onto PDA by puncture and incubated at 28°C for 7 d. Among these, 15 purified *Fusarium* spp. strains were retained for further identification. To preserve isolates, mycelial fragments were transferred to inclined agar tubes (Montesinos *et al*., 2015), incubated at 28°C for 7 d, and stored at 4°C.

Identification of *Fusarium* spp.

For *Fusarium* species identification, carnation leaf agar (CLA) at 2% was used to promote the formation of both macroconidia and microconidia (Duarte *et al*., 2016). Autoclaved carnation leaves (in five fragments) were added to 2% water agar and refrigerated for one day at 4°C. The 15 fungal isolates were inoculated by puncture and incubated at 28°C for 7 d. Conidial observations were conducted using traditional slide mounts, employing a mycological handle, a lactophenol blue stain, and a Primo Star ZEISS microscope, following the species descriptions of Leslie and Summerell (2006).

Fusarium spp. inoculum mass-production

The previously identified strains were tested for growth rate in PDA at 28°C, and the two isolates with the highest growth rate were selected for assays. For pathogen mass production, the methodology of Jarek *et al*. (2018) was followed. A test tube containing the isolate was supplemented with 1 ml of sterile distilled water and a drop of Tween 80. The fungal mycelium was scraped from the medium using a round inoculation handle and transferred to another tube. A 100 µl aliquot of the suspension was spread onto PDA plates using a Drigalski spatula in a spiral pattern. Five replicates were prepared and incubated at 28°C for 7 d.

Onion seed sowing

Sterilized peat (2 kg, autoclaved at 121°C for 1 h) was used as a substrate and placed in 72-cavity germination trays, which were isolated from the soil surface by a plastic-covered table. Onion seeds were surface-treated with 2% sodium hypochlorite for 1 min, followed by three rinses with distilled water. Then, using surface-sterile forceps, two seeds were sown in each tray cavity.

One month after germination, seedlings were transplanted into pots containing a mixed substrate (1:1 soil:sand) that had been double-sterilized in an autoclave at 121°C for 1 h. The peat substrate was gently removed from the roots with a spatula to minimize damage during transplant. Depending on the treatment, the fungal pathogen, the AMF, or both were placed in the center of the pot before transplanting the seedlings. Each pot contained a single seedling, and additional substrate was added to cover the roots without burying the foliage.

Experimental design

A completely randomized design was used with six treatments and ten plants per treatment (five per dosage), resulting in a total of 60 plants. The inhibitory effect of AMF on *Fusarium*-induced disease was evaluated based on bulb growth (mm), leaf length average (cm), and leaf count over 18 weeks. The treatments were performed as in Table 1.

TABLE 1. *Fusarium* and arbuscular mycorrhiza inoculation treatments on *A. cepa* plants in greenhouse conditions.

Treatment	AMF	*Fusarium*	Time of inoculation
T0	-	-	-
T1	x	-	Initial
T2	-	x	Initial
T3	x	x	*Fusarium* 7 d after AMF
T4	x	x	AMF 7 d after *Fusarium*
T5	x	x	Initial and simultaneous

Conventions: - = No inoculation, x = inoculation.

Three fertilization events were conducted using a 13-40-13 (NPK) formulation, following standard fertilization schedules for the onion crop. Additionally, plant mortality due to disease and phenotypic traits such as chlorosis and wilting was recorded.

For AMF inoculation, 250 µl of distilled water was added to each conical tube containing 40 AMF spores. Each tube suspension was then applied directly onto the roots per pot using a micropipette under a stereomicroscope, ensuring proper adhesion of the spores to the root surface. For *Fusarium* inoculation, mass culture conidia suspensions were adjusted to 1 × 10^8 conidia ml^{-1} using a Neubauer chamber. According to the treatment, two pathogens at both concentrations (75 µl and 420 µl) were applied directly to the roots, along with a control without conidia. Five plants per concentration were used per *Fusarium* treatment.

Data analysis

Normality and homoscedasticity tests were performed before statistical analysis. All analyses were conducted using SPSS v.27. Figures were generated with SigmaPlot v.12. A two-way ANOVA (weeks and pathogen concentration) was performed to assess the effect of *Fusarium* concentration (0, 75, 420 µl) on the evaluated variables and whether differences persisted over time. A post hoc Bonferroni test was applied at a 5% significance level if significant differences were detected.

A two-way ANOVA (weeks and treatment) was conducted to evaluate differences among AMF and *Fusarium* treatments over time. When significant differences were observed, a post-hoc Bonferroni test was applied (5% significance level). The interaction between time and treatment effects was also analyzed to determine the persistence of treatment effects over time.

Results

On each farm, 7 to 16 arbuscular mycorrhizal fungal species were identified, with the three most abundant being *Racocetra* sp., *Acaulospora* sp., and *Acaulospora morrowiae*, which were used in the consortium. Meanwhile, the phytopathogenic fungus used in the experiment corresponds to *Fusarium oxysporum* Schltdl.

We observed that the two pathogen concentrations did not differ in their effects on bulb growth and leaf number. However, significant differences arose when comparing these concentrations with those in the treatment in which the pathogen was absent from the soil. Additionally, no interactions between concentrations and time were detected (Fig. 2A and B).

However, when evaluating the interaction between average leaf length and the week of evaluation, a differential effect of *Fusarium* strain concentration was observed, where the absence of the pathogen resulted in shorter leaves. In the treatment inoculated exclusively with the AMF consortium, a differential resource allocation effect was observed, where plants allocate more carbon to the mycorrhizal symbiosis and root development (Smith & Read, 2008; Zheng *et al.*, 2015). This finding is consistent with previous observations (Jakobsen *et al.*, 2003; Smith *et al.*, 2009), and that AMF colonization can temporarily reduce shoot growth while the hyphal network is established and the root system is reinforced. Moreover, concentration interaction was detected only at the 17th week (Fig. 2C).

Leaf number increased precisely at the onset of bulb thickening (approximately at week 7), whereas the average leaf length did not follow the same trend; nevertheless, the mycorrhizal inoculation generally resulted in greater bulb diameter, leaf number, and leaf length than any other treatment (Fig. 3).

Although mycorrhizal and *Fusarium* inoculation did not exhibit an overall protective effect across treatments, after 18 weeks, bulb diameter and leaf number were similar among treatments with mycorrhizae and those where *Fusarium* and mycorrhizae were applied simultaneously (Fig. 3A and B).

Leaf number was significantly higher during the first 10 weeks following mycorrhizal application alone, a level that was eventually reached by the mycorrhizae + *Fusarium* treatment at 11 weeks (Fig. 3B). The treatment with AMF alone showed a gradual decrease in foliar parameters that became more noticeable as bulb size increased, and its behavior was similar to that of other therapies; however, in the last week, it showed an abrupt increase. Apparently, when *A. cepa* is inoculated only with AMF, without any other microorganism, physiological responses are faster and more pronounced during this final period (Fig. 3B and C).

Between weeks 16 and 17, a decline in both leaf number and length was observed across all treatments. In contrast, no such increase in bulb thickness was observed. This suggested a possible redistribution of energy resources toward the bulb rather than maintaining the photosynthetic area. Before this decline, an increase in average leaf length followed a consistent trend over time, progressing more rapidly in the control treatment than in the arbuscular mycorrhizae treatment, and finally in the simultaneous mycorrhizae + *Fusarium* treatment, compared to other treatments. However, this increase was followed by a subsequent decline after reaching its peak (Fig. 3C).

Treatments 2 (*Fusarium* sp.) and 4 (*Fusarium* sp. day 1 + Mycorrhizae day 7) lost one sample unit by week 5 due to the pathogenic fungus. Additionally, these treatments, along with treatment 3 (Mycorrhizae day 1 + *Fusarium* sp. day 7), exhibited symptoms of disease, such as progressive leaf chlorosis after 12 weeks.

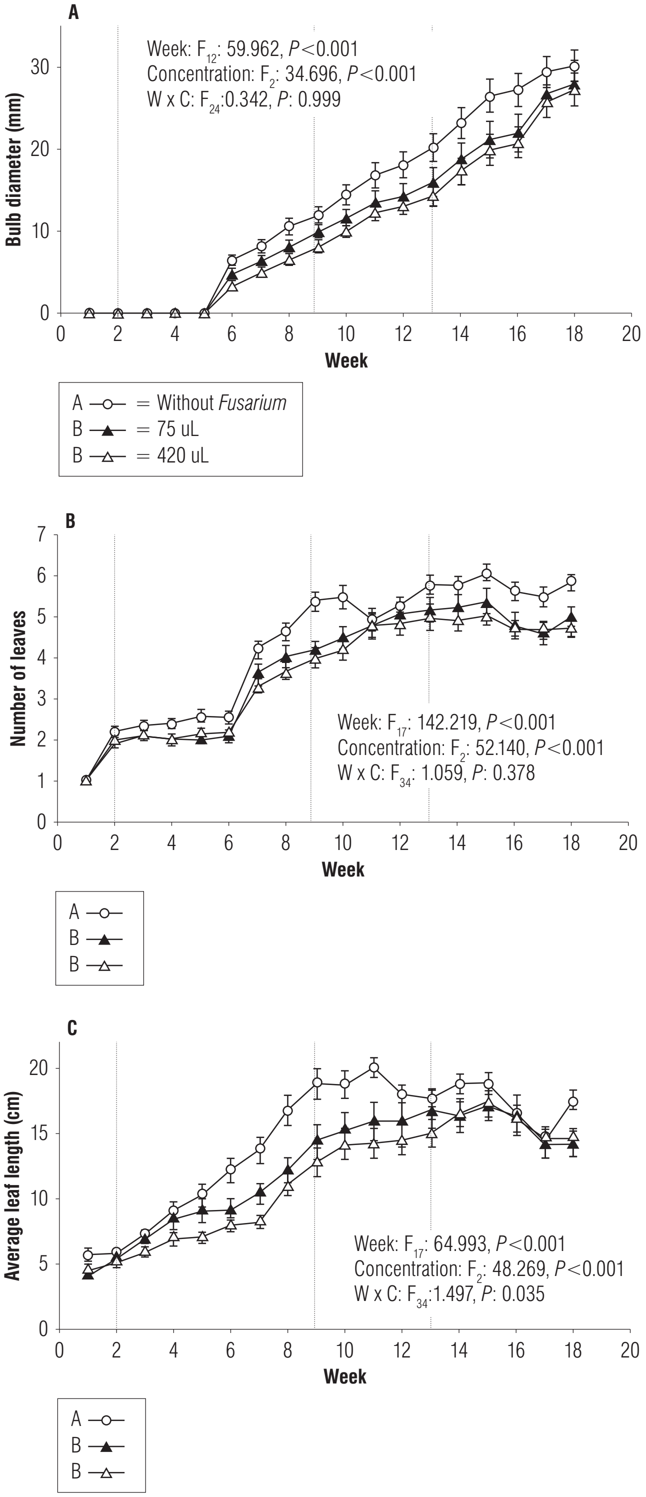

FIGURE 2. Effect of *Fusarium* sp. strain concentration on growth parameters and productivity of bulb onion (*A. cepa*) over 18 weeks. Bars correspond to the standard error. A) Bulb diameter, B) number of leaves, C) average leaf length. Uppercase letters within the labels correspond to the Bonferroni post hoc test for each strain concentration in each evaluated variable. The vertical dotted lines indicate fertilization events.

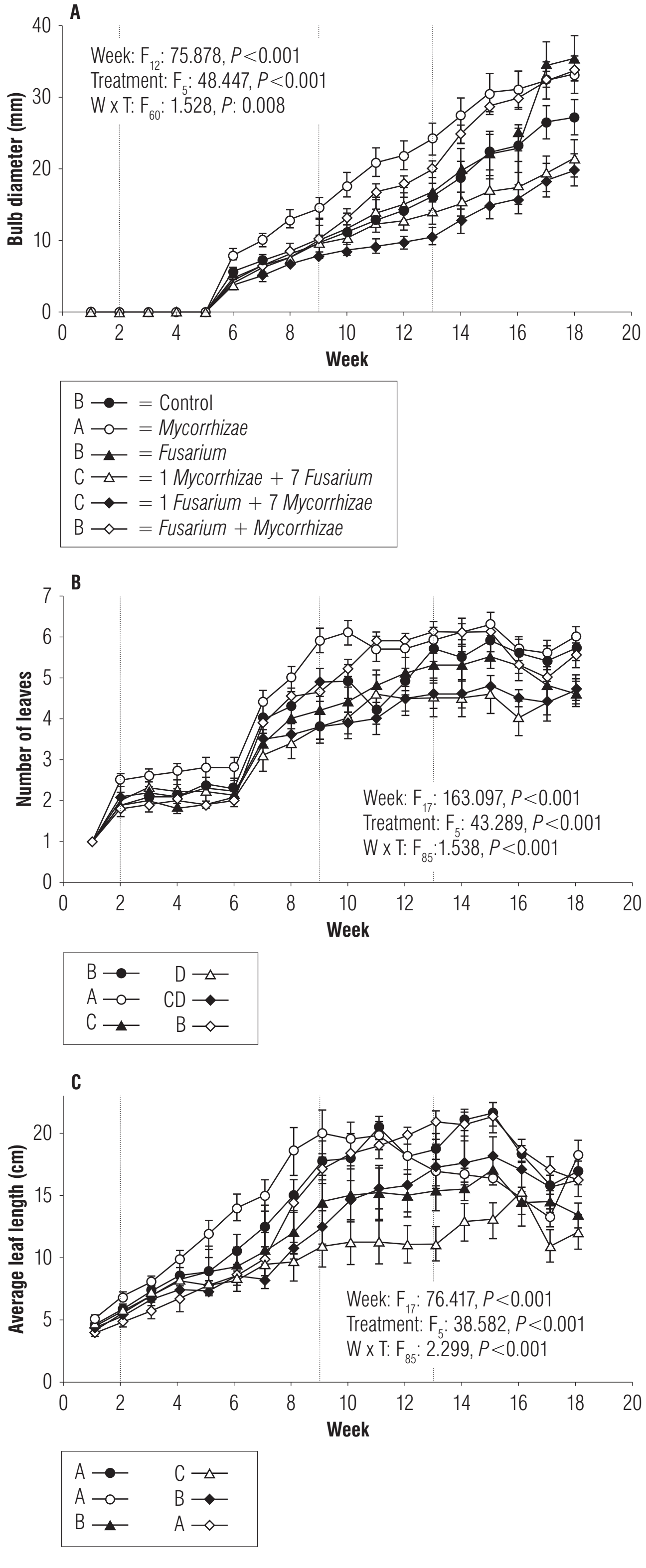

FIGURE 3. Effect of *Fusarium* sp. and arbuscular mycorrhizal treatments on bulb onion (*A. cepa*) growth parameters and the productivity over 18 weeks. Bars correspond to the standard error. A) Bulb diameter, B) number of leaves, C) average leaf length. Uppercase letters within the labels correspond to the Bonferroni post-hoc test for each strain treatment in each evaluated variable. The vertical dotted lines indicate fertilization events.

Discussion

Previous studies related to some AMF, such as *Glomus*, *Rhizophagus*, and *Funneliformis*, show effects against *F. oxysporum* wilt in onion. However, only Hu *et al*. (2010) and Tanwar *et al*. (2013) report the positive impact of *Acaulospora* spp against this disease. Evidence for *Racocetra* remains scarce and seems to be associated with mixed-species inoculum rather than effects specific to the genus.

There is a wide range of experiments aimed at reducing the impact of *Fusarium* spp. on different crops, including onions. These range from the use of one single antagonistic species, the use of known bacteria, fungi, or both groups of organisms in a synthetic consortium (such as the present), to the use of amendments (Habte & Dobo, 2025), plant extracts (Hegazy *et al*., 2024), or the use of undefined microorganism mixtures as in efficient microorganisms (Guigui *et al*., 2024). In the last-mentioned, the authors explore the antagonistic, suppressive, or resistance-inducing effects of products with different attributed properties, such that the set of components (in most cases undefined) can, simply or synergistically, contribute to controlling pathogenic microorganisms.

This study tested two hypotheses. The first proposed that pathogen concentration influences the level of Fusarium wilt severity in *A. cepa* plants, requiring an individual analysis of the evaluated parameters. Regarding bulb size and leaf number, all treatments inoculated with *F. oxysporum* showed similar averages over time regardless of pathogen concentration (Fig. 2A and B). All *F. oxysporum*-inoculated treatments displayed chlorosis and wilting symptoms, with two treatments also showing plant mortality (one at 75 µl and another at 420 µl). This indicated that *Fusarium* sp. concentrations did not significantly differ in their effect on Fusarium wilt severity, as assessed by measuring bulb size and leaf number. Both concentrations used likely exceeded the unknown minimum infectious dose of *F. oxysporum* for *A. cepa*, as evidenced by the appearance of disease symptoms. Similar results are reported by Manasa *et al*. (2017) working with carnations. Consequently, increasing the concentration does not significantly affect disease severity (Biswal *et al*., 2020; Wright *et al*., 1997) when evaluating by leaf number or bulb diameter.

For leaf length, an inverse relationship was observed between pathogen concentration and average leaf length, suggesting that pathogen concentration affects the plant's photosynthetic capacity. However, this effect was mitigated after 14 weeks, when the leaf length reached its maximum average in infected plants (Fig. 2C). Several authors have reported the severity of different *Fusarium* species in various crops, such as bean (*Phaseolus vulgaris*), where it affects plant growth and development (Biswal *et al*., 2020), or soybean (*Glycine max*), where it reduces productivity without significantly affecting other variables (Freitas *et al*., 2016).

Regarding the second hypothesis, because *F. oxysporum* concentration did not affect leaf number or bulb diameter (key productivity variables), all treatments were considered independent units, regardless of pathogen concentration. It was hypothesized that arbuscular mycorrhizal fungi promote a protective effect against Fusarium wilt, thereby enhancing the plant's productive characteristics—a detail that warrants close examination.

When comparing the simultaneous application of both microorganisms to the control (neither *Fusarium* sp. nor mycorrhizae) and considering that both outperformed *F. oxysporum* treatment alone in foliar measurements, a protective effect against the pathogen could be observed (Fig. 2B and C). Similar results are reported in *Citrullus* sp., where *Trichoderma viride* effectively suppressed *F. oxysporum* in *Solanum lycopersicum* plants (Ponsankar *et al*., 2023). Additionally, biocontrol effects of *Trichoderma harzianum* and *Glomus mosseae* against basal rot in onion plants (Ghanbarzadeh *et al*., 2016) are well documented. Comparing the control to the mycorrhiza-only treatment revealed a stimulatory effect of mycorrhizae on both leaf number and bulb diameter (Fig. 3A and B). This effect is widely recognized in crop plants associated with arbuscular mycorrhizal fungi (AMF), such as oat (*Avena sativa*) (Flores-Juárez *et al*., 2020) and banana (*Musa* sp.) (Bernal, 2020), among other commercially relevant species. Furthermore, onions fertilized with arbuscular mycorrhizal fungi have been reported to produce a higher quantity of indigestible oligosaccharides, which may be linked to protective mechanisms or potential medical applications (Lone *et al*., 2015).

However, the combined use of pathogenic and beneficial fungi reduced the individual effects of both microorganisms on the plant. While pathogen severity was minimized, productivity levels took longer to reach those observed with mycorrhizae alone. This represents a trade-off in protective benefit (Delgado-Oramas, 2020) and reflects the energetic balance between production and defensive processes (Cipollini & Heil, 2010; García *et al*., 2021). A similar trend was observed for average leaf length, with the effect becoming evident primarily after eight weeks of treatment (Fig. 2C). Interestingly, although average leaf length decreased first in the mycorrhiza treatment (week 12), this pattern was subsequently observed in the combined biological treatments and the control (week 16), once maximum average values were reached and, while leaf length declined, bulb diameter continued to increase (Fig. 3). This source-sink redistribution of nutrients, in which leaves act as sources and bulbs as sinks, is a common phenomenon across vegetation (Azcón-Bieto & Talón, 2013), particularly in short-cycle species like bulb onion once phenological maturity is reached. What makes this particularly interesting is that the maximum average leaf areas and the timing of their attainment appear to be closely linked to plant health status, being early in healthy plants and late in sick plants (Fig. 3B and C).

Finally, the staggered inoculation treatment—applying the mycorrhizal consortia first, followed by *F. oxysporum* —produced unexpected results, yielding the lowest productivity across all evaluated variables, even lower than *F. oxysporum* alone (Fig. 3). This suggested a greater metabolic burden that negatively impacted plant development. These findings indicated that mycorrhizal application should be performed preventively in pots rather than as a curative measure once *F. oxysporum* wilt has been established.

According to the results of this experiment, the best time to apply native arbuscular mycorrhizal fungi in onion crops is at the seedling stage, when the plants are developing their first roots, and in sterile soil conditions, without fertilizer, until the relationship between both members is established. In this way, the AMF application enhances productivity and provides protection against Fusarium wilt. Considering the potential of these AMF consortia to protect against *F. oxysporum*, it is essential to evaluate their impact on other *Fusarium* species that affect *A. cepa*. Moreover, further research is needed to determine their effectiveness in nonsterile conditions, where similar outcomes are expected due to the presence of the most abundant native AMF.

Conclusions

Under greenhouse sterile soil conditions, pathogen concentration did not significantly affect bulb growth or leaf number, but it negatively affected leaf length. The protective effect observed in onion plants inoculated with native AMF consortia (*Acaulospora* spp. and *Racocetra* sp.) from Boyacá supports their role in mitigating *F. oxysporum* wilt. This is the first report of *Racocetra* in consortia with other AMF as a biological control agent.

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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

RHP and SSS designed the experiment; SSS conducted the field and laboratory experiments; RHP and CJR contributed to data analysis; RHP, CJR, and SSS wrote the draft of the manuscript. All authors reviewed the final version of the manuscript.

Literature cited

Abdelrahman, M., Abdel-Motaal, F., El-Sayed, M., Jogaiah, S., Shigyo, M., Ito, S., & Tran, L.-S. P. (2016). Dissection of *Trichoderma longibrachiatum*-induced defense in onion (*Allium cepa* L.) against *Fusarium oxysporum* f. sp. *cepa* by target metabolite profiling. *Plant Science*, *246*, 128–138. https://doi.org/10.1016/j.plantsci.2016.02.008

Agudelo Becerra, M. Y., & Casierra-Posada, F. (2004). Efecto de la micorriza y gallinaza sobre la producción y la calidad de cebolla cabezona (*Allium cepa* L. 'Yellow Granex'). *Revista Facultad Nacional de Agronomía Medellín*, *57*(1), 2189–2202. https://revistas.unal.edu.co/index.php/refame/article/view/24174/24802

Amin, M. M., & Ahmed, M. F. A. (2023). Biological control of onion white rot disease using potential *Bacillus subtilis* isolates. *Egyptian Journal of Biological Pest Control*, *33*(1), Article 27. https://doi.org/10.1186/s41938-023-00673-4

Azcón-Bieto, J., & Talón, M. (Eds.) (2013). *Fundamentos de fisiología vegetal* (2nd ed.). McGraw-Hill Interamericana. https://exa.unne.edu.ar/biologia/fisiologia.vegetal/FundamentosdeFisiologiaVegetal2008Azcon..pdf

Badrbani, A. H., Amini, J., Sharifi, R., & Karimi, K. (2024). Arbuscular mycorrhizal fungi, induce resistance in tomato plant against Fusarium wilt through transferring underground warning signal. *Physiological and Molecular Plant Pathology*, *133*, Article 102380. https://doi.org/10.1016/j.pmpp.2024.102380

Bernal, M. A. (2020). *Efecto de microorganismos eficientes y micorrizas en la fertilización fosfórica del banano en alta densidad bajo condiciones edafoclimáticas de Apartadó-Colombia* [Undergraduate thesis, Universidad de Córdoba, Colombia]. https://repositorio.unicordoba.edu.co/handle/ucordoba/2892

Betancourth García, C., Salazar González, C., Lagos Mora, L. E., Díaz Rodríguez, V., & Mora Chaves, S. (2020). Caracterización de *Fusarium* spp. asociado con la pudrición basal de la cebolla de rama. *Revista U.D.C.A Actualidad* & *Divulgación Científica*, *23*(1), Article e1471. https://doi.org/10.31910/rudca.v23.n1.2020.1471

Biswal, K., Sahu, K. C., Ranasingh, N., & Moharana, R. L. (2020). *Fusarium oxysporum* causing Black gram wilt a new threat in the Western undulated zones of Odisha. *International Journal of Current Microbiology and Applied Sciences*, *9*(6), 3027–3031. https://doi.org/10.20546/ijcmas.2020.906.364

Błaszczyk, L., Siwulski, M., Sobieralski, K., Lisiecka, J., & Jędryczka, M. (2014). *Trichoderma* spp. – application and prospects for use in organic farming and industry. *Journal of Plant Protection Research*, *54*(4), 309–317. https://www.plantprotection.pl/pdf-93508-27523?filename=27523.pdf

Błaszkowski, J. (2012). Professor Janusz Błaszkowski's Glomeromycota collection. Zachodniopomorski University of Technology, Szczecin. http://www.zor.zut.edu.pl

Cipollini, D., & Heil, M. (2010). Costs and benefits of induced resistance to herbivores and pathogens in plants. *CABI Reviews*, *2010*, 1–25. https://doi.org/10.1079/PAVSNNR20105005

Couto, D., & Zipfel, C. (2016). Regulation of pattern recognition receptor signalling in plants. *Nature Reviews Immunology*, *16*(9), 537–552. https://doi.org/10.1038/nri.2016.77

Cramer, C. S. (2000). Breeding and genetics of *Fusarium* basal rot resistance in onion. *Euphytica*, *115*, 159–166. https://doi.org/10.1023/A:1004071907642

Datta, P., & Kulkarni, M. (2012). Arbuscular mycorrhizal fungal diversity in sugarcane rhizosphere in relation with soil properties. *Notulae Scientia Biologicae*, *4*(1), 66–74. https://doi.org/10.15835/nsb416567

Delgado-Oramas, B. P. (2020). La resistencia inducida como alternativa para el manejo de plagas en las plantas de cultivo. *Revista de Protección Vegetal*, *35*(1), 1–12. https://censa.edicionescervantes.com/index.php/RPV/article/view/1084/1570

Delgado-Ortiz, J. C., Ochoa-Fuentes, Y. M., Cerna-Chávez, E., Beltrán-Beache, M., Rodríguez-Guerra, R., Aguirre-Uribe, L. A., & Vázquez-Martínez, O. (2016). Patogenicidad de especies de *Fusarium* asociadas a la pudrición basal del ajo en el centro norte de México. *Revista Argentina de Microbiología*, *48*(3), 222–228. https://doi.org/10.1016/j.ram.2016.04.003

Dey, M., & Ghosh, S. (2022). Arbuscular mycorrhizae in plant immunity and crop pathogen control. *Rhizosphere*, *22*, Article 100524. https://doi.org/10.1016/j.rhisph.2022.100524

Duarte Leal, Y., Echevarría Hernández, A., & Martínez Coca, B. (2016). Identificación y caracterización de aislamientos de *Fusarium* spp. presentes en garbanzo (*Cicer arietinum* L.) en Cuba. *Revista de Protección Vegetal*, *31*(3), 173–183. https://censa.edicionescervantes.com/index.php/RPV/article/view/848/769

El-Sherbeny, T. M. S., Mousa, A. M., & El-Sayed, E.-S. R. (2022). Use of mycorrhizal fungi and phosphorus fertilization to improve the yield of onion (*Allium cepa* L.) plant. *Saudi Journal of Biological Sciences, 29*(1), 331–338. https://doi.org/10.1016/j.sjbs.2021.08.094

Flores-Juárez, D., Villegas-Aparicio, Y., Castro-Rivera, R., Gómez-Vázquez, A., Carrillo-Rodríguez, J., & Hidalgo, E. (2020). Inoculación de avena forrajera con hongos micorrízicos arbusculares. *Revista Mexicana de Ciencias Agrícolas*, (24), 191–199. https://doi.org/10.29312/remexca.v0i24.2369

Fontana, D. C., Paula, S., Torres, A. G., Souza, V. H. M., Pascholati, S. F., Schmidt, D., & Neto, D. D. (2021). Endophytic fungi: biological control and induced resistance to phytopathogens and abiotic stresses. *Pathogens*, *10*(5), Article 570. https://doi.org/10.3390/pathogens10050570

Frare, G. F., Silva-Junior, G. J., Lanza, F. E., Bassanezi, R. B., Ramires, T. G., & Amorim, L. (2019). Sweet orange fruit age and inoculum concentration affect the expression of Citrus black spot symptoms. *Plant Disease*, *103*(5), 913–921. https://doi.org/10.1094/PDIS-03-18-0492-RE

Freitas, R. M. S., Lima, L. E., Silva, R. S., Campos, H. D., & Perin, A. (2016). Fluxapyroxad in the Asian soybean rust control in the Cerrado biome. *Revista Caatinga*, *29*(3), 619–628. https://doi.org/10.1590/1983-21252016v29n312rc

Garcia, A., Martinez, M., Diaz, I., & Santamaria, M. E. (2021). The price of the induced defense against pests: a meta-analysis. *Frontiers in Plant Science*, *11*, Article 615122. https://doi.org/10.3389/fpls.2020.615122

Gardezi, A. K., Cetina Alcalá, V. M., Ferrera-Cerrato, R., Velásquez Mendoza, J., Pérez Mercado, C. A., & Larqué Saavedra, M. (2001). Hongos micorrízicos arbusculares como componente de control biológico de la pudrición causada por *Fusarium* sp. en gladiola. *Terra Latinoamericana*, *19*(3), 259–264. https://www.redalyc.org/pdf/573/57319307.pdf

Ghanbarzadeh, B., Safaie, N., Mohammadi Goltapeh, E., Rezaee Danesh, Y., & Khelghatibana, F. (2016). Biological control of *Fusarium* basal rot of onion using *Trichoderma harzianum* and *Glomus mosseae*. *Journal of Crop Protection*, *5*(3), 359–368. https://www.researchgate.net/publication/317545620_Biological_control_of_Fusarium_basal_rot_of_onion_using_Trichoderma_harzianum_and_Glomus_mosseae

Guigui, H. D., Zaouli, B. Z. P., Coulibaly, A. S., & Dedi, J. K. (2024). Influence of microorganisms effective against basal rot and on agronomic parameters of onion [*Allium cepa* L. (Amaryllidaceae)]. *Journal of Agricultural Chemistry and Environment*, *13*(3), 282–299. https://doi.org/10.4236/jacen.2024.133019

Habte, B., & Dobo, B. (2025). The effect of compost and biochar amendments, as well as the inoculation of arbuscular mycorrhizal fungi and *Trichoderma harzianum*, on the control of *Fusarium oxysporum* pathogen on tomato (*Solanum lycopersicum* L.) in the greenhouse. *International Journal of Agronomy*, *2025*(1), Article 7330396. https://doi.org/10.1155/ioa/7330396

Hao, J. J., Yang, M. E., & Davis, R. M. (2009). Effect of soil inoculum density of *Fusarium oxysporum* f. sp. *vasinfectum* race 4 on disease development in cotton. *Plant Disease*, *93*(12), 1324–1328. https://doi.org/10.1094/PDIS-93-12-1324

Hao, Z., Fayolle, L., van Tuinen, D., Chatagnier, O., Li, X., Gianinazzi, S., & Gianinazzi-Pearson, V. (2012). Local and systemic mycorrhiza-induced protection against the ectoparasitic nematode *Xiphinema index* involves priming of defence gene responses in grapevine. *Journal of Experimental Botany, 63*(10), 3657–3672. https://doi.org/10.1093/jxb/ers046

Hegazy, M. G. A., Ahmed, A.-R. M., Yousef, A. F., Ali, W. M., Nasr, A., Elshazly, E. H., Shalaby, M. E., Teiba, I. I., & Al-Bedak, O. A. M. (2024). Effectiveness of some plant extracts in biocontrol of induced onion basal rot disease in greenhouse conditions. *AMB Express*, *14*(1), Article 72. https://doi.org/10.1186/s13568-024-01721-4

Hernández Amasifuen, A. D., Pineda Lázaro, A. J., & Noriega-Córdova, H. W. (2019). Aislamiento e identificación de *Fusarium oxysporum* obtenidos de zonas productoras de "ají paprika" *Capsicum annumm* L. (Solanaceae) en el distrito de Barranca, Perú. *Arnaldoa*, *26*(2), 689–698. https://journal.upao.edu.pe/Arnaldoa/article/view/1210

Hu, J.-L., Lin, X.-G., Wang, J.-H., Shen, W.-S., Wu, S., Peng, S.-P., & Mao, T.-T. (2010). Arbuscular mycorrhizal fungal inoculation enhances suppression of cucumber *Fusarium* wilt in greenhouse soils. *Pedosphere*, *20*(5), 586–593. https://doi.org/10.1016/S1002-0160(10)60048-3

Hughes, M. A., Inch, S. A., Ploetz, R. C., Er, H. L., van Bruggen, A. H. C., & Smith, J. A. (2015). Responses of swamp bay, *Persea palustris*, and avocado, *Persea americana*, to various concentrations of the laurel wilt pathogen, *Raffaelea lauricola*. *Forest Pathology*, *45*(2), 111–119. https://doi.org/10.1111/efp.12134

International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (INVAM). (2024, July). INVAM – International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi. https://invam.wvu.edu

Islam, T., Danishuddin, Tamanna, N. T., Matin, M. N., Barai, H. R., & Haque, M. A. (2024). Resistance mechanisms of plant pathogenic fungi to fungicide, environmental impacts of fungicides, and sustainable solutions. *Plants*, *13*(19), Article 2737. https://doi.org/10.3390/plants13192737

Jakobsen, I., Smith, S. E., & Smith, F. A. (2003). Function and diversity of arbuscular mycorrhizae in carbon and mineral nutrition. In M. G. A. van der Heijden, & I. R. Sanders (Eds.), *Mycorrhizal ecology. Ecological studies* (Vol. 157, pp. 75–92). Springer. https://doi.org/10.1007/978-3-540-38364-2_3

Jaime, M. D. L. A., Hsiang, T., & McDonald, M. R. (2008). Effects of *Glomus intraradices* and onion cultivar on *Allium* white rot development in organic soils in Ontario. *Canadian Journal of Plant Pathology, 30*(4), 543–553. https://doi.org/10.1080/07060660809507554

Jarek, T. M., Santos, Á. F., Tessmann, D. J., & Vieira, E. S. N. (2018). Inoculation methods and aggressiveness of five *Fusarium* species against peach palm. *Ciência Rural, 48*(4). https://doi.org/10.1590/0103-8478cr20170462

Jones, J. D., & Dangl, J. L. (2006). The plant immune system. *Nature, 444*(7117), 323–329. https://doi.org/10.1038/nature05286

Kashyap, P., Sharma, I., Kashyap, S., & Agarwala, N. (2024). Arbuscular mycorrhizal fungi (AMF)-mediated control of foliar fungal diseases. In G. J. Ahammed, & R. Hajiboland (Eds.), *Arbuscular mycorrhizal fungi and higher plants* (pp. 193–223). Springer Nature. https://doi.org/10.1007/978-981-99-8220-2_9

Leifheit, E. F., Veresoglou, S. D., Lehmann, A., Morris, E. K., & Rillig, M. C. (2014). Multiple factors influence the role of arbuscular mycorrhizal fungi in soil aggregation – a meta-analysis. *Plant and Soil*, *374*(1–2), 523–537. https://doi.org/10.1007/s11104-013-1899-2

Leslie, J. F., & Summerell, B. A. (Eds.). (2006). *The Fusarium laboratory manual* (1st ed.). Wiley. https://doi.org/10.1002/9780470278376

Liu, X.-Q., Liu, Z., Zou, Y.-N., Alqahtani, M. D., & Wu, Q.-S. (2024). Defense responses and symbiotic functional initiation in trifoliate orange–arbuscular mycorrhizal fungi interaction. *Chemical and Biological Technologies in Agriculture, 11*(1), Article 3. https://doi.org/10.1186/s40538-023-00526-0

Lone, R., Shuab, R., Wani, K. A., Ganaie, M. A., Tiwari, A. K., & Koul, K. K. (2015). Mycorrhizal influence on metabolites, indigestible oligosaccharides, mineral nutrition and phytochemical constituents in onion (*Allium cepa* L.) plant. *Scientia Horticulturae*, *193*, 55–61. https://doi.org/10.1016/j.scienta.2015.06.032

Manasa, B. G., Somashekara, Y. M., Shankara, K., & Swamy, C. (2017). Efficacy of fungicides in control of *Fusarium oxysporum* f. sp. *dianthi*, the cause of wilt in carnation. *International Journal of Current Microbiology and Applied Sciences*, *6*(10), 2559–2565. https://doi.org/10.20546/ijcmas.2017.610.300

Martínez-Salgado, S. J., Andrade-Hoyos, P., Romero-Arenas, O., Villa-Ruano, N., Landeta-Cortés, G., & Rivera-Tapia, J. A. (2021). Control *in vitro* de *Fusarium* sp. asociado al cultivo de cebolla mediante *Trichoderma harzianum*. *Revista Mexicana de Fitopatología*, *39*(2), 314–328. https://doi.org/10.18781/r.mex.fit.2101-4

MinAgricultura. (2024, March 1). *Cien familias productoras de cebolla de Ábrego, en Norte de Santander, recibieron apoyos de MinAgricultura*. https://www.minagricultura.gov.co/noticias/Paginas/CienFamiliasProductorasCebollaAbrego.aspx

Montes-Belmont, R., Nava-Juárez, R. A., Flores-Moctezuma, H. E., & Mundo-Ocampo, M. (2003). Hongos y nemátodos en raíces y bulbos de cebolla (*Allium cepa* L.) en el estado de Morelos, México. *Revista Mexicana de Fitopatología*, *21*(3), 300–304. https://www.redalyc.org/pdf/612/61221309.pdf

Montesinos, R., Ayala, M., & Berlanga, A. (2015). *Manual para la conservación y mantenimiento de hongos entomopatógenos* (1st ed.). Servicio Nacional de Sanidad, Inocuidad y Calidad Agroalimentaria (SENASICA). https://www.researchgate.net/publication/304253307_Manual_para_la_conservacion_y_mantenimiento_de_hongos_entomopatogenos_Manual_for_the_conservation_and_maintenance_of_entomopathogenic_fungi

Morgan, K. T., & Timmer, L. W. (1984). Effect of inoculum density, nitrogen source and saprophytic fungi on Fusarium wilt of Mexican lime. *Plant and Soil, 79*(2), 203–210. https://doi.org/10.1007/BF02182342

Naeini, A., Ziglari, T., Shokri, H., & Khosravi, A. R. (2010). Assessment of growth-inhibiting effect of some plant essential oils on different *Fusarium* isolates. *Journal de Mycologie Médicale*, *20*(3), 174–178. https://doi.org/10.1016/j.mycmed.2010.05.005

Navarro, L., Camacho, R., López, J. E., & Saldarriaga, J. F. (2021). Assessment of the potential risk of leaching pesticides in agricultural soils: study case Tibasosa, Boyacá, Colombia. *Heliyon*, *7*(11), Article e08301. https://doi.org/10.1016/j.heliyon.2021.e08301

Navas-Cortés, J. A., Landa, B. B., Méndez-Rodríguez, M. A., & Jiménez-Díaz, R. M. (2007). Quantitative modeling of the effects of temperature and inoculum density of *Fusarium oxysporum* f. sp. *ciceris* races 0 and 5 on development of Fusarium wilt in chickpea cultivars. *Phytopathology, 97*(5), 564–573. https://doi.org/10.1094/PHYTO-97-5-0564

ONU. (2019, June 17). *La población mundial sigue en aumento, aunque sea cada vez más vieja*. https://news.un.org/es/story/2019/06/1457891#:~:text=Para%202050%2C%20una%20de%20cada,Am%C3%A9rica%20Latina%20y%20el%20Caribe

ONU. (2022, November 15). *Somos 8 mil millones de personas en el mundo*. https://onu-habitat.org/index.php/ya-somos-8-mil-millones-de-personas

Ponsankar, A., Senthil-Nathan, S., Vasantha-Srinivasan, P., Pandiyan, R., Karthi, S., Kalaivani, K., Chellappandian, M., Narayanaswamy, R., Thanigaivel, A., Patcharin, K., Mahboob, S., & Al-Ghanim, K. A. (2023). Systematic induced resistance in *Solanum lycopersicum* (L.) against vascular wilt pathogen (*Fusarium oxysporum* f. sp. *lycopersici*) by *Citrullus colocynthis* and *Trichoderma viride*. *PLoS ONE*, *18*(5), Article e0278616. https://doi.org/10.1371/journal.pone.0278616

Qu, L., Wang, M., & Biere, A. (2021). Interactive effects of mycorrhizae, soil phosphorus, and light on growth and induction and priming of defense in *Plantago lanceolata*. *Frontiers in Plant Science*, *12*, Article 647372. https://doi.org/10.3389/fpls.2021.647372

Rashid, M. I., Mujawar, L. H., Shahzad, T., Almeelbi, T., Ismail, I. M. I., & Oves, M. (2016). Bacteria and fungi can contribute to nutrients bioavailability and aggregate formation in degraded soils. *Microbiological Research*, *183*, 26–41. https://doi.org/10.1016/j.micres.2015.11.007

Rivera Méndez, Y. D., Galindo Castañeda, T., Caicedo, A. F., Navia, E. A., & Romero, H. M. (2014). *Hongos formadores de micorrizas arbusculares (HFMA) en palma de aceite.* Boletín *Técnico No. 35*. Centro de Investigación en Palma de Aceite - Cenipalma. https://publicaciones.fedepalma.org/index.php/boletines/article/view/10967/10954

Rojas Rodríguez, K., & Ortuño, N. (2007). Evaluación de micorrizas arbusculares en interacción con abonos orgánicos como coadyuvantes del crecimiento en la producción hortícola del Valle Alto de Cochabamba, Bolivia. *Acta Nova*, *3*(4), 697–719. http://www.scielo.org.bo/scielo.php?script=sci_arttext&pid=S1683-07892007000200005

Rozpądek, P., Rąpała-Kozik, M., Wężowicz, K., Grandin, A., Karlsson, S., Ważny, R., Anielska, T., & Turnau, K. (2016). Arbuscular mycorrhiza improves yield and nutritional properties of onion (*Allium cepa*). *Plant Physiology and Biochemistry*, *107*, 264–272. https://doi.org/10.1016/j.plaphy.2016.06.006

Saha, H., Kaloterakis, N., Harvey, J. A., Van der Putten, W. H., & Biere, A. (2022). Effects of light quality on colonization of tomato roots by AMF and implications for growth and defense. *Plants*, *11*(7), Article 861. https://doi.org/10.3390/plants11070861

Schütz, L., Saharan, K., Mäder, P., Boller, T., & Mathimaran, N. (2022). Rate of hyphal spread of arbuscular mycorrhizal fungi from pigeon pea to finger millet and their contribution to plant growth and nutrient uptake in experimental microcosms. *Applied Soil Ecology*, *169*, Article 104156. https://doi.org/10.1016/j.apsoil.2021.104156

Sieverding, E. (1984). *Manual de métodos para la investigación de la micorriza vesículo-arbuscular en el laboratorio* (1st ed.). Centro Internacional de Agricultura Tropical. http://ciat-library.ciat.cgiar.org/ciat_digital/CIAT/books/historical/198.pdf

Smith, F. A., Grace, E. J., & Smith, S. E. (2009). More than a carbon economy: nutrient trade and ecological sustainability in facultative arbuscular mycorrhizal symbioses. *New Phytologist, 182*(2), 347–358. https://doi.org/10.1111/j.1469-8137.2008.02753.x

Smith, S. E., & Read, D. J. (2008). *Mycorrhizal symbiosis* (3rd ed.). NY: Academic Press. https://www.sciencedirect.com/book/9780123705266/mycorrhizal-symbiosis

Stratton, C. A., Ray, S., Bradley, B. A., Kaye, J. P., Ali, J. G., & Murrell, E. G. (2022). Nutrition vs association: plant defenses are altered by arbuscular mycorrhizal fungi association not by nutritional provisioning alone. *BMC Plant Biology*, 22(1), Article 400. https://doi.org/10.1186/s12870-022-03795-3

Stürmer, S. L. (2025, July 25). Coleção internacional de cultura de Glomeromycota (CICG-FMA). https://sites.google.com/site/cicgfma/home

Tanwar, A., Aggarwal, A., & Panwar, V. (2013). Arbuscular mycorrhizal fungi and *Trichoderma viride* mediated *Fusarium* wilt control in tomato. *Biocontrol Science and Technology*, *23*(5), 485–498. https://doi.org/10.1080/09583157.2013.772561

Vergel, M., Martínez, J. J., & Zafra, S. L. (2016). Cultivo de cebolla (*Allium cepa* L.) en la provincia de Ocaña: factores asociados a la productividad y el rendimiento. *Revista Colombiana de Ciencias Hortícolas*, *10*(2), 333–344. https://doi.org/10.17584/rcch.2016v10i2.5070

Whipps, J. M. (2004). Prospects and limitations for mycorrhizas in biocontrol of root pathogens. *Canadian Journal of Botany*, *82*(8), 1198–1227. https://doi.org/10.1139/b04-082

Wilches Ortiz, W. A., Ramírez Gómez, M. M., Pérez Moncada, U. A., Serralde Ordoñez, D. P., Peñaranda Rolon, A. M., & Ramírez, L. (2019). Asociación de hongos micorrízicos arbusculares (HMA) con plantas de caña de azúcar (*Saccharum officinarum*) para la producción de panela en Colombia. *Terra Latinoamericana*, *37*(2), 175–184. https://doi.org/10.28940/terra.v37i2.481

Winona, B., Sawitri, W. D., Idris, M., Handayani, V. D. S., Wibowo, A., Matra, D. D., Doni, F., & Widiastuti, A. (2025). Dynamics of defense-related genes expression in shallot after UV-B-induced resistance against *Fusarium acutatum* showed time difference. *Physiological and Molecular Plant Pathology*, *136*, Article 102559. https://doi.org/10.1016/j.pmpp.2024.102559

Wright, G. F. K., Say, M., Pascoe, I. G., & Guest, D. I. (1997). Incidence and symptoms of *Fusarium* diseases of carnations in Victoria. *Australasian Plant Pathology*, *26*(1), 44–53. https://doi.org/10.1071/AP97007

Yağmur, A., Demir, S., Canpolat, S., Rezaee Danesh, Y., Farda, B., Djebaili, R., Pace, L., & Pellegrini, M. (2024). Onion *Fusarium* basal rot disease control by arbuscular mycorrhizal fungi and *Trichoderma harzianum*. *Plants, 13*(3), Article 386. https://doi.org/10.3390/plants13030386

Yin, Y., Miao, J., Shao, W., Liu, X., Zhao, Y., & Ma, Z. (2023). Fungicide resistance: progress in understanding mechanism, monitoring, and management. *Phytopathology*, *113*(4), 707–718. https://doi.org/10.1094/PHYTO-10-22-0370-KD

Zhang, W., Yu, L., Han, B., Liu, K., & Shao, X. (2022). Mycorrhizal inoculation enhances nutrient absorption and induces insect-resistant defense of *Elymus nutans*. *Frontiers in Plant Science*, *13*, Article 898969. https://doi.org/10.3389/fpls.2022.898969

Zheng, C., Ji, B., Zhang, J., Zhang, F., & Bever, J. D. (2015). Shading decreases plant carbon preferential allocation towards the most beneficial mycorrhizal mutualist. *New Phytologist, 205*(1), 361–368. https://doi.org/10.1111/nph.13025

Zhou, X. G., & Everts, K. L. (2003). Races and inoculum density of *Fusarium oxysporum* f. sp. *niveum* in commercial watermelon fields in Maryland and Delaware. *Plant Disease, 87*(6), 692–698. https://doi.org/10.1094/PDIS.2003.87.6.692

Effect of hydroethanolic extracts of *Eucalyptus globulus* and *Allium fistulosum* on strains of *Botrytis cinerea* in inoculated strawberries

Efecto de los extractos hidroetanólicos de *Eucalyptus globulus* and *Allium fistulosum* sobre cepas de *Botrytis cinerea* en fresas inoculadas

Magali Cañarejo-Antamba[1]*, Alexis Chandi Morales[2], and Julia K. Prado[1]

ABSTRACT

The strawberry (*Fragaria x ananassa*) faces sustainable production challenges due to its high susceptibility to pathogens like *Botrytis cinerea*. This research aimed to evaluate the inhibitory potential of eucalypt (*Eucalyptus globulus*) and Welsh onion (*Allium fistulosum*) extracts under *in vitro* and *in vivo* conditions in controlling *B. cinerea*. The *in vitro* mycelial inhibition of the extracts was evaluated by the diameter and the mycelial growth rate of the fungus for 5 d, with the application of low (5.6 $\mu l\ ml^{-1}$), medium (11.1 $\mu l\ ml^{-1}$), and high (16.7 $\mu l\ ml^{-1}$) concentrations of extracts. The antifungal activity of the extracts on the fruits was determined by quantifying the incidence and rate of decay in inoculated strawberries after 5 d of storage. The *in vitro* evaluation showed that the high concentration of the *A. fistulosum* extract was more efficient in controlling the mycelial growth of *B. cinerea*, showing averages of 31.66% inhibition, 68.33% mycelial growth rate, and 11.92 mm d^{-1} mycelial growth velocity, different ($P \leq 0.05$) in comparison to the other concentrations. The *in vivo* tests showed that the *E. globulus* extract had the lowest incidence on the fruits, with 81.11%, reduced fruit decomposition by 19% compared to the positive control, and evidenced the highest number of fruits without visible changes during the five evaluation days. The analysis of the extracts demonstrated a significant effect on the control of *B. cinerea*, as well as on the shelf life of the fruits.

Keywords: postharvest, vegetable extracts, inhibitory potential, maceration.

RESUMEN

La fresa (*Fragaria x ananassa*) enfrenta desafíos de producción sostenible, debido a su alta susceptibilidad a patógenos como *Botrytis cinerea*. El objetivo de esta investigación fue evaluar el potencial inhibitorio de los extractos de eucalipto (*Eucalyptus globulus*) y de cebolla de rama (*Allium fistulosum*) en condiciones *in vitro* e *in vivo* para el control de *B. cinerea*. La inhibición micelial *in vitro* de los extractos se evaluó mediante el diámetro y la velocidad de crecimiento micelial del hongo durante 5 d y con aplicación de concentraciones: baja (5,6 $\mu l\ ml^{-1}$), media (11,1 $\mu l\ ml^{-1}$) y alta (16,7 $\mu l\ ml^{-1}$) de extractos. La actividad antifúngica de los extractos en frutos se determinó cuantificando la incidencia y el índice de decaimiento en fresas inoculadas después de 5 d de almacenamiento. La evaluación *in vitro* evidenció que la concentración alta del extracto de *A. fistulosum* fue más eficiente en el control del crecimiento micelial de *B. cinerea,* con promedios de 31,66% de inhibición, de 68,33% de tasa de crecimiento del micelio y 11,92 mm d^{-1} de velocidad de crecimiento del micelio, y fueron diferentes ($P \leq 0.05$) con respecto a las demás concentraciones. Las pruebas *in vivo* demostraron que el extracto de *E. globulus* registró la menor incidencia en frutos con 81,11%, redujo la descomposición de los frutos en un 19% con respecto al control positivo, y evidenció el mayor número de frutos sin cambios visibles durante los cinco días de evaluación. Los extractos analizados demostraron tener un efecto significativo en el control de *B. cinerea*, así como en la vida útil de las frutas.

Palabras clave: poscosecha, extractos vegetales, potencial inhibidor, maceración.

Introduction

The strawberry (*Fragaria x ananassa*) is a highly consumed fruit in Ecuador. It is grown in open fields throughout the year due to the favorable climate conditions in the Andean highlands. According to the Food and Agriculture Organization of the United Nations (FAO) database, until 2022, Ecuador registered a harvested area of 102 ha and an average production of 1,448 t yr^{-1}. In recent years, strawberry cultivation has expanded significantly in Tungurahua, Cotopaxi, Pichincha, and Imbabura at altitudes between 2,500 and 2,750 m a.s.l. (Kirschbaum *et al*., 2017).

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However, this crop is susceptible to diseases caused by *Podosphaera xanthii*, *Botrytis cinerea*, *Colletotrichum* spp., *Phytophthora* spp. *Fusarium oxysporum* and *Verticillium dahliae* (Hernández-Martínez *et al*., 2023). In particular, gray mold caused by *Botrytis cinerea* is a destructive disease that grows rapidly in the organs of the strawberry plant and decomposes infected plant tissues (Petrasch *et al*., 2019). Therefore, finding ecological and economic alternatives to combat these diseases is important for small-scale producers. According to Vikas and Ranjan (2024), using natural production practices in disease control promotes environmental conservation, agricultural productivity, and food security. Among these alternatives are vegetable extracts used in agriculture due to their biological properties as biostimulants, insecticides, fungicides, and efficient pest control. Additionally, there is no evidence that these extracts generate resistance in pests, but they can be an economic and ecological tool.

Previous studies have shown morphological changes in fungal structures after treatments with compound substances, such as essential oils, plant extracts, and saline solutions, among others, due to secondary metabolites that induce physiological and morphological responses in the fungi (Badmi *et al*., 2023). These metabolites can alter the growth and structure of hyphae and the production of spores and other reproductive organs. For example, Dėnė and Valiuškaitė (2021) studied the inhibitory effect of plant extracts from cinnamon, bay leaves, and cloves on the fungus *B. cinerea*, which is the leading cause of low crop yield and fruit quality. Their results indicated that the fungus *B. cinerea* showed sensitivity to cinnamon extracts at concentrations of 800, 600, and 500 µl L^{-1}, as mycelium growth was reduced, and the laurel and clove extracts caused changes in the type of mycelium and the color of the fungal colony. Likewise, it has been shown that essential oils from several plants are potent antimicrobial and antifungal agents at high concentrations (Taghavi *et al*., 2018), and mixtures of these oils can completely inhibit pathogens such as *B. cinerea* (Šernaitė *et al*., 2020). The study reported by Oliveira Filho *et al*. (2021) revealed that the essential oils of *Mentha piperita*, *Mentha spicata, Cymbopogon martinii,* and *Cinnamomum camphora* could inhibit the mycelial growth of the fungus *B. cinerea* when applied in concentrations between 500 and 750 µl L^{-1} to wild strawberries. Also, Campa-Siqueiros *et al*. (2017) demonstrated that the hydroethanolic extract of garlic controls the development of decay in table grapes stored at 4°C and 25°C. This research aimed to evaluate the antifungal potential of plant extracts of *Allium fistulosum* L. and *Eucalyptus globulus* Labill. on strains of *B. cinerea* isolated from wild strawberries (*F. ananassa* var. Monterrey) collected from small-scale farmer crops in Otavalo, Ecuador.

Materials and methods

The study was carried out at the Physical, Chemical, and Microbiological Analysis Laboratory of the Faculty of Agricultural and Environmental Engineering of the Universidad Técnica del Norte located in Ibarra, a city in the

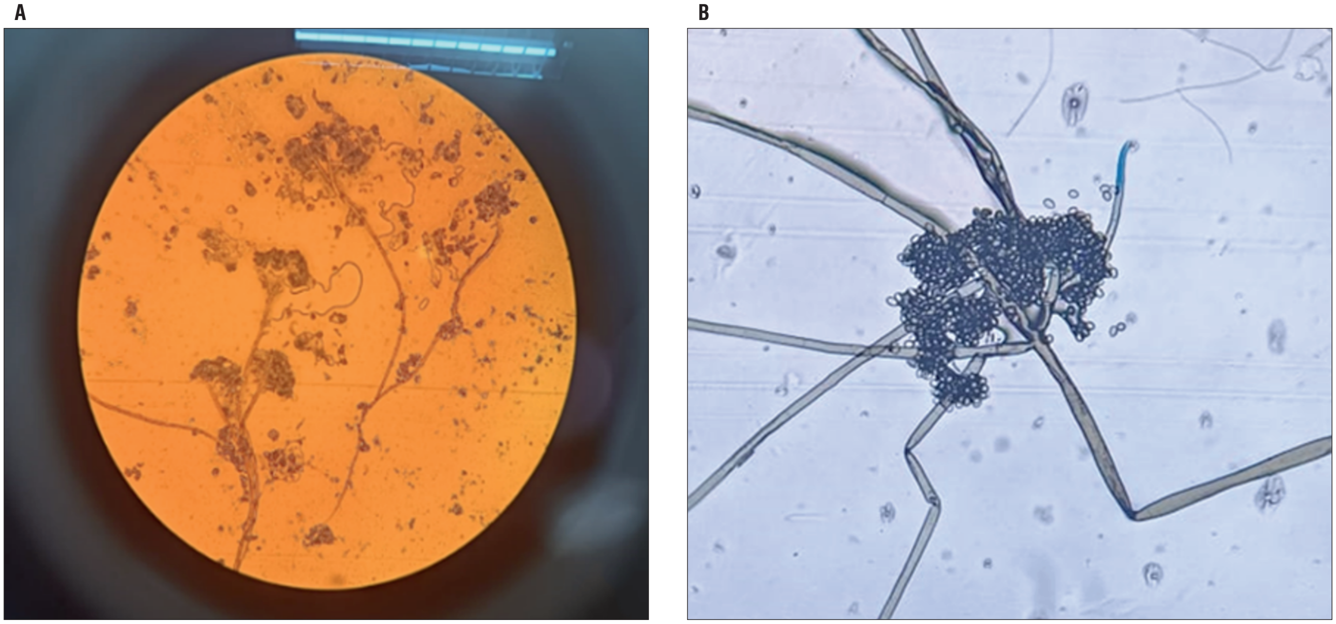

FIGURE 1. A) Preliminary identification of *Botrytis cinerea* colonies (10×), B) conidial structures of the fungus *B. cinerea* from purified strains (40×).

province of Imbabura, at an altitude of 2,250 m a.s.l., with a temperature of 20°C and a relative air humidity of 63%.

Plant material

The strawberry fruits were collected from small-scale farmers' land plots in the city of Otavalo (0°13'43" N, 78°15'49" W) at an average temperature of 16° C and an average annual rainfall of 500 mm. A sample of 30 kg of fruit at maturity stage 4 (75% red-colored and 25% green-colored) was collected. To obtain the fungal strain of *B. cinerea*, strawberries with gray mold symptoms were collected; the presence of the fungus was verified using an optical microscope at 10× magnification (Fig. 1A). During the observations, numerous hyphae, conidiophores, and conidia were identified, with ovoid-ellipsoid shapes resembling a bunch of grapes.

Obtainment of plant extracts

The process of obtaining plant extracts from eucalyptus (*Eucalyptus globulus*) leaves and Welsh onion (*Allium fistulosum*) residues is illustrated in Figure 2. The plant material (1 kg) was washed, disinfected, and placed in a dehydrator (Model WRH-100T) at 60°C for 5 h until reaching a 10% moisture content. Subsequently, the dried samples were crushed, ground (Mesh No. 20), and macerated in 1 L of ethanol and water (80:20, v/v) for 48 h. The resulting mixtures were filtered, and the residues were subjected to a second extraction. The filtrates obtained were combined, and the majority of the solvent was removed by rotary evaporation.

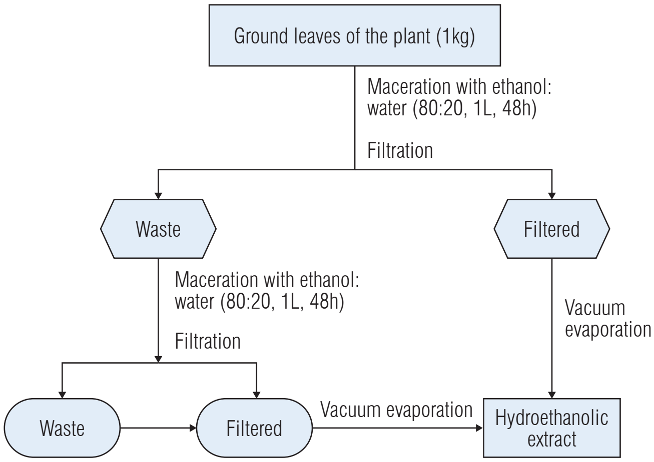

FIGURE 2. Process for obtaining hydroethanolic extracts from plant samples.

Isolation and purification of the *Botrytis* fungus

The isolation and purification of *B. cinerea* were carried out using the method described by Plascencia-Tenorio *et al.* (2012). Strawberries with gray mold were harvested from Otavalo. Subsequently, 1 cm^3 samples of diseased tissue with mycelium were taken and placed on a PDA medium, which was incubated at 18 ± 1°C for 48 h. At the end of the incubation, imprints of the fungal mycelium were taken with adhesive tape on a slide, which was stained with Lactophenol blue, and the conidial structures were observed under an optical microscope at 40× (Richter Optica HS-3T at 40×, Spain). The presence of the *B. cinerea* fungus was confirmed (Fig. 1B). Finally, a small sample of mycelium was taken and placed in Petri dishes with PDA, which were incubated again at 18°C ± 1 for 4 d. This procedure was repeated until the fungus was purified.

In vitro assay: antifungal activity of extracts on *B. cinerea* strains in Petri dishes

The extract concentrations were determined according to the method described by Larios-Palacios *et al.* (2020) with modifications. The plant extract and PDA were mixed with fungal inoculum at the following concentrations: low (5.55 $\mu l\ ml^{-1}$ of PDA), medium (11 $\mu l\ ml^{-1}$ of PDA), and high (16.66 $\mu l\ ml^{-1}$ of PDA); the mixtures were then incubated at 18 ±1°C for 5 d in the dark. Ultimately, the mycelium growth, growth rate, and inhibition were measured.

In this assay, the radial growth of the mycelium was measured every 24 h with a digital vernier for 5 d, during which the control plate was covered entirely with mycelium. The percentage of inhibition (*I*) was calculated using Equation 1 proposed by Larios-Palacios *et al.* (2020), where *D1* = diameter of the mycelial growth of the control (mm) and *D2* = diameter of the mycelial growth of the treatment (mm).

$$I\ (\%) = \frac{(D1 - D2)}{D1} * 100 \tag{1}$$

The mycelial growth rate was calculated by applying Equation 2 of Larios-Palacios *et al.* (2020), where
MG (%) = mycelial growth expressed as a percentage, *TG* = treatment growth (mm), and *CG* = control growth (mm).

$$MG\ (\%) = \frac{TG\ (100)}{CG} \tag{2}$$

The growth velocity of the mycelium (*GV*) was calculated using Equation 3, where *GV* = growth velocity (mm d^{-1}), *Df* = final growth diameter (mm), *Di* = initial growth diameter (mm), *Ti* = initial growth time (days), and *Tf* = final growth time (d).

$$GV = \frac{Df - Di}{Tf - Ti} \tag{3}$$

In vivo assay: antifungal activity of extracts on inoculated strawberries

Before inoculating the strawberries with the fungal strains, the fruits were selected to be free of mechanical damage and to at the maturity stage of 4. They were disinfected with 2% hypochlorite for 2 min and washed three times with distilled water (Hajji-Hedfi *et al*., 2024). To apply the inoculum to the fruits, a sample of purified fungal strains was collected and suspended in 10 ml of distilled water, shaken for 3 min, and filtered through sterile gauze. Subsequently, a 50 μl aliquot was taken, and the spore concentration was determined in a Neubauer chamber and adjusted to 1 ml. Spore suspensions containing 3.3×10^6 spores ml^{-1} and the plant extract at a concentration of 1.7% v/v were applied to each fruit. Finally, the fruits were stored at room temperature (22°C) for 5 d with a 12 h photoperiod.

In this second stage, the high concentrations of each plant extract from the first phase and two control treatments were evaluated: a negative control (fruits without inoculum and without extract application) and a positive control (fruits with plant extract). The disease incidence was calculated using Equation 4 proposed by Pazmiño-Miranda *et al*. (2017), where the number of infected fruits and the total number of fruits were expressed as a percentage.

$$Incidence\ (\%) = \frac{\#\ infected\ fruits}{\#\ total\ fruits} \times 100 \tag{4}$$

The percentage of affected areas in fruits was evaluated using the methodology proposed by Oliveira Filho *et al*. (2021), with modifications. This method is based on a scoring scale composed of five categories, from least to most significant damage, where category 0 indicates no visible changes, and category 5 indicates strong mycelial growth. Finally, the fruit decay was calculated using Equation 5, where N = total number of fruits measured per experimental unit and $N_{0,\ 1,\ 2,\ 3,\ 4,\ 5}$ = number of fruits for each category of the severity scale.

$$Decay\ (\%) = \frac{0xN_0 + 1xN_1 + 2xN_2 + 3xN_3 + 4xN_4 + 5xN_5}{5xN} \times 100 \tag{5}$$

Statistical analysis

The results are presented as mean values (±) standard deviation of three replicates per sample and were analyzed by analysis of variance (ANOVA) using the SAS System (version 9). The LSD test was used to compare the mean values, and differences were considered significant with $P \leq 0.05$.

Results and discussion

The biological potential of hydroethanolic extracts of eucalyptus (*Eucalyptus globulus* Labill.) and Welsh onion (*Allium fistulosum* L.) leaves was demonstrated by the percentage of inhibition of the extracts against *Botrytis* fungus strains isolated from the strawberries. Table 1 shows that the percentage of fungal inhibition was not statistically different (P>0.05) among the different concentrations of the eucalyptus extract. However, this extract inhibited the growth of the fungus between 24.33% and 30.44%. The high concentration (16.7 μl ml^{-1} of PDA) recorded a numerically lower growth rate (69.55%) than the other concentrations, as well as a reduced growth velocity (12-13 mm d^{-1}). On the other hand, the concentration of 16.7 μl ml^{-1} of the Welsh onion extract exhibited a different response, inhibiting the growth of the fungus by 31.66% and showing a statistically significant difference ($P \leq 0.05$) from the other concentrations. Additionally, this concentration recorded the same behavior in terms of growth rate and daily growth velocity, with average values of 68.33% and 11.92 mm d^{-1}, respectively, both lower than those observed at lower concentrations.

Fungal activity is evidenced by the presence or absence of mycelial proliferation. In Figure 3, the mycelial behavior of the *Botrytis* fungus under different concentrations of the two extracts on the 5th d of evaluation can be observed. The higher concentrations of the eucalyptus and Welsh onion extracts recorded the lowest mycelial growth of the fungus, with average values of 6.9 and 6.8 mm d^{-1}, respectively, approximately 30% less than the control treatment (sterilized PDA), which recorded an average value of 10 mm d^{-1}.

Eucalyptus species concentrate various bioactive compounds in their leaves, which have been associated with antifungal, medicinal, and aromatic properties, among others (Vuong *et al*., 2015). For example, the study by Yao *et al*. (2021) shows that 95% ethanolic extract of red eucalyptus (*Eucalyptus tereticornis*) can inhibit *B. cinerea* by more than 50% *in vitro*. Hajji-Hedfi *et al*. (2024) reported that *E. globulus* extract can inhibit the mycelial growth of the pathogens *Colletotrichum gloeosporioides* and *Alternaria alternata* in apples by 67–80%. The results of this study are lower than those reported by these authors and can be explained by the type of pathogen evaluated, since *B. cinerea*, due to its multiple infection strategies, is an aggressive and difficult to control fungus (Petrasch *et al*., 2019). Furthermore, this phytopathogenic fungus can catalyze the oxidation of metabolites present in the

TABLE 1. Mycelial growth inhibition (%), growth rate, and growth velocity of *Botrytis cinerea* by plant extracts at different concentrations.

Plant species	Extract concentration (μl ml^{-1})	Mycelial growth inhibition (%)	Growth rate (%)	Growth velocity (mm d^{-1})
Eucaliptus globulus	16.7	30.44 ± 4.66 a	69.55 ± 4.66 a	12.13 ± 0.91 a
	11.1	25.86 ± 1.88 a	77.61 ± 3.33 b	13.92 ± 0.50 b
	5.6	24.23 ± 6.85 a	78.79 ± 4.30 b	13.72 ± 0.47 b
	P-value	0.1755	0.102	0.0191
	LSD	8.41	8.04	0.93
Allium fistulosum	16.7	31.66 ± 5.19 a	68.33 ± 5.19 a	11.92 ± 0.88 a
	11.1	25.14 ± 3.90 b	75.84 ± 3.62 b	13.23 ± 0.69 b
	5.6	12.77 ± 4.55 c	86.13 ± 3.05 c	15.04 ± 0.69 c
	P-value	0.0003	0.0002	0.0004
	LSD	3.0	2.52	0.53

Means ± standard deviation followed by different letters are statistically different (LSD test, n = 3, $P \leq 0.05$). LSD = least significant difference.

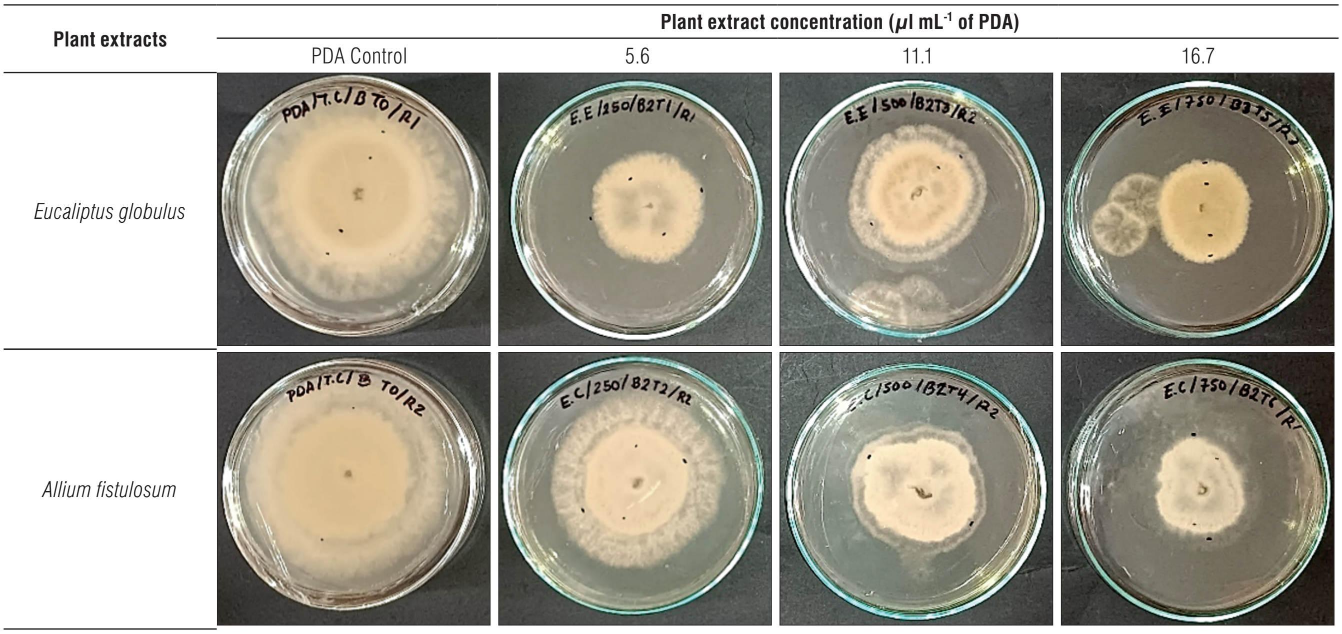

FIGURE 3. Effects of *E. globulus* and *A. fistulosum* extracts at different concentrations on mycelial growth of *B. cinerea* after 5 d.

extract by producing enzymes and, in turn, may develop resistance during the incubation period (Vio-Michaelis *et al*., 2012). Likewise, the efficiency of the plant extract as a natural control against a pathogen will also depend on the concentration of secondary metabolites present in the plant, as well as the environmental conditions and the incidence of the pathogen (Jiménez-Reyes *et al*., 2019).

Furthermore, essential oils of the *Allium* genus efficiently inhibit the germination of *B. cinerea* spores, the causal agent of gray mold (Taghavi *et al*., 2018). Similarly, Campa-Siqueiros *et al*. (2017) demonstrated that the hydroethanolic extract of garlic (*Allium sativum*) maintained total control of the disease in table grapes stored at 4°C for 14 d. In another study, Rouis-Soussi *et al*. (2014) evaluated the effect of different plant extracts and *Allium roseum* essential oil on inhibiting the mycelial growth of *B. cinerea*; the extract obtained with butyl alcohol recorded 46% inhibition, while the essential oil recorded 52% inhibition. The results obtained with the Welsh onion extract differ from those reported by the aforementioned authors, who observed between 46% and 100% control, while in this study, inhibition was 31.66%. This difference may be explained by the concentration of sulfur compounds present in garlic and onion, as well as the extraction method, temperature conditions, and fungal strain. Because it was isolated from strawberries obtained from conventional production, the strain could have developed resistance.

In the second stage of the research, the highest concentration (1.7%, v/v) of Welsh onion and eucalyptus extracts was evaluated in strawberries inoculated with the *Botrytis* isolate. Figure 4 shows the percentage of incidence on the strawberries after the eucalyptus and Welsh onion extracts were applied during the 5 d of evaluation. The eucalyptus extract presented greater disease control with an average value of 81.11% incidence on the strawberries, significantly lower ($P \leq 0.05$) compared to the other treatments. On the other hand, the Welsh onion extract showed significant differences compared to the negative control and reported average values of 90% and 94% incidence on the inoculated fruits, respectively.

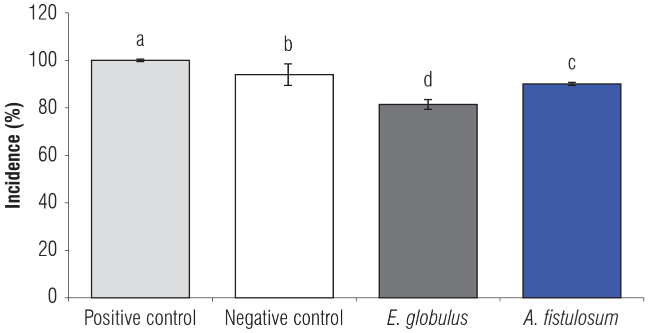

FIGURE 4. Effect of hydroethanolic extracts of *E. globulus* and *A. fistulosum* on *Botrytis* grey mold in inoculated strawberries. LSD test ($P \leq 0.05$), different letters between columns indicate statistically significant differences. The error bars represent the standard error (n=3).

In another study, the antifungal effect of *Cinnamomum zeylanicum* extract was higher than that of *Equisetum arvense* extract; the two extracts presented an average of 24% and 55% incidence of *B. cinerea*, respectively, on strawberries (Pazmiño-Miranda *et al*., 2017). In this study, eucalyptus and onion extracts inhibited the mycelial growth of the fungus *B cinerea* in strawberries. However, the average values are lower than those reported by the authors. This may be due to the variations in the content of secondary metabolites between species used, the polarity of the extraction solvents, and the experimental conditions.

The decay rate is essential to determine the shelf life of fresh products as it measures the speed at which the organoleptic and nutritional characteristics deteriorate. The results of the variance analysis indicate that there is an interaction between the treatments and the evaluation days (P=0.0416). Figure 5 shows the results of the decay rate of strawberries inoculated with the fungus *Botrytis cinerea* and treated with the two plant extracts. The most significant reduction in the rate of fruit decay caused by *Botrytis* was recorded with the application of eucalyptus extract, with an average value of 57.33% on day 5 of evaluation. This represented 19% fewer diseased fruits than the positive control (71.11% decay). On the other hand, the Welsh onion extract had no significant effect on *Botrytis* control in strawberry fruits compared to the negative control.

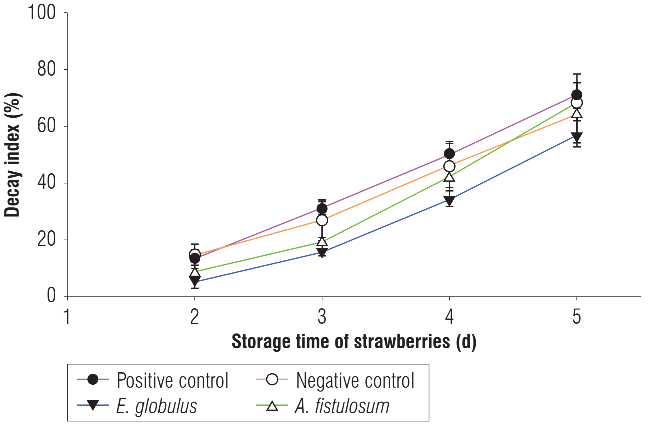

FIGURE 5. Decay rate of strawberries inoculated with *Botrytis* and treated with hydroethanolic extracts of *E. globulus* and *A. fistulosum* and stored at 22±1°C for 5 d. The error bars represent the standard error (n=3).

The findings of this study showed that *E. globulus* extract at a concentration of 1.7% had a significant effect on postharvest life and reduced decomposition of strawberry fruits. These results are directly related to the findings in Figures 6 and 7, which show the percentage of the area of the fruit affected by *Botrytis*, categorized using a score scale composed of five degrees. The *E. globulus* extract recorded the highest number of fruits without visible changes (scale 0) during the evaluation (19%), followed by the *A. fistulosum* extract with 10%. Meanwhile, the negative control (fruits without extracts) scored lowest on a scale 0 with an average of 9%.

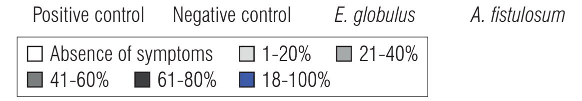

FIGURE 6. Percentage of affected areas in strawberries inoculated with *Botrytis* and treated with hydroethanolic extracts of *E. globulus* and *A. fistulosum* and stored at 22±1°C for 5 with a photoperiod of 12 h.

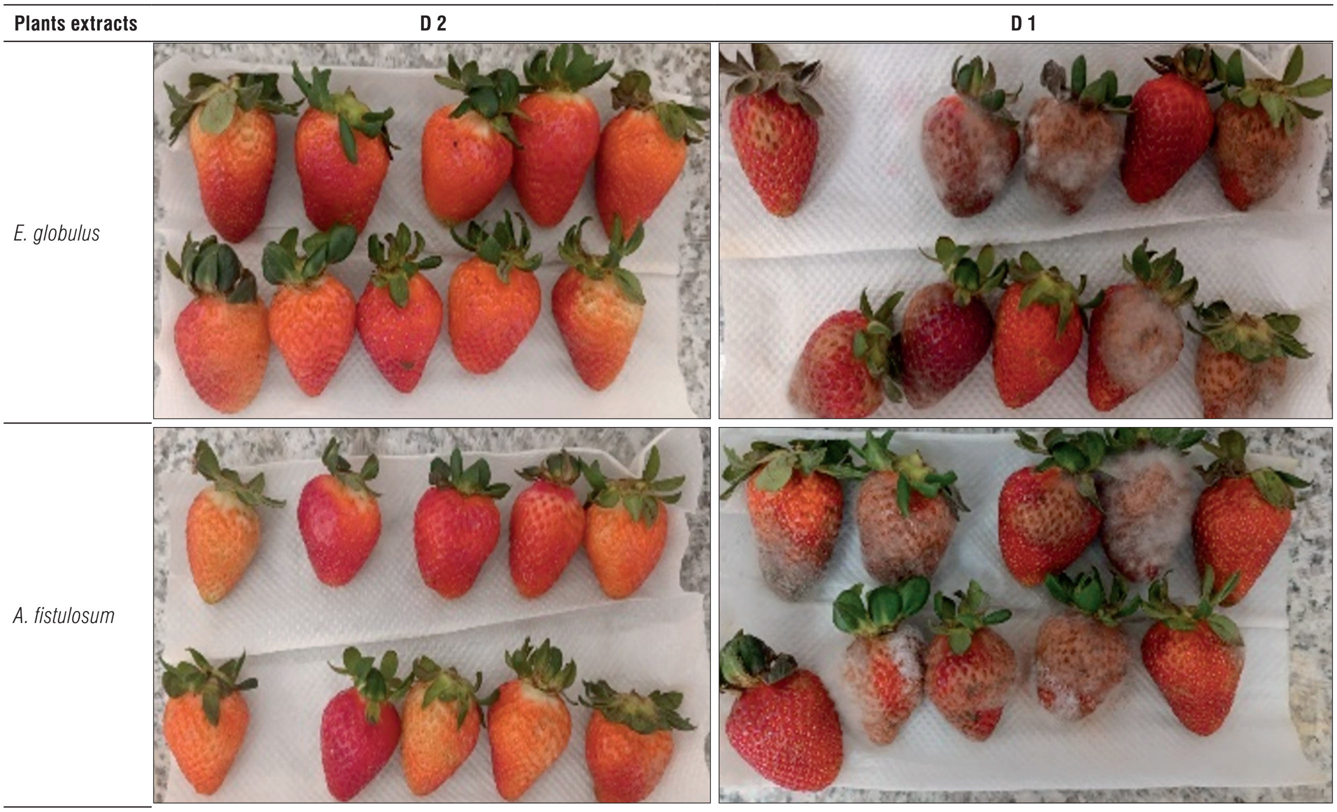

FIGURE 7. Strawberries inoculated with *Botrytis* treated with *E. globulus* and *A. fistulosum* extracts at a concentration of 1.7% and stored at 22±1°C for 5 d. D1 - control, D2 - treatment.

The scale of the affected area: 0%= no visible changes; 1-20% = slight brown discoloration; 21-40% = moderate discoloration; 41-60% = slight to moderate mycelial growth; 61-80% = moderate to intense mycelial growth; and 81-100% = characteristic sporulation and strong mycelial growth.

Various studies confirm the inhibitory potential of this plant species against phytopathogens. For example, Hajji-Hedfi *et al.* (2024) demonstrated that the essential oil of *E. globulus* efficiently controls *A. alternata* and *C. gloeosporioides* in apple fruits, since increasing the concentration (0.12, 0.25, 0.50, 1, 2, and 4 µl ml^{-1}) resulted in less damage to the fruits compared to the control. Similarly, Alemu *et al.* (2014) reported that eucalyptus extract at a concentration of 50% inhibits anthracnose development in naturally infected mango fruits. Extracts from the *Allium* genus have also been proven effective in controlling fungal diseases (Nxumalo *et al.*, 2021). In this study, the strawberry fruits with the application of eucalyptus extract at a concentration of 1.7% had the lowest percentage of decay during the 5 d of evaluation, with 19% of fruits remaining healthy during the post-harvest evaluation.

Furthermore, it is widely known that *Eucalyptus* has antifungal activity because it concentrates significant amounts of aromatic compounds. One of these is eucalyptol, a monoterpene linked to antimicrobial effects against different phytopathogens (Hajji-Hedfi *et al.*, 2024). In the same context, aqueous extracts of this species are an important source of phenolic compounds that accumulate in plant cellular structures and have been associated with fungal growth inhibitory effects (Vuong *et al.*, 2015). However, the effectiveness of plant extracts in fungus control varies depending on the plant species, the variety, the extraction method, and the experimental conditions, which could explain the variation in the results obtained in this research compared to other studies.

Conclusions

Hydroethanolic extracts of *E. globulus* and *A. fistulosum* inhibited *B. cinerea* mycelial growth in Petri dishes and strawberries. The *E. globulus* extract was more efficient in controlling gray mold under in vivo conditions, as it recorded the lowest percentage of incidence, reduced fruit decomposition by 19%, and presented the highest number of fruits without visible changes during the 5 d evaluation period. Therefore, these plant extracts could be used as ecological control alternatives, easily applied in small-scale strawberry crops.

Acknowledgments

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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

Project administration, research, and writing – original draft: MCA; methodology and research: ACM; formal analysis and writing – a review and editing: JKPB. All authors reviewed the manuscript and approved its final version.

Literature cited

Alemu, K., Ayalew, A., & Woldetsadik, K. (2014). Antifungal activity of plant extracts and their applicability in extending the shelf life on mango fruits. *Food Science and Quality Management, 33*, 48–54. https://www.iiste.org/Journals/index.php/FSQM/article/view/17612

Badmi, R., Gogoi, A., & Doyle Prestwich, B. (2023). Secondary metabolites and their role in strawberry defense. *Plants, 12*(18), Article 3240. https://doi.org/10.3390/plants12183240

Campa-Siqueiros, P., Vallejo-Cohen, S., Corrales-Maldonado, C., Martínez-Téllez, M. Á., Vargas-Arispuro, I., & Ávila-Quezada, G. (2017). Reducción en la incidencia de la pudrición gris en uva de mesa por el efecto de volátiles de un extracto de ajo. *Revista Mexicana de Fitopatología, 35*(3), 494–508. https://doi.org/10.18781/r.mex.fit.1707-1

Dėnė, L., & Valiuškaitė, A. (2021). Sensitivity of *Botrytis cinerea* isolates complex to plant extracts. *Molecules, 26*(15), Article 4595. https://doi.org/10.3390/molecules26154595

Hajji-Hedfi, L., Rhouma, A., Hlaoua, W., Dmitry, K. E., Jaouadi, R., Zaouali, Y., & Rebouh, N. Y. (2024). Phytochemical characterization of forest leaf extracts and application to control apple postharvest diseases. *Scientific Reports, 14*(1), Article 2014. https://doi.org/10.1038/s41598-024-52474-w

Hernández-Martínez, N. R., Blanchard, C., Wells, D., & Salazar-Gutiérrez, M. R. (2023). Current state and future perspectives of commercial strawberry production: A review. *Scientia Horticulturae, 312*, Article 111893. https://doi.org/10.1016/j.scienta.2023.111893

Jiménez-Reyes, M. F., Carrasco, H., Olea, A. F., & Silva-Moreno, E. (2019). Natural compounds: A sustainable alternative to the phytopathogens control. *Journal of the Chilean Chemical Society, 64*(2), 4459–4465. https://doi.org/10.4067/S0717-97072019000204459

Kirschbaum, D. S., Vicente, C. E., Cano-Torres, M. A., Gambardella, M., Veizaga-Pinto, F. K., & Antunes, L. E. C. (2017). Strawberry in South America: From the Caribbean to Patagonia. *Acta Horticulturae, 1156*, 947–956. https://doi.org/10.17660/ActaHortic.2017.1156.140

Larios-Palacios, O., López-Vázquez, É., Curiel Rodríguez, A., Ruiz-Espinoza, F., Solano-Vidal, R., & Serrato-Cruz, M. (2020). *In vitro* evaluation of methods against *Botrytis cinerea*. *Revista Mexicana de Ciencias Agrícolas, 11*(3), 593–606. https://doi.org/10.29312/remexca.v11i3.2077

Nxumalo, K. A., Aremu, A. O., & Fawole, O. A. (2021). Potentials of medicinal plant extracts as an alternative to synthetic chemicals in postharvest protection and preservation of horticultural crops: A review. *Sustainability, 13*, Article 5897. https://doi.org/10.3390/su13115897

Oliveira Filho, J. G., Silva, G. C., Aguiar, A. C., Cipriano, L., Azeredo, H. M. C., Bogusz Junior, S., & Ferreira, M. D. (2021). Chemical composition and antifungal activity of essential oils and their combinations against *Botrytis cinerea* in strawberries. *Journal of Food Measurement and Characterization, 15*(2), 1815–1825. https://doi.org/10.1007/s11694-020-00765-x

Pazmiño-Miranda, P., Velástegui-Espín, G. P., Curay, S., Yánez-Yánez, W., & Vásquez, C. (2017). Efecto de los extractos hidro-etanólicos de canela (*Cinnamomum zeylanicum* Blume) y cola de caballo (*Equisetum arvense* L.) sobre la incidencia y severidad de *Botrytis cinerea* en fresas. *Journal of the Selva Andina Biosphere, 5*(1), 29–38. http://www.scielo.org.bo/scielo.php?script=sci_arttext&pid=S2308-38592017000100004

Petrasch, S., Knapp, S. J., van Kan, J. A. L., & Blanco-Ulate, B. (2019). Grey mould of strawberry, a devastating disease caused by the ubiquitous necrotrophic fungal pathogen *Botrytis cinerea*. *Molecular Plant Pathology, 20*(6), 877–892. https://doi.org/10.1111/mpp.12794

Plascencia-Tenorio, R. I., Olalde-Portugal, V., Mena-Violante, H. G., Ceja-Torres, L. F., Venegas-González, J., Oyoque-Salcedo, G., & Angoa-Pérez, M. V. (2012). Antagonismo *in vitro* de aislados bacterianos de fresa comercial y silvestre vs. *Botrytis cinerea* y *Rhizopus stolonifer*. *Ra Ximhai, 8*(3), 103–110. https://doi.org/10.35197/rx.08.03.e1.2012.11.rp

Rouis-Soussi, L. A., Boughelleb-M'Hamdi, N., El Ayeb-Zakhama, A., Flamini, G., Ben Jannet, H., & Harzallah-Skhiri, F. (2014). Phytochemicals, antioxidant and antifungal activities of *Allium roseum* var. *grandiflorum* subvar. *typicum* Regel. *South African Journal of Botany, 91*, 63–70. https://doi.org/10.1016/j.sajb.2013.12.005

Šernaitė, L., Rasiukevičiūtė, N., Dambrauskienė, E., Viškelis, P., & Valiuškaitė, A. (2020). Biocontrol of strawberry pathogen *Botrytis cinerea* using plant extracts and essential oils. *Zemdirbyste-Agriculture, 107*(2), 147–152. https://doi.org/10.13080/z-a.2020.107.019

Taghavi, T., Kim, C., & Rahemi, A. (2018). Role of natural volatiles and essential oils in extending shelf life and controlling postharvest microorganisms of small fruits. *Microorganisms, 6*(4), Article 104. https://doi.org/10.3390/microorganisms6040104

Vikas., & Ranjan, R. (2024). Agroecological approaches to sustainable development. *Frontiers in Sustainable Food Systems, 8*, Article 1405409. https://doi.org/10.3389/fsufs.2024.1405409

Vio-Michaelis, S., Apablaza-Hidalgo, G., Gómez, M., Peña-Vera, R., & Montenegro, G. (2012). Antifungal activity of three Chilean plant extracts on *Botrytis cinerea*. *Botanical Science*, *90*(2), 179–183. https://www.scielo.org.mx/pdf/bs/v90n2/v90n2a6.pdf

Vuong, Q. V., Chalmers, A. C., Jyoti Bhuyan, D., Bowyer, M. C., & Scarlett, C. J. (2015). Botanical, phytochemical, and anticancer properties of the eucalyptus species. *Chemistry and Biodiversity, 12*(6), 907–924. https://doi.org/10.1002/cbdv.201400327

Yao, S., Xu, J., Zhang, H., Gao, H., Shang, S., & Wang, D. (2021). *In vitro* activity of extracts of five medicinal plant species on plant pathogenic fungi. *Frontiers of Agricultural Science and Engineering, 8*(4), 635–644. https://doi.org/10.15302/J-FASE-2020343

Biofertilizer enhances the expression of the *CaWRKY6* gene in water-stressed plants of *Capsicum annuum* L.

Biofertilizante mejora la expresión del gen *CaWRKY6* en plantas de *Capsicum annuum* L. sometidas a estrés hídrico

Santiago Amariles Barrera[1*], Diego Mauricio Martínez Rivillas[1], and Alejandra Vargas Ruiz[1]

ABSTRACT

Colombia has agroecological conditions for bell pepper (*Capsicum annuum* L.) cultivation. However, traditional management practices have depended mainly on agrochemicals. Organic production could enhance the value of products and facilitate their entry into international markets. Research indicates that biofertilizers and biostimulants can activate the gene expression that helps plants tolerate both biotic and abiotic stress. This study assessed the activation of the *CaWRKY6* gene in response to water stress tolerance in *C. annuum*, after applying a biofertilizer to greenhouse-grown young plants to mitigate the impacts of drought. Foliar samples were taken 48 h after the application of five treatments: biofertilizer at two concentrations (5 ml L^{-1} and 10 ml L^{-1}), salicylic acid (5 mg L^{-1}), and two controls (water), followed by RNA extraction and an RT-qPCR test to determine the relative expression of the gene *CaWRKY6*. To compare the adjuvant effect of biofertilizer and salicylic acid, treated young plants were exposed to water depletion for 21 d. The ANOVA indicated differences between treatments with 5 ml L^{-1} and 10 ml L^{-1} of biofertilizer, and they had the highest *CaWRKY6* gene expression, as well as higher growth and less wilting against water stress.

Keywords: organic agriculture, organic fertilizers, abiotic stress resistance, drought protection.

RESUMEN

Colombia presenta condiciones agroecológicas para la producción de pimentón (*Capsicum annuum* L.). Sin embargo, su manejo tradicional ha sido principalmente por medio del uso de agroquímicos. La producción orgánica podría dar valor agregado a los productos, y facilitar su entrada a los mercados internacionales. Se ha evidenciado que los biofertilizantes y bioestimulantes pueden activar la expresión de genes que ayudan a las plantas a tolerar estrés biótico y abiótico. En este estudio se evaluó la activación del gen *CaWRKY6*, uno de los más destacados para *C. annuum*, tras la aplicación de un biofertilizante sobre plántulas de pimentón cultivadas bajo invernadero. Se analizaron muestras foliares 48 h después de aplicar cinco tratamientos: biofertilizante a dos concentraciones (5 ml L^{-1} y 10 ml L^{-1}), ácido salicílico (5 mg L^{-1}) y dos controles (agua). Después se extrajo ARN y mediante una prueba RT-qPCR se determinó a la expresión relativa del gen *CaWRKY6*. Para comparar el efecto adyuvante del biofertilizante y el ácido salicílico, las plantas jóvenes tratadas se expusieron a la falta de agua durante 21 d. El ANOVA indicó diferencias entre los tratamientos con 5 ml L^{-1} y 10 ml L^{-1} de biofertilizante; además, estos tuvieron la mayor expresión del gen *CaWRKY6*, mayor crecimiento y menor marchitez por el estrés hídrico.

Palabras clave: agricultura orgánica, fertilizantes orgánicos, resistencia a estrés abiótico, protección contra sequía.

Introduction

Biotic and abiotic stresses are factors that can impact the productivity and quality of food crops worldwide, posing a threat to global food security (Abdou Zayan, 2020). Specifically, water deficit is the main abiotic factor in soils that limits plant growth and yields worldwide (Campos *et al*., 2014; Yaseen *et al*., 2024).

One of the leading causes of reduced soil water availability is climate change, which brings about increased temperatures and alterations in rainfall patterns. These changes can generate deficiencies in soil moisture, causing loss of biological functions in plants (Abdou Zayan, 2020). Consequently, the stress generated by these conditions may increase susceptibility in plants to disease and pest attack (Sinha *et al*., 2019).

Research conducted on pepper (*Capsicum annuum* L.) plants has shown that water and heat stress lead to decreased growth rate, decreased fruit yield and quality, and increased susceptibility to attack by pathogenic microorganisms (Jang *et al*., 2019; Lee *et al*., 2018; Ntanasi *et al*.,

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2025; Yaseen *et al*., 2024; Zhang Ma *et al*., 2024). This is why climatic conditions that promote water stress can actively contribute to the deterioration of crop phytosanitary conditions.

Colombia, a country with a strong agricultural tradition, has the potential to provide organic products with minimal reliance on chemical synthetic inputs. However, in bell pepper production, both fertilization and pest and disease management have historically depended on synthetic fertilizers and insecticides (Casilimas *et al*., 2012; Jaramillo Noreña *et al*., 2014). The use of these chemicals in agroecosystems poses a risk of toxicity for non-target organisms, leading to alterations in aquatic and terrestrial ecosystems (Campos-Soriano *et al*., 2020; Mandal *et al*., 2020) and adverse effects on human health (Elahi *et al*., 2019). For this reason, export markets are increasingly demanding standards aimed at reducing or eliminating the use of agrochemicals (Martínez Bernal *et al*., 2012). Countries such as the USA have a high demand for bell peppers, representing a significant export opportunity for emerging economies like Colombia. In international markets, value-added attributes related to the production system, particularly organic certification, play a key role in influencing sales prices and consumer preference (Agronegocios, 2015; MINCIT, 2019; Zhang *et al*., 2024). However, to access international organic markets, Colombian farmers must be certified in environmentally sustainable and agrochemical-free practices, and thus be able to give added value to their products (MADR, 2020; Martínez Bernal *et al*., 2012). To achieve this goal, farmers must fertilize their crops through the application of environmentally friendly methods that protect human health, among which are biofertilizers, effective microorganisms, and plant extracts (MADR, 2020).

The biofertilizers, besides containing nutrients of high bioavailability for plants (Goñi *et al*., 2018; Kour *et al*., 2020), include some biostimulant substances such as indole-acetic acid (Etesami *et al*., 2015), humic and fulvic acids, phosphites (Han *et al*., 2021), and free amino acids (Ahammed *et al*., 2020; Vijayakumar *et al*., 2019); these can activate genes and metabolic pathways that help plants to tolerate and/or to resist adverse environmental factors and pathogen attack that may alter their development (Malo *et al*., 2015; Orozco-Mosqueda *et al*., 2020; Xu *et al*., 2019; Yaseen *et al*., 2024). Accordingly, *CaWRKY* genes are one of the most representative gene families in *C. annuum* for protecting plants against biotic and abiotic stresses (Bulle *et al*., 2025; Hussain *et al*., 2019; Jingyuan *et al*., 2011; Zheng *et al*., 2019).

An extract of *Equisetum arvense* can act as a biostimulant in tomato (*Lycopersicum esculentum*) plants by activating action on *SlWRKY* genes (Malo *et al*., 2015). This species shares phylogenetic similarities with *C. annuum* (Park *et al*., 2011) and has comparable growing conditions and management practices (Roselló I Oltra & Porcuna, 2012). It can serve as a reference to evaluate the activation of *CaWRKY* genes in *C. annuum* and elucidate its relationship with protective activity against biotic and abiotic stresses.

It is necessary to find data that verify the effectiveness and supports the application of biofertilizers and/or biostimulant substances for the nutrition of *C. annuum* plants. Such applications will increase *CaWRKY6* gene expression, enhance drought tolerance, and improve growth under greenhouse conditions. This research aimed to evaluate the effect of the application of biofertilizers on young plants of *C. annuum* in order to investigate the activation of the *CaWRKY6* gene associated with water stress, as a strategy to face adverse environmental growing conditions.

Materials and methods

Plant material

Young plants of *Capsicum annuum* ‘California Wonder’ were obtained from a commercial supplier and established in the greenhouse facilities of CES University, Medellín, Colombia. The study was conducted in a greenhouse maintained under semi-controlled conditions. Relative air humidity ranged between 60% and 85%, with an average temperature of 24°C, reaching a maximum of 32°C and a minimum of 17°C. The greenhouse was equipped with a forced ventilation system consisting of an air injector and extractor to ensure adequate air exchange. The structure was covered with anti-thrips mesh to prevent pest entry. Plants were placed on welded wire mesh benches that allowed proper drainage and air circulation around the pots. The greenhouse had a concrete floor, which improved sanitization and weed control.

To ensure the quality of the potting soil substrate and verify that the aluminum content was within acceptable limits for planting, a physicochemical analysis of the substrate was performed. Additionally, a water retention curve was conducted to determine the field capacity of the substrate. Once the young plants reached an average height of 11 cm, they were subjected to the different treatments considered in this study.

Young plants of *Capsicum annuum* of approximately 30 d were used; at this stage they are more susceptible to changes in irrigation and water stress, because their root system is less developed than in adult plants (Bykova *et al*., 2019). This made it possible to detect changes in their growth more accurately when the young plants were subjected to different treatments.

The biofertilizer used for this research is a filtered extract derived from a fermentation process. The composition of the biofertilizer is as follows: oxidizable organic carbon 18.7 g L^{-1}, total nitrogen 1.10 g L^{-1}, phosphorus 1.16 g L^{-1}, potassium 5.75 g L^{-1}, calcium 3.14 g L^{-1}, magnesium 4.41 g L^{-1}, zinc 7.91 g L^{-1}, copper 0.32 g L^{-1}, iron 0.55 g L^{-1}, manganese 1.07 g L^{-1}, sulfur 8.70 g L^{-1}, boron 1.02 g L^{-1}. The potting soil used as substrate for planting had the following characteristics: pH 5.7, sandy loam texture, 17.24% organic matter, 7.63% organic carbon, and aluminum contents 1.2 cmol kg^{-1}.

Application of biofertilizer and salicylic acid

Thirty plants grown in the greenhouse were randomly selected and distributed using five treatments:

TABLE 1. Description of the five treatments applied to the plants.

Nomenclature	Treatment description
Bio 5	Liquid biofertilizer dissolved in water at a concentration of 5 ml L^{-1}
Bio 10	Liquid biofertilizer dissolved in water at a concentration of 10 ml L^{-1}
SA	Salicylic acid (SA) dissolved in water at a concentration of 5 mg L^{-1}, similar values have been proposed by other authors (Jingyuan *et al*., 2011)
CLI	Control - limited irrigation (CLI), a group of young plants to which water was applied and subsequently exposed to water depletion
CTI	Control - total irrigation (CTI), a group of young plants that had constant irrigation throughout the experiment to exclude the possibility that the environmental conditions of the greenhouse, the type of substrate in which they were planted, and the irrigation water did not have an undesirable effect on the plants

Only one application of the biofertilizer (5 ml L^{-1}, 10 ml L^{-1}) and salicylic acid was made at the beginning of the experiment. The application was sprayed on the leaves and added to the irrigation water, guaranteeing the same volume of prepared solution for all the plants.

RNA extraction and quantification

For the analysis of gene expression, the methodologies proposed by Han *et al*. (2021) and Verly *et al*. (2020) were considered; these suggest that the time required to show gene activation after the application of biostimulant substances and to proceed to take samples to extract RNA was between 48 and 72 h. Foliar samples were taken 48 h after the application of the treatments. RNA was extracted following the instructions of the Thermo Scientific™ GeneJET RNA Purification Kit. Subsequently, the RNA of each sample was quantified using the NanoDrop™ 2000 equipment.

Detection of the *CaWRKY6* gene expression

To determine the transcription of the *CaWRKY6* gene and its control, *CaActin*, the methodology proposed by Cai *et al*. (2015) was followed by performing RT-qPCR using the primers:

CaWRKY6 – Forward
5′GGTAGCTAGACAATTATGCTGC 3′

CaWRKY6 – Reverse
5′CAAAAAAAAATCTTATCAACTTG 3′

CaActin – Forward
5’AGGGATGGGTCAAAAGGATGC 3’

CaActin – Reverse
5’GAGACAACACCGCCTGAATAGC 3’

To detect *CaWRKY6* gene expression, the Verso 1-Step RT-PCR Kit ReddyMix from Thermo Fisher was used; the program suggested by Dang *et al*. (2013) was followed: 95°C for 30 s; 40 cycles of 95°C for 5 s; 60°C for 34 s; 95°C for 15 s; 60°C for 1 min; 95°C for 15 s; 60°C for 15 s; and 95°C for 15 s in an Eppendorf Mastercycler® ep realplex kit. Each reaction consisted of 1 µl of sample (100 ng μL^{-1}), 10 µl of enzyme, 1.6 µl of primers, and 7.4 µl of water, for a total volume of 20 µl.

For relative expression analysis, five replicates of each experiment were performed; the data were analyzed using the “Livak” $2^{-\Delta\Delta CT}$ method (Livak & Schmittgen, 2001). This quantifies the normalized relative expression level of the *CaWRKY6* gene of interest, comparing it to the actin reference gene *CaActin* that was previously reported in bell pepper (Cai *et al*., 2015; Dang *et al*., 2013).

Induction of water stress, plant height, and proportion of wilted leaves

Three days after the application of the Bio 5, Bio 10, SA, and CLI treatments the plants were subjected to water depletion, suppressing irrigation for 21 d (Ahammed *et al*. 2020; Goñi *et al*. 2018). The effect of water stress was evaluated by monitoring height growth and the proportion of wilted leaves during a 21-d water depletion period, with data collected on days 0, 3, 5, 8, 11, 14, 16, 18, and 21.

We assessed plant height using a tape measure; and the length in centimeters was determined by recording the distance from the plant base to the apical meristem of each plant. To quantify leaf wilting, we calculated the ratio of wilted leaves to the total number of leaves per seedling. Criteria for identifying wilted leaves included curled leaf margins and downward curvature below the horizontal plane (epinasty). We determined the presence or absence of wilting when one or both characteristics were observed.

Statistical analysis

For the analysis of relative *CaWRKY6* gene expression, plant height, and wilting, normality parameters were evaluated. An analysis of variance (ANOVA) was performed to identify differences between treatments, and Tukey's multiple range test was performed ($P<0.05$). The studies were conducted with R statistical software, R Studio 3.6.2 version.

Results and discussion

Relative expression of the *CaWRKY6* gene

Forty-eight hours after the application of the substances there were no significant differences in relative expression between biofertilizer treatments (P=0.797). Although the SA treatment had gene induction, it was significantly lower than the Bio 5 treatment (P=0.0001). Additionally, the expression of the SA treatment was different from that of the controls. There were no significant differences between the controls (P = 0.9986) (Fig. 1).

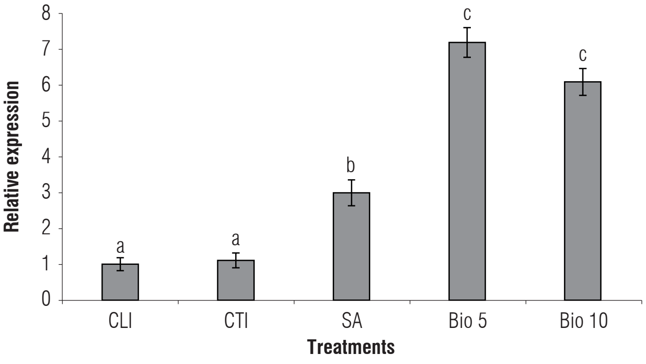

FIGURE 1. Relative expression of the *CaWRKY6* gene ("Livak" $2^{-\Delta\Delta CT}$) 48 h after application of the five treatments (Tab. 1). Different letters indicate significant differences between treatment means according to the Tukey's test ($P\leq0.05$); bars represent the standard error, n=6.

Plant height

From the beginning of the experiment until day 21, the increment in height of the biofertilizer treatments showed a nearly equal trend; there were no significant differences between them. The SA and CLI treatments had a similar growth pattern. However, from day 16 there was a slight increase in the growth of the plants of the SA treatment with no differences between them (Fig. 2).

On day 8 the treatments with biofertilizer had average heights of 15 cm with no significant differences between them (P=0.855). It is important to note that both treatments resulted in slightly greater heights than the CLI treatment; only the Bio 10 treatment showed significant differences with CLI (P=0.035). By day 11, the biofertilizer treatments and the CLI treatment showed an average height of 16 cm and showed no significant differences.

On day 14, the biofertilizer treatments showed an average height of 17 cm. This was lower than the 18.7 cm of the CTI treatment and was significantly different from the other treatments. From day 16 after water depletion, only the plants under the CTI treatment continued to grow. In contrast, those in the other treatments ceased their growth.

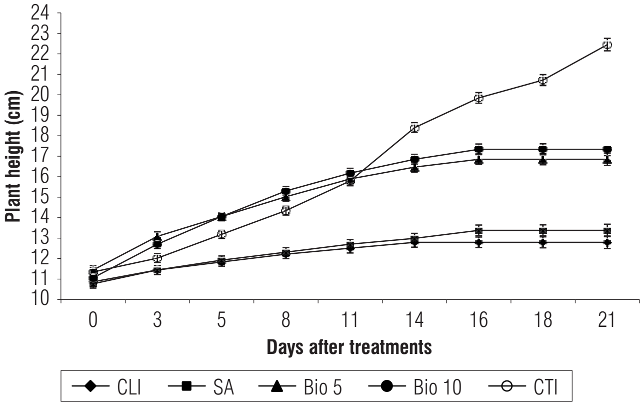

FIGURE 2. Average height (cm) of pepper plants for the five treatments (Tab. 1) during the 21 d after the onset of water depletion. Bars represent the standard error, n=6.

Proportion of wilted leaves

The five treatments showed no signs of wilt during the first 5 d. From day 5 to day 18, the percentage of biofertilizer treatments showed a similar trend and remained relatively constant during the 21 d there were no significant differences among them. A similar situation was observed between the SA and CLI treatments. It is important to note that the CTI treatment was constantly irrigated throughout the experiment; this may explain the absence of signs of wilting (Fig. 3).

On day 8 it is essential to note that there were no significant differences between the treatments with biofertilizer and CTI (P=0.99). However, the SA and CLI treatments showed differences (P=0.03); the SA treatment had a lower percentage of wilt of 15.2%. On day 11, the biofertilizer treatments showed values of 8%; in contrast, the SA and CLI treatments showed a wilting rate of approximately 55%.

By day 14, the treatments with biofertilizer showed an average wilt of 19%, while the SA and CLI treatments had values close to 100%. On day 18, all treatments showed values close to 100% wilt; at 21 d all plants appeared dry, except in the CTI control treatment with constant irrigation.

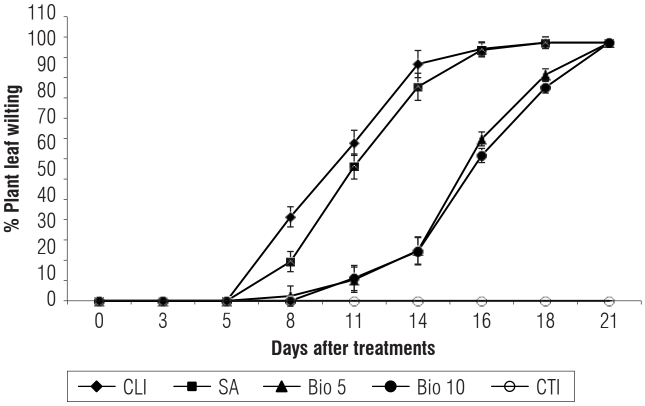

FIGURE 3. Average leaf wilting (± SE) (n = 6) recorded in pepper plants for the five treatments (Tab. 1) during the 21 d after the onset of water depletion.

Results indicated that the plants with a higher expression of *CaWRKY6* gene were those treated with biofertilizer, presenting greater height and fewer signs of wilting compared to the SA and CLI that had higher tolerances to water stress (Figs. 1 and 4). These results are consistent with the findings of other authors (Cai *et al*., 2015; Hussain *et al*., 2019; Zheng *et al*., 2019), who describe *CaWRKY6* as a gene involved in several responses of tolerance and resistance to both biotic and abiotic stresses in *C. annuum*. Hussain *et al.* (2019) propose that the *CaWRKY6* gene plays a significant role in signaling pathways mediated by jasmonates, ethylene, and abscisic acid, substances that are not only important in the defensive response of plants but also in the signaling network related to biotic and abiotic stresses. Therefore, the jasmonate, ethylene, and abscisic acid-mediated pathways constitute vital components of *CaWRKY6*-mediated induction of tolerance to heat stress, drought, and immune responses in peppers.

Additionally, Cai *et al.* (2015) found that, in *C. annuum*, the *CaWRKY6* gene partially induces the *CaWRKY40* gene by binding to its promoter and activating it, like how *CaWRKY40* participates in resistance to biotic stress conditions caused by pathogen attacks and tolerance to high temperatures. The preceding studies suggest that the

FIGURE 4. Pepper plants at day 14 after the onset of water depletion. Treatments from left to right: A) CLI, B) SA, C) Bio 5, D) Bio 10, and E) CTI (Tab. 1).

activation of *CaWRKY6* detected in our study is a possible mechanism that allowed biofertilizer-treated plants to tolerate water stress better than the SA and CLI treatments.

A possible correlation between the activation of *CaWRKY6* in *C. annuum* and enhanced height growth could be associated with the fact that the *CaWRKY6* gene is expressed in the root, stem, leaves, flower buds, and flowers, in addition to participating in the development of leaves and fruits (Zheng *et al*., 2019). Additionally, it supports the fact that the gene was expressed in the control treatments, as it is present in vegetative development.

About the application of the SA treatment, the relative expression of the *CaWRKY6* gene induced by salicylic acid had a value of 3.0 (Fig. 1). On day 8 of this experiment, comparing the proportion of wilted leaves of treatment SA 15.2% with CLI 32.5% (Fig. 3) a significant difference could be seen ($P = 0.032$). On days 11 and 14 although there was no significant difference between treatments SA and CLI (day 11: $P = 0.68$, day 14: $P = 0.77$), the wilting percentage of the SA treatment (day 11: 52.1%, day 14: 82.9%) was slightly lower than CLI (day 11: 61.2%, day 14: 91.7%). This shows that salicylic acid activated *CaWRKY6*, and with it there was a decrease in wilting signs during the experiment. However, activation by Bio 5 and Bio 10 treatments had a higher relative expression (Bio 5: 7.2, Bio 10: 6.1) and a higher protective effect against withering.

Salicylic acid has been considered as an inducer of response to biotic and abiotic stresses through the modulation of metabolic processes, acting as an elicitor of the systemic acquired resistance in plants (Estaji & Niknam, 2020; Thakur *et al*., 2019). In *C. annuum*, salicylic acid is shown to activate genes of the *CaWRKY* complex, which are related to protection against biotic and abiotic stresses. Jingyuan *et al*. (2011) report that two cultivars of *C. annuum* treated with salicylic acid had a rapid induction of transcripts and activation of the *CaWRKY30* gene, reflected in plant protection against pathogens such as *Meloidogyne incognita*, *Phytophthora capsici*, tobacco mosaic virus, *Ralstonia solanacearum*, and low-temperature stress. In addition, Dang *et al*. (2013) report that *CaWRKY40* transcripts are induced by signaling mechanisms mediated by salicylic acid, jasmonic acid, and ethylene, which have protective effects against heat stress, high relative humidity, and attack by *R. solanacearum* bacteria.

The results of the relative *CaWRKY6* expression show that, although the Bio 10 treatment had a higher concentration of biofertilizer, the Bio 5 treatment showed a higher expression of *CaWRKY6* (Fig. 1). This may be due to a possible saturation point in the plant by the mineral nutrients contained in the biofertilizer. Research by Campos-Soriano *et al*. (2020) and Melnikova *et al*. (2015) show that variation in nutrient concentration can affect the expression of genes related to protein and enzyme metabolism in plants.

The activation of the *CaWRKY6* gene, after the application of the biofertilizer, and its potential protective activity when activated before exposing the plants to 100% water stress, could be supported by the behavior of the plants that were treated with the biofertilizer and exposed to 11 d of drought, as they had similar growth to the plants with constant irrigation but were not treated with the biofertilizer. The description of the increase in height of the different treatments was noted on days 8, 11, and 14, considering that during this range of time the treatments had the following behavior: on day 8, Bio 5, and Bio 10 with values of 15.2 cm and 15.5 cm surpassed the height of 14.5 cm of the CTI treatment. Later on day 11, Bio 5 and Bio 10 had values of 16.1 cm and 16.4 cm, which were close to the 16 cm of the CTI treatment; and on day 14, the heights of the Bio 5 and Bio 10 treatments with values of 16.7 cm and 17.1 cm were surpassed by the 18.7 cm of the CTI treatment, which continued to increase until the end of the experiment (Fig. 2).

Day 5 was chosen to start describing the proportion of wilted leaves, because after this day, the five treatments began to show signs of wilting. By day 8, the plants treated with the biofertilizer showed tolerance to dehydration, such that Bio 10 had a total absence (0%) of wilted leaves and Bio 5 had a slight presence of wilting of 1.85% (Fig. 3). One of the possible factors that may be involved here, is the presence of indole-acetic acid detected in the composition of the biofertilizer; this growth regulator is reported in *C. annuum* plants as a promoter of water accumulation in leaf tissues under water stress conditions (Pérez-Jiménez *et al*., 2016).

In the plants of the CLI treatment, the growth of 12.9 cm up to day 14 and the absence of 0% wilting in the first 5 d after water depletion can be explained by the presence of water remaining in the substrate where the plants were planted, which helped to keep the roots hydrated, promoting their growth and resistance to dehydration. However, there was a marked difference in the treatments with biofertilizer, which supports its protective action against dehydration.

Conclusions

This study demonstrated that foliar application of biofertilizer, at 5 ml L^{-1} and 10 ml L^{-1}, significantly enhanced the expression of the *CaWRKY6* gene in *C. annuum* plants, conferring improved drought tolerance under greenhouse conditions. While salicylic acid, at 5 ml L^{-1}, also induced *CaWRKY6* activation, its protective effect against water stress was lower than that of the biofertilizer. These findings highlight the potential of biofertilizers as effective substances to mitigate water stress in bell pepper crops, offering a sustainable alternative to conventional agrochemical practices.

These results contribute to understanding the role of *CaWRKY* transcription factors in plant water stress responses and increase the agronomic value of biofertilizers to promote tolerance to climate-induced abiotic stress, such as drought in bell pepper crops.

Further research is needed to clarify the molecular mechanisms involved in the action of biofertilizers, as well as their potential synergies with other biostimulants. In addition, field trials are essential to validate their efficacy under diverse agroecological conditions and varying levels of abiotic stress. Moreover, incorporating studies focused on stress responses associated with pathogen pressure would contribute to a more comprehensive understanding of the range of conditions under which biofertilizers are effective. Taken together, these considerations could open new opportunities for the effective integration of biofertilizers into agricultural systems, particularly in regions vulnerable to drought and climate change.

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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

SAB and DMMR designed and developed the methodology. SAB performed field and laboratory experiments and data collection; AVR oversaw and contributed to the laboratory experiments. SAB conducted the data analysis. SAB and DMMR contributed to the result description. SAB wrote the draft. All authors reviewed the final version of the manuscript.

Literature cited

Abdou Zayan, S. (2020). Impact of climate change on plant diseases and IPM strategies. In S. Topolovec-Pintarić (Ed.), *Plant diseases – Current threats and management trends*. IntechOpen. https://doi.org/10.5772/intechopen.87055

Agronegocios. (2015). El futuro del pimentón que se produce en Colombia está en la más exclusiva gastronomía. https://www.agronegocios.co/agricultura/el-futuro-del-pimenton-esta-en-la-mas-exclusiva-gastronomia-2621159

Ahammed, G. J., Li, X., Wan, H., Zhou, G., & Cheng, Y. (2020). *SlWRKY81* reduces drought tolerance by attenuating proline biosynthesis in tomato. *Scientia Horticulturae, 270*, Article 109444. https://doi.org/10.1016/j.scienta.2020.109444

Bulle, M., Rahman, M. M., Islam, M. R., & Abbagani, S. (2025). Strategies to develop climate-resilient chili peppers: Transcription factor optimization through genome editing. *Planta, 262*(2), Article 30. https://doi.org/10.1007/s00425-025-04747-5

Bykova, O., Chuine, I., & Morin, X. (2019). Highlighting the importance of water availability in reproductive processes to understand climate change impacts on plant biodiversity. *Perspectives in Plant Ecology, Evolution and Systematics, 37*, 20–25. https://doi.org/10.1016/j.ppees.2019.01.003

Cai, H., Yang, S., Yan, Y., Xiao, Z., Cheng, J., Wu, J., Qiu, A., Lai, Y., Mou, S., Guan, D., Huang, R., & He, S. (2015). *CaWRKY6* transcriptionally activates *CaWRKY40*, regulates *Ralstonia solanacearum* resistance, and confers high-temperature and high-humidity tolerance in pepper. *Journal of Experimental Botany, 66*(11), 3163–3174. https://doi.org/10.1093/jxb/erv125

Campos, H., Trejo, C., Peña-Valdivia, C. B., García-Nava, R., Conde-Martínez, F. V., & Cruz-Ortega, M. R. (2014). Stomatal and non-stomatal limitations of bell pepper (*Capsicum annuum* L.) plants under water stress and re-watering: Delayed restoration of photosynthesis during recovery. *Environmental and Experimental Botany, 98*, 56–64. https://doi.org/10.1016/j.envexpbot.2013.10.015

Campos-Soriano, L., Bundó, M., Bach-Pages, M., Chiang, S., Chiou, T., & San Segundo, B. (2020). Phosphate excess increases susceptibility to pathogen infection in rice. *Molecular Plant Pathology, 21*(4), 555–570. https://doi.org/10.1111/mpp.12916

Casilimas, H., Monsalve, O., Bojacá, C. R., Gil, R., Villagrán, E., Arias, L. A., & Fuentes, L. S. (2012). Manejo de plagas y enfermedades. In C. Bojacá, & O. Monsalve (Eds.), *Manual de producción de pimentón bajo invernadero* (pp. 129–152). Universidad de Bogotá Jorge Tadeo Lozano. http://hdl.handle.net/20.500.12324/34356

Dang, F.-F., Wang, Y.-N., Yu, L., Eulgem, T., Lai, Y., Liu, Z.-Q., Wang, X., Qiu, A.-L., Zhang, T.-X., Lin, J., Chen, Y.-S., Guan, D.-Y., Cai, H.-Y., Mou, S.-L., & He, S.-L. (2013). *CaWRKY40*, a WRKY protein of pepper, plays an important role in the regulation of tolerance to heat stress and resistance to *Ralstonia solanacearum* infection. *Plant, Cell & Environment, 36*(4), 757–774. https://doi.org/10.1111/pce.12011

Elahi, E., Weijun, C., Zhang, H., & Nazeer, M. (2019). Agricultural intensification and damages to human health in relation to agrochemicals: Application of artificial intelligence. *Land Use Policy, 83*, 461–474. https://doi.org/10.1016/j.landusepol.2019.02.023

Estaji, A., & Niknam, F. (2020). Foliar salicylic acid spraying effect on growth, seed oil content, and physiology of drought-stressed *Silybum marianum* L. plant. *Agricultural Water Management, 234*, Article 106116. https://doi.org/10.1016/j.agwat.2020.106116

Etesami, H., Alikhani, H. A., & Hosseini, H. M. (2015). Indole-3-acetic acid (IAA) production trait, a useful screening to select endophytic and rhizosphere competent bacteria for rice growth promoting agents. *MethodsX, 2*, 72–78. https://doi.org/10.1016/j.mex.2015.02.008

Goñi, O., Quille, P., & O'Connell, S. (2018). *Ascophyllum nodosum* extract biostimulants and their role in enhancing tolerance to drought stress in tomato plants. *Plant Physiology and Biochemistry, 126*, 63–73. https://doi.org/10.1016/j.plaphy.2018.02.024

Han, X., Xi, Y., Zhang, Z., Mohammadi, M. A., Joshi, J., Borza, T., & Wang-Pruski, G. (2021). Effects of phosphite as a plant biostimulant on metabolism and stress response for better plant performance in *Solanum tuberosum*. *Ecotoxicology and Environmental Safety, 210*, Article 111873. https://doi.org/10.1016/j.ecoenv.2020.111873

Hussain, A., Noman, A., Khan, M. I., Zaynab, M., Aqeel, M., Anwar, M., Ashraf, M. F., Liu, Z., Raza, A., Mahpara, S., Bakhsh, A., & He, S. (2019). Molecular regulation of pepper innate immunity and stress tolerance: An overview of WRKY TFs. *Microbial Pathogenesis, 135*, Article 103610. https://doi.org/10.1016/j.micpath.2019.103610

Jang, J.-O., Kim, B.-H., Lee, J.-B., Joa, J.-H., & Koh, S. (2019). Evaluation of bacterial spot disease of *Capsicum annuum* L. in drought stress environment by high temperature. *Research in Plant Disease, 25*(2), 62–70. https://doi.org/10.5423/RPD.2019.25.2.62

Jaramillo Noreña, J. E., Aguilar Aguilar, P. A., Espitia Malagón, E. M., Tamayo Molano, P. J., & Guzmán Arroyave, M. (2014). Manejo integrado del cultivo de pimentón. In *Modelo productivo del cultivo de pimentón bajo condiciones protegidas en el oriente antioqueño* (1st ed., pp. 44–134). Corporación Colombiana de Investigación Agropecuaria - Corpoica. http://hdl.handle.net/20.500.12324/13753

Jingyuan, Z., Xuexiao, Z., Zhenchuan, M., & Bingyan, X. (2011). A novel pepper (*Capsicum annuum* L.) *WRKY* gene, *CaWRKY30*, is involved in pathogen stress responses. *Journal of Plant Biology, 54*(5), 329–337. https://doi.org/10.1007/s12374-011-9171-x

Kour, D., Rana, K. L., Yadav, A. N., Yadav, N., Kumar, M., Kumar, V., Vyas, P., Dhaliwal, H. S., & Saxena, A. K. (2020). Microbial biofertilizers: Bioresources and eco-friendly technologies for agricultural and environmental sustainability. *Biocatalysis and Agricultural Biotechnology, 23*, Article 101487. https://doi.org/10.1016/j.bcab.2019.101487

Lee, S. G., Kim, S. K., Lee, H. J., Lee, H. S., & Lee, J. H. (2018). Impact of moderate and extreme climate change scenarios on growth, morphological features, photosynthesis, and fruit production of hot pepper. *Ecology and Evolution, 8*(1), 197–206. https://doi.org/10.1002/ece3.3647

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. *Methods, 25*(4), 402–408. https://doi.org/10.1006/meth.2001.1262

Malo, I., Bernacchia, G., & Arévalo, P. (2015). Activación de genes de defensa en plantas de tomate de mesa *Lycopersicum esculentum* L., a través de la aplicación de sustancias químicas y naturales. *La Granja: Revista de Ciencias de la Vida, 21*(1), 61–68. https://doi.org/10.17163/lgr.n21.2015.05

Mandal, A., Sarkar, B., Mandal, S., Vithanage, M., Patra, A. K., & Manna, M. C. (2020). Impact of agrochemicals on soil health. In M. N. Vara Prasad (Ed.), *Agrochemicals detection, treatment and remediation* (pp. 161–187). Butterworth-Heinemann. https://doi.org/10.1016/B978-0-08-103017-2.00007-6

Martínez Bernal, L. F., Bello Rodríguez, P. L., & Castellanos Domínguez, O. F. (2012). Entorno normativo y certificación. In L. F. Bernal, P. L. Bello Rodríguez, & O. F. Castellanos (Eds.), *Sostenibilidad y desarrollo: el valor agregado de la agricultura orgánica* (1st ed., pp. 75–104). Universidad Nacional de Colombia. https://repositorio.unal.edu.co/handle/unal/10045

Melnikova, N. V., Dmitriev, A. A., Belenikin, M. S., Speranskaya, A. S., Krinitsina, A. A., Rachinskaia, O. A., Lakunina, V. A., Krasnov, G. S., Snezhkina, A. V., Sadritdinova, A. F., Uroshlev, L. A., Koroban, N. V., Samatadze, T. E., Amosova, A. V., Zelenin, A. V., Muravenko, O. V., Bolsheva, N. L., & Kudryavtseva, A. V. (2015). Excess fertilizer responsive miRNAs revealed in *Linum usitatissimum* L. *Biochimie*, *109*, 36-41. https://doi.org/10.1016/j.biochi.2014.11.017

MINCIT. (2019, May 9). Pimentón colombiano, a un paso de llegar a Estados Unidos, el mayor importador a nivel mundial. http://mincit.gov.co/prensa/noticias/comercio/pimenton-colombiano-a-un-paso-de-llegar-a-estados

Ministerio de Agricultura y Desarrollo Rural- MADR. (2020). Resolución No. 187 de 2006. https://www.minagricultura.gov.co/tramites-servicios/Documents/Resolucion_187_de_2006.pdf

Ntanasi, T., Karavidas, I., Savvas, D., Spyrou, G. P., Giannothanasis, E., Consentino, B. B., Papasotiropoulos, V., Sabatino, L., & Ntatsi, G. (2025). Physiological and yield responses of pepper (*Capsicum annuum* L.) genotypes to drought stress. *Plants, 14*(13), Arficle 1934. https://doi.org/10.3390/plants14131934

Orozco-Mosqueda, M. C., Glick, B. R., & Santoyo, G. (2020). ACC deaminase in plant growth-promoting bacteria (PGPB): An efficient mechanism to counter salt stress in crops. *Microbiological Research, 235*, Article 126439. https://doi.org/10.1016/j.micres.2020.126439

Park, M., Jo, S., Kwon, J.-K., Park, J., Ahn, J. H., Kim, S., Lee, Y.-H., Yang, T.-J., Hur, C.-G., Kang, B.-C., Kim, B.-D., & Choi, D. (2011). Comparative analysis of pepper and tomato reveals euchromatin expansion of pepper genome caused by differential accumulation of *Ty3/Gypsy*-like elements. *BMC Genomics, 12*, Article 85. https://doi.org/10.1186/1471-2164-12-85

Pérez-Jiménez, M., Pazos-Navarro, M., Piñero, M. C., Otálora-Alcón, G., López-Marín, J., & del Amor, F. M. (2016). Regulation of the drought response of sweet pepper (*Capsicum annuum* L.) by foliar-applied hormones, in Mediterranean-climate greenhouse conditions. *Plant Growth Regulation, 80*(2), 159–169. https://doi.org/10.1007/s10725-016-0153-3

Roselló I Oltra, J., & Porcuna, J. L. (2012). El manejo del cultivo en tomate y pimiento. In J. Roselló i Oltra, & J. L. Porcuna (Eds.), *Cultivo ecológico del tomate y del pimiento* (pp. 3–54). Cuadernos Técnicos SEAE, Sociedad Española de Agricultura

Ecológica. https://www.icia.es/icia/download/Agroecología/Material/Cultivo_ecologico.pdf

Sinha, R., Irulappan, V., Mohan-Raju, B., Suganthi, A., & Senthil-Kumar, M. (2019). Impact of drought stress on simultaneously occurring pathogen infection in field-grown chickpea. *Scientific Reports, 9*(1), Article 5577. https://doi.org/10.1038/s41598-019-41463-z

Thakur, M., Bhattacharya, S., Khosla, P. K., & Puri, S. (2019). Improving production of plant secondary metabolites through biotic and abiotic elicitation. *Journal of Applied Research on Medicinal and Aromatic Plants, 12*, 1–12. https://doi.org/10.1016/j.jarmap.2018.11.004

Verly, C., Djoman, A. C. R., Rigault, M., Giraud, F., Rajjou, L., Saint-Macary, M.-E., & Dellagi, A. (2020). Plant defense stimulator mediated defense activation is affected by nitrate fertilization and developmental stage in *Arabidopsis thaliana*. *Frontiers in Plant Science*, *11*, Article 583. https://doi.org/10.3389/fpls.2020.00583

Vijayakumar, S., Durgadevi, S., Arulmozhi, P., Rajalakshmi, S., Gopalakrishnan, T., & Parameswari, N. (2019). Effect of seaweed liquid fertilizer on yield and quality of *Capsicum annuum* L. *Acta Ecologica Sinica, 39*(5), 406–410. https://doi.org/10.1016/j.chnaes.2018.10.001

Xu, D., Deng, Y., Xi, P., Yu, G., Wang, Q., Zeng, Q., Jiang, Z., & Gao, L. (2019). Fulvic acid-induced disease resistance to *Botrytis cinerea* in table grapes may be mediated by regulating phenylpropanoid metabolism. *Food Chemistry, 286*, 226–233. https://doi.org/10.1016/j.foodchem.2019.02.015

Yaseen, A. A., Ahmed, S. J., & Bakr, T. D. (2024). Effect of biofertilizers in improving production of hot pepper (*Capsicum annuum* L.), and tolerating drought stress. *Zanco Journal of Pure and Applied Sciences, 36*(6), 104–117. https://doi.org/10.21271/ZJPAS.36.6.12

Zhang, L., Liu, D., Yin, Q., & Liu, J. (2024). Organic certification, online market access, and agricultural product prices: Evidence from Chinese apple farmers. *Agriculture, 14*(5), Article 669. https://doi.org/10.3390/agriculture14050669

Zhang, X., Ma, X., Wang, S., Liu, S., & Shi, S. (2024). Physiological and genetic aspects of resistance to abiotic stresses in *Capsicum* species. *Plants, 13*(21), Article 3013. https://doi.org/10.3390/plants13213013

Zheng, J., Liu, F., Zhu, C., Li, X., Dai, X., Yang, B., Zou, X., & Ma, Y. (2019). Identification, expression, alternative splicing and functional analysis of pepper *WRKY* gene family in response to biotic and abiotic stresses. *PLoS ONE, 14*(7), Article e0219775. https://doi.org/10.1371/journal.pone.0219775

The impact of global warming on fruit crops and mitigation strategies: A comprehensive review

El impacto del calentamiento global en los cultivos de frutales y las estrategias de mitigación: una revisión comprensiva

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ABSTRACT

Global warming poses a significant challenge to the agricultural sector, with fruit cultivation being particularly susceptible due to its intricate relationship with specific climatic conditions. The observed increases in global air temperatures, coupled with alterations in precipitation patterns and a higher frequency of extreme weather events, are fundamentally reshaping fruit production on a worldwide scale. This article explores the multifaceted impacts of global warming on fruit cultivation, highlighting key issues such as shifts in phenological phases, declines in crop yields, increased pressure from pests and diseases, and the growing scarcity of water resources. Furthermore, it provides a comprehensive analysis of adaptation and mitigation strategies, encompassing sustainable agricultural practices, the development of climate-resilient fruit varieties, and the implementation of effective water management strategies. Addressing these complex challenges is of paramount importance to ensure the long-term viability and economic sustainability of fruit production in the face of evolving climatic conditions.

Keywords: climate adaptation, phenological stages, yield losses, adaptation strategies.

RESUMEN

El calentamiento global plantea un desafío significativo para el sector agrícola, siendo el cultivo de frutales particularmente susceptible debido a su intrincada relación con condiciones climáticas específicas. Los aumentos observados en las temperaturas globales del aire, junto con las alteraciones en los patrones de precipitación y una mayor frecuencia de eventos climáticos extremos, están reconfigurando fundamentalmente la producción de frutales a escala global. Este artículo profundiza en los impactos multifacéticos del calentamiento global en el cultivo de frutales, explorando cuestiones críticas como los cambios en las fases fenológicas, la disminución de los rendimientos de los cultivos, la mayor presión de plagas y enfermedades, y la creciente escasez de recursos hídricos. Además, proporciona un análisis comprensivo de las estrategias de adaptación y mitigación, que abarcan las prácticas agrícolas sostenibles, el desarrollo de variedades de frutales resistentes al clima y la implementación de estrategias efectivas de gestión del agua. Abordar estos desafíos complejos es de suma importancia para asegurar la viabilidad a largo plazo y la sostenibilidad económica de la producción de frutales bajo las condiciones climáticas en evolución.

Palabras clave: adaptación al clima, fases fenológicas, pérdidas de rendimiento, estrategias de adaptación.

Introduction

Climate change is recognized as one of the most critical global-scale issues of the 21st century, exerting profound and widespread impacts on the agricultural sector (IPCC, 2021). The persistently rising global temperatures, coupled with unpredictable and variable weather conditions and an increasing frequency of extreme climatic events, significantly affect agricultural production, particularly fruit cultivation (Fischer *et al*., 2016). Fruit crops, due to their high sensitivity to specific climatic requirements, are highly vulnerable to these environmental changes (Ameen *et al*., 2023; Osorio-Marín *et al*., 2024) that adversely affect their growth cycles, overall yields, and final product quality.

The importance of thoroughly investigating and understanding the effects of climate change on fruit cultivation extends beyond the boundaries of mere food production. Fruits are recognized as an indispensable and fundamental component of the human diet, providing a range of vital nutrients such as vitamins, minerals, fiber, and antioxidants that are essential for human health (Ameen *et al*., 2023). The economic dimension of fruit production also holds a significant place on a global scale. This sector not only

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constitutes the livelihood for millions of farmers worldwide but also makes substantial contributions to international trade, forming a critical pillar of both local and global economies (Raju *et al.*, 2024). Therefore, given the systemic threats posed by a shifting climate, a deep understanding of how these changes affect fruit cultivation has become a vital necessity (Osorio-Marín *et al.*, 2024; Yadav *et al.*, 2023). This understanding is crucial to develop effective adaptation strategies aimed at sustainably maintaining production levels, ensuring global food security, and preserving the vitality of rural economies in a changing world (Karagatiya *et al.*, 2023).

The primary objective of this article is to comprehensively address the multifaceted impacts of global warming on fruit cultivation. In this context, issues such as changes occurring in phenological processes (*e.g.*, flowering timing and fruit ripening) (Fujisawa & Kobayashi, 2010; Funes *et al.*, 2016), reductions in crop yields (*e.g.*, fruit size and number) (Challinor *et al.*, 2014), declines in fruit quality (*e.g.*, sugar content and acidity) (Fujisawa & Kobayashi, 2010), and the increased susceptibility of plants to pests and diseases (*e.g.*, the emergence of new pest species) (Jones *et al.*, 2012; Moinina *et al.*, 2019) will be examined. Furthermore, adaptation strategies such as the adoption of sustainable agricultural practices, the modernization of irrigation techniques, and genetic improvement studies will also be discussed to provide applicable solutions to minimize climate-induced risks and make fruit cultivation more resilient to the adverse effects of climate change.

Climate change, with its primary drivers being temperature increase and altered precipitation patterns, has become one of the most significant sources of global concern in recent years (Fischer *et al.*, 2016; IPCC, 2021). Horticultural products, exhibiting a high degree of sensitivity to climatic conditions, are significantly affected by this change, which brings about significant challenges in this vital area of product production (Ameen *et al.*, 2023; Osorio-Marín *et al.*, 2024). Climate modeling studies predict that horticultural products are highly susceptible to the potential impacts of climate change, which could result in significant adverse effects (Karagatiya *et al.*, 2023; Yadav *et al.*, 2023). As both the frequency and severity of extreme weather events, such as tropical storms, hurricanes, and typhoons, are progressively increasing (Osorio-Marín *et al.*, 2024), the necessity and importance of being prepared for such events is becoming even more pronounced. Although researchers have a comprehensive understanding of the potential risks that climate change may pose in the future, thanks to significant advances in computer technologies, policymakers and planners have not yet taken sufficient steps towards effective actions to mitigate the potential damage that may arise in different sectors of agriculture (Fischer *et al.*, 2016; IPCC, 2021; Karagatiya *et al.*, 2023).

Fruit bearing plants are particularly vulnerable to the significant changes expected and projected in the climate due to their need for high chilling requirements to terminate the dormancy process (Luedeling, 2012; Luedeling *et al.*, 2011). Most fruit species from temperate regions need to be exposed to low temperatures for a certain period to eliminate physical and/or physiological dormancy (Luedeling *et al.*, 2011; Rodríguez *et al.*, 2021). This chilling period is critical for the regular opening of buds, the timely onset of flowering, and ultimately, the realization of fruit formation (Luedeling *et al.*, 2011; Rodríguez *et al.*, 2021). However, changing climatic conditions are expected to lead to significant changes in chilling hours and heat hour accumulation, which pose new and complex challenges for the fruit industry (Noorazar *et al.*, 2022; Osorio-Marín *et al.*, 2024; Ramírez & Kallarackal, 2015). Fruit trees, which are perennial plants, will be exposed to stress factors caused by changing climatic conditions for many years, unlike annual plants, where the effects of extreme weather events are limited to a single crop growing season. These long-term effects of climate change on perennial fruit plants have not yet been sufficiently studied in the scientific literature. This article aims to comprehensively examine the effects of climate change on the different stages of fruit cultivation. In this context, all stages, from chilling requirements to flowering, fruit set, fruit development, and postharvest processes, including changes in secondary metabolites, phytochemicals, and fruit nutritional value, will be discussed. In addition, adaptation recommendations in the form of various cultural practices are presented to overcome these unprecedented challenges and make fruit cultivation more resilient to climate change.

Effects of global warming on fruit cultivation

Global warming is projected to have complex and far-reaching effects on fruit cultivation. These effects can manifest themselves in a wide range, from the physiological processes of products to the dynamics of harmful organisms, from the availability of water resources to the geographical distribution of orchards.

Direct effects

Temperature effects

High temperature stress: Excessively high temperatures can negatively affect essential physiological processes in fruit crops, such as photosynthesis (Flórez-Velasco *et al.*, 2024), respiration, and enzyme activities (Ameen *et al.*, 2023; Osorio-Marín *et al.*, 2024). Photosynthetic activity significantly decreases under high temperature stress, which limits the plant capacity to produce sugars that are vital for fruit development (Ameen *et al.*, 2023; Yadav *et al.*, 2023). High temperatures can also reduce pollen viability and fruit set rates (Ameen *et al.*, 2023). The severity of temperature stress can vary greatly among fruit species and even among different varieties of the same species. For example, some apple varieties are more susceptible to heat-induced fruit drop than others, while some stone fruits, such as cherries and plums, may produce smaller and lower quality fruits under high temperature stress (Ameen *et al.*, 2023; Osorio-Marín *et al.*, 2024).

A less apparent but critical impact on plant physiology, particularly prevalent in fruit crops, is night-time heat stress. Elevated nocturnal temperatures increase plant respiration rates, leading to the consumption of a significant portion of carbohydrates (sugars) produced during daytime photosynthesis. This limits the net energy available for plant growth and fruit development. In apples, elevated night temperatures have been shown to suppress anthocyanin biosynthesis by down-regulating key genes (*e.g., MdCHS*, *MdF3H*, *MdDFR*, *MdANS*, and *MdUFGT*), resulting in reduced red pigmentation and diminished visual quality (Ryu *et al.*, 2017). In peaches, recent studies demonstrated that elevated day/night temperatures, such as 30°C/22 °C, significantly impair red flesh coloration and overall fruit quality, including reductions in fruit firmness, contents of soluble solids, and titratable acidity (Jayasooriya *et al.*, 2025). Physiologically, night-time heat stress may also lead to chlorophyll degradation, reduced photosynthetic efficiency, and hormonal imbalance during critical phases such as flowering and fruit set. Agronomically, it adversely affects fruit size, color, firmness, and flavor, leading to both yield and quality losses. Therefore, understanding the physiological mechanisms and agronomic consequences of night-time heat stress is a crucial component of climate change adaptation strategies (Ameen *et al.*, 2023; Yadav *et al.*, 2023).

Changes in chilling requirements: Many temperate fruit species, such as apples, pears, cherries, peaches, and plums, require a specific period of chilling hours (generally below 7°C) during the winter season (Ameen *et al.*, 2023; Yadav *et al.*, 2023). This chilling process is necessary for the plants to break dormancy and initiate regular and adequate flowering in the spring (Ramírez & Kallarackal, 2015; Rodríguez *et al.*, 2021). However, increasingly warmer winters can lead to a decrease in the accumulation of chilling hours required by the plants (Luedeling *et al.*, 2011; Ramírez & Kallarackal, 2015; Rodríguez *et al.*, 2021). This can cause untimely or insufficient bud break, irregular flowering, and consequently, lower fruit set rates and yield (Noorazar *et al.*, 2022; Salama *et al.*, 2021). This change in chilling hour requirements can lead to significant yield losses in fruit production. Chilling hour requirements between different fruit species and varieties can vary significantly (Noorazar *et al.*, 2022; Rodríguez *et al.*, 2021; Salama *et al.*, 2021).

Deviations in phenological stages: Rising global temperatures can cause phenological stages, such as flowering and bud break, to begin earlier and also result in their shorter duration in fruit trees (El Yaacoubi *et al.*, 2014; Fu *et al.*, 2022). This early onset can make fruit trees more vulnerable to late spring frosts, especially after mild winters followed by sudden cold snaps. Such changes in the timing and duration of phenological stages can disrupt the critical synchronization between fruit flowering and the activity of natural pollinators, negatively affecting fruit set (Fu *et al.*, 2022). In addition, increased temperatures can accelerate fruit development, thereby shortening the fruit filling period and reducing the time available for optimal sugar accumulation, which may ultimately diminish both yield and fruit quality (Jayasooriya *et al.*, 2025).

Water resources

Drought: The increasing frequency and severity of drought events can lead to severe water stress in fruit trees (Kumar *et al.*, 2019). From a physiological standpoint, water deficit disrupts stomatal conductance and reduces photosynthetic rates, leading to impaired carbon assimilation and limited biomass accumulation. It can also alter hormonal balances (*e.g.*, increased abscisic acid content), reduce cell expansion, and accelerate leaf senescence, ultimately limiting vegetative growth and reproductive success (Fischer *et al.*, 2016). These physiological constraints can slow down tree growth, reduce fruit size, decrease overall yield, and negatively affect fruit quality parameters such as texture, juiciness, and sugar content (Kumar *et al.*, 2019).

However, mild to moderate drought stress during specific stages of fruit development has been reported to promote the accumulation of sugars, anthocyanins, and other antioxidants in some species, potentially enhancing certain quality attributes such as fruit flavor and skin coloration (Jayasooriya *et al*., 2025; Silva *et al*., 2018). This duality underscores the complexity of drought responses in fruit crops. Drought stress can also compromise tree health by weakening structural defenses and metabolic resilience, thereby increasing vulnerability to insect pests and diseases (Kumar *et al*., 2019).

The magnitude of drought effects on fruit production varies significantly depending on the species, cultivar, developmental stage, and stress duration, with some fruit species (olive, fig, or pomegranate) exhibiting greater drought tolerance than others (Silva *et al*., 2018). Regions already prone to water scarcity, such as California, the Mediterranean basin, and parts of Australia, are especially at risk of long-term production losses due to these adverse impacts (El Jaouhari *et al*., 2018; Nath *et al*., 2018).

Flooding: More frequent occurrences of excessive rainfall and flooding can damage fruit crops. Especially in soils with insufficient drainage, problems such as excessive water accumulation and root rot can occur (Malhotra *et al*., 2017). From a physiological perspective, prolonged soil saturation creates hypoxic to anoxic conditions in the root zone, impairing root respiration, nutrient and water uptake, and triggering oxidative stress. Energy production shifts from aerobic to less-efficient anaerobic pathways, leading to toxic metabolite accumulation (ethanol, reactive oxygen species), decreased fine root growth, and compromised root integrity (McGee *et al*., 2022; Wurms *et al*., 2023).

These disruptions translate agronomically into reduced carbohydrate translocation, impaired photosynthesis, and hormonal imbalance—impacting flowering, fruit set, and growth duration. In peach trees, flooding has been shown to alter carbohydrate and nitrogen metabolism, increasing leaf sugar concentrations while simultaneously inhibiting nitrate assimilation and glycolytic enzyme activities, which ultimately impairs fruit filling and reduces fruit size and yield (McGee *et al*., 2022). Routine operations in orchards may also be disrupted, and long-term infrastructure damage can compound crop losses (Kourgialas & Karatzas, 2016; Malhotra *et al*., 2017).

Evapotranspiration: Rising temperatures directly increase evapotranspiration rates that can further exacerbate water scarcity even if rainfall patterns remain relatively constant. This increase in evapotranspiration creates an additional stress load on fruit trees, increasing irrigation needs (Zhang *et al*., 2013).

Extreme weather events

Heat waves, frost events, hail, and severe winds: Extreme weather events can cause direct physical damage to fruit trees, the flowers, and developing fruits. For example, heat waves can lead to sunburn and desiccation of fruits (Van Asten *et al*., 2011), while late spring frosts can completely destroy newly formed buds and flowers. Hail can damage the outer skins of fruits, reducing their market value (Bal *et al*., 2014) and increasing their susceptibility to rot. Severe winds can cause premature fruit drop, leading to significant yield losses (Chawla *et al*., 2011; Veste *et al*., 2020).

Indirect effects

Pests and diseases

Expansion of pest distribution areas: Rising global temperatures can expand the geographic distribution areas of some insect species that are considered pests (Sharma, 2014). This allows them to settle in regions where they could not previously survive due to the temperature factor. As a result, new pest species that have not been seen before may emerge in fruit-growing regions, which increases the complexity of pest management strategies (Moinina *et al*., 2019). Changes in rainfall patterns can also affect the life cycles of pests. For example, pest pressure may increase in some regions while decreasing in others (Moinina *et al*., 2019; Sharma, 2014).

Increased prevalence of diseases: Changes in temperature and humidity conditions can create more suitable microclimates for the development and spread of fungal and bacterial diseases affecting fruit trees (Jones *et al*., 2012; Yáñez-López *et al*., 2012). Rising temperatures can extend the active growing season of some pathogens, while increased humidity can promote the development of fungal diseases (Ghini, 2011). For example, in some fruit species such as strawberries, the frequency of fungal diseases can significantly increase in hot and humid climatic conditions (Yáñez-López *et al*., 2012).

Pest and disease interactions: Fruit trees that are weakened by factors such as high temperature or water stress become more vulnerable to both insect pest attacks and various diseases. For example, a tree that is already under stress due to drought may lose its normal resistance to fungal pathogens (Nawaz *et al*., 2020). This can trigger a chain reaction in which the primary stress factor increases the tree's overall vulnerability to other stress factors.

Disruptions in the pollination process

Disruptions in pollinator activity: The increase in global temperatures can disrupt the delicate balance between the flowering time of fruit trees and the activities of pollinators (especially bees), which play a critical role in the pollination of these trees (Forrest, 2015). Temperature changes can affect both the flowering time and the emergence time of pollinators, leading to a mismatch that negatively affects pollination success (Forrest, 2015; Millard *et al*., 2023). Changes in the behavior of pollinators, such as deviations in their foraging habits due to changing flower resources, can further negatively affect the pollination process (Millard *et al*., 2023; Scaven & Rafferty, 2013).

Deterioration of soil health

Soil erosion: The increase in the frequency and intensity of rainfall events, combined with the decrease in vegetation cover in some areas, such as due to drought-related plant deaths, can significantly increase soil erosion, especially in sloping lands or orchards with poor vegetation cover (Li & Fang, 2016). In some vulnerable regions, projected climate scenarios suggest that soil erosion rates could increase by 17% to 58% by the end of the 21st century under high-emission scenarios (Li & Fang, 2016). Soil erosion leads to the loss of the top fertile layer of the soil. This layer is rich in organic matter and nutrients necessary for plants, which leads to a decrease in soil fertility and water holding capacity (Kaye & Quemada, 2017).

Loss of soil organic matter and nutrients: Changes in temperature and humidity regimes can deeply affect the soil's organic matter content and nutrient cycling. High temperatures can increase the decomposition rate of organic matter, reducing the overall fertility of the soil (Sierra *et al*., 2015). Changes in rainfall patterns can affect the leaching of nutrients in the soil and their availability to plants. Drought conditions can also suppress the activities of microorganisms living in the soil, further negatively affecting nutrient cycling (Scavo *et al*., 2022).

Regional differences

The effects of global warming on fruit cultivation show significant differences between geographical regions. These differences stem from factors such as the region's basic climatic characteristics, its topographic structure, current agricultural techniques, and the diversity of fruit species grown (Del Pozo *et al*., 2019; Raju *et al*., 2024).

Mediterranean region: The Mediterranean climate zone is a critical region where important fruit products such as olives, grapes, citrus fruits, and stone fruits are grown. However, this region is expected to face an increased risk of drought in the future, experience more frequent and severe heat waves, and experience significant decreases in chilling hours (Del Pozo *et al*., 2019). These climate changes can lead to yield declines in fruit production in the region, changes in fruit quality, and geographical shifts in suitable areas for fruit cultivation (Del Pozo *et al*., 2019).

California (USA): California is an important region for the cultivation of high-value fruit products (including almonds, citrus fruits, grapes, and stone fruits) (Pathak *et al*., 2018). However, prolonged droughts and an increase in the frequency and intensity of heat waves pose serious challenges for fruit cultivation in this region. The decrease in snow cover in the Sierra Nevada mountains, which is the main source of irrigation water, also raises concerns about the sustainability of water resources (Pathak *et al*., 2018).

Tropical regions: Regions where tropical fruits such as mangoes, bananas, papayas, and pineapples are grown are adversely affected by changes in rainfall patterns, increases in both drought and flood risks, and the intensification of pest and disease pressure. These regions are also expected to experience an increase in the frequency and severity of extreme weather events (Lauri *et al*., 2013; Nath *et al*., 2018). In tropical highland areas, such as the Andes, fruit crops are additionally affected by altitude-dependent temperature shifts, which can alter photosynthetic efficiency and fruit development patterns (Flórez-Velasco *et al*., 2024).

Australia: In Australia, the decrease in winter cold has already begun to negatively affect apple production in some regions (Darbyshire *et al*., 2013). Other fruit species are experiencing yield losses due to more frequent and severe heat events. The state of water resources, especially the increasing risk of drought, is a major concern for Australian fruit cultivation (Head *et al*., 2014).

South Africa: South Africa is an important region for the production of grapes, citrus fruits, and stone fruits. However, shifts in rainfall patterns, temperature increases, and more frequent heat waves are negatively affecting fruit production in this region (Calzadilla, 2014). These effects of climate change are reducing both fruit yield and quality.

Sensitivity levels of some fruit species to climate change

Different fruit species exhibit varying levels of sensitivity to the diverse effects of climate change. Understanding these differences in sensitivity is crucial for developing effective and species-specific adaptation strategies.

Apple

Chilling hour requirements: Apple trees need to meet a specific period of chilling hours (usually below 7°C) during the winter season. This chilling process is vital for the trees to break dormancy and initiate regular flowering in the spring (Ramírez & Kallarackal, 2015; Salama *et al.*, 2021). In case of mild winters, the accumulation of necessary chilling hours may decrease (González-Martínez *et al.*, 2025; Ramírez & Kallarackal, 2015). This can lead to late or irregular opening of buds, reduced fruit set, and consequently, yield declines (Luedeling *et al.*, 2011; Rodríguez *et al.*, 2021; Salama *et al.*, 2021).

High temperature stress: High temperature stress can cause sunburn, a decrease in color development, and a reduction in sugar content in fruits. Prolonged exposure to high solar radiation—particularly during summer—can elevate fruit surface temperatures well above ambient air temperature, leading to sunburn, which is one of the visible symptoms of climate change impacts on fruit quality (Bacelar *et al.,* 2024).

Risk of frost damage: The early onset of spring temperatures due to global warming can lead to early bud break in apple trees. This significantly increases the risk of damage to sensitive apple blossoms from late spring frosts and can cause severe crop losses (Lamichhane, 2021).

Water scarcity: Apple trees need sufficient water for healthy growth and quality fruit development (El Jaouhari *et al.*, 2018). Drought conditions can lead to a reduction in fruit size, a decrease in yield, and a decline in fruit quality, including increased firmness, reduced soluble solids content, poor color development, and imbalanced acidity (Ameen *et al.*, 2023).

Pests and diseases: Warmer climatic conditions can facilitate the spread of some pests and diseases, such as the codling moth and apple scab (Moinina *et al.*, 2019). Temperature increases accelerate the life cycles of some pest species, allowing them to produce more generations per year. Changes in humidity levels can also affect the frequency of fungal diseases (Ghini *et al.*, 2011). For example, an increase in rainfall in some regions can increase the risk of apple scab disease (Moinina *et al.*, 2019). Therefore, it is of great importance to consider the complex interactions between temperature, humidity, and pest/disease dynamics (Moinina *et al.*, 2019).

Other fruit species

Stone fruits: Similar to apples, stone fruits such as peaches, cherries, and plums also require a certain chilling period (Fadón *et al.*, 2020) and are susceptible to high temperature stress, which can negatively affect their fruit set, fruit size, and fruit quality. Changes in rainfall patterns can also significantly affect stone fruit production (Kourgialas & Karatzas, 2016).

Citrus: Citrus species such as oranges, lemons, and grapefruits are generally more tolerant to higher temperatures compared to temperate climate fruits. However, climate change can also affect citrus production. The availability of water resources is of great importance for citrus cultivation, as citrus trees need significant amounts of water, especially through irrigation (Fares *et al.*, 2017). Deviations in rainfall patterns and an increased risk of drought can significantly reduce citrus yields. In addition, some citrus pests and diseases, such as citrus greening disease, can be further exacerbated by temperature increases (Ghini *et al.*, 2011).

Berries: Berry species such as strawberries, blueberries, and raspberries are particularly sensitive to temperature fluctuations (Nezhadahmadi *et al.*, 2015). Temperature changes can affect fruit set, size, quality, and yield (Nezhadahmadi *et al.*, 2015). The adequacy of water resources is also a critical factor for berry production. Since different berry species need different climatic conditions, the specific requirements of each species should be evaluated separately (Nezhadahmadi *et al.*, 2015).

Grapes (wine and table grapes): Grapevines are quite sensitive to temperature changes. Temperature can affect the composition of grape berries (sugar content, acidity, etc.) and therefore wine quality. Changes in rainfall patterns can also affect grape yields (Rogiers *et al.*, 2022).

Tropical fruits (mango, banana, papaya, avocado): Tropical fruit species such as mango, banana, papaya, and avocado exhibit a wide spectrum of sensitivity to the effects of climate change (Nath *et al.*, 2018). While some tropical fruits, such as mango (Lauri *et al.*, 2013), are relatively tolerant to high temperatures, others, such as bananas (Van Asten *et al*, 2011), are more sensitive to changes in rainfall patterns. Avocado, on the other hand, while showing a certain tolerance to high temperatures, needs a significant amount of water and is therefore vulnerable to drought and flood conditions (Taleb *et al*., 2022).

Adaptation and mitigation strategies

Coping with the complex and multidimensional challenges of global warming on fruit production requires a two-pronged strategic approach: mitigation efforts focused on reducing greenhouse gas emissions and adaptation strategies aimed at adapting to changing climatic conditions.

Climate change mitigation strategies (reducing greenhouse gas emissions)

While adaptation strategies aim to adapt to the inevitable effects of climate change, mitigation strategies focus on the root causes of climate change by reducing greenhouse gas emissions (Malhi *et al*., 2021). In the agricultural sector, mitigation strategies to reduce greenhouse gas emissions include various practices:

Adoption of sustainable agricultural practices: The widespread adoption of sustainable farming techniques can significantly reduce greenhouse gas emissions from agricultural activities (Baldock *et al*., 2012):

- **No-till or reduced tillage:** Practices that minimize or completely eliminate tillage reduce the amount of carbon dioxide released from the soil into the atmosphere and improve soil health. No-till or reduced tillage methods help carbon to be stored in the soil for longer and minimize soil erosion (Powlson *et al*., 2014);
- **Cover cropping:** Planting cover crops between fruit tree rows or during fallow periods after crop harvest significantly improves soil health, reduces soil erosion, and sequesters carbon from the atmosphere into the soil (Kaye & Quemada, 2017). Cover crops also positively affect soil water permeability and nutrient cycling (Scavo *et al*., 2022);
- **Efficient fertilizer use:** Optimizing the application rates and timing of fertilizers is an effective method to reduce emissions of nitrous oxide (N_2O), which is a potent greenhouse gas (Thapa *et al*., 2016). Precision agriculture technologies and soil-based fertilization allow farmers to use fertilizers more efficiently;
- **Improved manure management techniques:** Proper storage and processing of livestock manure can reduce greenhouse gas emissions such as methane (CH_4) and nitrous oxide (N_2O) (Thapa *et al*., 2016). Technologies such as anaerobic digestion and composting are effective manure management techniques for reducing greenhouse gas emissions (Zhang, Dou *et al*., 2013).

Use of renewable energy sources: Shifting to renewable energy sources such as solar energy, wind energy, or biogas in agricultural enterprises can significantly reduce greenhouse gas emissions from agricultural activities by reducing dependence on fossil fuels (Bilgili *et al*., 2024):

- **Solar energy systems:** Solar panels can be used to meet the energy needs of irrigation systems, farm buildings, and other on-farm energy needs, thereby reducing dependence on fossil fuel-based electricity (Bilgili *et al*., 2024);
- **Wind energy plants:** Wind turbines can generate electricity to meet the energy needs of farms, especially in regions with high wind energy potential (Bilgili *et al*., 2024);
- **Biogas production facilities:** Biogas production from anaerobic fermentation of agricultural waste and animal manure provides a renewable energy source that can be used for heating, electricity generation, and even as a transportation fuel (Alengebawy *et al*., 2024).

Preventing food loss and waste: Minimizing losses and waste occurring throughout the food chain plays a critical role in reducing greenhouse gas emissions from food production, processing, transportation, and disposal processes (Tang, 2020):

- **Improving harvesting and storage techniques:** Using advanced harvesting techniques, establishing modern storage facilities, and developing efficient transportation infrastructure to reduce postharvest crop losses can significantly reduce food waste (Moretti *et al*., 2010; Siddiqui *et al*., 2015);
- **Consumer awareness and education efforts:** Raising consumer awareness about food waste and disseminating practical information to reduce food waste at home can significantly contribute to reducing the overall amount of food waste (Reisch *et al*., 2021).

Atmospheric carbon sequestration in soil: Implementing practices to increase carbon sequestration in agricultural soils is an important strategy in the fight against climate change (Powlson *et al*., 2011). Methods such as afforestation, reforestation, and agroforestry contribute to the mitigation of climate change by sequestering carbon dioxide from the atmosphere into the soil (Baldock *et al*., 2012; Ghale *et al*., 2022):

- **Agroforestry systems:** Integrating trees into fruit orchards or vineyard areas (agroforestry) increases carbon sequestration as well as improving soil health and providing various ecological benefits (Ghale *et al.*, 2022). Agroforestry systems support biodiversity and create suitable habitats for beneficial insects and pollinators (Baldock *et al.*, 2012; Ghale *et al.*, 2022);
- **Afforestation and reforestation projects:** Planting trees on agriculturally unsuitable or marginal lands or converting idle agricultural lands into forest areas can significantly increase the storage of carbon from the atmosphere in the soil (Doelman *et al.*, 2020; Wang *et al.*, 2022).

Climate change adaptation strategies (adapting to changing climatic conditions)

Crop management strategies: Implementing effective crop management strategies to adapt to changing climatic conditions is crucial. These strategies must be informed by detailed ecophysiological studies that explore how different fruit species respond to variables such as temperature, water availability, and solar radiation (Fischer *et al.*, 2016). For instance, research on apple trees has shown that elevated temperatures reduce photosynthetic rates and increase respiration, affecting fruit quality and yield (Ameen *et al.*, 2023). Likewise, in peaches, high day and night temperatures have been linked to impaired fruit coloration and firmness (Jayasooriya *et al.*, 2025). Therefore, integrating findings from plant ecophysiology into crop management—such as adjusting planting dates, selecting climate-resilient cultivars, or optimizing irrigation schedules—is essential to ensure sustainable fruit production under changing climatic conditions.

- **Development and use of heat and drought-resistant varieties:** Developing and disseminating fruit varieties that exhibit high tolerance to temperature and drought stress is one of the basic adaptation strategies (Haokip *et al.*, 2020; Sayyad-Amin, 2022). This process includes the following approaches:
  - » **Traditional breeding methods:** Conventional breeding techniques can be effectively used to select and cross fruit varieties with desirable traits such as temperature and drought tolerance (Chapman *et al.*, 2012);
  - » **Marker-assisted selection (MAS):** Molecular markers can be used to identify gene regions associated with stress tolerance in plants (Chapman *et al.*, 2012; Gogorcena *et al.*, 2020). In this way, breeding programs can be carried out more quickly and efficiently;
  - » **Genetic engineering and CRISPR-Cas9 technology:** Gene editing technologies such as CRISPR-Cas9 offer the potential to increase stress tolerance by precisely altering the genetic structure of fruit trees (Bacelar *et al.*, 2024; Ndudzo *et al.*, 2024). These technologies can accelerate the development of climate-resistant fruit varieties.
- **Increasing irrigation efficiency:** In regions experiencing water scarcity, in particular, the implementation of water-saving irrigation systems such as drip irrigation and micro-sprinkling is very important (Peng *et al.*, 2024; Romero *et al.*, 2006). Rainwater harvesting and efficient water storage can also contribute to sustainable water management for fruit production (Zhang *et al.*, 2019; Zhang, Zhang *et al.*, 2018). In addition, the use of Regulated Deficit Irrigation (RDI)—a strategy that applies water below full crop evapotranspiration during less sensitive growth stages—has shown promising results in fruit crops such as pear, enhancing water use efficiency without significantly compromising yield or fruit quality (Vélez-Sánchez *et al.*, 2023).
  - » **Drip irrigation systems:** Drip irrigation minimizes water losses through evaporation and surface runoff by delivering water directly to the plant root zone (Peng *et al.*, 2024). This method significantly increases water use efficiency;
  - » **Micro-sprinkling systems:** Micro-sprinkling systems provide effective irrigation using less water than traditional sprinkler systems and contribute to water conservation (Taleb *et al.*, 2022);
  - » **Rainwater harvesting and storage:** Collecting and storing rainwater provides an additional water source for irrigation, reducing dependence on groundwater and surface water resources. Rainwater harvesting is a crucial practice in sustainable water management (Ward, 2014; Zhang *et al.*, 2019; Zhang, Zhang *et al.*, 2018);
  - » **Development of water storage infrastructure:** Improving water storage infrastructure such as dams, ponds, and groundwater storage facilities can help ensure a reliable supply of water during drought periods (Shakoor & Ullah, 2024).
- **Shading structures and other protective measures:** The use of shading nets, reflective mulches, and other protective materials can help reduce heat stress by protecting fruit trees from extreme heat and adverse weather conditions (Mditshwa *et al.*, 2019).

  - **Shading nets:** Shading nets can reduce the temperature of the canopy above fruit trees by reducing solar radiation, thereby alleviating heat stress (Kalcsits *et al.*, 2017);
  - **Reflective mulches:** Reflective mulch materials can reduce soil temperature and heat stress on plants by reflecting sunlight away from trees and soil (Kalcsits *et al.*, 2017);
  - **Windbreakers:** Planting windbreak rows of plants around orchards protects trees from strong winds, reducing fruit drop and physical damage to trees (Chawla *et al.*, 2011; Veste *et al.*, 2020);
  - **Hail nets:** Protective nets against hail effectively protect fruits from hail damage, preventing significant crop losses that may occur during hailstorms (Bal *et al.*, 2014; Brglez Sever *et al.*, 2015).
- **Adjusting planting times and growing seasons:** Optimizing planting dates and growing seasons can help synchronize fruit production with more suitable climatic conditions. However, such adjustments must be based on the systematic collection and analysis of long-term climatic data, including temperature trends, rainfall patterns, and the timing of frost events. Agroclimatic modeling tools and decision support systems are increasingly used to assess the suitability of specific planting windows, helping to avoid critical stress periods and align phenological stages with favorable environmental conditions (IPCC, 2021; Osorio-Marín *et al.*, 2024). For example, in temperate regions, shifting planting schedules by even one or two weeks has been shown to reduce exposure to early-season heatwaves or late frosts, thereby improving fruit set and quality (Fischer *et al.*, 2016).
  - **Delayed planting practices:** In some regions, delaying planting dates beyond the regular schedule can help avoid late spring frosts or extreme heat stress in the summer months (Campoy *et al.*, 2011; Chawla *et al.*, 2011);
  - **Flexibility in variety selection:** Choosing fruit varieties with different chilling requirements or different ripening times is one way to better adapt to changing climatic conditions (Malhotra, 2017);
  - **Second crop or intercropping systems:** In regions where climatic conditions are suitable, implementing second crop or intercropping systems can maximize resource use and potentially increase overall productivity (Burgess *et al.*, 2022).
- **Strengthening integrated pest and disease management (IPM) strategies:** In changing climatic conditions, implementing effective pest and disease management strategies is vital for sustainable fruit production.
  - **Integrated pest management (IPM) approaches:** IPM strategies offer significant advantages to fruit growers in coping with the effects of climate change (Bacelar *et al.*, 2024; Moinina *et al.*, 2019):
    - **Biological control methods:** The use of natural enemies such as predatory insects and parasitoids should be encouraged to control pest populations naturally (Bacelar *et al.*, 2024);
    - **Cultural measures:** Cultural methods such as crop rotation (where appropriate), sanitation practices, and the use of resistant varieties play an important role in preventing pest and disease outbreaks (Gruda *et al.*, 2019);
    - **Targeted pesticide applications:** When the use of chemical pesticides is unavoidable, only necessary and targeted applications should be preferred, in a way that minimizes adverse effects on the environment and protects beneficial organisms (Zhou *et al.*, 2024).
- **Promoting biological control:** Expanding the use of natural enemies to control pests offers a more sustainable approach by reducing dependence on chemical pesticides (Bacelar *et al.*, 2024; Thomson *et al.*, 2010):
  - **Use of predatory insects and mites:** Increasing the populations of predatory insect and mite species that feed on fruit pests or releasing them into orchards can help reduce pest pressure naturally (Thomson *et al.*, 2010);
  - **Use of parasitoids:** Beneficial organisms such as parasitoid wasps can be effective in controlling fruit pests by parasitizing them and controlling their populations (Prasad & Bambawale, 2010; Thomson *et al.*, 2010);
  - **Biological control with microbial agents:** Microbial agents such as bacteria like *Bacillus thuringiensis* (Bt), beneficial fungi, or viruses can be used as biological pesticides against pests and offer an environmentally friendly alternative (Sangiorgio *et al.*, 2020; Thomson *et al.*, 2010).
- **Soil health improvement management:** Adopting practices to improve soil health is critical to increase the resistance of fruit trees to climate change.

  - **Strategies to increase soil organic matter:** Increasing soil organic matter content forms the basis of soil health and resilience to climate change (Lal, 2013). Healthy soils are more resistant to drought and erosion and contribute to long-term sustainability for fruit production (Sierra *et al.*, 2015):
    - **Cover crops:** Cover crops significantly enrich soil organic matter content in addition to preventing soil erosion and increasing carbon sequestration (Kaye & Quemada, 2017; Scavo *et al.*, 2022);
    - **Compost applications:** Regularly applying compost to orchards and vineyards increases the water-holding capacity of the soil, improves soil structure, and raises soil organic matter levels (Montanaro *et al.*, 2017);
    - **No-till or reduced tillage:** Agricultural techniques that do not till the soil or till it at a minimum level help protect organic matter without disrupting the soil structure and support soil health (Montanaro *et al.*, 2017; Powlson *et al.*, 2014).
- **Policy and economic support mechanisms:** The effective use of policy and economic tools is of great importance to promote and support adaptation to climate change in the fruit growing sector.
  - **Subsidy and incentive programs:** Governments can implement various subsidy and incentive programs to encourage farmers to adopt sustainable agricultural practices and invest in climate-resistant technologies. These programs help farmers finance the adaptation process and manage risks:
    - **Financial support for sustainable practices:** Subsidies, grant programs, and cost-sharing mechanisms play a crucial role in alleviating the financial burden faced by farmers as they transition to sustainable agricultural practices and invest in climate-resistant technologies (Piñeiro *et al.*, 2020);
    - **Tax advantages for climate-friendly investments:** Practices such as tax deductions and tax exemptions can encourage farmers to modernize irrigation systems, build shading structures, and invest in other adaptation measures (Beddington *et al.*, 2011).
- **Increasing research and development (R&D) investments:** Continuous investment in R&D activities is essential for promoting innovation in precision agriculture and smart farming techniques in fruit cultivation (Lee *et al.*, 2014). These investments accelerate the development of new technologies and methods, thereby enhancing the sector's competitiveness.
  - **Increasing public R&D funds:** Increasing public R&D funds for research in critical areas such as developing climate-resistant fruit varieties, improving water efficiency techniques, and effective pest and disease management strategies is vital (Beddington *et al.*, 2011);
  - **Public-private sector collaborations:** Collaboration and partnerships between public research institutions and private sector companies can accelerate innovation in smart agriculture technologies, ensuring that the developed new technologies reach farmers more quickly (Manos *et al.*, 2014; Zhang *et al.*, 2018).
- **Developing climate risk insurance systems:** Establishing and disseminating climate insurance programs can help fruit growers manage financial risks arising from climate change-related crop losses.
  - **Crop insurance against extreme weather events:** Insurance programs that cover crop losses caused by extreme weather events such as heatwaves, droughts, frost events, and hailstorms can provide an important safety net for fruit producers (Mârza *et al.*, 2015);
  - **Index-based insurance systems:** Index-based insurance systems that make payments based on specific weather parameters, such as rainfall amounts or temperature values, can offer a more efficient and cost-effective alternative to traditional insurance methods (Ricome *et al.*, 2017).
- **Strengthening education and extension activities:** Effectively delivering information and technology to farmers and capacity building efforts are among the keys to a successful adaptation process.
  - **Modernization of agricultural extension services:** Strengthening agrarian extension services and supporting them with modern methods play a critical role in delivering up-to-date information and training on climate-smart agriculture practices to farmers (Asfaw *et al.*, 2019);
  - **Farmer field schools and practical training:** Farmer field schools contribute to the dissemination of sustainable agricultural techniques and climate adaptation strategies by providing practical training opportunities and promoting the sharing of knowledge (Davis *et al.*, 2012);
  - **Online information resources and decision support systems:** Developing online information platforms and decision support tools can provide farmers with easily accessible climate data, best practice examples, and information to assist them in decision-making processes (Zhai *et al.*, 2020).

Future perspectives and necessary measures

The future of fruit production in an increasingly warming world depends on implementing effective adaptation strategies and joint efforts to reduce greenhouse gas emissions. The following key recommendations should be considered to increase the long-term sustainability and resilience of fruit production to climate change:

Priority should be given to research on climate-resistant fruit varieties: Future research and development efforts should focus on developing fruit varieties that are particularly tolerant to temperature and drought, resistant to pests and diseases, able to use water and nutrient resources efficiently, and able to maintain or improve fruit quality even under changing climatic conditions (Chapman *et al*., 2012; Nath *et al*., 2018). Advanced breeding technologies such as marker-assisted selection and genetic engineering should be used effectively to accelerate the development and dissemination of climate-resistant varieties (Gogorcena *et al*., 2020; Muranty *et al*., 2014).

Investments should be made in efficient irrigation and water management infrastructure: Increasing water storage capacity through dams, ponds, and groundwater storage projects is of critical importance (Shakoor & Ullah, 2024). The adoption of water-saving irrigation systems such as drip irrigation and micro-sprinkling should be supported and disseminated through incentive programs and technical support services (Peng *et al*., 2024; Romero *et al*., 2006). Rainwater harvesting projects should be encouraged and supported at both the farm and community level (Zhang, Zhang *et al*., 2018; Zhang *et al*., 2019). Regulatory and economic mechanisms such as water pricing policies and water audit programs should be implemented to increase water use efficiency in agriculture (Peng *et al*., 2024; Romero *et al*., 2006).

The adoption of sustainable agricultural practices should be encouraged: Practices to protect and improve soil health should be disseminated through financial incentives and technical assistance mechanisms to encourage farmers to adopt reduced tillage, cover crops, composting, and other soil health-improving practices (Beddington *et al*., 2011; Piñeiro *et al*., 2020). Integrated pest management (IPM) strategies should be promoted and facilitated through research studies, extension services, and supportive legal regulations (Gvozdenac *et al*., 2022). The potential of supporting and disseminating organic farming systems should be evaluated. Organic farming offers an approach that prioritizes soil health, biodiversity, and the reduction of synthetic inputs (Muller *et al*., 2017).

Cooperation between researchers, extension experts, policy makers and fruit growers should be strengthened: Interdisciplinary research projects involving experts from different disciplines (breeders, agricultural engineers, soil scientists, entomologists, plant pathologists, economists and social scientists) should be encouraged to tackle the complex challenges of climate change in the fruit growing sector (Reyes-García *et al*., 2019). Extension and information networks should be strengthened to effectively disseminate research results and best practices to fruit growers (Below *et al*., 2012). Actively involving fruit growers, other stakeholders in the sector, and policymakers in the development and implementation of climate adaptation strategies is critical to increasing the relevance and adoption of these strategies.

Development and implementation of policies supporting adaptation to climate changes in the fruit sector: Climate adaptation plans should be prepared for the fruit growing sector at national and regional level, and sector-specific targets, strategies and actionable steps should be defined in detail (Zhang *et al*., 2018). Policy incentives such as subsidies, tax breaks and insurance schemes should be used effectively to promote climate adaptation and greenhouse gas (GHG) emissions reductions (Del Pozo *et al*., 2019; Ghale *et al*., 2022). Regulatory frameworks should be established that prioritise environmental sustainability objectives in fruit production, such as sustainable management of water resources, reduction of pesticide use, and protection of soil health.

Conclusions

Global warming presents significant challenges to fruit production worldwide. Rising temperatures, shifting precipitation patterns, and increased frequency of extreme weather events are impacting fruit yields, quality, and geographic distribution. Adapting to these changes is crucial for ensuring the long-term sustainability of the fruit industry. This requires a multifaceted approach, including the development of heat- and drought-tolerant varieties, improving irrigation efficiency, implementing sustainable farming practices, and adopting integrated pest and disease management strategies. Mitigating climate change through the reduction of greenhouse gas emissions is also essential. Policy interventions, such as subsidies, incentives, and research funding, can play a critical role in supporting farmers in their efforts to adapt to a changing climate. By taking proactive steps to address these challenges, we can help ensure the future of fruit production and the availability of nutritious and delicious fruits for generations to come.

Conflict of interest statement

The author declares that there is no conflict of interests regarding the publication of this article.

Author´s contributions

AI prepared the manuscript and approved its final version.

Literature cited

Alengebawy, A., Ran, Y., Osman, A. I., Jin, K., Samer, M., & Ai, P. (2024). Anaerobic digestion of agricultural waste for biogas production and sustainable bioenergy recovery: A review. *Environmental Chemistry Letters, 22*(1), 2641–2668. https://doi.org/10.1007/s10311-024-01789-1

Ameen, M., Mahmood, A., Ahmad, M., Javaid, M., Nadeem, M. A., Asif, M., Balal, R. M., & Khan, B. A. (2023). Impacts of climate change on fruit physiology and quality. In M. Hasanuzzaman (Ed.), *Climate-resilient agriculture* (Vol. 1, pp. 93–124). Springer Cham. https://doi.org/10.1007/978-3-031-37424-1_5

Asfaw, A., Simane, B., Bantider, A., & Hassen, A. (2019). Determinants in the adoption of climate change adaptation strategies: Evidence from rainfed-dependent smallholder farmers in north-central Ethiopia (Woleka sub-basin). *Environment, Development and Sustainability, 21*, 2535–2565. https://doi.org/10.1007/s10668-018-0150-y

Bacelar, E., Pinto, T., Anjos, R., Morais, M. C., Oliveira, I., Vilela, A., & Cosme, F. (2024). Impacts of climate change and mitigation strategies for some abiotic and biotic constraints influencing fruit growth and quality. *Plants, 13*(14), Article 1942. https://doi.org/10.3390/plants13141942

Bal, S. K., Saha, S., Fand, B. B., Singh, N. P., Rane, J., & Minhas, P. S (Eds.). (2014). *Hailstorms: Causes, damage and post-hail management in agriculture* (Technical bulletin No. 5). National Institute of Abiotic Stress Management. https://doi.org/10.13140/2.1.4841.7922

Baldock, J. A., Wheeler, I., McKenzie, N., & McBrateny, A. (2012). Soils and climate change: Potential impacts on carbon stocks and greenhouse gas emissions, and future research for Australian agriculture. *Crop and Pasture Science, 63*(3), 269–283. https://doi.org/10.1071/CP11170

Beddington, J. R., Asaduzzaman, M., Fernandez, A., Clark, M., Guillou, M., Jahn, M., Erda, L., Mamo, T., Bo, N. D., Nobre, C. A., Scholes, R., Sharma, R., & Wakhungu, J. (2011). *Achieving food security in the face of climate change: Summary for policy makers from the Commission on Sustainable Agriculture and Climate Change*. CGIAR Research Program on Climate Change, Agriculture and Food Security. https://hdl.handle.net/10568/10701

Below, T. B., Mutabazi, K. D., Kirschke, D., Franke, C., Sieber, S., Siebert, R., & Tscherning, K. (2012). Can farmers' adaptation to climate change be explained by socio-economic household-level variables? *Global Environmental Change, 22*(1), 223–235. https://doi.org/10.1016/j.gloenvcha.2011.11.012

Bilgili, M., Tumse, S., & Nar, S. (2024). Comprehensive overview on the present state and evolution of global warming, climate change, greenhouse gases, and renewable energy. *Arabian Journal for Science and Engineering, 49*, 14503–14531. https://doi.org/10.1007/s13369-024-09390-y

Brglez Sever, M., Tojnko, S., & Unuk, T. (2015). Impact of various types of anti-hail nets on light exposure in orchards and quality parameters of apples: A review. *Agricultura, 12*(1/2), 25–31. https://www.researchgate.net/publication/309623554_Impact_of_various_types_of_anti-hail_nets_on_light_exposure_in_orchards_and_quality_parameters_of_apples-_a_rewiev

Calzadilla, A., Zhu, T., Rehdanz, K., Tol, R. S. J., & Ringler, C. (2014). Climate change and agriculture: Impacts and adaptation options in South Africa. *Water Resources and Economics, 5*, 24–48. https://doi.org/10.1016/j.wre.2014.03.001

Campoy, J. A., Ruiz, D., & Egea, J. (2011). Dormancy in temperate fruit trees in a global warming context: A review. *Scientia Horticulturae, 130*(2), 357–372. https://doi.org/10.1016/j.scienta.2011.07.011

Chapman, S. C., Chakraborty, S., Dreccer, M. F., & Howden, S. M. (2012). Plant adaptation to climate change – Opportunities and priorities in breeding. *Crop and Pasture Science, 63*(3), 251–268. https://doi.org/10.1071/CP11303

Chawla, R., Sheokand, A., Rai, M. R., & Sadawarti, R. K. (2011). Impact of climate change on fruit production and various approaches to mitigate these impacts. *The Pharma Innovation, 10*(3), 564–571. https://www.thepharmajournal.com/archives/2021/vol10issue3/PartH/10-2-96-689.pdf

Davis, K., Nkonya, E., Kato, E., Mekonnen, D. A., Odendo, M., Miiro, R., & Nkuba, J. (2012). Impact of farmer field schools on agricultural productivity and poverty in East Africa. *World Development, 40*(2), 402–413. https://doi.org/10.1016/j.worlddev.2011.05.019

Del Pozo, A., Brunel-Saldias, N., Engler, A., Ortega-Farias, S., Acevedo-Opazo, C., Lobos, G. A., Jara-Rojas, R., & Molina-Montenegro, M. A. (2019). Climate change impacts and adaptation strategies of agriculture in Mediterranean-climate regions (MCRs). *Sustainability, 11*(10), Article 2769. https://doi.org/10.3390/su11102769

Doelman, J. C., Stehfest, E., van Vuuren, D. P., Tabeau, A., Hof, A. F., Braakhekke, M. C., Gernaat, D. E. H. J., van den Berg, M., van Zeist, W.-J., Daioglou, V., van Meijl, H., & Lucas, P. L. (2020). Afforestation for climate change mitigation: Potentials, risks and trade-offs. *Global Change Biology, 26*(3), 1576–1591. https://doi.org/10.1111/gcb.14887

El Jaouhari, N., Abouabdillah, A., Bouabid, R., Bourioug, M., Aleya, L., & Chaoui, M. (2018). Assessment of sustainable deficit irrigation in a Moroccan apple orchard as a climate change adaptation strategy. *Science of the Total Environment, 642*, 574–581. https://doi.org/10.1016/j.scitotenv.2018.06.108

El Yaacoubi, A., Malagi, G., Oukabli, A., Hafidi, M., & Legave, J.-M. (2014). Global warming impact on floral phenology of fruit tree species in Mediterranean region. *Scientia Horticulturae, 180*, 243–253. https://doi.org/10.1016/j.scienta.2014.10.041

Fadón, E., Herrera, S., Guerrero, B. I., Guerra, M. E., & Rodrigo, J. (2020). Chilling and heat requirements of temperate stone fruit trees (*Prunus* sp.). *Agronomy, 10*(3), Article 409. https://doi.org/10.3390/agronomy10030409

Fares, A., Bayabil, H. K., Zekri, M., Mattos-Jr, D., & Awal, R. (2017). Potential climate change impacts on citrus water requirement across major producing areas in the world. *Journal of Water and Climate Change, 8*(4), 576–592. https://doi.org/10.2166/wcc.2017.182

Fischer, G., Ramírez, F., & Casierra-Posada, F. (2016). Ecophysiological aspects of fruit crops in the era of climate change: A review. *Agronomía Colombiana, 34*(2), 190–199. https://doi.org/10.15446/agron.colomb.v34n2.56799

Flórez-Velasco, N., Fischer, G., & Balaguera-López, H. E. (2024). Photosynthesis in fruit crops of the high tropical Andes: A systematic review. *Agronomía Colombiana, 42*(2), 1–18. https://doi.org/10.15446/agron.colomb.v42n2.113887

Forrest, J. R. K. (2015). Plant–pollinator interactions and phenological change: What can we learn about climate impacts from experiments and observations? *Oikos, 124*(1), 4–13. https://doi.org/10.1111/oik.01386

Fu, Y. S., Prevéy, J. S., & Vitasse, Y. (2022). Plant phenology shifts and their ecological and climatic consequences. *Frontiers in Plant Science, 13*, Article 1071266. https://doi.org/10.3389/fpls.2022.1071266

Fujisawa, M., & Kobayashi, K. (2010). Apple (*Malus pumila* var. domestica) phenology is advancing due to rising air temperature in northern Japan. *Global Change Biology, 16*(10), 2651–2660. https://doi.org/10.1111/j.1365-2486.2009.02126.x

Funes, I., Aranda, X., Biel, C., Carbó, J., Camps, F., Molina, A. J., de Herralde, F., & Grau, B. (2016). Future climate change impacts on apple flowering date in a Mediterranean subbasin. *Agricultural Water Management, 164*, 19–27. https://doi.org/10.1016/j.agwat.2015.06.013

Ghale, B., Mitra, E., Sodhi, H. S., Verma, A. K., & Kumar, S. (2022). Carbon sequestration potential of agroforestry systems and its potential in climate change mitigation. *Water, Air, & Soil Pollution, 233*(7), Article 228. https://doi.org/10.1007/s11270-022-05689-4

Ghini, R., Bettiol, W., & Hamada, E. (2011). Diseases in tropical and plantation crops as affected by climate changes: Current knowledge and perspectives. *Plant Pathology, 60*(1), 122–132. https://doi.org/10.1111/j.1365-3059.2010.02403.x

Gogorcena, Y., Sánchez, G., Moreno-Vázquez, S., Pérez, S., & Ksouri, N. (2020). Genomic-based breeding for climate-smart peach varieties. In C. Kole (Ed.), *Genomic designing of climate-smart fruit crops* (pp. 271–331). Springer Cham. https://doi.org/10.1007/978-3-319-97946-5_8

González-Martínez, E., Munné-Bosch, S., & Lordan, J. (2025). Chilling and heat requirements of apple cultivars: Future perspectives in a global climate change context. *Scientia Horticulturae, 345*, Article 114134. https://doi.org/10.1016/j.scienta.2025.114134

Gruda, N., Bisbis, M., & Tanny, J. (2019). Influence of climate change on protected cultivation: Impacts and sustainable adaptation strategies – A review. *Journal of Cleaner Production, 225*, 481–495. https://doi.org/10.1016/j.jclepro.2019.03.210

Gvozdenac, S., Dedić, B., Mikić, S., Ovuka, J., & Miladinović, D. (2022). Impact of climate change on integrated pest management strategies. In N. Benkeblia (Ed.), *Climate change and agriculture: Perspectives, sustainability and resilience* (pp. 311–372). John Wiley & Sons, Ltd. https://doi.org/10.1002/9781119789789.ch14

Haokip, S. W., Shankar, K., & Lalrinngheta, J. (2020). Climate change and its impact on fruit crops. *Journal of Pharmacognosy and Phytochemistry, 9*(1), 435–438. https://www.phytojournal.com/archives/2020/vol9issue1/PartG/8-6-492-960.pdf

Harvell, C. D., Mitchell, C. E., Ward, J. R., Altizer, S., Dobson, A. P., Ostfeld, R. S., & Samuel, M. D. (2002). Climate warming and disease risks for terrestrial and marine biota. *Science, 296*(5570), 2158–2162. https://doi.org/10.1126/science.1063699

Head, L., Adams, M., McGregor, H. V., & Toole, S. (2014). Climate change and Australia. *WIREs Climate Change, 5*(2), 175–197. https://doi.org/10.1002/wcc.255

IPCC. (2021). *Climate change 2021: The physical science basis*. Cambridge University Press. https://doi.org/10.1017/9781009157896

Jayasooriya, L. S. H., Kwack, Y. B., Shin, M. H., Wijethunga, W. M. U. D., Kim, G. H., Moon, Y. J., Kim, S. H., Cho, J. G., & Kim, J. G. (2025). Assessing the impact of elevated day and night temperatures on flesh red coloration and diminished quality in peaches. *Horticultural Science and Technology, 43*(2), 165–181. https://doi.org/10.7235/HORT.20250015

Jones, R. A. C., & Barbetti, M. J. (2012). Influence of climate change on plant disease infections and epidemics caused by viruses and bacteria. *CABI Reviews, 2012*, 1–33. https://doi.org/10.1079/PAVSNNR20127022

Kalcsits, L., Musacchi, S., Layne, D. R., Schmidt, T., Mupambi, G., Serra, S., Mendoza, M., Asteggiano, L., Jarolmasjed, S., Sankaran, S., Khot, L. R., & Zúñiga Espinoza, C. (2017). Above and below-ground environmental changes associated with the use of photoselective protective netting to reduce sunburn in apple. *Agricultural and Forest Meteorology, 237–238*, 9–17. https://doi.org/10.1016/j.agrformet.2017.01.016

Karagatiya, F. P., Patel, S., Parasana, J. S., Vasava, H. V., Chaudhari, T. M., Kanzaria, D. R., & Paramar, V. (2023). Adapting fruit crops to climate change: Strengthening resilience and implementing adaptation measures in fruit crops. *The Pharma Innovation Journal, 12*(7), 3159–3164. https://www.thepharmajournal.com/archives/?year=2023&vol=12&issue=7&ArticleId=21776

Kaye, J. P., & Quemada, M. (2017). Using cover crops to mitigate and adapt to climate change: A review. *Agronomy for Sustainable Development, 37*, Article 4. https://doi.org/10.1007/s13593-016-0410-x

Kourgialas, N. N., & Karatzas, G. P. (2016). A flood risk decision making approach for Mediterranean tree crops using GIS; climate change effects and flood-tolerant species. *Environmental Science & Policy, 63*, 132–142. https://doi.org/10.1016/j.envsci.2016.05.020

Kumar, R., Berwal, M. K., & Saroj, P. L. (2019). Morphological, physiological, biochemical and molecular facet of drought stress in horticultural crops. *International Journal of Bio-resource and Stress Management, 10*(5), 545–560. https://www.researchgate.net/publication/337982716_Morphological_Physiological_Biochemical_and_Molecular_Facet_of_Drought_Stress_in_Horticultural_Crops

Lamichhane, J. R. (2021). Rising risks of late-spring frosts in a changing climate. *Nature Climate Change, 11*(7), 554–555. https://doi.org/10.1038/s41558-021-01090-x

Lee, D. R., Edmeades, S., De Nys, E., McDonald, A., & Janssen, W. (2014). Developing local adaptation strategies for climate change in agriculture: A priority-setting approach with application to Latin America. *Global Environmental Change, 29*, 78–91. https://doi.org/10.1016/j.gloenvcha.2014.08.002

Li, Z., & Fang, H. (2016). Impacts of climate change on water erosion: A review. *Earth-Science Reviews, 163*, 94–117. https://doi.org/10.1016/j.earscirev.2016.10.004

Luedeling, E. (2012). Climate change impacts on winter chill for temperate fruit and nut production: A review. *Scientia Horticulturae, 144*, 218–229. https://doi.org/10.1016/j.scienta.2012.07.011

Luedeling, E., Girvetz, E. H., Semenov, M. A., & Brown, P. H. (2011). Climate change affects winter chill for temperate fruit and nut trees. *PLoS ONE, 6*(5), Article e20155. https://doi.org/10.1371/journal.pone.0020155

Malhi, G. S., Kaur, M., & Kaushik, P. (2021). Impact of climate change on agriculture and its mitigation strategies: A review. *Sustainability, 13*(3), Article 1318. https://doi.org/10.3390/su13031318

Malhotra, S. K. (2017). Horticultural crops and climate change: A review. *The Indian Journal of Agricultural Sciences, 87*(1), 12–22. https://doi.org/10.56093/ijas.v87i1.67138

Manos, B., Partalidou, M., Fantozzi, F., Arampatzis, S., & Papadopoulou, O. (2014). Agro-energy districts contributing to environmental and social sustainability in rural areas: Evaluation of a local public–private partnership scheme in Greece. *Renewable and Sustainable Energy Reviews, 29*, 85–95. https://doi.org/10.1016/j.rser.2013.08.080

Mârza, B., Angelescu, C., & Tindeche, C. (2015). Agricultural insurances and food security. The new climate change challenges. *Procedia Economics and Finance, 27*, 594–599. https://doi.org/10.1016/S2212-5671(15)01038-2

McGee, T., Schaffer, B., Shahid, M. A., Chaparro, J. X., & Sarkhosh, A. (2022). Carbon and nitrogen metabolism in peach trees on different *Prunus* rootstocks in response to flooding. *Plant and Soil, 475*(1–2), 427–441. https://doi.org/10.1007/s11104-022-05377-6

Millard, J., Outhwaite, C. L., Ceaușu, S., Carvalheiro, L. G., Silva e Silva, F. D., Dicks, L. V., Ollerton, J., & Newbold, T. (2023). Key tropical crops at risk from pollinator loss due to climate change and land use. *Science Advances, 9*(41), Article eadh0756. https://doi.org/10.1126/sciadv.adh0756

Moinina, A., Lahlali, R., & Boulif, M. (2019). Important pests, diseases and weather conditions affecting apple production in Morocco: Current state and perspectives. *Revue Marocaine des Sciences Agronomiques et Vétérinaires, 7*(1), 71–87. https://www.agrimaroc.org/index.php/Actes_IAVH2/article/view/669

Montanaro, G., Xiloyannis, C., Nuzzo, V., & Dichio, B. (2017). Orchard management, soil organic carbon and ecosystem services in Mediterranean fruit tree crops. *Scientia Horticulturae, 217*, 92–101. https://doi.org/10.1016/j.scienta.2017.01.012

Moretti, C. L., Mattos, L. M., Calbo, A. G., & Sargent, S. A. (2010). Climate changes and potential impacts on postharvest quality of fruit and vegetable crops: A review. *Food Research International, 43*(7), 1824–1832. https://doi.org/10.1016/j.foodres.2009.10.013

Muller, A., Schader, C., El-Hage Scialabba, N., Brüggemann, J., Isensee, A., Erb, K.-H., Smirh, P., Klocke, P., Leiber, F., Stolze, M., & Niggli, U. (2017). Strategies for feeding the world more sustainably with organic agriculture. *Nature Communications, 8*(1), Article 1290. https://doi.org/10.1038/s41467-017-01410-w

Muranty, H., Jorge, V., Bastien, C., Lepoittevin, C., Bouffier, L., & Sanchez, L. (2014). Potential for marker-assisted selection for forest tree breeding: Lessons from 20 years of MAS in crops. *Tree Genetics & Genomes, 10*(6), 1491–1510. https://doi.org/10.1007/s11295-014-0790-5

Nath, V., Kumar, G., Pandey, S. D., & Pandey, S. (2018). Impact of climate change on tropical fruit production systems and its mitigation strategies. In S. Sheraz Mahdi (Ed.), *Climate change and agriculture in India: Impact and adaptation* (pp. 129–146). Springer, Cham. https://doi.org/10.1007/978-3-319-90086-5_11

Nawaz, R., Abbasi, N. A., Hafiz, I. A., & Khalid, A. (2020). Increasing level of abiotic and biotic stress on Kinnow fruit quality at different ecological zones in climate change scenario. *Environmental and Experimental Botany, 171*, Article 103936. https://doi.org/10.1016/j.envexpbot.2019.103936

Ndudzo, A., Makuvise, A. S., Moyo, S., & Bobo, E. D. (2024). CRISPR–Cas9 genome editing in crop breeding for climate change resilience: Implications for smallholder farmers in Africa. *Journal of Agriculture and Food Research, 16*, Article 101132. https://doi.org/10.1016/j.jafr.2024.101132

Nezhadahmadi, A., Faruq, G., & Rashid, K. (2015). The impact of drought stress on morphological and physiological parameters of three strawberry varieties in different growing conditions. *Pakistan Journal of Agricultural Sciences, 52*(1), 79–92. https://www.cabidigitallibrary.org/doi/full/10.5555/20153183022

Noorazar, H., Kalcsits, L., Jones, V. P., Jones, M. S., & Rajagopalan, K. (2022). Climate change and chill accumulation: Implications for tree fruit production in cold-winter regions. *Climatic Change, 171*(1), Article 34. https://doi.org/10.1007/s10584-022-03339-6

Normand, F., Lauri, P.-E., & Legave, J. M. (2013). Climate change and its probable effects on mango production and cultivation. *Acta Horticulturae, 1075*, 21–23. https://doi.org/10.17660/ActaHortic.2015.1075.1

Osorio-Marín, J., Fernandez, E., Vieli, L., Ribera, A., Luedeling, E., & Cobo, N. (2024). Climate change impacts on temperate fruit and nut production: A systematic review. *Frontiers in Plant Science, 15*, Article 1352169. https://doi.org/10.3389/fpls.2024.1352169

Pathak, T. B., Maskey, M. L., Dahlberg, J. A., Kearns, F., Bali, K. M., & Zaccaria, D. (2018). Climate change trends and impacts on California agriculture: A detailed review. *Agronomy, 8*(3), Article 25. https://doi.org/10.3390/agronomy8030025

Peng, X., Chen, D., Zhen, J., Wang, Y., & Hu, X. (2024). Greenhouse gas emissions and drivers of the global warming potential of vineyards under different irrigation and fertilizer management practices. *Science of The Total Environment, 950*, Article 175447. https://doi.org/10.1016/j.scitotenv.2024.175447

Piñeiro, V., Arias, J., Dürr, J., Elverdin, P., Ibáñez, A. M., Kinengyere, A., Morales Opazo, C., Owoo, N., Page, J. R., Prager, S. D., & Torero, M. (2020). A scoping review on incentives for adoption of sustainable agricultural practices and their outcomes. *Nature Sustainability, 3*(10), 809–820. https://doi.org/10.1038/s41893-020-00617-y

Powlson, D. S., Stirling, C. M., Jat, M. L., Gerard, B. G., Palm, C. A., Sanchez, P. A., & Cassman, K. G. (2014). Limited potential of no-till agriculture for climate change mitigation. *Nature Climate Change, 4*(8), 678–683. https://doi.org/10.1038/nclimate2292

Powlson, D. S., Whitmore, A. P., & Goulding, K. W. (2011). Soil carbon sequestration to mitigate climate change: A critical re-examination to identify the true and the false. *European Journal of Soil Science, 62*(1), 42–55. https://doi.org/10.1111/j.1365-2389.2010.01342.x

Prasad, Y. G., & Bambawale, O. M. (2010). Effects of climate change on natural control of insect pests. *Indian Journal of Dryland Agricultural Research and Development, 25*(2), 1–12. https://www.indianjournals.com/ijor.aspx?target=ijor:ijdard&volume=25&issue=2&article=001

Raju, C., Pazhanivelan, S., Perianadar, I. V., Kaliaperumal, R., Sathyamoorthy, N. K., & Sendhilvel, V. (2024). Climate change as an existential threat to tropical fruit crop production – A review. *Agriculture, 14*(11), Article 2018. https://doi.org/10.3390/agriculture14112018

Ramírez, F., & Kallarackal, J. (2015). Climate change and chilling requirements. In F. Ramírez, & J. Kallarackal (Eds.), *Responses of fruit trees to global climate change* (pp. 31–34). Springer, Cham. https://doi.org/10.1007/978-3-319-14200-5_9

Reisch, L. A., Sunstein, C. R., Andor, M. A., Doebbe, F. C., Meier, J., & Haddaway, N. R. (2021). Mitigating climate change via food consumption and food waste: A systematic map of behavioral interventions. *Journal of Cleaner Production, 279*, Article 123717. https://doi.org/10.1016/j.jclepro.2020.123717

Reyes-García, V., García-del-Amo, D., Benyei, P., Fernández-Llamazares, Á., Gravani, K., Junqueira, A. B., Labeyrie, V., Li, X., Matias, D. M. S., McAlvay, A., Mortyn, P. G., Porcuna-Ferrer, A., Schlingmann, A., & Soleymani-Fard, R. (2019). A collaborative approach to bring insights from local observations of climate change impacts into global climate change research. *Current Opinion in Environmental Sustainability, 39*, 1–8. https://doi.org/10.1016/j.cosust.2019.04.007

Ricome, A., Affholder, F., Gérard, F., Muller, B., Poeydebat, C., Quirion, P., & Sall, M. (2017). Are subsidies to weather-index insurance the best use of public funds? A bio-economic farm model applied to the Senegalese groundnut basin. *Agricultural Systems, 156*, 149–176. https://doi.org/10.1016/j.agsy.2017.05.015

Rodríguez, A., Pérez-López, D., Centeno, A., & Ruiz-Ramos, M. (2021). Viability of temperate fruit tree varieties in Spain under climate change according to chilling accumulation. *Agricultural Systems, 186*, Article 102961. https://doi.org/10.1016/j.agsy.2020.102961

Romero, P., García, J., & Botía, P. (2006). Cost–benefit analysis of a regulated deficit-irrigated almond orchard under subsurface drip irrigation conditions in Southeastern Spain. *Irrigation Science, 24*(3), 175–184. https://doi.org/10.1007/s00271-005-0008-6

Ryu, S., Han, H.-H., Jeong, J. H., Kwon, Y., Han, J. H., Do, G. R., Choi, I.-M., & Lee, H. J. (2017). Night temperatures affect fruit coloration and expressions of anthocyanin biosynthetic genes in 'Hongro' apple (*Malus domestica*) fruit skins. *European Journal of Horticultural Science, 82*(5), 232–238. https://doi.org/10.17660/eJHS.2017/82.5.2

Salama, A.-M., Ezzat, A., El-Ramady, H., Alam-Eldein, S. M., Okba, S. K., Elmenofy, H. M., Hassan, I. F., Illés, A., & Holb, I. J. (2021). Temperate fruit trees under climate change: Challenges for dormancy and chilling requirements in warm winter regions. *Horticulturae, 7*(4), Article 86. https://doi.org/10.3390/horticulturae7040086

Sangiorgio, D., Cellini, A., Donati, I., Pastore, C., Onofrietti, C., & Spinelli, F. (2020). Facing climate change: Application of microbial biostimulants to mitigate stress in horticultural crops. *Agronomy, 10*(6), Article 794. https://doi.org/10.3390/agronomy10060794

Sayyad-Amin, P. (2022). A review on breeding fruit trees against climate changes. *Erwerbs-Obstbau, 64*(4), 697–701. https://doi.org/10.1007/s10341-022-00737-z

Scavo, A., Fontanazza, S., Restuccia, A., Pesce, G. R., Abbate, C., & Mauromicale, G. (2022). The role of cover crops in improving soil fertility and plant nutritional status in temperate climates. A review. *Agronomy for Sustainable Development, 42*(5), Article 93. https://doi.org/10.1007/s13593-022-00825-0

Shakoor, A., & Ullah, Z. (2024). Review of agricultural water management techniques for drought resilience and water conservation. *International Journal of Research and Advances in Agricultural Sciences, 3*(1), 31–45.

Sharma, H. C. (2014). Climate change effects on insects; implications for crop protection and food security. *Journal of Crop Improvement, 28*(2), 229–259. https://doi.org/10.1080/15427528.2014.881205

Siddiqui, M. W., Patel, V. B., & Ahmad, M. S. (2015). Effect of climate change on postharvest quality of fruits. In M. L. Choudhary, V. B. Patel, M. W. Siddiqui, & S. Sheraz Mahdl (Eds.), *Climate dynamics in horticultural science: Principles and applications* (Vol. 1, pp. 313–326). Apple Academic Press. https://doi.org/10.1201/b18035

Sierra, C. A., Trumbore, S. E., Davidson, E. A., Vicca, S., & Janssens, I. (2015). Sensitivity of decomposition rates of soil organic matter with respect to simultaneous changes in temperature and moisture. *Journal of Advances in Modeling Earth Systems, 7*(1), 335–356. https://doi.org/10.1002/2014MS000358

Taleb, H. B., Brhadda, N., & Ziri, R. (2022). A study of the effect of replacing drip irrigation system by micro-sprinklers in avocado (*Persea americana* Mill.) in Morocco. *Revista Electronica de Veterinaria, 23*(3), 61–68. https://www.cabidigitallibrary.org/doi/full/10.5555/20220536619

Thapa, R., Chatterjee, A., Awale, R., McGranahan, D. A., & Daigh, A. (2016). Effect of enhanced efficiency fertilizers on nitrous oxide emissions and crop yields: A meta-analysis. *Soil Science*

Society of America Journal, 80(5), 1121–1134. https://doi.org/10.2136/sssaj2016.06.0179

Thomson, L. J., Macfadyen, S., & Hoffmann, A. A. (2010). Predicting the effects of climate change on natural enemies of agricultural pests. *Biological Control, 52*(3), 296–306. https://doi.org/10.1016/j.biocontrol.2009.01.022

Van Asten, P. J. A., Fermont, A. M., & Taulya, G. (2011). Drought is a major yield loss factor for rainfed East African highland banana. *Agricultural Water Management, 98*(4), 541–552. https://doi.org/10.1016/j.agwat.2010.10.005

Vélez-Sánchez, J., Casierra-Posada, F., & Fischer, G. (2023). Effect of regulated deficit irrigation (RDI) on the growth and development of pear fruit (*Pyrus communis* L.), var. Triunfo de Viena. *Sustainability, 15*(18), Article 13392. https://doi.org/10.3390/su151813392

Veste, M., Littmann, T., Kunneke, A., du Toit, B., & Seifert, T. (2020). Windbreaks as part of climate-smart landscapes reduce evapotranspiration in vineyards, Western Cape Province, South Africa. *Plant, Soil and Environment, 66*(3), 119–127. https://doi.org/10.17221/616/2019-PSE

Wang, B., Waters, C., Anwar, M. R., Cowie, A., Liu, D. L., Summers, D., Paul, K., & Feng, P. (2022). Future climate impacts on forest growth and implications for carbon sequestration through reforestation in southeast Australia. *Journal of Environmental Management, 302*(Part A), Article 113964. https://doi.org/10.1016/j.jenvman.2021.113964

Ward, F. A. (2014). Economic impacts on irrigated agriculture of water conservation programs in drought. *Journal of Hydrology, 508*, 114–127. https://doi.org/10.1016/j.jhydrol.2013.10.024

Wurms, K. V., Reglinski, T., Buissink, P., Ah Chee, A., Fehlmann, C., McDonald, S., Cooney, J., Jensen, D., Hedderley, D., McKenzie, C., & Rikkerink, E. H. A. (2023). Effects of drought and flooding on phytohormones and abscisic acid gene expression in kiwifruit. *International Journal of Molecular Sciences, 24*(8), 7580. https://doi.org/10.3390/ijms24087580

Yadav, S., Korat, J. R., Yadav, S., Mondal, K., Kumar, A., Homeshvari., & Kumar, S. (2023). Impacts of climate change on fruit crops: A comprehensive review of physiological, phenological, and pest-related responses. *International Journal of Environment and Climate Change, 13*(11), 363–371. https://doi.org/10.9734/ijecc/2023/v13i113179

Zhai, Z., Martínez, J. F., Beltran, V., & Martínez, N. L. (2020). Decision support systems for agriculture 4.0: Survey and challenges. *Computers and Electronics in Agriculture, 170*, Article 105256. https://doi.org/10.1016/j.compag.2020.105256

Zhang, D., Liu, X., & Hong, H. (2013). Assessing the effect of climate change on reference evapotranspiration in China. *Stochastic Environmental Research and Risk Assessment, 27*(6), 1871–1881. https://doi.org/10.1007/s00477-013-0723-0

Zhang, L., Hu, J., Li, Y., & Pradhan, N. S. (2018). Public-private partnership in enhancing farmers' adaptation to drought: Insights from the Lujiang Flatland in the Nu River (Upper Salween) valley, China. *Land Use Policy, 71*, 138–145. https://doi.org/10.1016/j.landusepol.2017.11.034

Zhang, S., Dou, Z., He, P., Ju, X.-T., Powlson, D., Chadwick, D., Norse, D., Zhang, Y., Wu, L., Chen, X.-P., Cassman, K. G., & Zhang, F.-S. (2013). New technologies reduce greenhouse gas emissions from nitrogenous fertilizer in China. *Proceedings of the National Academy of Sciences, 110*(21), 8375–8380. https://doi.org/10.1073/pnas.1210447110

Zhang, S., Zhang, J., Jing, X., Wang, Y., Wang, Y., & Yue, T. (2018). Water saving efficiency and reliability of rainwater harvesting systems in the context of climate change. *Journal of Cleaner Production, 196*, 1341–1355. https://doi.org/10.1016/j.jclepro.2018.06.133

Zhang, S., Zhang, J., Yue, T., & Jing, X. (2019). Impacts of climate change on urban rainwater harvesting systems. *Science of The Total Environment, 665*, 262–274. https://doi.org/10.1016/j.scitotenv.2019.02.135

Zhang, W., Dou, Z., He, P., Ju, X.-T., Powlson, D., Chadwick, D., Norse, D., Zhang, Y., Wu, L., Chen, X.-P., Cassman, K. G., & Zhang, F.-S. (2013). New technologies reduce greenhouse gas emissions from nitrogenous fertilizer in China. *Proceedings of the National Academy of Sciences, 110*(21), 8375–8380. https://doi.org/10.1073/pnas.1210447110

Zhou, W., Arcot, Y., Medina, R. F., Bernal, J., Cisneros-Zevallos, L., & Akbulut, M. E. (2024). Integrated pest management: An update on the sustainability approach to crop protection. *ACS Omega, 9*(40), 41130–41147. https://doi.org/10.1021/acsomega.4c06628

Application of bokashi improves the agronomic quality and bioactive compounds of radish cv. Quiron

La aplicación de bocashi mejora la calidad agronómica y los compuestos bioactivos del rábano cv. Quiron

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ABSTRACT

The aim of the study was to compare horticultural variables of radish cv. Quiron using bokashi, boiled chicken manure (BCM), and a mineral fertilizer. Experiments were conducted in a plastic greenhouse with the following treatments: fertigation with BCM concentrations in water (2.5%, 5.0%, 7.5%, and 10%), bokashi, mineral fertilizer in substrate, and control (water). The agronomic variables evaluated were: storage root biomass, storage root volume, leaf biomass, and chlorophyll index. Bioactive compounds, total polyphenol content, antioxidant capacity, and nitrate contents in storage root were also quantified. All agronomic variables were influenced by the treatments. For storage root biomass, the highest averages were obtained with bokashi and mineral fertilization, surpassing the other treatments. Root volume significantly increased with all treatments except for BCM 7.5%, with bokashi application resulting in the highest mean, followed by mineral fertilization. Leaf biomass was significantly enhanced by BCM 5.0%, BCM 7.5%, bokashi, and mineral fertilizer, with the latter showing the highest mean. The chlorophyll index increased with bokashi and mineral treatments. Total polyphenol contents significantly increased with all treatments, with bokashi, mineral, and BCM 5.0% and 7.5% showing the highest averages. Both DPPH and nitrate levels significantly increased with all treatments, with bokashi having the highest mean, followed by mineral fertilizer. FRAP levels were significantly elevated by all treatments, with bokashi and mineral fertilizer resulting in the highest means.

Keywords: chemical fertilizer, *Raphanus sativus* L., organic fertilizer, total polyphenols.

RESUMEN

El objetivo del estudio fue comparar variables hortícolas del rábano cv. Quirón utilizando bocashi, estiércol hervido de pollo (EPH) y un fertilizante mineral. Los experimentos se llevaron a cabo en un invernadero de plástico, con los siguientes tratamientos: fertirrigación con concentraciones de EPH en agua (2,5; 5,0; 7,5 y 10%); bocashi, fertilizante mineral en el sustrato; y control (agua). Las variables agronómicas evaluadas fueron: biomasa de raíz tuberosa, volumen de raíz tuberosa, biomasa foliar e índice de clorofila. Se cuantificaron también compuestos bioactivos, contenido total de polifenoles, capacidad antioxidante y cantidad de nitratos en la raíz tuberosa. Todas las variables agronómicas fueron influenciadas por los tratamientos. Para la biomasa de raíz tuberosa, las medias más altas se observaron con bocashi y fertilización mineral, superando a los otros tratamientos. El volumen de la raíz tuberosa aumentó significativamente con todos los tratamientos, excepto para el EPH 7,5%, con bocashi mostrando la media más alta, seguido de la fertilización mineral. La biomasa foliar se incrementó significativamente con EPH 5,0%, EPH 7,5%, bocashi y fertilizante mineral, el cual tuvo la media más alta. El índice de clorofila aumentó con los tratamientos de bocashi y fertilizante mineral. Los contenidos totales de polifenoles aumentaron significativamente con todos los tratamientos, siendo bocashi, fertilizante mineral y EPH 5,0% y 7,5% los que tuvieron las medias más altas. Tanto los niveles de DPPH como los de nitratos aumentaron significativamente con todos los tratamientos, con bocashi teniendo la media más alta, seguido de fertilizante mineral. Los niveles del FRAP se incrementaron significativamente en todos los tratamientos, resultando en las medias más altas con bocashi y fertilizante mineral.

Palabras clave: fertilizante químico, *Raphanus sativus* L., fertilizante orgánico, polifenoles totales.

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Introduction

The radish (*Raphanus sativus* L.) is a Brassicaceae vegetable that is usually consumed raw in salads. The storage roots and especially the leaves are rich in bioactive compounds such as glucosinolates, flavonoids, phenols, terpenes, and fatty acids (Gamba *et al.*, 2021).

Plants need a balanced uptake of nutrients for their development, and each plant species and cultivar require different amounts of these elements. In organic agriculture and agroecology, the use of synthetic fertilizers is prohibited; hence, farmers often depend on organic matter, amendments, and fertilizers to enhance and sustain soil fertility (Altieri *et al.*, 2015). Furthermore, low-input smallholders must rely on the existing natural resources available on the farm for soil fertilization. Therefore, studies on low-cost organic sources are crucial for sustainable agricultural models.

Organic agriculture uses a variety of fertilizer sources, both solid and liquid, to improve crop yield. Bovine composted manure (Maia *et al.*, 2018), cow urine (Furlanetto *et al.*, 2020), plant-based compost, green manure (Aboyeji, 2019), organic amendments such as bokashi (Erdal *et al.*, 2025; Hata *et al.*, 2021; Hata, Ventura *et al.*, 2021), and liquid biofertilizers (Cavalcante *et al.*, 2019) are low-cost options for smallholders. These studies normally evaluate only agronomic variables; few have focused on the influence of organic amendments/fertilizers on bioactive compounds. Effective microorganisms (EM) and IMO bokashi increased the phenolic content in potato tubers while also increasing the plant yield (Mbouobda *et al.*, 2014). EM manure increased the total phenolics of taro (*Colocasia esculenta* L.) (Mbouobda *et al.*, 2013) and the antioxidant activity of various tomato (*Solanum lycopersicum* L.) cultivars (Tommonaro *et al.*, 2021).

Therefore, the aim of this study was to assess the impact of low-cost organic amendments on agronomic variables and bioactive compound content in radish storage roots.

Materials and methods

The experiment was conducted in a plastic-covered greenhouse (23º20'28'' S, 51º12'34'' W; 548 m a.s.l.) in Londrina, Paraná State, Brazil. Radish (cv. Quiron) seeds were sown directly in the substrate on April 20, 2020. The cultivation was carried out in horizontal bags measuring 1.5 m length, 0.5 m width and 55 dm^3 of total volume. The substrate consisted of 40% soil (Oxisols, clay texture), 30% commercial substrate (Carolina Soil: Sphagnum peat, expanded vermiculite, dolomitic limestone, agricultural gypsum), 15% vermicompost, and 15% sand. Chemical analysis of the substrate was determined using the methodologies described by Teixeira *et al.* (2012). The analysis revealed the following values: pH_{H20} = 5.10, P = 7.50 mg kg^{-1}, K^+ = 0.94 $cmol_c$ kg^{-1}, Ca^{+2} = 1.67 $cmol_c$ kg^{-1}, Mg^{+2}= 1.50 $cmol_c$ kg^{-1}, Al^{+3} = 0.0, H+Al^{+3}= 2.63 $cmol_c$ kg^{-1}, and organic matter (%) = 1.80.

Boiled chicken manure (BCM) was prepared by boiling 30 kg of chicken manure on December 10, 2019. The BCM was stored and the necessary dilutions were prepared for each treatment. The dilution was prepared based on the farmer traditional use. In Brazil, farmers typically use a 5.0% concentration of BCM; the present study evaluated the increase and decrease of this dose. The pure BCM concentrations of nutrients were: N = 3.80 g kg^{-1}; P = 0.01 g kg^{-1}; K^+ = 0.002 g kg^{-1}; Ca^{+2} = 0.31 g kg^{-1} and Mg^{+2} = 0.11 g kg^{-1}.

Bokashi was prepared by dry fermentation using wheat (25%), rice (25%), maize (25%) and soybean brans (25%), sugarcane molasses (3 ml kg^{-1} dry weight) and effective microorganisms (EM) according to a previous study (Hata, Ventura *et al.*, 2021). After fermentation, bokashi had the following chemical characteristics: N = 37.67 g kg^{-1}; P = 14.36 g kg^{-1}; K = 21.01 g kg^{-1}; Ca = 12.00 g kg^{-1}; and Mg = 8.8 g kg^{-1}.

Treatments were as follows: fertigation with boiled chicken manure concentrations in water (2.5%, 5.0%, 7.5%, or 10%); bokashi (15 g per plant), mineral fertilizer (N-P-K 4-14-8) (Heringer, Brazil) (6 g per plant) in the substrate; and a control (water).

Irrigation management was carried out twice daily, at 10 am and 4 pm. All bags were irrigated with water until reaching field capacity. BCM fertigation was applied daily with 100 ml per plant per day. Bokashi and mineral fertilizer were applied 7 d before the experiment onset and 15 d after the seedling transplant.

The agronomic variables were: storage root biomass (RB) (g), storage root volume (RV) (cm^3), leaf biomass (LB) (g), and chlorophyll index (CI) (Falker index). A Falker ClorofiLOG® CFL1030 device was used to read the indirect chlorophyll index, with three readings taken from young leaves of each plant, using five plants per treatment. RB, RV, and LB were determined 39 d after seeding transplant. CI was determined 30 d after seeding transplant.

For the analysis of bioactive compounds and nitrate, storage root tissues were used as samples. The roots were collected after harvesting and agronomic measurements. Fresh samples were homogenized, and the dried samples were ground into powder for the quantification of bioactive compounds. One gram of sample was ultrasonically extracted three times for 30 min at 40°C in 10 ml of 80% aqueous methanol (w:v= 1:10). The extracts were centrifuged for 5 min at 1500 g, and the supernatant was collected and used for total polyphenol and antioxidant activity assays.

The total polyphenol contents were measured with adaptations using absorbance at 750 nm at room temperature (Bobo-García *et al.*, 2015). The results were expressed as mg of gallic acid equivalent (GAE) per 100 g of dry weight (mg GAE 100 g^{-1} dry weight).

Two antioxidant methods (2,2 diphenyl-1-picrylhydrazyl - DPPH and ferric reducing antioxidant power - FRAP) were used to determine the antioxidant capacity of radish roots. For DPPH, the determination using the stable radical 2,2'-diphenyl-1-picrylhydrazyl was performed according to the method of Brand-Williams *et al.* (1995). The results were expressed in micromoles of Trolox equivalents per gram of leaf biomass (μmol TE g^{-1} dry weight). The FRAP was determined by using the potassium ferricyanide–ferric chloride method (Benzie & Strain, 1996). The results were expressed in micromoles of Trolox equivalents (TE) per gram of leaf biomass (μmol TE g^{-1} dry weight). Nitrate content was quantified as described by Cataldo (1975) and expressed in mg NO^{-3} kg^{-1} dry weight.

Statistical analysis

For agronomic and bioactive compound assays, a completely randomized design with five and three replicates, respectively, was used. Each plant was considered a replicate. Variance homogeneity (F test) and normality (Shapiro-Wilk test) were performed. Once the prerequisites were met, the data were subjected to analysis of variance (ANOVA), and means were compared using Tukey's test ($P<0.05$). A Pearson correlation matrix ($P<0.05$) was used to analyze the relationships between variables.

Results

All the agronomic variables were affected by the treatments when compared to the control (water only). For storage root biomass, the highest means were observed for bokashi (111.20 g) and mineral fertilization (89.60 g), which were similar to each other and higher than the other treatments (Tab. 1, Fig. 1). Still, for BCM doses, only 2.5% and 5.0% (8.80 and 11.60 g, respectively) were higher than the control (2.60 g). Storage root volume was significantly increased by all the treatments, compared to the control, except for BCM 7.5%. Bokashi was the treatment with the highest mean (842.36 g), followed by mineral fertilization (626.65 g). Compared to the control (4.60 g), leaf biomass was significantly increased by BCM 5.0% and 7.5%, bokashi, and mineral fertilizer (12.80, 11.20, 159.00, and 202.00 g, respectively). The mineral treatment had the highest mean, followed by bokashi. The chlorophyll index was significantly increased by bokashi and mineral fertilizer treatments (44.93 and 48.67, respectively) compared to the control. In general, the BCM doses did not increase the agronomic variables in radish.

TABLE 1. Means of storage root biomass (RB) (g), storage root volume (RV) (cm^3), leaf biomass (LB) (g), and chlorophyll index (CI) (Falker index) in radish plants cv. Quiron subjected to various concentrations of boiled chicken manure (BCM), bokashi and mineral fertilizer in a greenhouse (Londrina, Paraná State, Brazil, 2020).

Treatments	RB	RV	LB	CI
Control	2.60 d	11.89 e	4.60 d	36.87 c
BCM 2.5%	8.80 bc	61.88 c	10.40 cd	39.33 bc
BCM 5.0%	11.60 b	84.87 c	12.80 c	35.80 c
BCM 7.5%	4.00 cd	21.72 de	11.20 c	37.87 c
BCM 10%	6.00 bcd	44.55 cd	8.40 cd	38.93 bc
Bokashi	111.20 a	842.36 a	159.00 b	44.93 ab
Mineral fertilizer	89.60 a	626.65 b	202.00 a	48.67 a
CV (%)	13.02	11.85	10.47	7.87
F	142.63	219.50	250.30	10.88

CV: Coefficient of variation; means followed by the same letter in the column do not differ significantly from each other according to Tukey's test ($P>0.05$).

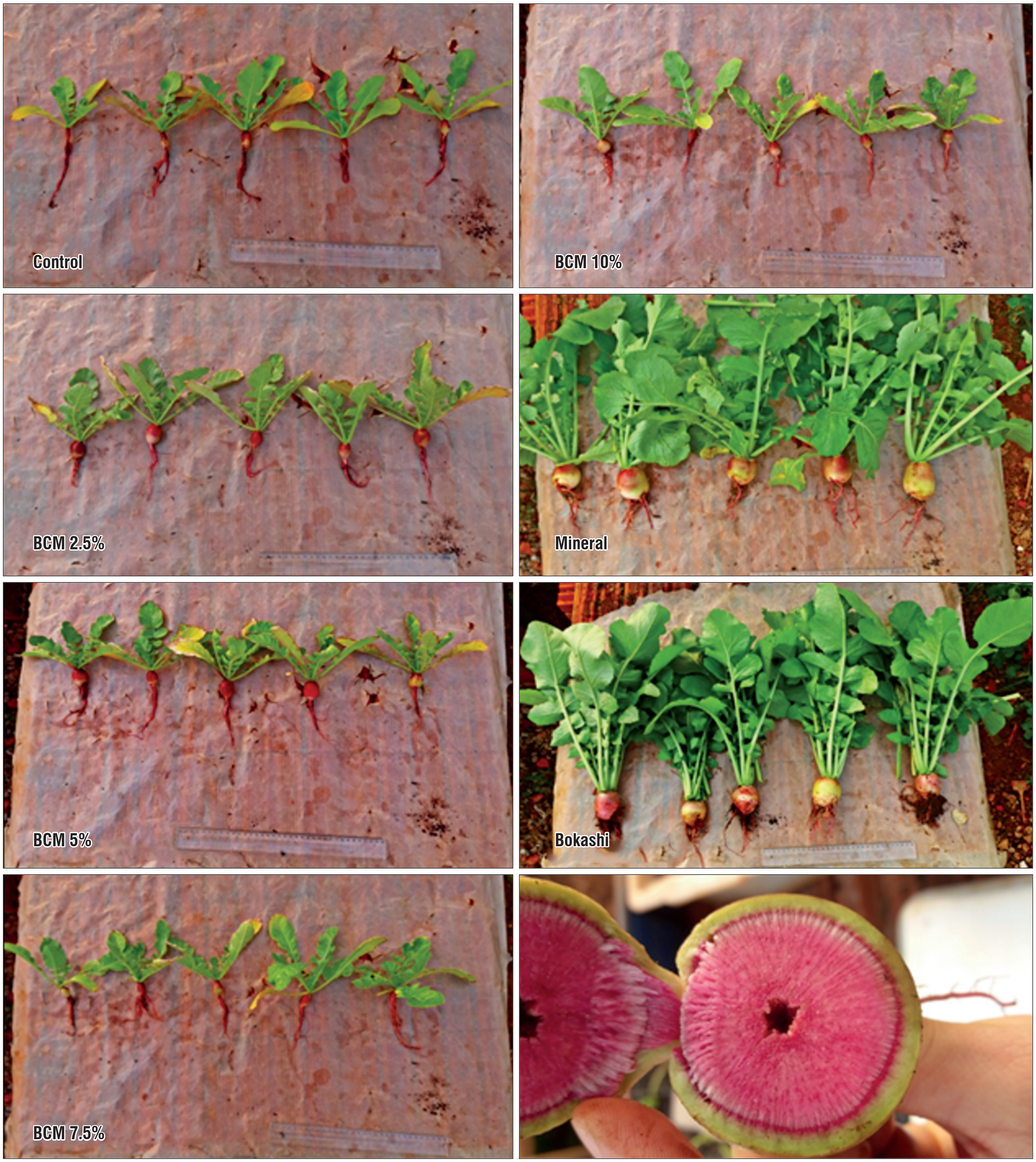


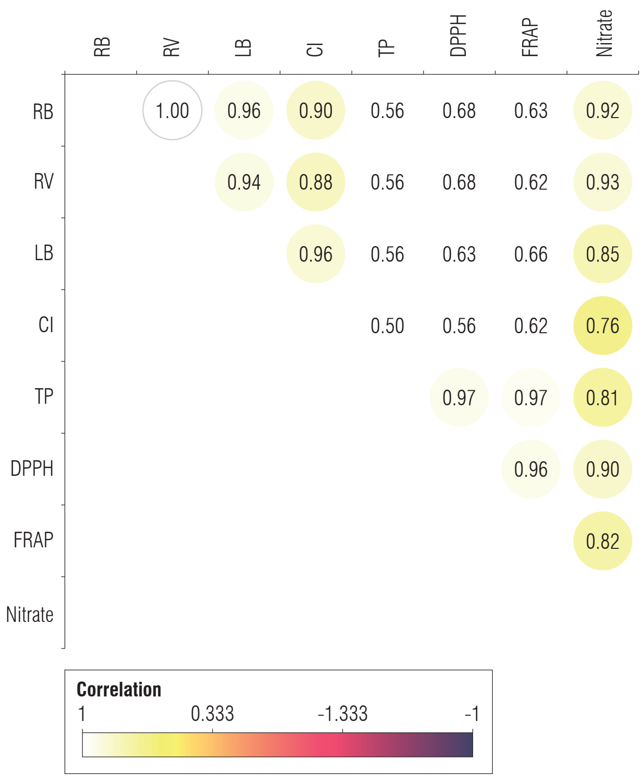
FIGURE 1. Radish plants cv. Quiron under different fertilizer and organic amendment treatments. BCM – Boiled chicken manure.

The bioactive compounds and nitrate contents were affected by the treatments, compared to the control (Tab. 2). The highest total polyphenol contents were found in plants treated with bokashi and mineral fertilizer (975.18 and 959.89 mg GAE 100 g^{-1} dry weight, respectively). DPPH values were significantly increased by all treatments compared to control. The highest DPPH means were found in the bokashi treatment followed by the mineral fertilizer treatment and BCM 5.0% (118.26, 109.44, and 105.32 µmol TE g^{-1} dry weight, respectively). FRAP was significantly

TABLE 2. Mean contents of total phenolics, antioxidant activity (DPPH and FRAP) and nitrate in storage roots of radish plants cv. Quiron subjected to various concentrations of boiled chicken manure (BCM), bokashi and mineral fertilizer in a greenhouse (Londrina, Paraná State, Brazil, 2020).

Treatments	Total polyphenols	DPPH	FRAP	Nitrate
Control	497.91 d	61.71 e	5.21 d	180.53 f
BCM 2.5%	689.54 c	85.53 d	9.77 c	1622.05 e
BCM 5.0%	897.09 ab	105.32 b	12.71 b	4407.37 c
BCM 7.5%	938.50 ab	99.11 c	12.91 b	3079.75 d
BCM 10%	834.91 b	96.73 c	11.02 c	3393.10 cd
Bokashi	975.18 a	118.26 a	13.92 ab	9679.85 a
Mineral fertilizer	959.89 a	109.44 b	15.28 a	7212.81 b
CV (%)	5.06	2.05	4.44	9.11
F	2.87	2.85	128.76	215.26

CV: Coefficient of variation; means followed by the same letter in the column do not differ significantly from each other according to Tukey's test ($P > 0.05$). Total phenolics expressed in mg GAE 100 g^{-1} dry weight; DPPH: 2,2 diphenyl- 1- picrylhydrazyl radical (μmol TE g^{-1} dry weight); FRAP: ferric reducing antioxidant power (μmol TE g^{-1} dry weight). Nitrate content expressed in mg kg^{-1} dry weight.



	RB	RV	LB	CI	TP	DPPH	FRAP	Nitrate
RB		1.00	0.96	0.90	0.56	0.68	0.63	0.92
RV			0.94	0.88	0.56	0.68	0.62	0.93
LB				0.96	0.56	0.63	0.66	0.85
CI					0.50	0.56	0.62	0.76
TP						0.97	0.97	0.81
DPPH							0.96	0.90
FRAP								0.82
Nitrate								

FIGURE 2. Pearson correlation between variables in Radish plants cv. Quiron subjected to various concentrations of boiled chicken manure, bokashi, and mineral fertilizer in a greenhouse. White/yellow represents the "+1" correlation coefficient and black/red color represents the "-1" correlation coefficient. Significant correlations ($P < 0.05$) are shown with circles. Storage root biomass (RB), storage root volume (RV), leaf biomass (LB), chlorophyll index (CI), total phenolic content (TP), 2,2 diphenyl-1-picrylhydrazyl radical (DPPH), Ferric reducing antioxidant power (FRAP), and nitrate content.

increased in all treatments, with the greatest increase in bokashi and mineral fertilizer (13.92 and 15.28 µmol TE g^{-1} dry weight, respectively). For nitrate, bokashi showed the highest mean followed by mineral fertilizer-treated plants (9679.85 and 7212.81 mg kg^{-1} dry weight, respectively).

The relationships among the variables are shown in a correlation matrix (Fig. 2). In general, a positive and significant correlation was observed among the agronomic variables. A similar trend was observed for the bioactive compounds. The nitrate content was positively correlated with all variables. The chlorophyll index was positively correlated with nitrate content and agronomic variables.

Discussion

The bokashi treatment exhibited similar results to those of mineral fertilizer, indicating that this organic amendment can be used as a replacement or to reduce mineral fertilization use in agriculture. The storage root volume was significantly increased by all treatments except BCM 7.5%, with bokashi showing the highest mean, followed by mineral fertilizer. Similar results were observed in other studies (De Guzman & Dagupan, 2022; Hata *et al.*, 2019; Mendivil-Lugo *et al.*, 2020; Suthamathy & Seran, 2013). This increase can be explained by the fact that bokashi enhances soil structure and aeration (Olle, 2021) promoting efficient water and nutrient absorption by plant roots. Additionally, the bokashi aerobic fermentation process breaks down organic matter into simple, plant-accessible nutrients, ensuring supply of nitrogen, phosphorus and potassium (Ombita *et al.*, 2024).

Furthermore, the microbial community associated with bokashi contributes to its effectiveness in promoting plant growth. The production of 3-phenyllactic acid by microorganisms associated with bokashi has also been reported—a compound recognized for its role in promoting root development (Maki *et al.*, 2021). This suggests that bokashi not only enhances nutrient availability but may also stimulate root development through biochemical pathways.

Taken together, these factors indicate that bokashi acts as a biofertilizer with both physical and biochemical mechanisms that enhance soil fertility and promote plant growth, contributing to the observed improvements in agronomic variables.

Studies on lettuce and arugula intercropping showed that higher plant height, number of leaves, fresh and dry biomass were observed in bokashi-fertilized plants (Oliveira *et al.*, 2010). Bokashi promoted higher fresh biomass, head diameter and yield of lettuce, with an increase of more than 250% compared to the control without fertilization (Goulart *et al.*, 2018). In three-cycle experimentation with arugula, an increase of 137% in leaf biomass production was obtained with bokashi (Hata *et al.*, 2019). In kale (*Brassica oleracea* var. acephala), bokashi-fertilized plants had similar productivity to chemically fertilized plants (Shingo & Ventura, 2009). These results can be explained by the increase in chlorophyll index in bokashi and mineral fertilizer treatments. Chlorophyll content is strongly related to the nitrogen present in the leaf (Jumrani *et al.*, 2024), resulting in a higher production of photosynthates and an increased biomass of tubers.

Regarding bioactive compounds and nitrate contents, these variables were affected by all treatments. This may be a result of the improved soil health and subsequent plant yields triggered by the application of bokashi. The benefit provided to microbial communities, such as lactic acid bacteria and yeasts, bolster soil health, suppress pathogens and enhance nutrient uptake, ensuring plant health and higher photosynthesis rates, which is a limiting factor for the production of these compounds (Octavia *et al.*, 2023; Roig-Coll, 2020).

The total polyphenol contents were increased by all treatments compared to the control. Similar results have been observed in other experiments, which showed that organic fertilizer and amendments alter the polyphenol profile and the antioxidant capacity (Cojocaru *et al.*, 2020; Frías-Moreno *et al.*, 2021; Machado *et al.*, 2020). In Romaine and frisée lettuce cultivars, total phenolic contents increased by 30% and 35%, respectively, with the use of bokashi amendment in comparison to the control (water only) (Hata *et al.*, 2023). DPPH and nitrate contents were significantly increased by all treatments, with bokashi resulting in the highest average, followed by mineral fertilizer. FRAP also increased in plants, with bokashi and mineral fertilizer showing the best results. The results of this study demonstrate that bokashi application improves agronomic variables and enhances the accumulation of bioactive compounds in radish plants, with effects comparable to those of mineral fertilization. These findings suggest that bokashi can serve as a partial or complete substitute for mineral fertilizers, depending on the production context. Moreover, as an organic amendment, bokashi may provide not only essential nutrients but also a beneficial microbial community that contributes to improving plant yield and quality (Maki *et al.*, 2021; Scotton *et al.*, 2017; Tommonaro *et al.*, 2021).

Conclusion

Bokashi represents a sustainable alternative for integrated nutrient management in agricultural systems.

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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

FTH: Conception and design, acquisition of data, analysis and interpretation of data, and preparation of the manuscript draft; GVG, IAS, CEPP: Preparation of the manuscript draft and critical review of the manuscript; NNYH: Preparation of the manuscript draft, acquisition of data, analysis and interpretation of data; MAQC: Acquisition of data, analysis and interpretation of data; MCR, VHCS, LCPG: Acquisition of data, preparation of the manuscript draft; MUV, WAS: Conception and design, analysis and interpretation of data, and preparation of the manuscript draft. All authors have read and approved the final version of the manuscript.

Literature cited

Aboyeji, C. M. (2019). Impact of green manures of *Vernonia amygdalina* and *Chromolaena odorata* on growth, yield, mineral and proximate composition of radish (*Raphanus sativus* L.). *Scientific Reports, 9*, Article 17659. https://doi.org/10.1038/s41598-019-54071-8

Altieri, M. A., Nicholls, C. I., Henao, A., & Lana, M. A. (2015). Agroecology and the design of climate change-resilient farming systems. *Agronomy for Sustainable Development, 35*(3), 869–890. https://doi.org/10.1007/s13593-015-0285-2

Benzie, I. F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Analytical Biochemistry, 239*(1), 70–76. https://doi.org/10.1006/abio.1996.0292

Bobo-García, G., Davidov-Pardo, G., Arroqui, C., Vírseda, P., Marín-Arroyo, M. R., & Navarro, M. (2015). Intra-laboratory validation of microplate methods for total phenolic content and antioxidant activity on polyphenolic extracts, and comparison with conventional spectrophotometric methods. *Journal of the Science of Food and Agriculture, 95*(1), 204–209. https://doi.org/10.1002/jsfa.6706

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT – Food Science and Technology, 28*(1), 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5

Cataldo, D. A., Maroon, M., Schrader, L. E., & Youngs, V. L. (1975). Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. *Communications in Soil Science and Plant Analysis, 6*(1), 71–80. https://doi.org/10.1080/00103627509366547

Cavalcante, L. F., Bezerra, F. T. C., Souto, A. G., Bezerra, M. A. F., Lima, G. S., Gheyi, H. R., Pereira, J. F. S., & Beckmann-Cavalcante, M. Z. (2019). Biofertilizers in horticultural crops. *Comunicata Scientiae*, *10*(4), 415–428. https://doi.org/10.14295/cs.v10i4.3058

Cojocaru, A., Vlase, L., Munteanu, N., Stan, T., Teliban, G. C., Burducea, M., & Stoleru, V. (2020). Dynamic of phenolic compounds, antioxidant activity, and yield of rhubarb under chemical, organic and biological fertilization. *Plants, 9*(3), Article 355. https://doi.org/10.3390/plants9030355

De Guzman, R. S., & Dagupan, J. R. (2022). Growth and yield performance of radish as affected by different amount of EM-Bokashi in lahar soil. *European Journal of Agricultural and Rural Education, 3*(4), 9–14. https://doi.org/10.5281/zenodo.6473209

Erdal, I., Ekinci, K., Kumbul, B. S., & Madenli, E. C. (2025). Effect of dairy manure-derived bokashi prepared from different organic materials on lettuce growth and mineral nutrition. *Journal of Soil Science and Plant Nutrition, 25*, 2923–2936. https://doi.org/10.1007/s42729-025-02309-y

Frías-Moreno, M. N., Parra-Quezada, R. A., González-Aguilar, G., Ruíz-Canizales, J., Molina-Corral, F. J., Sepúlveda, D. R., Salas-Salazar, N., & Olivas, G. I. (2021). Quality, bioactive compounds, antioxidant capacity, and enzymes of raspberries at different maturity stages, effects of organic vs. conventional fertilization. *Foods, 10*(5), Article 953. https://doi.org/10.3390/foods10050953

Furlanetto, A. D., Martinez, R. A. S., Lima, M. V. G., Santi, A., Vendruscolo, M. C., & Magalhães, M. O. L. (2020). Agroecological management in production of radish fertilized with cow urine. *Revista Verde de Agroecologia e Desenvolvimento Sustentável, 15*(4), 353–359. https://doi.org/10.18378/rvads.v15i4.8082

Gamba, M., Asllanaj, E., Raguindin, P. F., Glisic, M., Franco, O. H., Minder, B., Bussler, W., Metzger, B., Kern, H., & Muka, T. (2021). Nutritional and phytochemical characterization of radish (*Raphanus sativus*): A systematic review. *Trends in Food Science* & *Technology, 113*, 205–218. https://doi.org/10.1016/j.tifs.2021.04.045

Goulart, R. G. T., Santos, C. A., Oliveira, C. M., Costa, E. S. P., Oliveira, F. A., Andrade, N. F., & Carmo, M. G. F. (2018). Desempenho agronômico de cultivares de alface sob adubação orgânica em Seropédica, RJ. *Revista Brasileira de Agropecuária Sustentável, 8*(3), 66–72. https://doi.org/10.21206/rbas.v8i3.3011

Hata, F. T., Paula, M. T., Moreira, A. A., Ventura, M. U., Lima, R. F., Fregonezi, G. A. F., & Oliveira, A. L. M. (2021). Adubos orgânicos e fertirrigação com esterco aviário fervido para o cultivo de morangueiro. *Revista de la Facultad de Agronomía de la Universidad del Zulia, 38*(2), 342–359. https://doi.org/10.47280/RevFacAgron(LUZ).v38.n2.07

Hata, F. T., Silva, D. C., Yassunaka-Hata, N. N., Cancian, M. A. Q., Sanches, I. A., Poças, C. E. P., Ventura, M. U., Spinosa, W. A., & Macedo, R. B. (2023). Leafy vegetables' agronomic variables, nitrate, and bioactive compounds have different responses to bokashi, mineral fertilization, and boiled chicken manure. *Horticulturae, 9*(2), Article 194. https://doi.org/10.3390/horticulturae9020194

Hata, F. T., Ventura, M. U., Fregonezi, G. A. F., & Lima, R. F. (2021). Bokashi, boiled manure and penergetic applications increased agronomic production variables and may enhance powdery mildew severity of organic tomato plants. *Horticulturae, 7*(2), Article 27. https://doi.org/10.3390/horticulturae7020027

Hata, F. T., Ventura, M. U., Sousa, V., & Fregonezi, G. A. F. (2019). Low-cost organic fertilizations and bioactivator for arugula–radish intercropping. *Emirates Journal of Food and Agriculture, 31*(10), 773–778. https://www.cabidigitallibrary.org/doi/pdf/10.5555/20209902596

Jumrani, K., Bhatia, V. S., Kataria, S., & Rastogi, A. (2024). The interactive effect of high temperature and water deficit stress on nitrogen fixation, photosynthesis, chlorophyll fluorescence, seed yield and quality in soybean (*Glycine max*). *Plant Physiology Reports, 29*(1), 125–140. https://doi.org/10.1007/s40502-023-00763-3

Kruker, G., Guidi, E. S., Santos, J. M. D. S. D., Mafra, Á. L., & Almeida, J. A. D. (2023). Quality of bokashi-type biofertilizer formulations and its application in the production of vegetables in an ecological system. *Horticulturae, 9*(12), Article 1314. https://doi.org/10.3390/horticulturae9121314

Machado, R. M. A., Alves-Pereira, I., Lourenço, D., & Ferreira, R. M. A. (2020). Effect of organic compost and inorganic nitrogen fertigation on spinach growth, phytochemical accumulation and antioxidant activity. *Heliyon, 6*(9), Article e05085. https://doi.org/10.1016/j.heliyon.2020.e05085

Maia, A. H., Souza, M. E., Silva, F. C., Rebelatto, B. F., Silva, T. O., Souza, V. S., & Ferreira, L. S. (2018). Productivity of radish fertilized with different doses of bovine manure. *African Journal of Agricultural Research, 13*(18), 963–968. https://doi.org/10.5897/AJAR2018.13140

Maki, Y., Soejima, H., Kitamura, T., Sugiyama, T., Sato, T., Watahiki, M. K., & Yamaguchi, J. (2021). 3-Phenyllactic acid, a root-promoting substance isolated from Bokashi fertilizer, exhibits synergistic effects with tryptophan. *Plant Biotechnology, 38*(1), 9–16. https://doi.org/10.5511/plantbiotechnology.20.0727a

Mbouobda, H. D., Fotso, J. C. D., Djeuani, C. A., Baliga, M. O., & Omokolo, N. D. (2014). Comparative evaluation of enzyme activities and phenol content of Irish potato (*Solanum tuberosum*) grown under EM and IMO manures Bokashi. *International Journal of Biological and Chemical Sciences, 8*(1), 157–166. https://doi.org/10.4314/ijbcs.v8i1.15

Mbouobda, H. D., Fotso, J. C. D., Djeuani, C. A., Fai, K., & Omokolo, N. D. (2013). Impact of effective and indigenous microorganisms manures on *Colocassia esculenta* and enzymes activities. *African Journal of Agricultural Research, 8*(12), 1086–1092. https://doi.org/10.5897/AJAR12.1867

Mendivil-Lugo, C., Nava-Pérez, E., Armenta-Bojórquez, A. D., Ruelas-Ayala, R. D., & Félix-Herrán, J. A. (2020). Elaboración de un abono orgánico tipo bocashi y su evaluación en la germinación y crecimiento del rábano. *Biotecnia, 22*(1), 17–23. https://doi.org/10.18633/biotecnia.v22i1.1120

Octavia, D., Murniati., Suharti, S., Hani, A., Mindawati, N., Suratman., Swestiani, D., Junaeid, A., Undaharta, N. K. E., Santosa, P. B., Wahyuningtyas, R. S., & Faubiany, V. (2023). Smart agroforestry for sustaining soil fertility and community livelihood. *Forest Science and Technology, 19*(4), 315–328. https://doi.org/10.1080/21580103.2023.2269970

Oliveira, E. Q., Souza, R. J., Cruz, M. C. M., Marques, V. B., & França, A. C. (2010). Produtividade de alface e rúcula, em sistema consorciado, sob adubação orgânica e mineral. *Horticultura Brasileira, 28*(1), 36–40. https://doi.org/10.1590/S0102-05362010000100007

Olle, M. (2021). Review: Bokashi technology as a promising technology for crop production in Europe. *Journal of Horticultural Science & Biotechnology, 96*(2), 145–152. https://doi.org/10.1080/14620316.2020.1810140

Ombita, S. N., Mwendwa, S. M., & Mureithi, S. M. (2024). Influence of organic fertilization on growth and yield of strawberry (*Fragaria × ananassa*) in Kabete and Mbooni areas, Kenya. *Heliyon, 10*(3), Article e25324. https://doi.org/10.1016/j.heliyon.2024.e25324

Quiroz, M., & Céspedes, C. (2019). Bokashi as an amendment and source of nitrogen in sustainable agricultural systems: a review. *Journal of Soil Science and Plant Nutrition, 19*, 237–248. https://doi.org/10.1007/s42729-019-0009-9

Roig-Coll, S., & Kechagia, A. (2020). Fermenting locally to improve soil fertility: Global lessons to the future of food sovereignty. In N. Betoret, & E. Betoret (Eds.), *Sustainability of the food system: Sovereignty, waste, and nutrients bioavailability* (pp. 25–31). Academic Press. https://doi.org/10.1016/B978-0-12-818293-2.00002-1

Scotton, J. C., Pereira, J. S., Campos, A. A. B., Pinto, D. F. P., Costa, W. L. F., & Homma, S. K. (2017). Different sources of inoculum to the bokashi provides distinct effects on the soil quality. *Revista Brasileira de Agropecuária Sustentável, 7*(3), 32–38. https://doi.org/10.21206/rbas.v7i3.411

Shingo, G. Y., & Ventura, M. U. (2009). Collard greens yield with mineral and organic fertilization. *Semina: Ciências Agrárias, 30*(3), 589–594. https://doi.org/10.5433/1679-0359.2009v30n3p589

Suthamathy, N., & Seran, T. H. (2013). Residual effect of organic manure EM Bokashi applied to proceeding crop of vegetable cowpea (*Vigna unguiculata*) on succeeding crop of radish (*Raphanus sativus*). *Research Journal of Agriculture and Forestry Sciences, 1*(1), 2–5. https://www.isca.me/AGRI_FORESTRY/Archive/v1/i1/1.ISCA-RJAFS-2013-001.php

Teixeira, P. C., Donagemma, G. K., Fontana, A., & Teixeira, W. G. (2017). *Manual de métodos de análise de solo* (3th ed.). Embrapa. https://www.infoteca.cnptia.embrapa.br/handle/doc/1085209

Tommonaro, G., Abbamondi, G. R., Nicolaus, B., Poli, A., D'Angelo, C., Iodice, C., & De Prisco, R. (2021). Productivity and nutritional trait improvements of different tomatoes cultivated with effective microorganisms technology. *Agriculture, 11*(2), Article 112. https://doi.org/10.3390/agriculture11020112

Morpho-molecular characterization of fungi in harvested fruits of *Theobroma cacao* L.

Caracterización morfo-molecular de hongos en frutos cosechados de *Theobroma cacao* L.

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ABSTRACT

In the present research, various isolates of pathogenic, endophytic, and epiphytic fungi were molecularly identified from diseased fruits collected in the harvest phase of *Theobroma cacao* L. coming from two different farms of Norte de Santander and Bolívar departments, Colombia. Standard microbiological procedures were used to phenotypically and molecularly characterize 26 fungi. *Moniliophthora roreri*, *Scopulariopsis* spp., 7 species belonging to the *Fusarium solani* complex, 6 species of *Aspergillus*, *Neocosmospora tonkinensis*, *Penicillium paneum*, *Pestalotiopsis microspora,* and *Geotrichum candidum* were found. Besides this, the identification of *Neofusicoccum parvum*, PQ721376.1, the causal agent of cocoa pod rot in the analyzed samples, is one of the most remarkable results since it is the first time that this recognized destructive fungal pathogen is reported in the country. We concluded that cocoa crops from two geographically different farms located in Colombia harbor a wide variety of fungi, most of them with pathogenic behavior, which could influence their low productivity. This finding supports the need to implement differentiated strategies for disease management depending on the regions where *T. cacao* L. is cultivated and the pathogenicity profile of the fungus found, to improve crop productivity.

Keywords: cocoa, pathogens, fungal infection, agroecology.

RESUMEN

En la presente investigación se identificaron molecularmente varios aislados de hongos patógenos, endófitos y epífitos a partir de frutos enfermos, recolectados en la fase de cosecha de *Theobroma cacao* L. provenientes de dos fincas diferentes ubicadas en los departamentos de Norte de Santander y Bolívar, Colombia. Se emplearon procedimientos microbiológicos y moleculares estándar para caracterizar fenotípica y genotípicamente 26 hongos. Se encontraron *Moniliophthora roreri*, *Scopulariopsis* spp., 7 especies pertenecientes al complejo *Fusarium solani*, 6 especies de *Aspergillus*, *Neocosmospora tonkinensis, Penicillium paneum, Pestalotiopsis microspora* y *Geotrichum candidum*. Además de esto, la identificación de *Neofusicoccum parvum*, PQ721376.1, el agente causal de la pudrición de la mazorca del cacao en las muestras analizadas, es uno de los resultados más notables, debido a que es la primera vez que este reconocido patógeno fúngico destructor es reportado en el país. Se concluyó que los cultivos de cacao de dos fincas geográficamente diferentes ubicadas en Colombia, albergan una gran variedad de hongos, la mayoría de ellos con comportamiento patógeno, lo que podría estar influyendo en su baja productividad. Este hallazgo apoya la necesidad de implementar estrategias diferenciadas para el manejo de enfermedades dependiendo de las regiones donde se encuentre establecido el cultivo de *T. cacao* L. y del perfil de patogenicidad de los hongos encontrados, con el fin de mejorar la productividad de los cultivos.

Palabras clave: cacao, patógenos, infección fúngica, agroecología.

Introduction

Cocoa (*Theobroma cacao* L.) is a plant species native to equatorial tropical moist forests of South America. It is established in warm, humid regions at latitudes spanning 10°N and 10°S of the equator. Its beans are primarily used as raw materials to produce chocolate, and lipids are used in the food, pharmaceutical, and cosmetics industries (Arvelo *et al*., 2016). Worldwide, cocoa production is led by the African continent with 70.3% followed by the American continent with 17.4% and finally Asia and Oceania with 12% (Ministerio de Desarrollo Agrario y Riego, 2023).

Colombian cocoa stands out internationally for its aroma and delicate flavor, characteristics present in nearly 95% of its national production (Fernández Niño *et al*., 2021),

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providing higher quality and price in the foreign market (Antolinez *et al*., 2020). It is cultivated throughout the national territory, approximately in 30 of the 32 departments of the country. Its production and export increased 28% over the last decade, increasing from 46,730 t in 2013 to 73,000 t in 2024 (Fedecacao, 2025). The sector has had a significant socioeconomic impact on the nation, thus becoming an alternative for more than 65 thousand families (MADR, 2021).

Theobroma cacao crops are established in pantropical regions, characterized by two rainy and two dry annual periods. This causes variables such as high temperatures and relative humidity to facilitate the growth of fungi and contribute to the spread of diseases, affecting the quality of the final product (Delgado-Ospina *et al*., 2021). At a global level, it has been estimated that cocoa infections with pathogenic microorganisms can cause losses exceeding 30% of worldwide productivity (ICCO, 2021). But if the infection occurs during the initial twelve weeks of its development, the losses can exceed 60% (Albores *et al*., 2022). Some of the most impactful disease-causing agents, such as *Phytophthora palmivora,* responsible for root rot, have global distribution.

In contrast, others, such as *Moniliophthora roreri*, the causal agent of moniliasis, are restricted to Latin America and the Caribbean (Marelli *et al*., 2019). It is estimated that approximately 20,000 fungi cause diseases in plants, including those with phytopathogenic and endophytic behavior. The latter promotes the vital functions of the plant. But, under conditions of physiological stress, they can change their behavior to opportunistic pathogens (Jain *et al*., 2019). In the case of *T. cacao*, it is estimated that there are up to 30 species of fungi that significantly affect its production, both globally and in more specific local contexts (Bailey & Meinhardt, 2016).

The aforementioned supports the need to carry out characterization studies of phytopathogenic fungi in the different geographical areas where the cultivation of *T. cacao* is established and to learn more about its agroecology, especially in those regions with low production rates (Amaro *et al*., 2021). To our knowledge, this is the first research carried out on the phenotypic and molecular characterization of fungal species present in different diseased cocoa biological materials, collected in the harvest phase, from the Norte de Santander and Bolívar departments of Colombia, where national production is not higher than 454 and 436 kg ha^{-1} per year, respectively (Fedecacao, 2021). This study aims to identify the primary fungal pathogens that may be affecting the productivity of small producers and to propose mitigation strategies in the future.

Materials and methods

Collection of plant material

Between eight and ten fruits were randomly selected in the harvest phase, which presented morphological alterations such as humps, oily spots, presence of necrotic tissue, superficial white mycelium, etc. These were randomly chosen (Villamizar-Gallardo *et al.,* 2019). We carried out sampling in two cocoa plantations whose characteristics are shown in Table 1. We packed the collected samples independently in plastic bags. We placed them in expanded polystyrene cellars with cooling gels for subsequent transport and processing at the laboratory of the group of research in Nanotechnology and Sustainable Management (NANOSOST) at the University of Pamplona, Norte de Santander, Colombia.

TABLE 1. Characteristics of the sampling sites.

Description/ Sampling sites	S1	S2
Georeferencing	8°16´24” N - 72°26´46” W	8°02´02” N - 74°04´23” W
Farm	Finca Miraflores	Finca los Pinos
Village	Vereda la Floresta	Vereda Juan Pablo II
Town	Cúcuta	Santa Rosa del Sur
Department	Norte de Santander	Bolívar
Elevation	64 m a.s.l.	734 m a.s.l.
Air temperature	21°C - 33°C	22°C - 30°C
Precipitation	878 mm	1000 mm
Pest control	Chemical and cultural	Chemical and cultural
Average annual yield (kg ha^{-1})	377	475
Cocoa accesiones	FSA-13, FTA-2, FEAR-5, CCN-51, ICS-95, SCC-61, and FLE3	ICS-95, CCN-51, and ICS-60

Fungal isolation

We carried out abundant superficial washing with sterile distilled water on each of the fruits to remove impurities. The isolation technique was based on direct cultivation, which consisted of making superficial cuts of the fruit of approximately 5 mm^2 to 10 mm^2 from the observed lesions. The samples were placed in Petri dishes with potato dextrose agar culture medium (PDA-Condolab®) and incubated at 25°C for 5-14 d, due to the heterogeneous growth observed in the crops. Subcultures of the different morphospecies were carried out until axenic cultures were obtained. To avoid redundancy and ensure representativeness, a single strain was selected for each group of isolates with similar morphological characteristics (Villamizar-Gallardo *et al*., 2019).

Macroscopic characterization

To evaluate the macroscopic characteristics, monosporic cultures were subcultured in APD medium and incubated at 25°C for 5-14 d. The parameters proposed by Muñoz and collaborators were evaluated to determine the color, appearance, and growth rate of the colony (Muñoz *et al*., 2020). The latter was obtained by measuring colony growth daily in APD medium for 10 d. For this purpose, a 7 mm diameter disc was placed in the center of the plate that contained the inoculum of each isolate, and incubated at 25-27°C. Daily photographic records of the growth of each isolate were taken along with a known scale and then processed in ImageJ™ software to obtain growth values. The procedure was done in triplicate for each isolate. The growth rate was obtained with the following formula:

$$GR = \frac{\Delta d}{\Delta t} = \frac{fd - id}{ft - it} \qquad (1)$$

where:

GR: Growth rate; *fd*: final diameter; *id*: initial diameter; *ft*: final time; *it*: initial time. To determine the statistical differences between the growth rates of the different fungi, the Kruskal-Wallis test was used with a *P*-value < 0.05. To identify the groups that presented significant differences, Dunn's post hoc test was applied. All analyses were performed using RStudio statistical software, version 4.3.3. The characterization information was contrasted with the classification guide proposed by Garcés de Granada *et al.* (2015).

Microscopic characterization

To evaluate the microscopic characteristics, monosporic cultures were grown in microcultures using APD culture medium. Concentrated suspensions of conidia were prepared, and 2 µl were taken from these to inoculate 1 cm² of medium. They were incubated at 25°C for 5-7 d, stained with lactophenol blue, and observed under the Zeiss Primo Star binocular microscope with 40x and 100x objectives. Different characteristics, such as shape and size of conidia, presence or absence of septate hyphae, and characterization of fruiting bodies, were evaluated as described by De Hoog *et al.* (2020). The size of the conidia was measured using the Zeiss® ocular micrometer, and the respective photographic record was taken for processing in the ImageJ software.

Molecular characterization

DNA extraction

DNA extraction started from cultures in liquid medium (Sabouraud dextrose broth) incubated at 28°C for 3-5 d with constant agitation, to promote the growth of mycelium. For DNA extraction, the commercial Exgene® Plant SV mini kit (GeneAll Biotechnology Co., Korea) was used following the manufacturer's instructions. The quality of the DNA obtained was visualized in a 0.8% agarose gel and was subsequently quantified by spectrophotometry (Agilent BioTek Epoch, CA, USA).

Polymerase chain reaction (PCR)

The ITS region of the ribosomal DNA was used as an amplification target using the oligonucleotides ITS-1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS-4 (5'-TCCTCCGCTTATTGATATGC-3') (Luo & Mitchel, 2002). To amplify, a 1X buffer was used: 2 mM $MgCl_2$, 0.25 mM dNTPs, 0.3 µM oligonucleotides, 3.5 U of Taq DNA polymerase, 50 ng of DNA for a total reaction volume of 50 µl. One cycle at 95°C for 3 min was used; 30 cycles of 95°C/30 s, 55°C/30 s, 72°C/30 s and 1 final cycle at 72°C/3 min were also used. This procedure was carried out using the Corbett Research thermal cycler. The amplification products were visualized on a 1.5% agarose gel and subsequently purified using the DNA Clean & Concentrator kit or the Zymoclean® Gel DNA Recovery kit (Zymo Research, USA) for the subsequent sequencing process.

Sequencing

The purified fragments were sent for sequencing to the Molecular Biology Laboratory of the Institute of Cellular Physiology of the UNAM, using the Sanger chain termination method, on an Applied Biosystems 3500 Series Genetic Analyzer 3500 (Thermo Fisher Scientific, USA).

Identity by homology

From the sequences obtained, bioinformatic analyses were performed using the BLAST (Basic Local Alignment Search Tool) of the National Center for Biotechnology Information (NCBI, https://www.ncbi.nlm.nih.gov/) and the MycoBank database (https://www.mirri.org/upcp_product/mycobank/). The objective of this procedure was to determine the percentage of identity (%I) by comparing it with curated sequences deposited in these databases, specifically for the ITS region, which is widely recognized as a reliable molecular marker for the identification of fungal species (Tekpinar & Kalmer, 2019). The selection criteria considered included an E-value equal to 0.0 and an identity percentage between 99% and 100%, as reported by Suárez-Contreras and Peñaranda-Figueredo (2022) (Tab. 2).

Representation of the phylogenetic relationship

For the analysis of the phylogenetic relationship, the "Molecular Evolutionary Genetic Analysis" software (MEGA 11.v) was used to understand the evolutionary relationships between the molecularly characterized organisms. The sequences obtained were aligned with a sequence used as an "Outgroup", corresponding to the gene that encodes the ITS region of the basidiomycete fungus *Amanita muscaria*, selected as an external reference point not related to the "Ingroup". The alignment of the DNA sequences was performed using the MUSCLE algorithm. The sequences were aligned using the MUSCLE algorithm for DNA alignment. Subsequently, a phylogram was constructed using the Neighbor-Joining statistical method, with a bootstrap analysis based on 1000 replications (Suárez & Peñaranda, 2022).

Results

It was possible to identify 26 axenic isolates from the samples taken of which 15 (H1 to H15), that is, 58% of the total, were obtained from Norte de Santander department (S1) (Fig. 1). In comparison, 11 isolates (H16 to H26), corresponding to 42% were obtained from Bolívar department (S2) (Tab. 2). From S1 isolates, we found that H2-H6, H8, H11 and H13 presented white and beige colonies. In the majority, the center of the colonies presented a denser growth of light brown color (except in isolates H11 and H13). The texture of most colonies was cottony on the periphery and more compact in the center. Isolate H7 showed a greenish-yellow color, with a powdery appearance. Isolates H10, H12, and H14 presented an olive-green color, with irregular

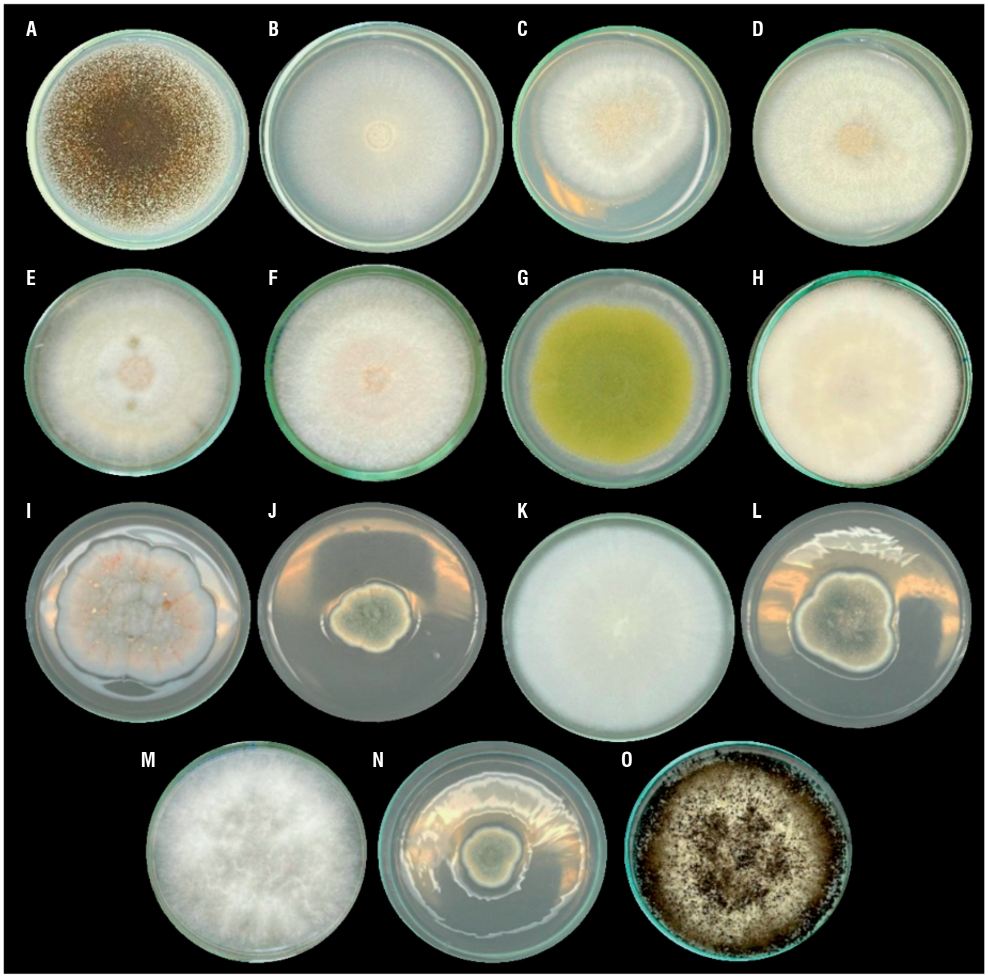

FIGURE 1. Macroscopic characterization of strains isolated from S1. The strains are labeled as follows: (A) H1, (B) H2, (C) H3, (D) H4, (E) H5, (F) H6, (G) H7, (H) H8, (I) H9, (J) H10, (K) H11, (L) H12, (M) H13, (N) H14, and (O) H15. All strains were photographed after 5-14 d at 25°C on PDA medium.

white edges and a velvety appearance. Isolate H9 showed orange pigmentation on the surface of the white mycelium. Finally, isolates H1 and H15 presented light and dark brown colonies, respectively, with a powdery texture.

Eleven isolates were obtained from the Bolívar region (S2) (Fig. 2) of which H16, H18-H20, H22, H24-H26 showed white to beige colonies with a cottony texture and regular edges. Isolates H18 and H19 showed irregular and rhizoid borders. Isolate H17 presented a colony with variable shades from yellow to light brown with asymmetric concentric rings and a cottony texture. Isolate H21 showed a colony with yellow tones on the edge and orange to reddish in the center, including a cottony texture. Finally, isolate H23 presented a green colony with white edges and a yellow center, with a powdery texture.

Regarding the growth rate, Figure 3 showed the significant differences in the 26 isolates. When applying the Kruskal-Wallis statistical test, a *P*-value of 5.212 e^{-07} was obtained, which was much lower than the established level of significance, making it possible to identify statistically significant differences in at least one of the fungi evaluated. Dunn's post hoc test indicated that isolates H17 and H23 were significantly different from the others, having the highest and lowest growth rate, with values of 22.48 mm d^{-1} and 3.28 mm d^{-1}, respectively.

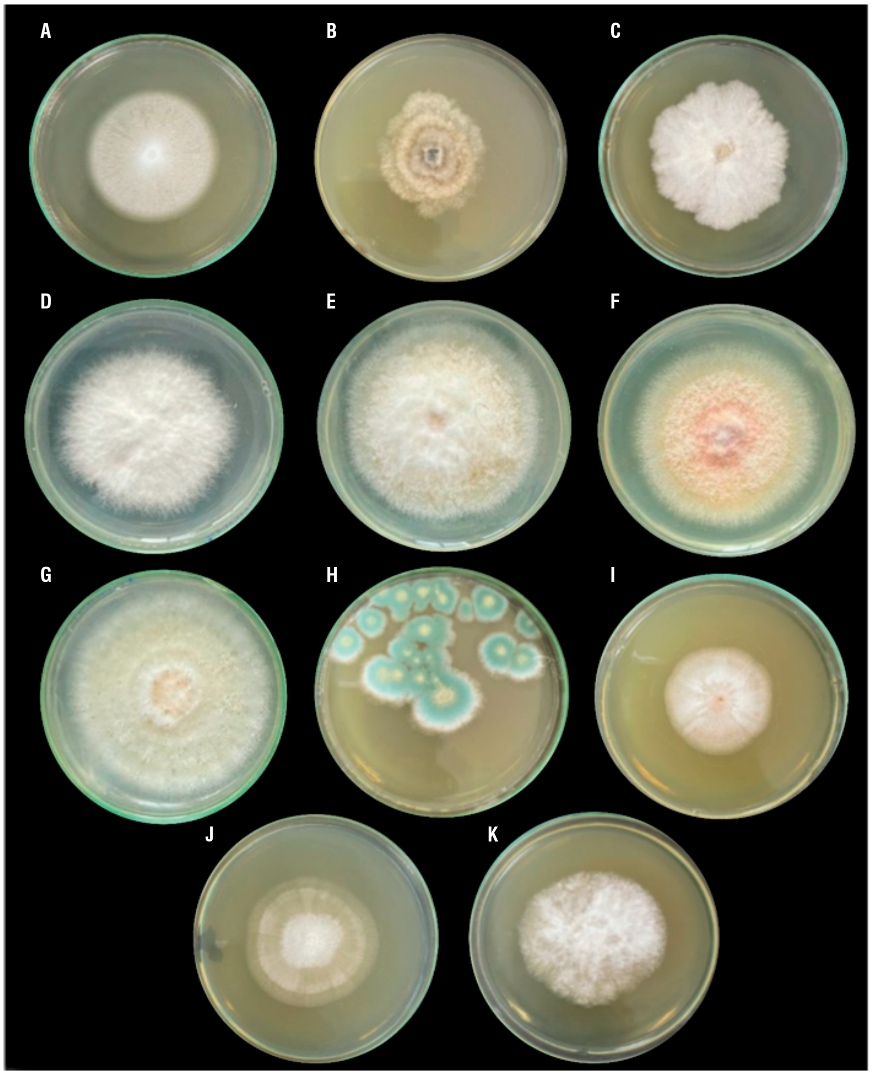

FIGURE 2. Macroscopic characterization of strains isolated from S2. The strains are labeled as follows: (A) H16, (B) H17, (C) H18, (D) H19, (E) H20, (F) H21, (G) H22, (H) H23, (I) H24, (J) H25, (K) H26. All strains were photographed after 5-14 d at 25°C on PDA medium.

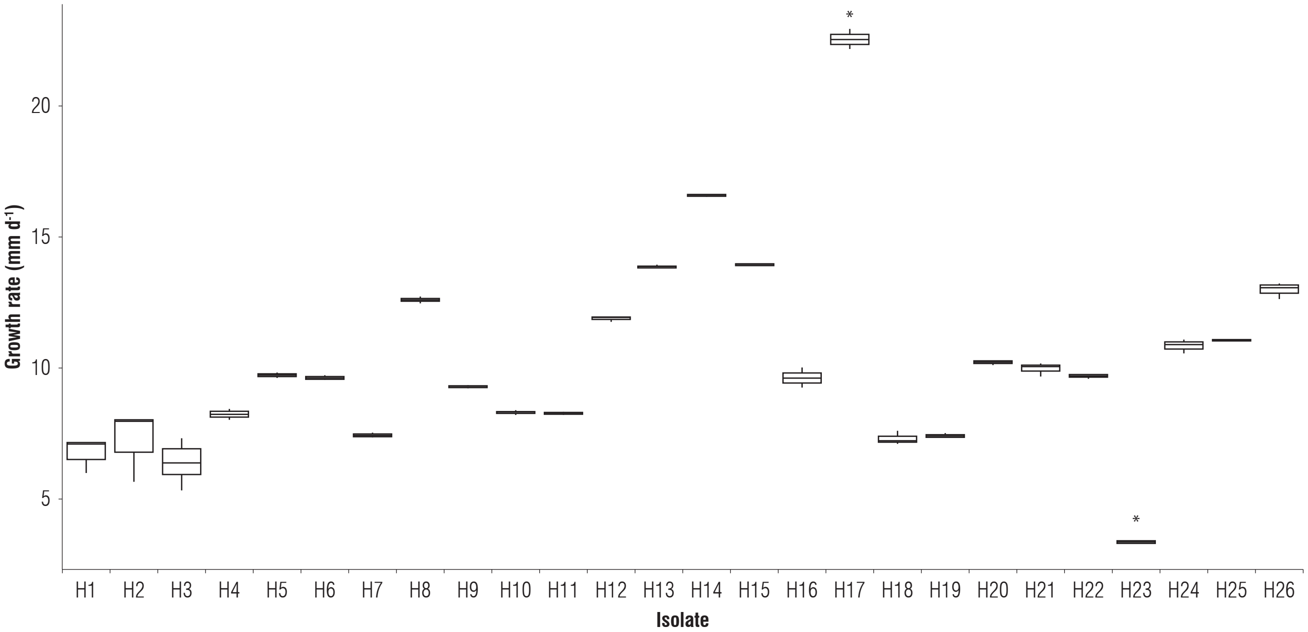

FIGURE 3. Differences between fungal growth rates. *: This represents statistically significant differences according to the Kruskal–Wallis test ($P = 5.21\ e^{-07}$).

TABLE 2. Identification of the isolates obtained in S1 and S2 with their respective E-value from the Mycobank database.

Code	Species	Percentage of identity
Isolates from Norte de Santander (S1)		
H1, H15	*Aspergillus niger*	100
H2, H11	*Geotrichum candidum*	100
H3, H4, H6	*Fusarium solani* complex (FSCS)	100
H5	*Neocosmospora tonkinensis*	100
H7	*Aspergillus flavus*	100
H8	*Moniliopthora* spp.	NR*
H9	*Scopulariopsis* spp.	NR*
H10-H12	*Aspergillus versicolor*	100
H13	*Sterile mycelium*	NR*
H14	*Aspergillus amoenus*	100
Isolates from Bolivar (S2)		
H16, H25	*Geotrichum candidum*	100
H17	*Neofusicoccum parvum*	100
H18	*Clonostachys rosea*	100
H19	*Bionectria wenpingii*	100
H20, H21, H22, H24	*Fusarium solani* complex (FSCS)	100
H23	*Penicillium paneum*	100
H26	*Pestalotiopsis microspora*	100

NR*: Not recovered.

Regarding the microscopic characterization, we observed that H1 and H15, isolated from S1 (Fig. 4), showed wide conidiophores with thick light brown walls, with conidia of 3 to 5 µm, which arise from a conidial apparatus consisting of globose vesicles and radial phialides. Isolates H2 and H11 exhibited thick, septate hyphae that formed hyaline, rectangular arthroconidia, with rounded ends, with a size of 10 to 20 µm. Isolates H3, H4, H5, and H6 showed septate hyphae, forming monophialides, with ovoid microconidia of 3 to 6 µm and fusiform, septate macroconidia of variable length (between 6 and 30 µm), in addition to the presence of chlamydoconidia.

Isolate H7 exhibited long conidiophores, with a conidial apparatus consisting of a biseriate spherical vesicle and globose conidia of 3 to 5 µm. At the same time, H8 showed globose, ellipsoid, and catenulate conidia of 8 to 20 µm, forming a chain, as well as septate hyphae. H9 was characterized by having thin, long, septate hyphae that stained a light blue tone. The conidiophores were short and branched, from which spherical conidia emerged, arranged in a chain, with a diameter of 4 to 6 µm. Isolates H10 and H12 presented septate and hyaline hyphae. Hyaline conidiophores emerge from these hyphae, ending in vesicles with radially arranged phialides, with spherical conidia of 4 to 6 µm. Finally, in H13, only sterile mycelium was observed.

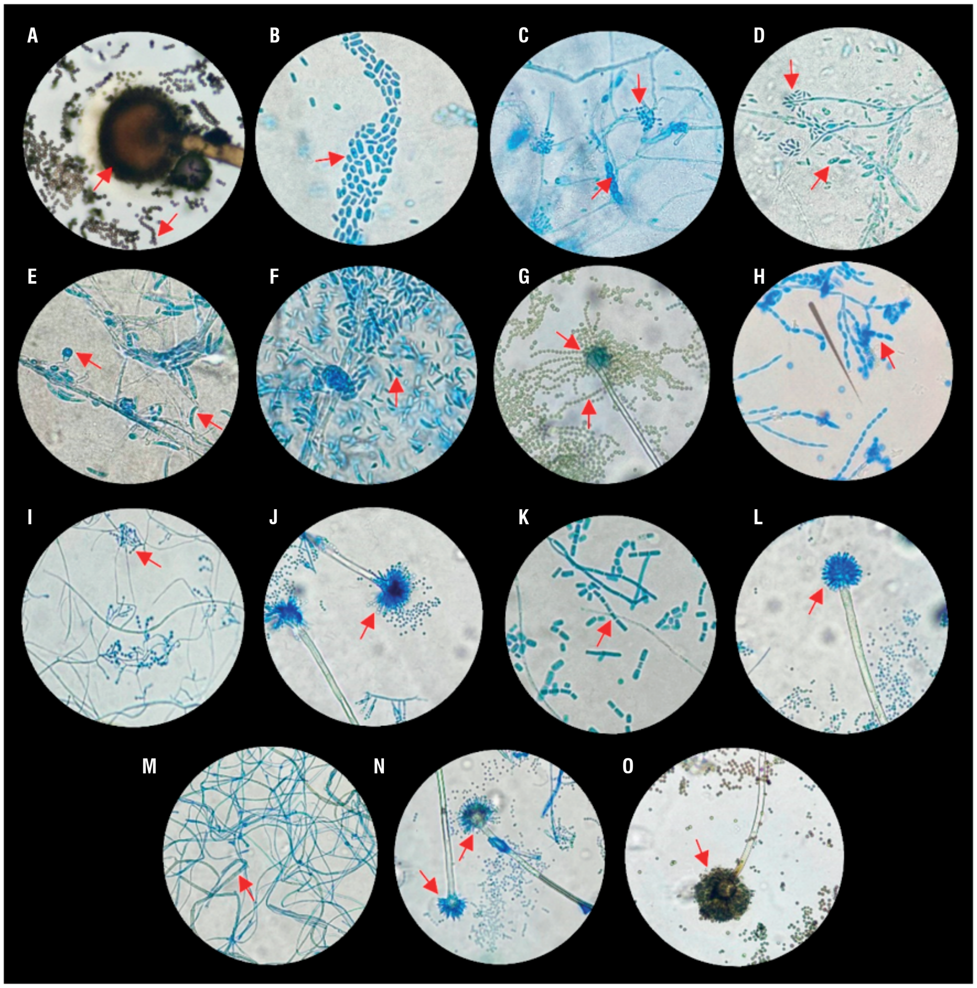

FIGURE 4. Microscopic characterization of strains isolated from S1. The strains are labeled as follows: (A) H1-40X, (B) H2-100X, (C) H3-100X, (D) H4-100X, (E) H5-100X, (F) H6-100X, (G) H7-40X, (H) H8-100X, (I) H9-40X, (J) H10-40X, (K) H11-100X, (L) H12-40X, (M) H13-100X, (N) H14-40X, and (O) H15-40X. The arrows indicate the presence of structures of interest, such as reproductive structures.

In the isolates from site S2, various morphological characteristics were observed (Fig. 5). The H16 and H25 showed conidial structures formed by the fragmentation of hyphae (arthroconidia), with a size between 3 and 6 µm, and a rectangular shape. In H17, we observed septate, pigmented hyphae without conidia. The H18 presented septate hyphae and whorled phialides, with ovoid conidia, 5 to 11 µm long. The H19 showed septate hyphae with secondary branches and ellipsoid conidia between 13 and 20 µm. H20, H21, H22, and H24 presented hyaline, septate hyphae and conidiophores with fusiform conidia between 20 and 30 µm long. On the other hand, the H23 showed septate hyphae, conidiophores with metulas and phialides, and round conidia forming chains 3 to 5 µm long. The H26 showed initially hyaline and then pigmented, septate hyphae, forming pigmented conidia, with three to four septa, with hyaline cells at the ends, 3 µm wide by 10 µm long.

For the molecular study, it was not possible to recover isolates H8, H9, and H13 in subculture, so the first two were only identified at the genus level, and the third was not identified. From the DNA extracted from the remaining 23 isolates and previously phenotypically characterized, a PCR was performed to amplify a fragment of the ITS region (Fig. 6). Amplicons ranging between 450 and 750 bp were obtained.

Molecular analyses allowed the identification of 23 isolates at the species level, all with identity values of 100% in both databases (Genbank & Mycobank).

The phylogenetic analysis of the described species is shown in Figure 7. It represented the evolutionary relationship between the different recovered fungal isolates and several reference species. As an "outgroup", an ITS

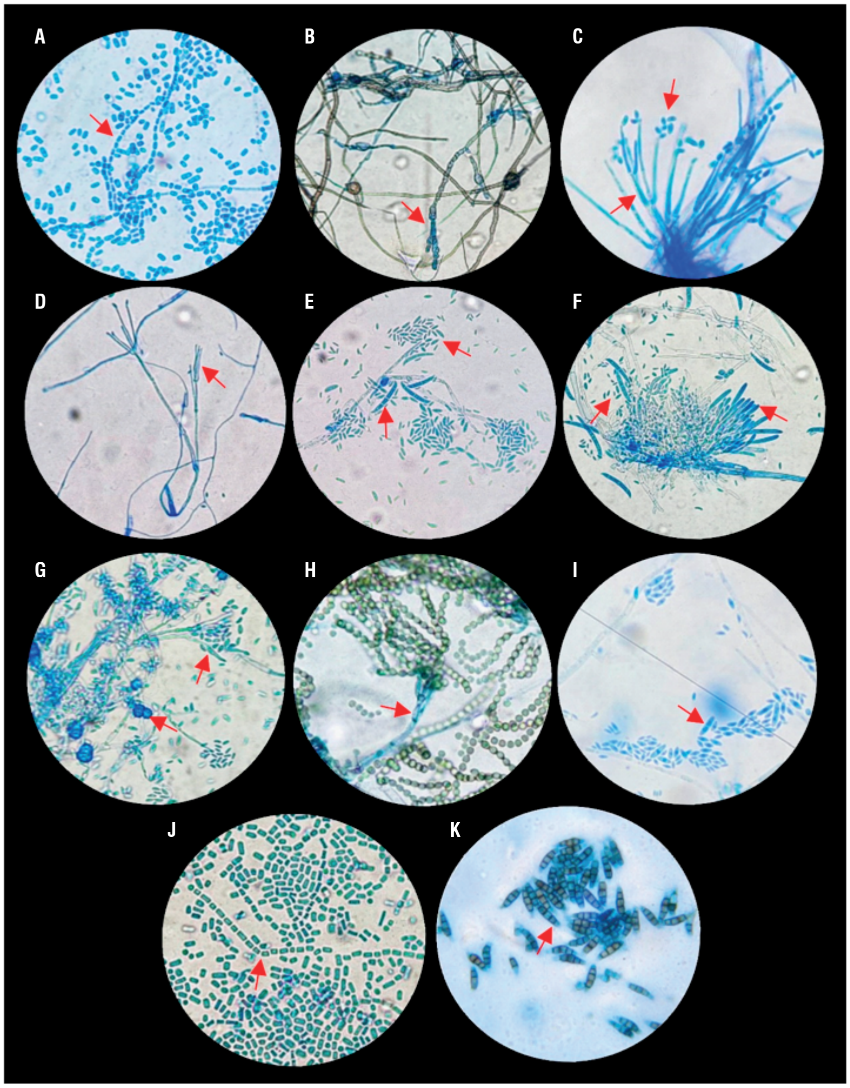

FIGURE 5. Microscopic characterization of strains isolated from S2. The strains are labeled as follows: (A) H16-100X, (B) H17100X, (C) H18-100X, (D) H19-40X, (E) H20-100X, (F) H21-100X, (G) H22-100X, (H) H23-100X, (I) H24-100X, (J) H25-100X, (K) H26-100X.

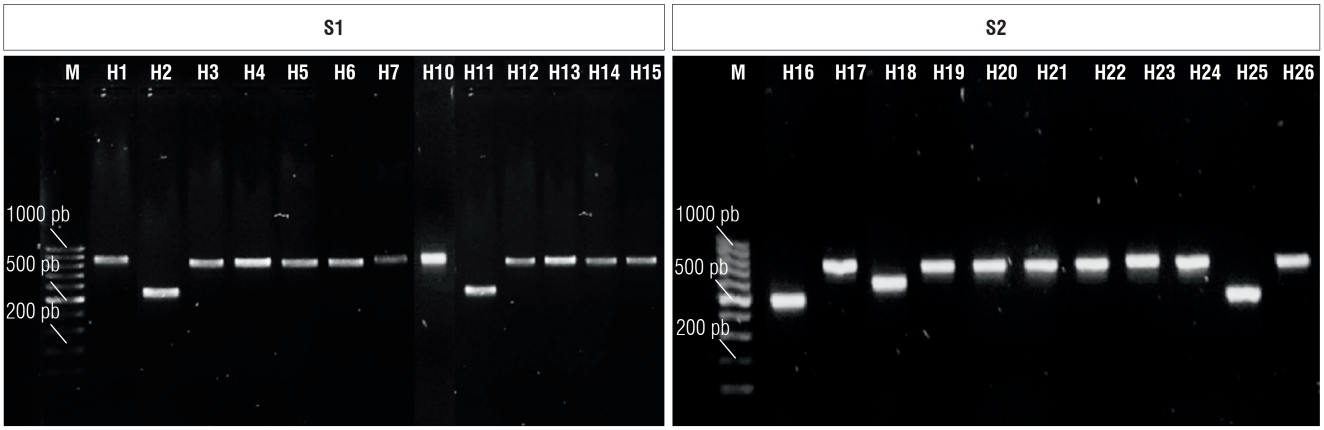

FIGURE 6. The 1.5% agarose gel electrophoresis product of the ITS region amplicons obtained from samples S1 and S2.

sequence corresponding to the Basidiomycete fungus *Amanita muscaria* was used to root the phylogenetic tree. The results showed that all isolates belong to the phylum Ascomycota, grouped into the classes Sordariomycetes, Dothideomycetes, Eurotiomycetes, and Saccharomycetes, distributed in five orders: Hypocreales, Amphisphaeriales, Botryosphaeriales, Eurotiales, and Saccharomycetales. A clear separation was observed between the different fungal species identified, with coherent groupings by genus, which highlighted the effectiveness of this marker for the taxonomic classification of fungi. The group formed by the isolates identified as *Fusarium solani* (H20, H21, H22, H24, H3, H4, H6) was grouped with high support values (bootstrap of up to 95), forming a consistent clade corresponding to *Fusarium solani species complex* (FSSC), which showed a close phylogenetic relationship between these isolates. This group, in turn, is related to related genera such as *Clonostachys* and *Bionectria*.

On the other hand, the isolates belonging to the genus *Aspergillus* (H12, H14, H10, H1, H15, H7) were grouped in different well-supported clades (bootstrap between 94 and 99), showing a clear separation between species such as *A. niger, A. flavus, A. versicolor,* and *A. amoenus*. Likewise, *Penicillium paneum* (H23) was positioned as a close sister group to the clade of *Aspergillus*, which agreed with its belonging to the same taxonomic family. The isolates classified as *Geotrichum candidum* (H2, H11, H16, H25) formed a clade with a high support (bootstrap up to 99), reflecting a close evolutionary relationship between them. As for the isolates H26, H17, and H5, identified as *Pestalotiopsis microspora, Neofusicoccum parvum,* and *Neocosmospora tonkinensis,* respectively; these formed independent clades, which indicated that they corresponded to differentiated species, a result that was also supported by high percentages of identity in the comparison with reference sequences. However, their location within the tree did not have high bootstrap values (19-29), so it was not possible to establish solid evolutionary inferences for these branches, although their identity was recognized at a specific level. Finally, the tree was rooted using *Amanita muscaria*, a basidiomycete fungus, as an outgroup, which allowed establishing the evolutionary direction of the lineages analyzed, all belonging to the phylum Ascomycota.

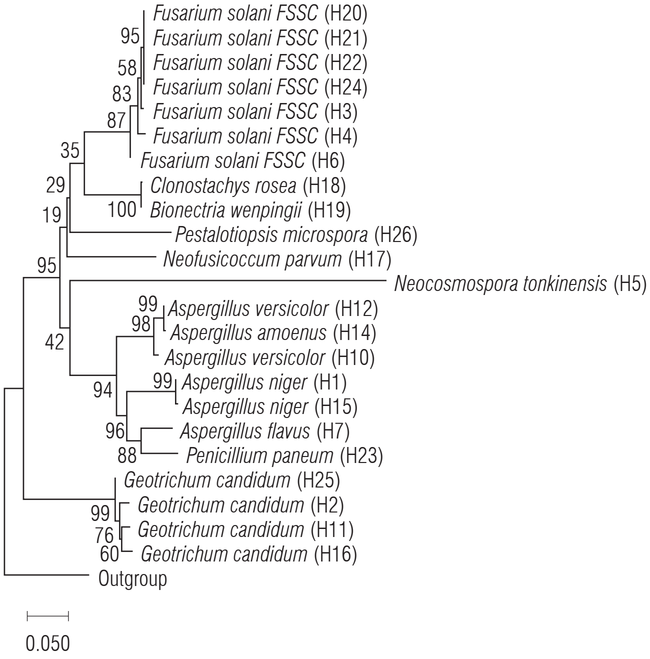

FIGURE 7. Phylogram of the amplified sequences of the ITS region of the species obtained from S1 and S2 using the Neighbor-Joining method. The values above the branches represent the bootstrap support percentages.

Discussion

Through this study, it was possible to identify 2 genera and 23 species of fungi from diseased fruits of *Theobroma cacao* in the harvest phase from two regions of Colombia. Among the isolates obtained from a cocoa farm located in the department of Norte de Santander (S1), it is worth highlighting that of *Moniliophthora* spp., although it could not be characterized molecularly because it was difficult to recover in synthetic culture medium, its phenotypic characteristics corresponded to *Moniliophthora roreri*, which was previously reported by Villamizar-Gallardo *et al.* (2019) from cocoa fruits obtained from the exact geographical location. This pathogen is characterized by producing the disease called moniliasis, which affects only fruits and is endemic to this crop (ICCO, 2021). Aime and Phillips-Mora (2005) note that cocoa moniliasis may have occurred for the first time in Colombia, specifically in the department of Norte de Santander, in 1817. This could explain its isolation in the S1 zone and not in S2, although its distribution is ample in the national territory. The presence of this disease in only one of the study areas, despite having favorable environmental conditions in both, could be due to several factors, including the presence of susceptible plant material, the degree of incidence of the disease, or inadequate crop management by farmers.

In contrast, in zone S2, the isolation of the species *Neofusicoccum parvum* was achieved for the first time in the department of Bolívar in Colombia. This strain was deposited in GenBank and can be evidenced through the accession number PQ721376.1. This species stands out for its high growth rate, 22.48 mm d^{-1}, which is related to its ability to adapt to the environment and its pathogenic potential. Recently, this pathogen has been identified as the causal agent of pod rot in cocoa crops in Hawaii (Puig *et al*., 2019, 2021). In Colombia, this disease is usually associated with *Phytophthora palmivora*, which affects not only the pods but also the root and stem of the cocoa tree, causing significant losses of up to 30% of production (Rodríguez-Polanco *et al*., 2020). Therefore, the identification of *Neofusicoccum parvum* as the etiological agent of this pathology in the country generates new knowledge for the agroecology of the crop.

In the culture phase, it is also common to find endophytic fungi of the genus *Aspergillus* spp.*, Fusarium* spp.*, Penicillium* spp.*, Pestalotiopsis* spp., as reported by Delgado-Ospina and collaborators (Delgado-Ospina *et al*., 2021). In this study, 7 of the 26 isolates, which are about 27% of the total, corresponded to the *Fusarium solani* complex (FSCS) with 100% identity percentages. More than 60 phylogenetically distinct species belonging to this complex are known for their pathogenic action in plants (Coleman, 2016; Hamzah *et al*., 2018), particularly in cocoa cultivation. *Fusarium solani* was described by Sosa del Castillo *et al*. (2016) as a species characterized by aggressive behavior. Villamizar-Gallardo *et al*. (2017) isolated and molecularly characterized various species of pathogenic fungi in cocoa and reported *F. solani* as a secondary pathogen of this crop. Kagezi *et al*. (2017) show this species comes from beetles, and they inoculated it into cocoa seedlings, observing that it caused wilting. This finding allowed us to identify that *F. solani* can establish symbiotic relationships with vectors that can transmit this disease to this crop. Likewise, Huda-Shakirah *et al*. (2024) identify and associate *F. solani* as the etiological agent of stem cancer, a disease that affects cocoa.

The second most abundant group of fungi in this research corresponds to the genus *Aspergillus,* comprising six species that account for approximately 23% of the total isolates, including *A. niger*, *A. flavus*, *A. versicolor*, and *A. amoenus*. The presence and impact of those fungi are indirect, since it has been shown that they significantly affect the quality of the final product, but not the development of the crop. Delgado-Ospina *et al.* (2022) report the presence of mycotoxins, specifically Ochratoxin A, produced by *Aspergillus niger* in cocoa beans. Similarly, *A. flavus* is reported as a fungus that affects cocoa cultivation in the post-harvest phase (Sukmawati *et al*., 2024), while *A. versicolor* is reported by Romero-Cortes *et al.* (2019) as a fungus responsible for general food spoilage. In general terms, the presence of all these fungal species is related to their mycotoxigenic potential, with a carcinogenic effect when they reach the consumer through the final processed product. The species identified as *Penicillium paneum* also represents a risk for the crop, especially during the grain fermentation process. This fungus, like the *Aspergillus* species mentioned above, produces mycotoxins, which represent a public health problem as reported by Subroto *et al.* (2023).

In addition, we identified *Pestalotiopsis micrmisporo*a. This is an endophytic fungus characterized by its ability to remain dormant in the plant until it is stressed. At this time, it adopts a pathogenic lifestyle, triggering the development of diseases. EFSA Panel on Plant Health (PLH) *et al.* (2023) reported more than 100 endophytic fungi, including *P. microspora*. It evaluated their effect on stimulating or inhibiting the growth of two high-impact diseases in cocoa: moniliasis and Witch's Broom. We found that *P. microspora* promotes the growth of cocoa fruits when it behaves as an endophytic fungus but also promotes the development of moniliasis when it changes its behavior to a pathogenic mode.

In contrast, the epiphytic species *Clonostachys rosea* is identified, coinciding with the reports made by Krauss *et al.* (2013), who report the presence of the same pathogen and characterize it as the native mycoparasite most commonly isolated in cocoa trees. The species identified as *Geotrichum candidum* has been reported in the literature and is usually found on the surface of cocoa fruits (Copetti *et al.,* 2011; Oussou *et al*., 2022). The genus *Scopulariopsis* spp. is been identified as the anamorphic phase of the genus *Microascus*, both of which are recognized as agents responsible for biodeterioration, characterized by being infectious opportunists (Paterson & Lima, 2015). In cocoa crop, this genus can be present in different stages of processing, especially during fermentation and drying (Schwan *et al*., 2014). Finally, the genus *Bionectria* spp. is described as the teleomorphic phase of the genus *Clonostachys* (Han *et al*., 2020), which explains the closeness obtained in the phylogram carried out, which groups them into the same clade. *Bionectria* is found in the necrotic tissue of plants. In its latest taxonomic classifications, *Bionectria* is reported as a subgenus of *Clonostachys* (Moreira *et al*., 2016).

Conclusions

In the present study, various species of phytopathogenic, endophytic, and epiphytic fungi were identified associated with diseased fruits and in the harvest phase of the *Theobroma cacao* crop in the Norte de Santander and Bolívar departments, regions characterized by their low productivity. In Norte de Santander, the presence of *Moniliophthora roreri*, a fungus responsible for moniliasis, one of the most devastating diseases for this crop, was highlighted, which reaffirms its endemic nature and historical impact in this region. On the other hand, in Bolívar, the species *Neofusicoccum parvum*, recognized as responsible for black ear rot, was identified and characterized for the first time. This is a novel and highly relevant finding, since it expands the knowledge we have about the etiological agents responsible for diseases associated with cocoa cultivation in Colombia, traditionally attributed to oomycetes of the genus *Phytophthora*. This discovery has significant implications at a regional and national scale for the management and control of diseases in this crop. The identification of the *F. solani* complex, as well as the different species of *Aspergillus* and *Penicillium*, supports the need for integrated management of the crop, to avoid potential public health risks, due to the presence of traces of mycotoxins that can resist the grain benefit process and reach the final consumer in the transformed product. In contrast, the identification of *Geotrichum candidum, Clonostachys rosea*, and *Bionectria wenpingii* demonstrated the presence of fungi with beneficial functions for the crop, either contributing to plant growth or as potential biocontrollers against pathogens.

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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

Article writing: OJPP, RAVG, RGC. Experimental design: RAVG, RGC, FHH. Experimental setups: OJPP, ECM. Data analysis: OJPP, RAVG, RGC, FHH. All authors reviewed the final version of the manuscript.

Literature cited

Aime, M. C., & Phillips-Mora, W. (2005). The causal agents of witches' broom and frosty pod rot of cacao (chocolate, *Theobroma cacao*) form a new lineage of Marasmiaceae. *Mycologia*, *97*(5), 1012–1022. https://doi.org/10.1080/15572536.2006.11832751

Albores Flores, V. J., Gómez Rodríguez, L., López García, J. A., & Grajales Conesa, J. (2022). Mecanismos de infección endógena en frutos de cacao con *Moniliophthora roreri*. *Polibotánica*, (53), 197–209. https://doi.org/10.18387/polibotanica.53.13

Amaro, T. M. M. M., Cope, J., & Franco-Orozco, B. (2021). Current status of plant pathogens of agricultural importance for Colombia. A review. *Revista Colombiana de Ciencias Horticolas*, *15*(2), Article e12425. https://doi.org/10.17584/rcch.2021v15i2.12425

Antolinez Sandoval, E. Y., Almanza Merchán, P. J., Baraona Rodriguez, A. F., Polanco Díaz, E., & Serrano Cely, P. A. (2020). Estado actual de la cacaocultura: una revisión de sus principales limitantes. *Ciencia y Agricultura*, *17*(2), 1–11. https://doi.org/10.19053/01228420.v17.n2.2020.10729

Arvelo, M. Á., Delgado, T., Maroto, S., Rivera, J., Higuera, I., & Navarro, A. (2016). *Estado actual sobre la producción, el comercio y cultivo del cacao en América*. Instituto Interamericano de Cooperación para la Agricultura, San José, Costa Rica,. http://hdl.handle.net/11159/3773

Bailey, B. A., & Meinhardt, L. W. (Eds.) (2016). *Cacao diseases: A history of old enemies and new encounters*. https://doi.org/10.1007/978-3-319-24789-2

Coleman, J. J. (2016). The *Fusarium solani* species complex: Ubiquitous pathogens of agricultural importance. *Molecular Plant Pathology*, *17*(2), 146–158. https://doi.org/10.1111/mpp.12289

Copetti, M. V., Iamanaka, B. T., Frisvad, J. C., Pereira, J. L., & Taniwaki, M. H. (2011). Mycobiota of cocoa: From farm to chocolate. *Food Microbiology*, *28*(8), 1499–1504. https://doi.org/10.1016/j.fm.2011.08.005

De Hoog, G. S., Guarro, J., Gené, J., Ahmed, S. A., Al Hatmi, A. S. S., Figueras, M. J., & Vitale, R. G. (2020). *Atlas of clinical fungi* (4th ed.). https://www.atlasclinicalfungi.org

Delgado-Ospina, J., Molina-Hernández, J. B., Chaves-López, C., Romanazzi, G., & Paparella, A. (2021). The role of fungi in the cocoa production chain and the challenge of climate change. *Journal of Fungi*, *7*(3), Article 202. https://doi.org/10.3390/jof7030202

Delgado-Ospina, J., Molina-Hernandez, J. B., Viteritti, E., Maggio, F., Fernández-Daza, F. F., Sciarra, P., Serio, A., Rossi, C., Paparella, A., & Chaves-López, C. (2022). Advances in understanding the enzymatic potential and production of ochratoxin A of filamentous fungi isolated from cocoa fermented beans. *Food Microbiology*, *104*, Article 103990. https://www.sciencedirect.com/science/article/abs/pii/S0740002022000144

EFSA Panel on Plant Health (PLH), Bragard, C., Baptista, P., Chatzivassiliou, E., Di Serio, F., Gonthier, P., Miret, J. A. J., Justesen, A. F., MacLeod, A., Magnusson, C. S., Milonas, P., Navas-Cortes, J. A., Parnell, S., Potting, R., Stefani, E., Thulke, H. H., Van der Werf, W., Civera, A. V., Yuen, J., …, & Reignault, P. L. (2023). Pest categorisation of *Pestalotiopsis microspora*. *EFSA Journal*, *21*(12), Article e8493. https://doi.org/10.2903/j.efsa.2023.8493

Fedecacao. (2021a). *Caracterización de productores de cacao 2017-2021*. Federacion Nacional de Cacaoteros. https://www.fedecacao.com.co/documentos-tecnicos

Fedecacao. (2021b). *Economía nacional*. Federacion Nacional de Cacaoteros. https://www.fedecacao.com.co/economianacional

Fedecacao. (2025). *Colombia reportó en 2024 producción histórica de cacao*. Federacion Nacional de Cacaoteros. https://www.fedecacao.com.co/post/colombia-report%C3%B3-en-2024-producci%C3%B3n-hist%C3%B3rica-de-cacao

Fernández-Niño, M., Rodríguez-Cubillos, M. J., Herrera-Rocha, F., Anzola, J. M., Cepeda-Hernández, M. L., Aguirre Mejía, J. L., Chica, M. J., Olarte, H. H., Rodríguez-López, C., Calderón, D., Ramírez-Rojas, A., Portillo, P., Restrepo, S., & González Barrios, A. F. (2021). Dissecting industrial fermentations of fine flavour cocoa through metagenomic analysis. *Scientific Reports*, *11*, Article 8638. https://doi.org/10.1038/s41598-021-88048-3

Garcés de Granada, E., Correa de Restrepo, M., Coba de Gutierrez, B., Orozco de Amézquita, B., Zapata, A. C., Anacona Chingana, A., & Sabogal, S. P. (2015). *Morfología y clasificación de los hongos*. Universidad Nacional de Colombia. https://repositorio.unal.edu.co/handle/unal/79887

Hamzah, T. N. T., Lee, S. Y., Hidayat, A., Terhem, R., Faridah-Hanum, I., & Mohamed, R. (2018). Diversity and characterization of endophytic fungi isolated from the tropical mangrove species, *Rhizophora mucronata*, and identification of potential antagonists against the soil-borne fungus, *Fusarium solani*. *Frontiers in Microbiology*, *9*, Article 1707. https://doi.org/10.3389/fmicb.2018.01707

Han, P., Zhang, X., Xu, D., Zhang, B., Lai, D., & Zhou, L. (2020). Metabolites from *Clonostachys* fungi and their biological activities. *Journal of Fungi*, *6*(4), Article 229. https://doi.org/10.3390/jof6040229

Huda-Shakirah, A. R., Zakaria, L., & Mohd, M. H. (2024). Morphology, phylogeny and pathogenicity of fungal species associated with leaf blight and stem canker of *Theobroma cacao* in Malaysia. *Plant Pathology*, *73*(6), 1382–1399. https://doi.org/10.1111/ppa.13900

ICCO. (2021). *Pests & diseases*. International Cocoa Organization. https://www.icco.org/pests-diseases/#toggle-id-39

Jain, A., Sarsaiya, S., Wu, Q., Lu, Y., & Shi, J. (2019). A review of plant leaf fungal diseases and its environment speciation. *Bioengineered*, *10*(1), 409–424. https://doi.org/10.1080/21655979.2019.1649520

Kagezi, G. H., Sseruyange, J., Kucel, P., Kobusinge, J., Nakibuule, L., Kabole, C., & Wagoire, W. W. (2017). *Fusarium* spp. associated with *Xylosandrus compactus* causing wilting in cocoa. *Journal of Plant Pathology*, *99*(2), 542–542. https://www.jstor.org/stable/44686824

Krauss, U., ten Hoopen, M., Rees, R., Stirrup, T., Argyle, T., George, A., Arroyo, C., Corrales, E., & Casanoves, F. (2013). Mycoparasitism by *Clonostachys byssicola* and *Clonostachys rosea* on *Trichoderma* spp. from cocoa (*Theobroma cacao*) and implication for the design of mixed biocontrol agents. *Biological Control*, *67*(3), 317–327. https://doi.org/10.1016/j.biocontrol.2013.09.011

Luo, G., & Mitchell, T. G. (2002). Rapid identification of pathogenic fungi directly from cultures by using multiplex PCR. *Journal of Clinical Microbiology, 40*(8), 2860–2865. https://pmc.ncbi.nlm.nih.gov/articles/PMC120665/pdf/1281.pdf

MADR. (2021). *Cadena de cacao. Dirección de Cadenas Agrícolas y Forestales*. Ministerio de Agricultura y Desarrollo Rural. https://sioc.minagricultura.gov.co/Cacao/Documentos/2021-03-31%20Cifras%20Sectoriales.pdf

Marelli, J. P., Guest, D. I., Bailey, B. A., Evans, H. C., Brown, J. K., Junaid, M., Barreto, R. W., Lisboa, D. O., & Puig, A. S. (2019). Chocolate under threat from old and new cacao diseases. *Phytopathology*, *109*(8), 1331–1343. https://doi.org/10.1094/PHYTO-12-18-0477-RVW

Ministerio de Desarrollo Agrario y Riego. (2023). *Observatorio de commodities de cacao*. Boletín trimestral No. 1. Ministerio de Desarrollo Agrario y Riego. https://cdn.www.gob.pe/uploads/document/file/5264502/ Commodities Cacao%3A ene-mar 2023.pdf?v=1697059698#:~:text=En el primer semestre del,%2C Junín%2C Huánuco y Cusco

Moreira, G. M., Abreu, L. M., Carvalho, V. G., Schroers, H. J., & Pfenning, L. H. (2016). Multilocus phylogeny of *Clonostachys* subgenus *Bionectria* from Brazil and description of *Clonostachys chloroleuca* sp. nov. *Mycological Progress*, *15*(10–11), 1031–1039. https://doi.org/10.1007/s11557-016-1224-6

Muñoz, V., Cisterna, V., & France, A. (2020). Aislamiento de microorganismos fitopatogenos. In J. F. Castro (Ed.), *Conformación de colecciones de cultivos microbianos* (Boletín INIA No. 428, pp. 75–91). Instituto Nacional de Investigaciones Agropecuarias, Chile. https://hdl.handle.net/20.500.14001/6945

Oussou, K. F., Guclu, G., Kelebek, H., & Selli, S. (2022). Elucidating the contribution of microorganisms to the spontaneous fermentation and the quality of Ivorian cacao (*Theobroma cacao*) beans: The quality of Ivorian cacao (*Theobroma cacao*) beans. *Quality Assurance and Safety of Crops & Foods*, *14*(4), 23–35. https://doi.org/10.15586/qas.v14i4.1078

Paterson, R. R. M., & Lima, N. (Eds.) (2015). *Molecular biology of food and water borne mycotoxigenic and mycotic fungi*. CRC Press. https://doi.org/10.1201/b18645

Puig, A. S., Keith, L. M., Matsumoto, T. K., Gutierrez, O. A., & Marelli, J. P. (2021). Virulence tests of *Neofusicoccum parvum*, *Lasiodiplodia theobromae*, and *Phytophthora palmivora* on *Theobroma cacao*. *European Journal of Plant Pathology*, *159*(4), 851–862. https://doi.org/10.1007/s10658-021-02210-1

Puig, A. S., Marelli, J. P., Matsumoto, T. K., Keith, L. M., & Gutierrez, O. A. (2019). First report of *Neofusicoccum parvum* causing pod rot on cacao in Hawaii. *Plant Disease*, *103*(6), 1416–1416. https://doi.org/10.1094/PDIS-10-18-1719-PDN

Rodríguez-Polanco, E., Morales, J. G., Muñoz-Agudelo, M., Segura, J. D., & Carrero, M. L. (2020). Morphological, molecular and pathogenic characterization of *Phytophthora palmivora* isolates causing black pod rot of cacao in Colombia. *Spanish Journal of Agricultural Research*, *18*(2), Article e1003. https://doi.org/10.5424/sjar/2020182-15147

Rodriguez Polanco, E., Parra Alferes, E. B., Bermeo Fuquene, P. A., Segura Amaya, J. D., & Rodríguez Polanco, L. A. (2020). Manejo de la pudrición parda de la mazorca (*Phytophthora palmivora*) en cacao por aplicación conjunta de prácticas culturales y químicas. *Revista Facultad de Ciencias Básicas*, *16*(1), 79–94. https://doi.org/10.18359/rfcb.4887

Romero-Cortes, T., López-Pérez, P. A., Pérez España, V. H., Medina-Toledo, A. K., Aparicio-Burgos, J. E., & Cuervo-Parra, J. A. (2019). Confrontation of *Trichoderma asperellum* VSL80 against *Aspergillus niger* via the effect of enzymatic production. *Chilean Journal of Agricultural and Animal Sciences*, *35*(1), 68–80. https://doi.org/10.4067/s0719-38902019005000202

Schwan, R. F., Pereira, G. V. M., & Fleet, G. H. (2014). Microbial activities during cocoa fermentation. In R. F. Schwan, & G. H. Fleet (Eds.), *Cocoa and coffee fermentations* (pp. 129–192). CRC Press. https://doi.org/10.1201/b17536

Sosa del Castillo, D. S., Parra, D., Noceda, C., & Pérez-Martínez, S. (2016). Co-occurrence of pathogenic and non-pathogenic *Fusarium decemcellulare* and *Lasiodiplodia theobromae* isolates in cushion galls disease of cacao (*Theobroma cacao* L.). *Journal of Plant Protection Research*, *56*(2), 129–138. https://www.plantprotection.pl/Co-occurrence-of-pathogenic-and-non-pathogenic-Fusarium-decemcellulare-and-Lasiodiplodia,94178,0,2.html

Suárez Contreras, L. Y., & Peñaranda Figueredo, F. A. (2022). Identificación molecular de hongos filamentosos y su potencial biotecnológico. *Biotecnología en el Sector Agropecuario e Industrial*, *20*(1), 194–206. https://doi.org/10.18684/rbsaa.v20.n1.2022.1914

Subroto, E., Djali, M., Indiarto, R., Lembong, E., & Baiti, N. (2023). Microbiological activity affects post-harvest quality of cocoa (*Theobroma cacao* L.) beans. *Horticulturae*, *9*(7), Article 805. https://doi.org/10.3390/horticulturae9070805

Sukmawati, D., Firhandini, A., Nurkhasanah, S., Khumaiya, U., Gunadi, S. A., Supiyani, A., Nida Al Husna, S., El Enshasy, H., Dailin, D. J., & Sriherwanto, C. (2024). The effect of yeast antagonist isolated from the fermentation of cocoa beans (*Theobroma cacao*) from Lampung, Indonesia, on the growth of *Aspergillus flavus* UNJCC F-55. *KnE Life Sciences*, *8*(1), 75–84. https://doi.org/10.18502/kls.v8i1.15398

Tekpinar, A. D., & Kalmer, A. (2019). Utility of various molecular markers in fungal identification and phylogeny. *Nova Hedwigia*, *109*(1–2), 187–224. https://doi.org/10.1127/nova_hedwigia/2019/0528

Villamizar-Gallardo, R. A., Ortíz-Rodriguez, O. O., & Escobar, J. W. (2017). Symbiotic and endophytic fungi as biocontrols against cocoa (*Theobroma cacao* L.) phytopathogens. *Summa Phytopathologica*, *43*(2), 87–93. https://doi.org/10.1590/0100-5405/2175

Villamizar-Gallardo, R., Osma, J. F., & Ortíz-Rodriguez, O. O. (2019). Regional evaluation of fungal pathogen incidence in Colombian cocoa crops. *Agriculture*, *9*(3), Article 44. https://doi.org/10.3390/agriculture9030044

Zhao, W., Hong, S.-Y., Kim, J.-Y., & Om, A.-S. (2024). Effects of temperature, pH, and relative humidity on the growth of *Penicillium paneum* OM1 isolated from pears and its patulin production. *Fungal Biology*, *128*(4), 1885–1897. https://doi.org/10.1016/j.funbio.2024.05.005

Agroecological analysis of traditional practices in the Yaquivá Indigenous Resguardo (Inzá, Cauca, Colombia)

Análisis desde la agroecología de prácticas tradicionales en el resguardo indígena de Yaquivá (Inzá, Cauca, Colombia)

Marco Helí Franco Valencia[1]*

ABSTRACT

Colombian ethnographic literature reveals a significant gap in systematized documentation of traditional practices. This study examines agroecological practices within the Yaquivá community, focusing on those at risk of disappearing, as a contribution to the socio-agricultural revalorization of local knowledge. Using Participatory Action Research (PAR) methodology, the research documented both the underlying causes of practice decline and potential recovery strategies. The study differentiates between practices experiencing gradual loss and those demonstrating resilience. The Community Educational Project (CEP) of the Jiisa Fxiw Agroecological School emerges as a crucial platform for strengthening and recontextualizing traditional practices, particularly the *minga* (collective work system), hand-to-hand labor exchange, and the *Tul* (ancestral household garden), elements that integrate technological, social, and spiritual dimensions. Findings demonstrate that the erosion of biological diversity parallels the loss of cultural diversity. Food production related to cultural heritage, intrinsically linked to the community's worldview, has diminished substantially, creating dependency on external inputs with severe economic, environmental, and social implications. Results indicate that the Yaquivá educational community recognizes threats to its cultural legacy and underscore the need to counter the expansion of conventional agriculture.

Keywords: ancestral knowledge, worldview, rural development, indigenous communities, reinterpretation.

RESUMEN

Dentro de la literatura etnográfica colombiana, se carece de registros que sistematicen las prácticas tradicionales. Se planteó como objetivo analizar desde la agroecología las prácticas tradicionales, con énfasis en aquellas amenazadas en desaparecer, como aporte a la resignificación socioagricultural en la comunidad yaquiveña. La metodología de investigación utilizada se basó en Investigación Acción Participativa (IAP); se documentó la razón de pérdida de las prácticas tradicionales y algunas alternativas de recuperación; las prácticas que se están perdiendo y las que todavía se realizan. El Proyecto Educativo Comunitario (PEC) del colegio agroecológico Jiisa Fxiw está contemplado como oportunidad para fortalecer y resignificar las prácticas tradicionales, entre ellas, la *minga* (el sistema del trabajo colectivo), el intercambio de trabajo mano a mano y el *Tul* (el jardín ancestral del hogar) como componentes que integran lo tecnológico, social y espiritual. Se concluyó que aunada a la pérdida de la diversidad biológica ha sucedido también la pérdida de la diversidad cultural. Se ha reducido el acervo cultural ligado a la producción de alimentos asociado a su cosmovisión y como consecuencia se ha incrementado el uso de insumos externos, con graves consecuencias económicas, ambientales y sociales. Los resultados señalan que la comunidad educativa yaquiveña es consciente de las amenazas y riesgos que penden sobre su cultura y que el énfasis de sus respuestas debe enfocarse en tratar de revertir este tipo de agricultura convencional.

Palabras clave: conocimiento ancestral, cosmovisión, desarrollo rural, comunidades indígenas, resignificación.

Introduction

The knowledge possessed by indigenous communities, with respect to their traditional practices, involves very complex interactions, socially constructed, that are integral, difficult to fragment and transversal to areas such as health, education, governance, environment, spirituality and production systems (including the *Tul,* defined as an ancestral household garden), within the indigenous logic and worldview. According to Mendoza Hernández *et al.* (2017), the knowledge accumulated from practice and oral transmission is going through a very critical moment, a vision shared by the inhabitants themselves, so it requires great encouragement for its strengthening; beyond its rescue and transmission, it is important to revalue and motivate its practice, in such a way that it contributes to

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the existence of a culture according to its own concepts of life and worldview (Franco-Valencia & Sánchez de Prager, 2017).

Furthermore, the present of a community is the manifestation of historical cultural processes and spatiotemporal variables linked to the current socio-economic and political context at the global, national, regional and local levels (Caparrós, 2014; Harari, 2014; He, 2012). Hence, there is a need to specify, in the first instance, the features of local culture, the gradual changes implied by the imposition and adoption of the green revolution model vs. traditional production systems (Karplus & Deng, 2008), and their consequences on the society/culture/nature relationship in the notion of good living (León Sicard, 2010; Molina *et al*., 2017; Rojas *et al*., 2017; Sánchez de Prager, Rojas *et al*., 2017).

Agroecology as a science, practice and social movement welcomes analytical approaches, such as Participatory Action Research (PAR), to encourage appropriation by the communities of their problems and construction of endogenous solutions; that is, overcoming the vision of formal academia based on documents with an academia that accompanies processes of change that remain in the communities, regardless of whether academia is present or not (Fals Borda, 2008; Torre, 2014; Wezel *et al*., 2011).

The present research is contextualized in the framework of agroecological processes developed in indigenous territories of Colombia. The objective was to study from an agroecological perspective, the traditional practices, with emphasis on those threatened with disappearance, as a contribution to the socio-agricultural reinterpretation in the Yaquivá community. The work was carried out in the Tierradentro region, in a community belonging to the Yaquivá Indigenous Resguardo (Inzá, Cauca). This community is undergoing a process of change to make better use of natural resources, in accordance with the legacy of their ancestors and, above all, with their social reality, the defense of their territories and the care of Mother Earth (Uma Kiwe), to provide sovereignty, dignity and food autonomy to all its inhabitants.

Materials and methods

Location

The Yaquivá Resguardo is located to the east of the central mountain range, in the municipality of Inzá, department of Cauca, Colombia (Fig. 1). It includes the villages of Mesopotamia, Cabuyo, Chichucue, Coscuro, Dos Quebradas, La Milagrosa, El Guadual and Yaquivá. It has a population of approximately 3,900 inhabitants, located in a total area of 16,184 ha at an altitude between 1,600 and 4,000 m a.s.l.,

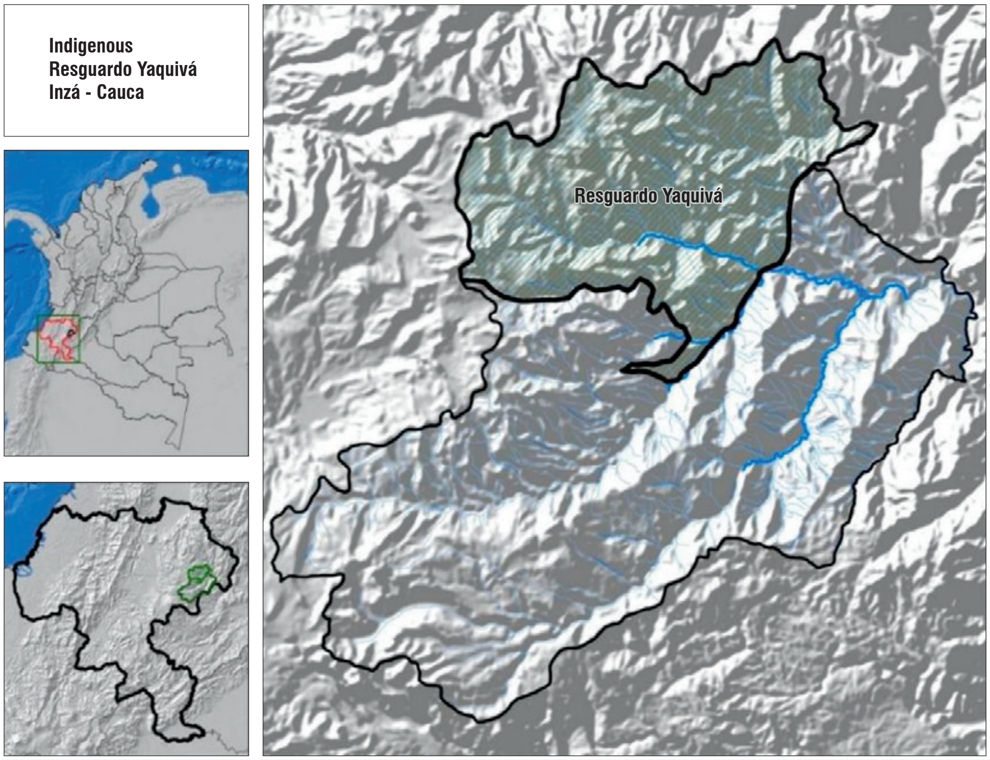

FIGURE 1. Map showing the geographic location of the Yaquivá Indigenous Resguardo. Reproduced with modification from Franco-Valencia and Sánchez de Prager (2018), with permission by Agronomía Colombiana.

which includes the paramo zone. The average annual precipitation and temperature are between 1,000 and 2,000 mm and 18°C, respectively.

In order to bring institutions and academia closer to rural communities (peasant and indigenous), participatory research has been carried out since 2008 between the Universidad Nacional de Colombia and the indigenous community of the Yaquivá Resguardo, in the municipality of Inzá, department of Cauca. The methodology used was Participatory Action Research (PAR), and the methodological tools used were participant observation, discussion groups, participatory diagnoses, surveys and interviews, and dialogic discussions (dialogues of knowledge and practice). Other techniques used to collect information included meetings, visits, pedagogical tulpas (a word derived from the ancestral *Tul* production system, where training events are held), days of hand-to-hand labor exchange and mingas (collective work in the villages) to fix roads and aqueducts, build houses, health centers and schools, and/or assist with sowing; in which all members of the Resguardo participate. The mingas are as much about work as they are about thinking.

Analysis of socio-agricultural clustering data

Analyses were performed using the InfoStat program (Di Rienzo *et al.*, 2017), with the support of the biostatistics unit of the graduate school of the Tropical Agricultural Research and Higher Education Center (CATIE) in Turrialba, Costa Rica. To group the interviewees according to socio-agricultural variables, a cluster analysis was carried out using the Ward grouping method and Gower distance.

The variables used were level of schooling, age composition of the family nucleus, and agricultural information on the farm. The variable composition of the family nucleus was transformed using a Shannon-Weaver diversity index; this index measures the diversity of the family nucleus (Sana *et al.*, 2010). Subsequently, the groups formed with the socio-agricultural variables were associated with the different perceptions of the interviewees. The association was made through Multiple Correspondence Analysis (MCA) for each of the questions. From the worldview of the Nasa people and their own thinking, they were asked: what is agroecology? what traditional and ancestral practices are being lost? why are they being lost? how can they be recovered? what practices are still carried out in the Resguardo? what is the spiritual meaning of the *Tul*?; and what is the participation in the minga and hand-to-hand labor exchange and their relation with the loss of the *Tul*, among others.

Results and discussion

Grouping of respondents according to socio-agricultural variables

Five groups (clusters) were formed according to socio-agricultural variables. The groups are mainly characterized by the following activities: Group 1 (G1), cultivation of pastures and livestock production (cows, guinea pigs, and pigs). Groups 2 (G2) and 5 (G5) are engaged in coffee cultivation, day labor and commerce. Group 3 (G3) includes those who cultivate *Tul*, corn and beans. In group 4 (G4), the main activity is the cultivation of bananas, sugarcane, and poultry (Fig. 2).

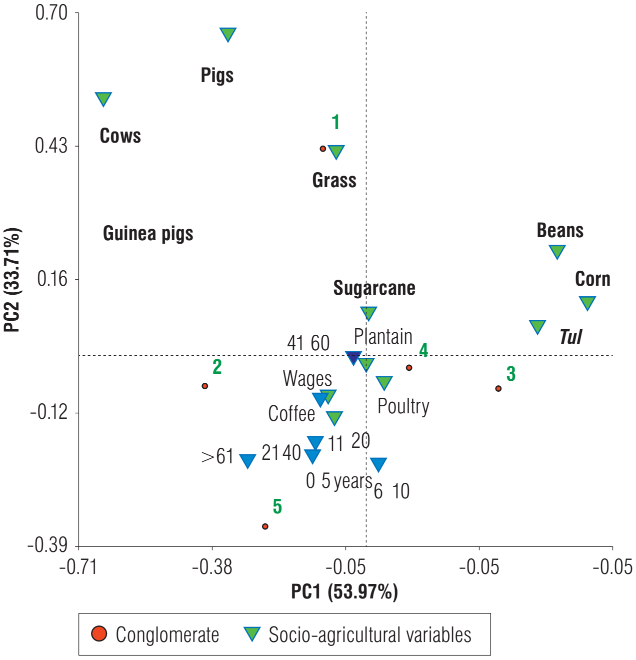

FIGURE 2. Association between groups, age ranges, and socio-agricultural variables.

In the descriptions of the Nasa (Paeces) communities of Tierradentro from the last century to the present, these have been fundamentally agricultural-horticultural, using the slash-and-burn system and cultivating, in the different thermal floors, a diversity of products, including corn, wheat, potato, ulluco, majua, achira, arracacha, and plantations of cabuya, coffee and sugarcane (Rappaport, 1982). Agriculture is based on a calendar around the cultivation of maize, whose labors involve cultural systems of the indigenous worldview (Sanabria Diago, 2016).

Regarding the age composition of the family group, G4 is more associated with people between 41 and 60 years of age; G3, between 6 and 10 years; in G2 those older than 61 years; while G5 presents the highest family composition, in all age ranges, and G1 does not present a defined trend (Fig. 3). In this sense, Franco Valencia (2010) affirms that, for the Nasa people, the family is a vital component of the community and the nucleus of the same, and it will be the school, based on their own education and the worldview of this people, that will be able to nurture this concept and the meaning it has in the children and youth, who, in the future, will be the bearers and transmitters of these values, when they become adults, elders, spiritual leaders (*the Wala*) and indigenous authorities of their communities.

Of the 163 community members interviewed, 41 (25.15%) belong to Cabuyo, 50 (30.68%) to Yaquivá and 72 (44.17%) to La Milagrosa. A review of the distribution of the community members interviewed in the groups obtained from the cluster analysis shows that: 60 (36.81%) are in G4; 34 (20.86%) in G2; 28 (17.18%) in G3; 23 (14.11%) in G5 and 18 (11.04%) in G1 (Tab. 1). In addition, 80 are female and 83 are male; that is, 49.08% and 50.92%, respectively.

Association between typologies, villages and knowledge of agroecology

Community members who know the least about agroecology are those in the G4 group, mainly in the Yaquivá and Cabuyo villages. In contrast, those who know the most are found in groups G1, G2, G3, and G5, in the villages of La Milagrosa, Yaquivá and Cabuyo (Fig. 3). Agroecology and its practices are often spoken of as something relatively new, but it is evident that many of these practices are ancestral; it would be more accurate to say that they have been reused, rediscovered, or better yet, have regained validity and recovered their meaning. In this sense we speak of "resignifying".

There are countless indigenous communities and native peoples whose subsistence depends on ancestral knowledge and traditional practices and who enjoy an intimate familiarity with their culture, forged over hundreds or even thousands of years. Their existence makes sense thanks to this millenary knowledge and "doing". It is their way of life, although they call it differently, distinct from the word "agroecology", which is new to them and unknown to the vast majority; however, agroecological processes and practices are immersed in their daily work and daily life.

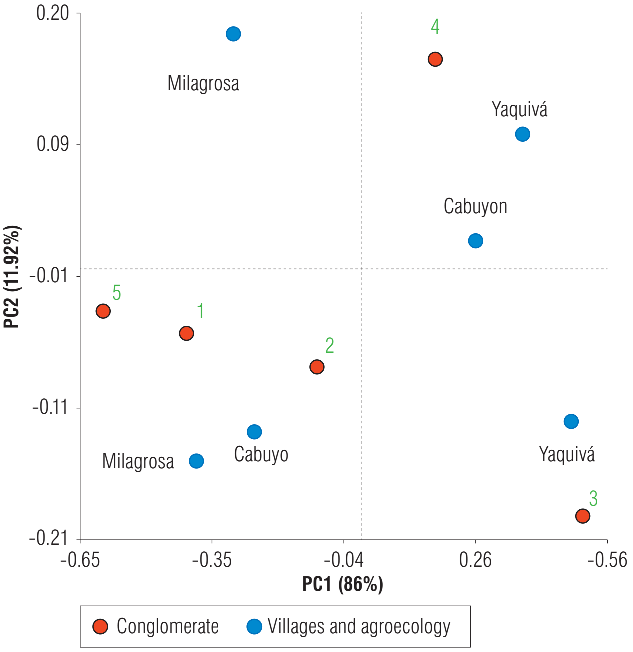

FIGURE 3. Association between typologies, villages, and knowledge of agroecology.

According to Chate (2016, pers. commun.), "with the arrival of the Spaniards and the subsequent colonization policies of the Colombian government, the Paez culture, like other cultures of the country and the continent, has suffered a process of forced assimilation that has resulted in the loss of many elements of traditional culture." In the Yaquivá Resguardo, although it is one of the Resguardos that has existed since the conquest, the process of assimilation and cultural confrontation is one of the strongest in the Nasa communities of Tierradentro. In this sense, Mendoza Hernández *et al.* (2017) state: "The loss of the cultural base would bring enormous consequences in terms of identity, governance, social and natural control, and territorial management, among others, given that the process of knowledge transmission has been weakened because of the current distancing of youth from traditional activities". In addition, we must implement agroecological production systems to

TABLE 1. Number and percentage distribution according to the typologies formed with the socio-agricultural variables.

	G1		G2		G3		G4		G5		Total	
Village	**Number**	**%**	**Number**	**%**	**Number**	**%**	**Number**	**%**	**Number**	**%**	**Number**	**%**
Cabuyo	4	22.22	9	26.47	8	28.57	15	25	5	21.74	41	25.15
Milagrosa	11	61.11	16	47.06	6	21.43	23	38.33	16	69.56	72	44.17
Yaquivá	3	16.67	9	26.47	14	50	22	36.67	2	8.70	50	30.68
Total	18	11.04	34	20.86	28	17.18	60	36.81	23	14.11	163	100

avoid the loss of biodiversity, dependence on external inputs, environmental pollution (soil, water, air) and the loss of biocultural memory (Toledo & Barrera-Basolls, 2008).

The agroecological approach proposes a change in the technological orientations developed by conventional agricultural sciences, seeking to offer farmers contributions that go beyond the technological circuits (Altieri, 1999); therefore, agroecology is considered as a way of producing and living while respecting and loving nature. Furthermore, it is not only a change of inputs but a different way of understanding the complexity of systems. Agroecology takes on the challenge of understanding and applying the principles of nature in agriculture and animal husbandry. To do this, it is necessary to understand a little more about nature. People who live in the countryside know more about nature, but they were induced to abandon this knowledge and adopt a technology that disregarded both knowledge and nature (Sevilla Guzmán, 2006).

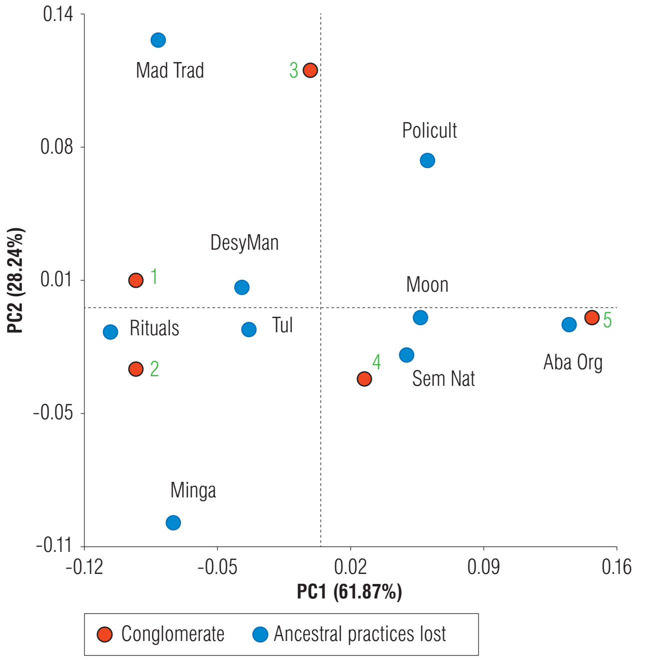

FIGURE 4. Association between typologies and ancestral practices lost

Association between typologies, villages and the ancestral practices being lost

The traditional and ancestral practices that are being lost in G1 and G2 are mainly rituals, minga, manual weeding and *Tul*. In addition, activities linked to the phases of the moon, native seeds and organic fertilizers are being lost in G4 and G5, while traditional doctors (*the Wala*) and polycultures are being lost in G3 (Fig. 4).

In the Resguardo, ancestral and agroecological production practices are being lost because conventional agriculture, the green revolution until 1990, the genetic revolution (since 1990 until today) and input-dependent agriculture (both yesterday and today) have managed to permeate the traditional production systems (peasant, indigenous and Afro). According to Ceccon (2008), the green revolution emerged as a standard-bearer of development, and its main purpose was to generate large-scale yields from agricultural activities based on extensive production and the use of high technology, especially genetic manipulation through bioengineering. It has been the Western concept of progress that has invaded every corner of the world. This has been one of the immediate or specific causes for the Resguardo to have lost the vision of agroecological production, bringing with it social, economic and environmental consequences.

Andoque and Castro (2012) highlight the importance of local participatory research and consider that the strengthening of traditional practices ensures the welfare of indigenous communities and becomes a strategy to cope with global climate change; therefore, they highlight the vision of local knowledge associated with agricultural production systems and, on the other hand, the role of women in the care of crops, the home and the beings of nature. According to Nicholls *et al.* (2017), many of the traditional agroecological strategies that reduce vulnerability to climate variability include crop diversification, maintenance of local genetic diversity, animal integration, biological control, addition of organic matter to the soil, water conservation and harvesting.

Currently, it is difficult to find traditional production systems that manage all these practices; the changes in these production systems reflect the processes of forced acculturation that peasant and indigenous communities are undergoing (Van der Hammen, 1992). By forced or imposed acculturation, we refer to the phenomena of imposition of elements foreign to a culture that imply a loss of elements proper to that culture. From the political ecology perspective, these transformations should be seen not only from a local perspective, since global economic and political processes have been determinant (González de Molina *et al.*, 2014).

Therefore, the traditional *Tul Nasa* production system (traditional ancestral orchard) replicates, from its ancestral knowledge and experience, agroecological practices that have been recognized today, such as crop rotation and association, minimal soil intervention, use of cover crops, allelopathic relationships between the plants, natural biological control, conservation of animal and plant genetic wealth and rational use of water, among others (Franco Valencia, 2010; Sanabria Diago, 2006).

In this way, the *Tul*, which is a fundamental part of the *Nasa* culture and preserves ancestral and agroecological practices, continues to be replicated in a life cycle that respects and preserves life (Franco-Valencia & Sánchez de Prager, 2018). Consequently, the approach from food security and sovereignty towards dignity and food autonomy seeks to promote the development of sustainable production systems, aimed at the well-being of indigenous families. These systems do not generate irreparable consequences to culture and nature; on the contrary, they recover and preserve knowledge along with culture, worldview, vegetation, fauna, water and soils (Franco & Chate, 2016).

From the local level and through the impulse of the *Tul*, an environmental and cultural fabric is reconstructed, where the *Wala* and the elders guide what plants to sow, how to sow, and how much to sow, as exercises of autonomy, territoriality, culture and unity. Additionally, according to Franco Valencia (2010), the environmental program has defined strategies for the conservation of the natural base, articulated with productive and extractive proposals for the Resguardo, in which four components stand out: 1) territorial planning and management; 2) establishment of biological corridors; 3) rescue and improvement of the *Tul Nasa* production system; and 4) community regulation.

According to Vásquez (2004), in parallel to the biological corridors, corridors for food sovereignty have been established, structured based on the improvement of production system models and towards the configuration of a model that reflects the worldview of the *Nasa* people while responding to current ecological and economic needs. In this sense, the rescue and improvement of the *Tul Nasa* model has been proposed as an important strategy for the food sovereignty of the indigenous family and as productive base for the reconstruction of the *Nasa* worldview, which integrates differentiated productive processes with simultaneous processes of conservation of natural resources and strategic ecosystems (Sánchez de Prager, Barrera *et al.*, 2017).

Association between typologies, villages and ancestral practices still performed

For the five groups (G1, G2, G3, G4, and G5), the practices that are still performed in the Resguardo are the minga, hand-to-hand labor exchange and rituals. However, it is worth noting that bartering is mainly carried out in G2 (Fig. 5).

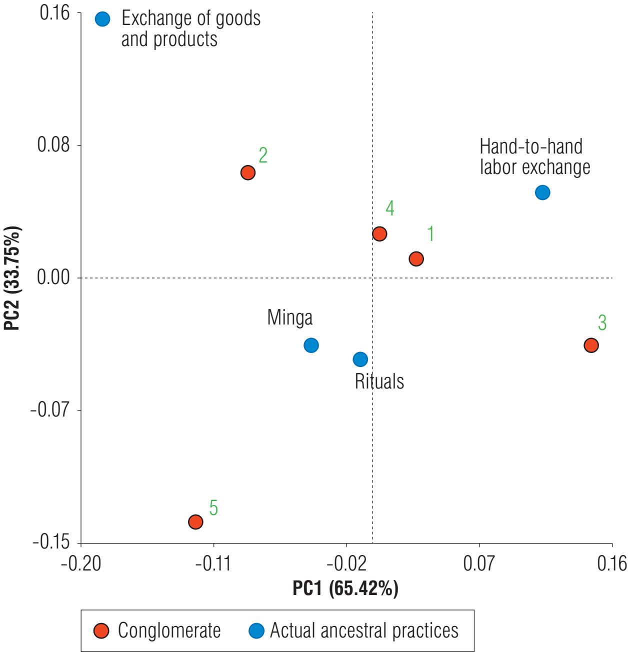

FIGURE 5. Association between typologies and current ancestral practices.

According to Bermúdez Guerrero *et al.* (2005), the indigenous person does not represent the world, but makes it symbolically present through ritual and celebration; he/she knows it through his/her own life, in an experiential way. This is clearly seen in the rites carried out on the summer solstice or in the celebration in honor of the Rainbow that the Ingas perform to guarantee good harvests and also in the mingas and their celebrations. The minga is understood as the feast of planting and harvesting, where the members of the community gather to make the furrows, sow, talk and thank Mother Earth for the fruits and food provided. For the Iku, the "pagamentos" (payments) are rituals of gratitude and return to Mother Earth for everything received. Human societies have survival strategies that are mediated by cosmologies, mythical structures, rituals, habits and customs (Reichel-Dolmatoff, 1997). However, "the relations of communities with the environment are not always adaptive strategies determined by materialistic rationality, and not all practices are aimed at preserving the homeostasis of the system", as Rappaport (1982) stated when studying rituals.

Taking into account that there are several types of mingas (family and community; work and thinking, among others), these acquire certain particularities; in the family minga, it is the heads of the household who are in charge of the convocation and organization, while in the community minga, it is the authorities of the Resguardo who do these tasks (Franco & Chate, 2016). According to Mendoza Hernández *et al.* (2017): "These mingas are of great value, as they allow the transmission of a wealth of information regarding work, knowledge of nature (vegetation, animals, soils, etc.), norms of social and natural conduct, etc.". The practice of the minga as a work, learning and social relationship strategy has been undermined by the individualization of work due to personal interests, less respect for traditional authorities and the loss of traditional knowledge. The decline of this work strategy contributes, to some extent, to community disintegration, as the opportunity for space and time for social and cultural exchange is lost. Thus, a form of community work of great importance is disappearing, despite being socially and environmentally sustainable (Mendoza Hernández *et al*., 2017).

Finally, it is worrying that the elderly, considered the current support of culture, already at the end of their life cycle, may take with their passing an accumulation of knowledge and information that has not been transmitted; their advanced age and small number make this concern even greater. Therefore, it is urgent to promote an internal dialogue in an analytical and reflective environment, establishing actions and strategies that achieve in the immediate, the reunion of these two generations and their genders, so that the agreements can be established that transcend into facts and reactivate the flow of knowledge.

Participation in the minga, the hand-to-hand labor exchange and their relation to the loss of the *Tul*

Groups G1, G3 and G4 are those that participate the most in the minga and in the hand-to-hand labor exchange (Fig. 6). In contrast, G2 and G5 are those with the least participation in these activities and are also the groups that have lost the *Tul*. The minga and the labor exchange are two forms of work that allow sharing resources and forces to carry out a task; in addition, the knowledge acquired during these activities not only has to do with material work but also includes spiritual guidance.

The *Tul* is the main source of food and medicinal plants; it is also a symbol of social cohesion among the community, *as* it is also used in minga. However, the process of globalization and modernization has pressured the adoption of foreign consumption, uses and customs, which have been detrimental to ancestral knowledge and practices, leading to the loss or disuse of both their traditional plant species and the knowledge and practices associated with them.

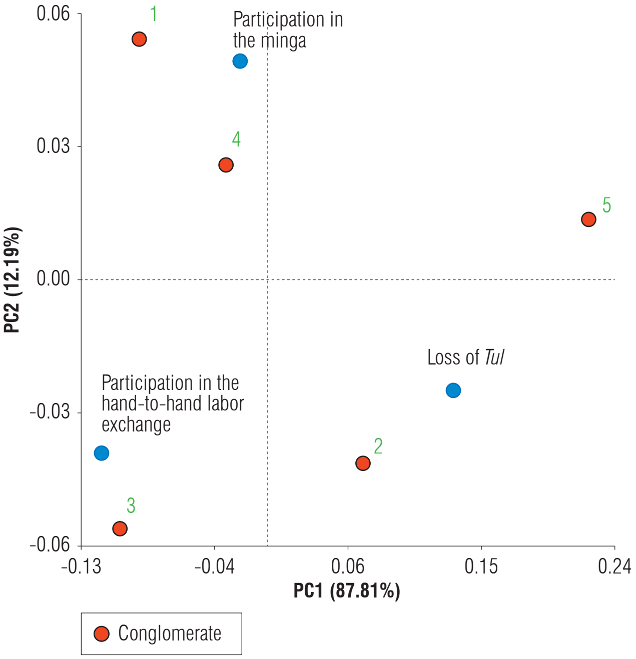

FIGURE 6. Participation in the minga, hand-to-hand labor exchange and their relation to the loss of the *Tul*.

According to Franco Valencia (2010), the *Tul* is considered the expression of the capacity for domestication and technological adaptation to the diversity of Andean agroecological conditions, where the passes or meeting places of basins and sub-basins constituted points of Andean economic dynamization, for a living local territory. These dynamization points (which are more than 500 years old and have resisted the onslaught of green revolution production models) continue to provide benefits to the communities, where the reciprocity and exchange systems of products and food remain dynamic and intense. This testimony of historical permanence is a reliable indicator of their sustainability (Vásquez, 2004).

Spiritual meaning of the *Tul* from the *Nasa* worldview

Groups G2 and G5 mainly associate the spiritual meaning of the Tul with ancestry, while groups G1, G3, and G4 associate it with good living, food sovereignty and autonomy (Fig. 7). For Franco-Valencia and Sánchez de Prager (2018), despite the western socio-cultural onslaught, the *Nasa* skillfully maintained much of the cultural legacy such as: thought through language, spirituality manifested through rituals, traditional medicine, minga as a form of collective work and the *Tul* as part of food sovereignty, dignity, and autonomy. In addition, it is in the *Tul* where a whole process of spiritual relationship between the indigenous and nature is manifested and expressed, to guarantee food production and good living (Chate & Franco, 2016).

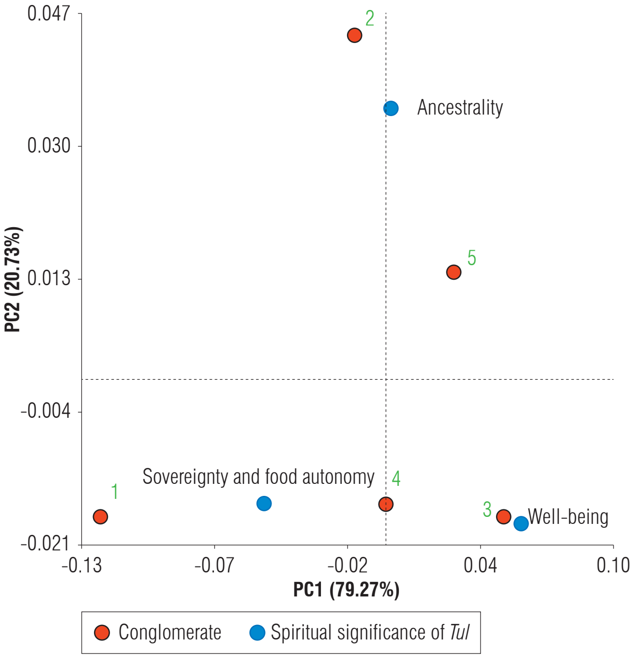

FIGURE 7. Spiritual meaning of the *Tul* from the Nasa worldview.

Like the *Tul*, Román (2007) considers that "the practice of the *chagra* entails a whole work protocol that is related to myths, which support incantations to take care of physical and spiritual health; the *chagra* is the strength of spiritual and physical work". Myths, legends and rituals constitute the cosmogony through which indigenous people interpret their environment, organize it and give the communities regulatory guidelines for the use of the territory. For example, the conservation of sacred or mythological places, modeled by supernatural beings, whose violation may be subject to punishment by these beings (Rappaport, 1982; Van der Hammen, 1992).

For Mendoza Hernández *et al*. (2017), "The construction of a healthy person, physically, spiritually and morally, depends on the food consumed; the product of each species sown contributes with its nutrients to this construction. Hence the importance of the variety of species and their conservation to ensure a healthy and integral diet." The traditional strategy for the conservation of the species is the transfer of the seeds to a new *chagra*, together with the exchange that takes place through community work (*minga*). The consumption of their products is also a guarantee for their conservation; as long as they are used, production is encouraged. Additionally, an important particularity that defines the *chagra* is the existence of a spiritual relationship between the different elements of nature and human beings, which guarantees the success of production and its benefits. In this regard, Van der Hammen (1992) refers to the importance of the magical-religious knowledge that sustains the proper functioning of traditional productive systems.

Mendoza Hernández *et al*. (2017) also evaluated in a general way the content of traditional species in the *chagras* and found that traditional species have lost almost half of their presence in the *chagra*. Consequently, the situation is critical from three perspectives: from the loss of the cultural value that is related to their identity; from the loss of the opportunity to consume healthy and nutritious food; and from the risk of economic dependence on processed products of external origin. All the above impacts the loss of knowledge that has been forged through practice and transmitted orally for hundreds of years. In short, food sovereignty and autonomy, provided by the *Tul*, would be strongly affected.

Conclusions

The study of traditional practices, visualized from the interaction with the community, corroborates that together with the loss of biological diversity, there has been a loss of cultural diversity. The cultural heritage linked to food production associated with their worldview has been reduced and the use of external inputs (pesticides and fertilizers) has increased, with serious economic, environmental and social consequences. In contrast, the Yaquivá community is trying to retake the most important aspects of their worldview to maintain and survive in the territory for many years, leaving a legacy for future generations.

Despite the above, globalizing modernity has permeated the community, and the socioeconomic decisions they have made are immersed in this vision rather than in their culture. This modernization is also reflected in the transformation of the agricultural sector and its traditional practices of production (*Tul*) and collective work (minga and hand-to-hand labor exchange), which are intimately linked to education and preserved through time. Today, they are decreasingly practiced by the new generations. However, the ethno-education component of the Jiisa Fxiw Agroecological School has become a defense of the traditional Yaquivá culture and has sought the socio-agricultural reinterpretation of these practices.

Finally, this research process, extended over fifteen years, allows us to reflect on the role of academia in the development of communities and, from agroecology, to understand with full knowledge of the facts that the academia is a qualified companion that can be present in community processes of feedback such as the one experienced and, at the same time, provide feedback. However, decision making and actions correspond to the autonomy and identity of the Yaquivá community.

Conflict of interest statement

The author declares that there is no conflict of interests regarding the publication of this article.

Author's contributions

MHFV: writing, review and editing. The author approved the final version of the manuscript.

Literature cited

Altieri, M. A. (1999). *Agroecología. Bases científicas para una agricultura sustentable*. Editorial Nordan-Comunidad. https://www.biodiversidadla.org/Documentos/AGROECOLOGIA_Bases_cientificas_para_una_agricultura_sustentable

Andoque, I., & Castro, H. (2012). *La vida de la chagra, saberes tradicionales y prácticas locales para la adaptación al cambio climático en la comunidad El Guacamayo*. Tropenbos Internacional Colombia, Bogotá D.C. https://tropenboscol.org/recursos/publicaciones/the+life+of+the+chagra:+local+traditional+knowledge+and+practices+for+climate+change+adaptation

Bermúdez Guerrero, O. M., Mayorga Rodríguez, M. L., Jacanamijoy Tisoy, B., Seigundiba Quigua, A., & Fajardo Epinayu, T. (2005). *El diálogo de saberes y la educación ambiental*. Universidad Nacional de Colombia. Instituto de Estudios Ambientales, Bogotá. https://books.google.com.co/books?id=EvH4o_LGK4QC&printsec=frontcover&hl=es&source=gbs_ge_summary_r&cad=0#v=onepage&q&f=false

Caparrós, M. (2014). *El hambre: ninguna enfermedad, ninguna guerra, ninguna plaga han sido tan letales y, al mismo tiempo, tan evitables como el hambre* (1st ed.). Editorial Planeta Colombia.

Ceccon, E. (2008). La revolución verde tragedia en dos actos. *Ciencias*, *1*(91), 21–29. https://www.redalyc.org/pdf/644/64411463004.pdf

Chate, R., & Franco, M. (2016). El cambio de mano en el Resguardo indígena de Yaquivá, Inzá (Cauca). *IX Simposio Nacional de Agroecología, V Seminario Internacional de Agroecología, VII Feria de Intercambio de Experiencias y Productos de la Agricultura Ecológica*. Universidad Nacional de Colombia, Palmira. Colombia. Octubre 17–23, 2016.

Di Rienzo, J. A., Casanoves, F., Balzarini, M. G., Gonzalez, L., Tablada, M., & Robledo, C. W. (2017). InfoStat versión 2017. Grupo InfoStat, Universidad Nacional de Córdoba, Argentina. http://www.infostat.com.ar

Fals Borda, O. (2008). Orígenes universales y retos actuales de la IAP. *Análisis Político*, (38), 73–90. https://revistas.unal.edu.co/index.php/anpol/article/view/79283

FAO. (2014). *Simposio Internacional sobre Agroecología para la Seguridad Alimentaria y Nutrición*. Rome, September 18–19, 2014. Organización de las Naciones Unidas para la Agricultura y la Alimentación. https://www.fao.org/about/meetings/afns/es/

Franco, M., & Chate, R. (2016). La minga: practica ancestral agroecológica en el Resguardo indígena de Yaquivá, Inzá (Cauca). *IX Simposio Nacional de Agroecología, V Seminario Internacional de Agroecología, VII Feria de Intercambio de Experiencias y Productos de la Agricultura Ecológica*. Universidad Nacional de Colombia, Palmira, Colombia. October 17–23, 2016.

Franco Valencia, M. H. (2010). *Análisis participativo agroecológico del "Plan de Vida" del Resguardo indígena de Yaquivá* [Master thesis, Universidad Internacional de Andalucía, Universidad de Córdoba /Universidad Pablo de Olavide]. https://dspace.unia.es/bitstream/handle/10334/1774/0277_Franco.pdf?sequence=1

Franco-Valencia, M. H., & Sánchez de Prager, M. (2018). Life plan for the Yaquivá indigenous reservation in the municipality of Inza, Cauca Colombia, from the perspective of Agroecology. *Agronomía Colombiana*, *36*(2), 143–151. https://doi.org/10.15446/agron.colomb.v36n2.71996

González de Molina, M., Infante-Amate, J., & Herrera González de Molina, A. (2014). Cuestionando los relatos tradicionales: desigualdad, cambio liberal y crecimiento agrario en el Sur peninsular (1752–1901). *Historia Agraria*, (63), 55–88. https://dialnet.unirioja.es/servlet/articulo?codigo=4800748

Harari, Y. N. (2014). *Sapiens. De animales a dioses. Breve historia de la humanidad*. Debate.

He, C. (2012). Field-specific modernization. In C. He (Ed.), *Modernization science. The principles and methods of national advancement* (pp. 347–508). Springer Berlin. https://doi.org/10.1007/978-3-642-25459-8_6

Karplus, V. J., & Deng, X. W. (2008). Modern science on the farm: The green revolution. In V. J. Karplus, & X. Wang Deng (Eds.), *Agricultural biotechnology in China: Origins and prospects* (pp. 17–26). Springer.https://doi.org/10.1007/978-0-387-71139-3_2

León Sicard, T. (2010). Agroecología: desafíos de una ciencia ambiental en construcción. In M. A. Altieri (Ed.), *Vertientes del pensamiento agroecológico: fundamentos y aplicaciones* (pp. 45–67). Sociedad Científica Latinoamericana de Agroecología, Medellín, Colombia. https://media2.utp.edu.co/oficinas/61/vertientes-del-pensamiento-agroecologico-fundamentos-y-aplicaciones.pdf

Mendoza Hernández, D., Rodriguez Uaroke, O., Mendoza Rivera, C., Mendoza Rivera, E., Gómez, A., Kutdo, L., Ortiz Valencia, J., & Ortiz, J. C. (2017). *Moniya ringo. Mujer de abundancia y reproducción: Estudio de caso de la chagra de la Gente de Centro, Resguardo Indígena de Monochoa*. Instituto Amazónico de Investigaciones Científicas "Sinchi". Editorial Kimpres. https://sinchi.org.co/index.php/moniya-ringo-mujer-de-abundancia-y-reproduccion

Molina, J. P., Aranda Camacho, Y., Parra, J. E., Montoya, J. N., Montoya, I., Lesmes, A., Bayona, M., Castaño, G., Parrado, A., & Manzo Ramos, F. (2017). Formulación de un plan de desarrollo rural con enfoque territorial (PDRET). In F. R. Leiva (Ed.), *Territorio en vilo. Desarrollo rural para el posconflicto* (pp. 43–64). Editorial Universidad Nacional de Colombia, Bogotá D.C. https://www.unidadvictimas.gov.co/documentos_bibliotec/territorio-en-vilo-desarrollo-rural-para-el-postconflicto/

Nicholls, C. I., Altieri, M. A., & Vázquez, L. L. (2017). Agroecología: principios para la conversión y el rediseño de sistemas agrícolas. *Agroecología*, *10*(1), 61–72. https://revistas.um.es/agroecologia/article/view/300741

Rappaport, J. (1982). *Territory and tradition: the ethnohistory of the Paez of Tierradentro, Colombia* [Doctoral dissertation, University of Illinois Urbana-Champaign]. https://hdl.handle.net/2142/70687

Reichel-Dolmatoff, G. (1997). Discurso pronunciado por Gerardo Reichel-Dolmatoff el 16 de diciembre de 1987, al recibir el doctorado Honoris Causa de la Universidad Nacional de Colombia. In *Gerardo Reichel-Dolmatoff, antropólogo de Colombia* (pp. 11–14). Universidad Nacional de Colombia - Banco de la República Museo del Oro. Bogotá.

Román, G. (2007). Formas de producción y conocimiento tradicional de las mujeres Huitoto, Colombia. In L. M. Donato, E. M. Escobar, P. Escobar, A. Pazmiño, & A. Ulloa (Eds.), *Mujeres indígenas, territorialidad y biodiversidad en el contexto latinoamericano* (pp. 165–168). Universidad Nacional de Colombia. https://repositorio.unal.edu.co/bitstream/handle/unal/3084/01prel01.pdf?sequence=1&isAllowed=y

Sana, E. A. (Ed.) (2010). *Teaching and learning in the health sciences*. University of the Philippines Press. http://nast.dost.gov.ph/images/pdf%20files/Publications/Outstanding-Awardees%20BOOKS/2011/Teaching%20and%20Learning%20in%20the%20Health%20Sciences.pdf

Sanabria Diago, O. L. (2006). *Manejo de germoplasma nativo en agroecosistemas tradicionales de la región andina de Tierradentro, Cauca, Colombia, Suramérica* [Doctoral dissertation, Universidad Nacional Autónoma de México]. https://ru.dgb.unam.mx/bitstream/20.500.14330/TES01000609819/3/0609819.pdf

Sanabria Diago, O. L. (2016). La agrobiodiversidad como patrimonio biocultural de los pueblos: de los saberes ancestrales a las políticas de conservación. In C. M. Caetano, & C. I. Cardozo Conde (Eds.), *Cuadernos Recursos Fitogenéticos Neotropicales*, *8*, 48–49. Memorias II Simposio de Recursos Fitogenéticos Neotropicales. Palmira, Colombia. https://simposiorecursosfi.wixsite.com/ii-simposio-rfgn/copia-de-comites-1

Sánchez de Prager, M., Barrera, N., Prager, M., Zuluaga, I., De la Cruz, G., & Ángel, D. (2017). La agroecología: opción de bienestar. In *Universidad y territorio* (pp. 252–279). Universidad Nacional de Colombia, Colección Sesquicentenario.

Sánchez de Prager, M., Rojas, L. J., Sabogal, J., Ortiz, J. C., Franco, M. H., De la Cruz, G., & Guetocue, E. M. (2017). La soberanía alimentaria garante de la seguridad alimentaria, la biodiversidad y la conservación de la riqueza natural. In F. Leiva (Ed.), *Territorio en vilo. Desarrollo rural para el posconflicto* (pp. 83–112). Editorial Universidad Nacional de Colombia, Bogotá D.C. https://www.unidadvictimas.gov.co/documentos_bibliotec/territorio-en-vilo-desarrollo-rural-para-el-postconflicto/

Sevilla Guzmán, E. (2006). *De la sociología rural a la agroecología*. Icaria Editorial, Barcelona. https://icariaeditorial.com/perspectivas-agroecologicas/3780-de-la-sociologia-rural-a-la-agroecologia.html

Toledo, V. M., & Barrera-Bassols, N. (2008). *La memoria biocultural. La importancia ecológica de las sabidurías tradicionales*. Icaria Editorial, Barcelona. https://www.uv.mx/orizaba/mgas/files/2016/03/memoria-biocultural.pdf

Torre, M. E. (2014). Participatory action research. In T. Teo (Ed.), *Encyclopedia of critical psychology* (pp. 1323–1327). Springer. https://link.springer.com/rwe/10.1007/978-1-4614-5583-7_211

Van der Hammen, M. C. (1992). *El manejo del mundo. Naturaleza y sociedad entre los Yukuna de la Amazonia colombiana* (2nd ed.). Tropenbos-Colombia. https://tropenboscol.org/recursos/publicaciones/managing+the+world%3B+nature+and+society+by+the+yukuna+of+the+colombian+amazonia.?language=es

Vásquez, L. (2004). Los planes de vida de los Resguardos indígenas Paeces del Norte del Cauca, como planes de desarrollo integral. *Tierra*, *3*, 96–100.

Wezel, A., & Soldat, V. (2009). A quantitative and qualitative historical analysis of the scientific discipline of agroecology. *International Journal of Agricultural Sustainability*, *7*(1), 3–18. https://doi.org/10.3763/ijas.2009.0400

Social aspects of peasantry. Case studies in two villages of Cundinamarca (Colombia)

Aspectos sociales del campesinado. Estudios de caso en dos veredas de Cundinamarca (Colombia)

Diego Alejandro López Melo[1] and Juan Carlos Barrientos Fuentes[2]

ABSTRACT

This study analyzed the social aspects of peasantry in two villages in Cundinamarca (Roble Hueco and Rincón Santo) using a mixed-methods approach with interviews (seven experts from the study site), surveys (eight per community), and direct observation. Findings revealed small and aging families, with increasing female leadership in productive units. Rural-urban migration and gender equality trends have reshaped traditional family structures. Most farmers identify as food producers, deeply connected to the land and aware of their responsibility for national food security. However, weak leadership, low trust, and poor cohesion hinder community organization, associativity, and the preservation of cultural activities. Rincón Santo shows stronger political participation and organization due to its proximity to urban areas. Government services (education, health, technical assistance, and infrastructure) remain inadequate, as do access to land and decent housing. The study concluded that strengthening peasant communities requires comprehensive policies that recognize their role in rural development, promoting generational renewal and facilitate better living conditions to support territorial permanence.

Keywords: family, housing, networks, organization, education, health.

RESUMEN

El estudio analizó los aspectos sociales del campesinado en dos veredas de Cundinamarca (Roble Hueco y Rincón Santo) mediante una metodología mixta con entrevistas (siete expertos del lugar de estudio), encuestas (ocho por comunidad) y observación directa. Los resultados muestran familias pequeñas y envejecidas, con una creciente participación femenina en la dirección de las unidades productivas. La migración rural-urbana y la búsqueda de igualdad de género han transformado la estructura familiar y los roles tradicionales. La mayoría de los campesinos se autodefinen como productores de alimentos, arraigados a la tierra y conscientes de su papel en la seguridad alimentaria nacional. Sin embargo, la pérdida de liderazgo, la débil cohesión social y la falta de confianza limitan la organización comunitaria, la asociatividad y la preservación de actividades culturales rurales. En Rincón Santo existe mayor organización y participación política que en Roble Hueco, gracias a su cercanía al centro urbano. Los servicios estatales (educación, salud, asistencia técnica e infraestructura vial) son insuficientes, al igual que las oportunidades de acceso a tierra y vivienda digna. El estudio concluyó que el fortalecimiento de la comunidad campesina requiere políticas integrales que reconozcan su papel en el desarrollo rural, promuevan el relevo generacional y facilitar mejores condiciones de vida para su permanencia en el territorio.

Palabras clave: familia, vivienda, redes, organización, educación, salud.

Introduction

In Colombia, agricultural activities, important for the country's food sovereignty and security, have been carried out by a specialized community called the peasant community. In pre-Columbian times, the Muisca, an indigenous group of the central region of the country, had a hierarchical system based on agriculture. They owned communal and family lands; the former for the upper classes, the latter for family consumption, with orchards of representative species. Pre-Hispanic agriculture influenced today's efficient agriculture, with associative polycultures and the implementation of orchards for food and barter mechanisms between families (Sierra, 2019; Villate Santander, 1998). During the Spanish colonial era, indigenous systems gave way to feudalism and slavery. Monocultures of grain crops, especially wheat, replaced agricultural diversity. Creoles and mestizos emerged as modern peasants, ensuring the colonial food supply. Rural emancipation confronted feudal exploitation until the 19th century in the departments

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of Cundinamarca, Antioquia, and Bolivar. The hacienda prevailed, with Creole landowners and highly precarious indigenous labor. The 19th century lacks records on peasantry, which was overshadowed by coffee production and political ups and downs. Export production, such as tobacco and cotton, promoted rural-urban migration and semi-servile labor, fostering settlements and small-scale agriculture. The expansion of the agricultural frontier generated intermediate and small urban centers (Bejarano, 1983; Fajardo Montaña, 1981; Preciado Beltrán, 2006). In the twentieth century, the coffee industry and the economic crisis of 1929, known as the Great Depression, transformed the rural structure of Colombia. The violence that emerged in the 1950s reflected the abandonment of the State and the lack of a long-awaited rural reform. During the period 1960-1980, unconventional military forces and private military groups sought to control rural areas, while the State implemented inadequate security policies. Caught in the middle of these disputes were the peasants, who suffered the most serious consequences: threats, kidnapping, death, forced displacement, dispossession of land, and increased migration to the cities. In the 21st century, the Democratic Security Policy (2002-2010), the signing of the Peace Agreement with the FARC (2016), and the Comprehensive Rural Reform (2016) seek to improve the situation in rural areas, strengthening productive activity and highlighting the role of peasants in Colombian society and economy (Alarcón Meneses, 2010; Cruz Rodríguez, 2019; Fajardo Montaña, 1981; Suárez, 2015).

In the history of agriculture, the peasantry has played a central role. However, its role and therefore its concept have changed as the context has changed. The green revolution and technological advances changed the concept of the peasant, which became obsolete from a capitalist perspective, generating academic debate. Leeds (1977) and Kearney (1996) questioned its accuracy and proposed neologisms. Kearney (1996) suggested "polybian" to reflect non-agricultural activities. However, his theory was challenged by the peasant and indigenous revolution in Chiapas, Mexico, the focus of his research. The variety of rural economic roles and dynamics suggested a conceptual reassessment of the peasantry in the modern era, where subsistence gave way to competitiveness and labor diversification in rural communities (Edelman, 2022; Kearney, 1996; Leeds, 1977; Shanin, 1979). Contemporary agriculture has blurred the line between peasantry and agribusiness. Van der Ploeg (2016) proposes a new rurality where both coexist. Thus, the peasantry prioritizes cost reduction, crop diversification and cooperation (Edelman, 2022). Since its creation in 1993, *La Vía Campesina* has gradually redefined the peasant as someone who has a direct and special connection to the land, relies on family labor and organizes on a small-scale. In this sense, peasants are rooted in their communities and care for the natural environment. Thus, more recent definitions expand the term "campesino" to include those who work in agriculture, livestock, transhumance and related crafts, including indigenous people (La Via Campesina 2009, 8–9, cited in Edelman, 2022). In 2020, the Colombian Institute of Anthropology and History (ICAH) defined the Colombian peasant as an intercultural subject committed to direct work on the land and nature, living in forms of social organization based on family and community work. This definition highlights the rural multidimensionality, where peasant life goes beyond production (Saade Granados, 2020). Considering the diverse views of authors who have enriched the concept of peasantry, the peasant can be defined as an intercultural subject, politically active, rooted to the land and engaged in economic dynamics complementary to capitalism. Their territoriality is fundamental, going beyond formal tenure (with property titles) of land ownership (Edelman, 2022; Hernández, 1993; Shanin, 1979).

From a sociological approach, Sevilla-Guzmán and Pérez Yruela (1977) refer to a diversity of social structures in rural communities and their influence on peasant behavior, highlighting the importance of characterizing the different peasant groups within their social context in order to advance the theoretical debate on peasantry. For his part, Edelman (2013) discusses the diversity of peasant experiences in different parts of the world and the interaction between traditional agricultural practices and modern economic and social changes. His study highlights peasant management and resistance to market forces and state policies, and the effect that globalization and industrialization have had on peasant ways of life and identity. In the same vein, Hervieu and Purseigle (2015) regard the coexistence of different agricultural models and practices in a country, between traditional and modern, giving rise to sociological characteristics of each peasant group or community.

There have also been changes in agricultural activity in Colombia, and consequently, in the lives of peasants. In this sense, and considering that there are changes throughout society and especially among the peasantry, we propose the following research question: what are the current social characteristics of the peasantry in Colombia, specifically in the department of Cundinamarca and in municipalities near a large city such as Bogotá? To answer this question, this article aims to describe and analyze the social aspects of the peasants of Cundinamarca based on case studies of two villages: Roble Hueco and Rincón Santo.

Materials and methods

The study uses a mixed methodological design, combining qualitative and quantitative approaches, to understand, through two case studies (Stake, 1995), the perception and realities of the farmers of the Roble Hueco and Rincón Santo villages (Figs. 1 and 2) in the municipalities of Bojacá and Zipacón, respectively, in the region of Cundinamarca.

The choice of the aforementioned villages was based on their productive structure, their proximity to the country's capital Bogotá D.C. and the researchers' previous knowledge of the region. The target population was peasant families involved in agricultural activities and rooted in their territories. Of the total population of 100 families, 40 from Roble Hueco and 60 from Rincón Santo, eight peasant families were selected from each village, representing 44.4% of the 18 families from Roble Hueco and 47.05% of the 17 families from Rincón Santo. The sample was small, and the sampling was non-probabilistic and convenience-based. The selection criteria were: being from the village, being a peasant, and being willing to be interviewed. Data collection was conducted during 2021 and 2022 primarily through semi-structured interviews and surveys, supplemented by direct observation and informal conversations. Interviews were conducted in a fluid manner, allowing interviewees to share their perception and understanding of the environment. The surveys provided basic information for the characterization of the family units. Direct observation helped identify additional considerations to the interviews, while conversations with local actors (one technical assistant for each UMATA (Municipal Agricultural Technical Assistance Unit), one secretary of economic development per municipality, the director of the UMATA in Zipacón and two non-farmers per municipality) provided context about the situation in each village.

The analysis focused on factors and indicators such as:

- Characterization of the peasant family unit (name and age of interviewee, name and description of property, family members and roles);
- Housing and land tenure (land size and ownership, basic housing services, material the house is made of);
- Family configuration (number of members, organization and roles);
- Being a peasant (a definition);

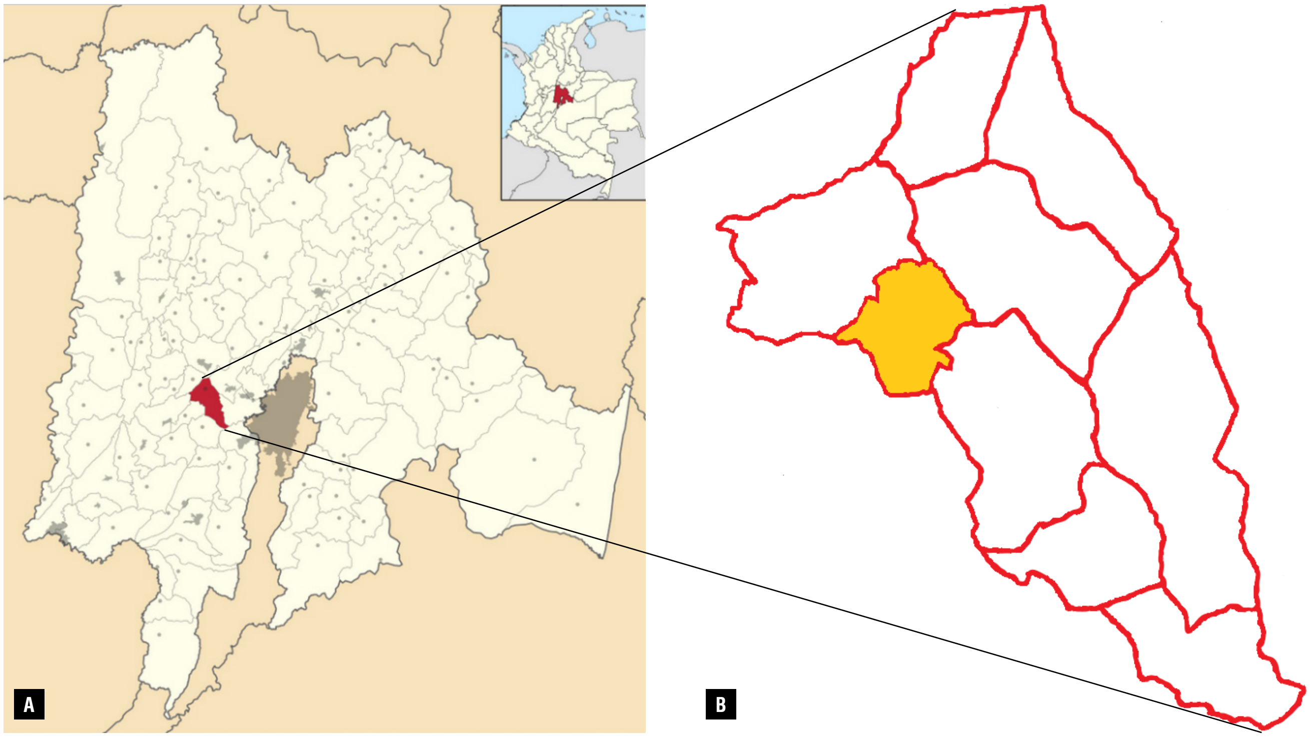

FIGURE 1. Location of Roble Hueco (orange) in the municipality of Bojacá (red) in the region of Cundinamarca (left). Source: López Melo and Barrientos Fuentes (2023), published with permission by Agronomía Colombiana.

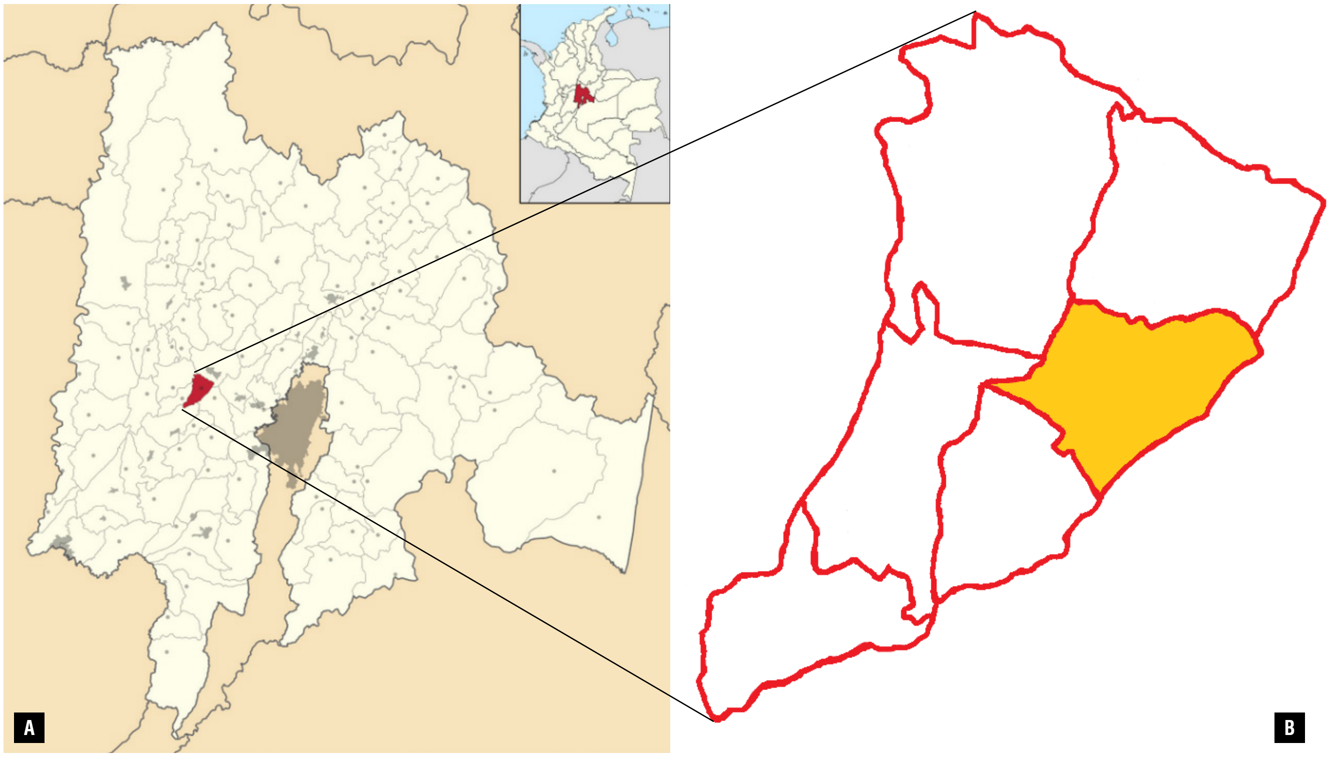

FIGURE 2. Location of Rincón Santo (orange) in the municipality of Zipacón (red) in the region of Cundinamarca (left). Source: López Melo and Barrientos Fuentes (2023), published with permission by Agronomía Colombiana.

- Participation in rural cultural activities (community organization and activities, celebrations and commemorations);
- Peasant community (roles and networks, social capital and organization);
- Relationship with the State (services of education, health, and technical assistance, rural infrastructure, local celebrations and political participation.

For the analysis of the information, matrices were used to describe each indicator and a situational analysis was carried out based on the responses of the interviewees (Ander-Egg, 2000; FAO, 2019). Relational graphs were created for each village to analyze community relationships (see Community roles and networks) and determine the centrality of the actors involved in the research. Finally, the results also underwent content analysis following a systematic process (Krippendorff, 2013).

Results and discussion

Characterization of the peasant family

In Roble Hueco, families had an average size of 2.75 members, with a tendency towards older couples whose children migrated to urban areas, thus deviating from the traditional family structure that prioritized family labor. The ageing of the population was evident, with 64% of the population over 45 years of age and only 24% in the economically active age group (18 to 65). The lack of generational replacement threatened the sustainability of the productive units. In Rincón Santo, the average family size was 2.25 members, with an unusual phenomenon of female heads of household raising children without a partner, attributed to early marriages of men migrating for work. This change reflected an emerging female empowerment in rural areas, with women leading productive projects. In addition, most of the population was at economically active ages, suggesting a potential labor pool for local economic projects (Cuéllar, 1990; Van der Ploeg, 2016).

The closeness to the urban area of the municipality facilitated access to education for young people in Rincón Santo who developed agricultural and livestock projects, such as the production of ornamental species. This indicated a process of "re-peasantization" (preserving and strengthening farmers and their culture) in Zipacón, focused on agricultural projects without the logic of capital accumulation, which promoted peasant forms of production and generated hope for generational replacement and local sustainability (Acevedo, 2021; Acuña, 2021; Contreras Román, 2021).

Land tenure and housing

Access to land for the peasantry in Colombia is crucial for their livelihood and forms a central part of the comprehensive rural reform stemming from the peace agreement between the FARC EP guerrillas and the government in 2016. However, in the country, access to land is determined more by the economic purchasing power than by the productive efficiency of rural dwellers. This has led to a part of the peasantry being forced to work on other people's land without having sufficient property of their own, which relegates them to a situation of dependency. In municipalities such as Zipacón, the rural-urban migration has generated a real estate bubble, raising the prices of agricultural land and making it inaccessible to peasants. In the Rincón Santo and Roble Hueco hamlets, land tenure is a problem, since most of the peasants do not own the deeds to their property. In addition, the aging of the rural population and the division of land among heirs are decreasing the productive capacity of family units. In terms of public services, there are disparities among the villages, with Rincón Santo enjoying greater coverage because of its proximity to the head municipality. In general, the lack of access to land and adequate basic services remains a challenge for the peasantry in Colombia (Cucó i Giner & Juan i Fenollar, 1979; Montañez *et al*., 1994; Vergara, 2020).

Family configuration

The analysis of families in the villages reveals a trend towards a reduction in the family size, 62.5% in Roble Hueco and 50% in Rincón Santo. Many families consist of only one or two members living on rural property. This change is reflected in the gender roles within the household, where women have assumed more diverse roles, including productive work such as livestock and agriculture, breaking with the traditional paradigm in which the man was the main provider and the woman was in charge of household chores. This shift towards greater female independence is evidenced in testimonies such as that of a peasant woman from Roble Hueco, who describes how she can now generate her own income and make economic decisions without depending on her husband. In Zipacón, women's independence is even more marked, with 80% playing agricultural and wage-earning roles, which has contributed to maintaining agricultural traditions and strengthening the productive force of the municipality. These changes in family configuration may reflect broader gender equality processes in rural communities, challenging traditional patriarchal structures and reconceptualizing the peasant family as a multidimensional unit focused on the quality of life of all its members and on permanence in the territory (González Moreno, 2022).

Being a peasant

The concept of peasantry has been the subject of conceptual debate for years, encompassing not only agricultural production but also its connection to territory and its traditions (Saade, 2020). However, a single, permanent definition remains elusive, suggesting that peasantry is a dynamic concept, shaped by experiences and relationships in the community over time. To understand peasant ways of life, it is crucial to ask those (interviewees) who identify themselves as peasants, which reveals a number of key considerations:

- Being a peasant implies a deep love for the land and an integral connection with the environment, participating in its preservation and protection;
- Peasants are skilled in food production, showing a high degree of ingenuity and problem solving although they often lack formal recognition;
- There is a strong sense of social responsibility among peasants, who perceive their contribution to food security as fundamental to society;
- Resistance is a central characteristic of the peasantry, facing both external and internal threats to the farm and the village, such as abandonment by the State and the rest of Colombian society;
- Peasant identity is perceived as a conscious choice, linked to processes of resistance to adversity and territorial development.

These aspects suggest that peasantry is not limited to a defined demographic group but represents a diverse range of experiences and traditions in rural territories. Therefore, public policies should recognize this heterogeneity and support community processes to promote sustainable territorial development (Botero & Duque, 2023; Edelman, 2022).

Participation in rural cultural activities

The concept of culture, from an anthropological-sociological perspective, encompasses activities resulting from social dynamics, fulfilling collective needs and linked to processes of creation, inheritance and reinterpretation. In the rural context, these activities, celebrations and commemorations reinforce the collective historical memory, strengthen identity and generate social cohesion in the territories. However, in the Roble Hueco village there is a loss of these cultural activities due to several reasons: lack of community leadership, difficult relationships between families, difficulties of mobilization within the village and the ineffectiveness of the Community Action Board (CAB). On the other hand, in Rincón Santo, the community shows greater unity in the organization of events and celebrations, such as Farmer's Day, religious festivities and municipal fairs, as well as hosting bazaars to raise funds. This is attributed to proximity to the urban center and effective political representation in the community, with a councilwoman and a well-functioning CAB. Generally speaking, while some rural areas experience a loss of cultural activities due to internal and leadership problems, others benefit from good political representation and proximity to urban centers, which facilitate the organization of and participation in community events (Colombres, 1983).

Peasant community

The development of the social network has a direct relationship with the development of the territory as a village (Galindo Cubillos & Guavita Moreno, 2018). Here, the peasant community is analyzed through networks and by interpreting social aspects such as trust, cohesion and cooperation. The community aspects of the villages under study are detailed below.

Community roles and networks

In Roble Hueco, intra-community relationships play a central role. They are mainly concentrated around the food transporter (collector) and the farming couple, who are leaders in agricultural production and also take on organizational roles. Other people, such as the food seller, also play a central role; in this case, their place of sale is also a meeting site. Three other community members are recognized for their role in selling milk and dairy products, as well as for their contribution to teaching and supporting new members of the village. Relationships are mediated by function and expertise in specific activities that generate community recognition and foster the development of useful skills for the community. In Roble Hueco, the centrality of relationships is concentrated in people who have political roles as councilman and president of the Community Action Board, respectively. Another relevant community member is the transporter of agricultural products. Unlike Roble Hueco, transportation is not vital due to the availability of vehicles among community members and their proximity to the main municipality. In Rincón Santo, the economy focuses on the commercial exchange of food products and the sale of labor services, which drive the local economy and promote productive growth. In addition, the community is organized into solid structures that allow participation in activities for the collective well-being, generating an environment of organizational growth and strengthening the social structure.

Social capital

The concept of social capital refers to the social functions and forms that a group of people uses to satisfy individual and collective needs (Ostrom & Ahn, 2003). In the studied communities, social cohesion varies significantly. In the Roble Hueco village, there is a perceived lack of strong relationships and sense of belonging, which hinders organization for the common good. In contrast, in the Rincón Santo village, there is an organizational climate that promotes endogenous development, with defined roles oriented toward collective progress. Cooperation, measured by the capacity for mutual support, also differs in the two villages. In Roble Hueco, the practice of "mano devuelta" is common, which involves peasants paying for labor with labor. Road repair days are also organized, while in Rincón Santo, cooperation focuses on public activities such as road maintenance and drinking water system maintenance, as well as solidarity events in response to individual economic needs. In terms of trust, this is fundamental to promote collaboration and common well-being in a community (Lockward Dargam, 2011). In Roble Hueco, the lack of cohesion results in decreased trust among inhabitants, which affects the development of teamwork and cooperation. In Rincón Santo, trust is greater because it is based on better organization, cohesion and cooperation.

Organization

In the Roble Hueco village, reduced cohesion and cooperation among members have resulted in individualism that hinders the formation of productive organizations such as associations or cooperatives. The absence of leadership and defined roles in community projects has impeded the initiative to create these structures, which in turn has hindered the institutional approach and the execution of government projects that require associativity to receive support and investment. Thus, a vicious circle has been generated that disfavors the community. The only attempt at organization in Roble Hueco focuses on the management of the San Carlos potable water system, which provides water to some families in the village. However, this effort is limited to the maintenance of the service and does not extend to other productive or collaborative activities. In the Rincón Santo village, despite having a better social cohesion, there are also no formal community organizations due to lack of trust and previous bad experiences with this type of initiative. Previous associations were dissolved due to the lack of teamwork and the absence of immediate economic benefits for the members. This case illustrates the importance of social capital in the formation of associations and cooperatives. Without a foundation of trust and collaboration among community members, community organizing initiatives are hampered by individualism and lack of synergy.

Local cultural activities

In Roble Hueco, there has been a decline in participation in celebrations and cultural events due to transportation problems and the centralization of festivities in the municipal capital. This lack of celebrations in rural areas affects community cohesion. In contrast, in Rincón Santo, participation in local events is 75% facilitated by the proximity to the urban center. Peasant celebrations strengthen community cohesion and identity, as evidenced by collaboration in events (town halls, CAB, etc.), and the organization of activities to generate income. These festivities not only have a religious or cultural significance but also contribute to the construction of identity and a sense of belonging to the territory. These differences in social dynamics between the two communities reflect the importance of community cohesion in the participation in peasant celebrations, both externally organized and internally initiated (Torres, 2015).

Relationship of peasants with the State

The development of rural territories, where peasants also live, depends in part on State contributions to education, health, road infrastructure, technical assistance, basic services and housing services (Ramírez González, 2020; Tamayo, 2012). These peasant-related aspects are detailed as follows:

Education

Rural public education service in Roble Hueco and Rincón Santo is limited to primary school, with a single teacher following Paulo Freire's Nova Escuela methodology (Vallory, 2016). To attend public school, children and adolescents in the rural area of Rincón Santo must travel to the municipal capital, while in Roble Hueco some students attend a boarding school in Tena, another municipality, where they do not need to commute daily. Regarding public higher education, technical and professional courses are offered in agriculture, confectionery and dairy products with the collaboration of SENA (National Apprenticeship Service), but their impact is minimal due to the lack of dissemination of information about this offer and the community's lack of interest. This drives youth migration to urban educational centers, leading them to abandon rural areas. Migration is critical in Roble Hueco, which currently has a mostly elderly population.

Health

The public health service in rural areas depends on urban municipal centers, which makes it difficult to request medical appointments and affects access to regular medical care. Peasants prefer to use the service only in emergencies due to transportation difficulties and costs. They turn to ancestral medicine and private doctors due to the perceived ineffectiveness of the municipal health system. Although they have lost knowledge of medicinal plants, they still use them. Hiring private doctors implies high costs, but they prefer this option over a system they consider ineffective and unreliable in diagnosis.

Technical assistance

Agricultural technical assistance is scarce in Roble Hueco and Rincón Santo, given that rural support laws are not enforced. One hundred percent of respondents do not receive adequate assistance according to their needs. Training initiatives are temporary and are not integrated into territorial development processes. This contradicts Colombian laws on special protection for food production and the guarantee of associated services such as rural technical assistance (Law 607 of 2000 and the current Law 1876 of 2017). The lack of structural support hinders productive improvement and access to formal markets for peasants in the region.

Road infrastructure in the villages

The road infrastructure in the Roble Hueco village is deficient due to its rugged topography, which makes access difficult during the months of high rainfall. The lack of regular transportation services increases transportation costs and causes community isolation, affecting local governance. In contrast, the Rincón Santo village in Zipacón has optimal road infrastructure due to its proximity to the urban center. Although most residents have vehicles to get around, they still lack important services such as collection centers, food processing, and internet access. The lack of rural infrastructure development, especially roads, reduces agricultural competitiveness, increases dependence on imports and intermediation and negatively impacts the rural economy and can lead to the abandonment of agricultural activities (Lozano-Espitia & Restrepo-Salazar, 2016).

Participation in politics

Political participation in both communities is limited to voting and party-sponsored political events. In Zipacón, there is strong political representation, with councilwoman D. M. promoting projects for her village. The Community Action Board (CAB) in Rincón Santo is active in infrastructure improvements and municipal projects. In contrast, in Roble Hueco, the lack of unity makes it difficult to identify leadership, hindering representation and community development. The political problem lies in the lack of cohesion and empathy, which prevents effective representation for territorial governance. The importance of politics for the peasantry lies in the struggle against adversities, which motivates their organization in favor of their interests and those of their class (Tamayo Castro, 2012).

Conclusions

It can be said that peasant families are small and aging, with increasing female leadership and weak community organization.

The farming families in both villages are characterized by being mostly small, with one to two members present on the farm-home, a population that tends to age. Another aspect to highlight is the increased participation of women in the leadership of the productive unit. This characteristic is partly a consequence of rural-urban migration and the trend to equalize the rights of men and women in rural areas. Regarding the self-perception of the farmers in the two villages, they perceive themselves as individuals dedicated to food production, rooted in the land and aware of their important responsibility in the country's food security. In relation to peasant cultural activities, these are being lost mainly due to a lack of leadership and organization among the community. Likewise, the social networking and social capital within the peasant communities of the two villages are weak due to weak leadership and organization. Also, associativity is a strategy that has not been developed in either village due to a lack of trust and collaboration among community members. Political participation through the Community Action Boards (CAB) is greater in Rincón Santo than in Roble Hueco, although in both villages there is still room for improvement. From the point of view of the State, it can be said that education, health, and technical assistance services, as well as road infrastructure, have not yet reached the required level of quality and coverage. In the same sense, land acquisition by peasants is restricted, mainly due to the low purchasing power of peasants. In terms of housing, the availability of public services and quality housing has not yet reached the expected level, although the closer they are to urban centers, the better, as in the case of Rincón Santo.

Conflict of interest statement

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

Author's contributions

DALM provided new information, systematized the data, and collaborated in the writing. JCBF structured the manuscript and collaborated in the writing and translation. Both authors reviewed the final version of the manuscript.

Literature cited

Acevedo-Osorio, Á., & Schneider, S. (2020). *Agricultura campesina, familiar y comunitaria: una perspectiva renovada del campesinado para la construcción de paz en Colombia. Luna Azul*, (50), 132–148. https://doi.org/10.17151/luaz.2020.50.7

Acuña-Rodríguez, O. Y., Acuña-Rodríguez, B. O., Albesiano-Fernández, L. E., Pinzón-Camargo, L. C., & Cobo-Mejía, E. A. (2021). *Identidad y familia campesina en torno a la producción de queso en el municipio de Paipa (Colombia). Mundo Agrario, 22*(51), Article e177. https://doi.org/10.24215/15155994e177

Alarcón Meneses, L. (2010). Educar campesinos y formar ciudadanos en Colombia durante la república liberal (1930-1946). *Investigación y Desarrollo*, *18*(2), 296–313.http://www.scielo.org.co/scielo.php?script=sci_abstract&pid=S0121-32612010000200005&lng=e&nrm=iso&tlng=es

Ander-Egg, E. (2000). *Metodología y práctica del desarrollo de la comunidad: Tomo II (Cómo hacer)* (33rd ed.). Buenos Aires, Argentina: Grupo Editorial Lumen / Humanitas. https://es.scribd.com/document/363262944/Ander-Egg-Ezequiel-Metodologia-y-Practica-Del-Desarrollo-de-La-Comunidad-Tomo-II

Bejarano, J. A. (1983). Campesinado, luchas agrarias e historia social: notas para un balance historiográfico. *Anuario Colombiano de Historia Social y de la Cultura, 11*, 251–304. https://revistas.unal.edu.co/index.php/achsc/article/view/31272

Botero, R. S., & Duque, Q. S. P. (2023). Comunidades campesinas en Colombia, principio de equidad y derecho fundamental a una pensión. *Equidad y Desarrollo, 1*(41), Article e1502. https://doi.org/10.19052/eq.vol1.iss41.4

Colombres, A. (1983) (Comp.). La cultura popular. Premiá Editora. https://laresolana.wordpress.com/wp-content/uploads/2014/08/1-2-colombres-cultura-popular.pdf

Contreras Román, R. (2021). Recampesinizar el futuro. La alternativa campesina ante el colapso del sistema agroalimentario global. *Perspectivas Rurales Nueva Época*, *19*(37), 1–22. https://doi.org/10.15359/prne.19-37.1

Cruz Rodríguez, E. (2019). La recomposición del movimiento campesino en Colombia (2013-2016). *Revista Via Iuris*, (26), 103–124. https://doi.org/10.37511/viaiuris.n26a6

Cucó i Giner, J., & Juan i Fenollar, R. (1979). La proletarización con el campesinado y su relación con el desarrollo capitalista: El caso del País Valenciano. *Agricultura y Sociedad*, (12), 145–168. https://dialnet.unirioja.es/servlet/articulo?codigo=82405

Cuéllar, O. (1990). Las familias campesinas numerosas viven menos mal. *Demos*, (3), 8–9. http://revistas.unam.mx/index.php/dms/article/view/6549

Edelman, M. (2013). *What is a peasant? What are peasantries? A briefing paper on issues of definition*. Intergovernmental working group on a United Nations declaration on the rights of peasants and other people working in rural areas, Geneva, 15-19 July 2013, 1–18. https://www.ohchr.org/sites/default/files/Documents/HRBodies/HRCouncil/WGPleasants/MarcEdelman.pdf

Edelman, M. (2022). ¿Qué es un campesino? ¿Qué son los campesinados? Un breve documento sobre cuestiones de definición. *Revista Colombiana de Antropología*, *58*(1), 153–173. https://doi.org/10.22380/2539472X.2130

Fajardo Montaña, D. (1981). *Campesinado y capitalismo en Colombia*. Cinep.

FAO – Food and Agriculture Organization of the United Nations. (2019). *Guide to context analysis: Informing FAO decision-making – Approaches to working in fragile and conflict-affected contexts.* Rome: FAO. https://www.fao.org/3/ca5968en/ca5968en.pdf

Galindo Cubillos, L. S., & Guavita Moreno, R. N. (2018). *Construcción de tejido social entre víctimas del conflicto armado. Una experiencia de los campesinos de la localidad de Sumapaz* [Master thesis, Universidad de La Salle]. https://ciencia.lasalle.edu.co/items/04584efa-51b8-44c8-be9d-3d762bbfc07e

González Moreno, C. (2022). *Feminismo campesino: las mujeres de Inzá Tierradentro* [Master thesis, Universidad Andina Simón Bolívar]. https://repositorio.uasb.edu.ec/handle/10644/8502

Hernández, R. (1993). Teorías sobre campesinado en América Latina: una evaluación crítica. *Revista Chilena de Antropología*, (12), 179–200. https://revistadeantropologia.uchile.cl/index.php/RCA/article/view/17606/

Hervieu, B., & Purseigle, F. (2015). The sociology of agricultural worlds: from a sociology of change to a sociology of coexistence. *Review of Agricultural and Environmental Studies*, *96*(1), 59–90. https://doi.org/10.22004/ag.econ.276761

Kearney, M. (1996). *Reconceptualizing the peasantry: anthropology in global perspective* (1st ed.). Routledge. https://www.routledge.com/Reconceptualizing-The-Peasantry-Anthropology-In-Global-Perspective/Kearney/p/book/9780813309880?srsltid=AfmBOoodG1Cv8qf5RLPQ9wraXguoC2vmUZOC_3NnWRWxULzixSQ7lzzR

Krippendorff, K. (2013). *Content analysis: An introduction to its methodology* (3rd ed.). Thousand Oaks, CA: Sage Publications. https://web.stanford.edu/class/comm1a/readings/krippendorff-ch1and4.pdf

Leeds, A. (1977). *Mythos and pathos: some unpleasantries on peasantries*. In R. Halperin, & J. Dow (Eds.), *Peasant livelihood: Studies in economic anthropology and cultural ecology (*pp. 227–256). St. Martin's Press.

Lockward Dargam, A. M. (2011). El rol de confianza en las organizaciones a través de los distintos enfoques o pensamientos de la administración. *Ciencia y Sociedad*, *36*(3), 464–502. http://www.redalyc.org/articulo.oa?id=87022526005

López Melo, D. A., & Barrientos Fuentes, J. C. (2023). Characterization of the peasant economy in two small villages of Cundinamarca (Colombia): Case studies. *Agronomía Colombiana*, *41*(3), Article 110435. https://doi.org/10.15446/agron.colomb.v41n3.110435

Lozano-Espitia, I., & Restrepo-Salazar, J. C. (2016). El papel de la infraestructura rural en el desarrollo agrícola en Colombia. *Coyuntura Económica*, *46*(1), 107–147. https://www.repository.fedesarrollo.org.co/handle/11445/3351

Montañez Gómez, G., Arcila Niño, O., Pacheco Giraldo, J. C., Hernández, Y., Gracia, J., & Lancheros Murillo, H. (1994). *Hacia dónde va la sabana de Bogotá: modernización, conflicto, ambiente y sociedad.* Universidad Nacional de Colombia, Centro de Estudios Sociales – SENA. https://repositorio.unal.edu.co/handle/unal/2989

Ostrom, E., Ahn, T. K., & Olivares, C. (2003). Una perspectiva del capital social desde las ciencias sociales: capital social y acción colectiva. *Revista Mexicana de Sociología*, *65*(1), 155–233. https://doi.org/10.2307/3541518

Preciado Beltrán, J. (2006). Ruralidad y conflicto en Colombia: retos y desafíos para reorientar el escenario rural. *Tecnogestión: Una Mirada al Ambiente*, *3*(1), 14–22. https://revistas.udistrital.edu.co/index.php/tecges/article/view/4334

Ramírez González, J. I. (2020). *El campesinado como sujeto de derechos en el siglo XXI: una mirada desde el caso colombiano* [Undergraduate thesis, Universidad de Antioquia]. https://bibliotecadigital.udea.edu.co/bitstream/10495/19344/1/RamirezJose_2020_CampesinadoSujetoDerecho.pdf

Saade Granados, M. (Ed.). (2020). *Conceptualización del campesinado en Colombia: Documento técnico para su definición, caracterización y medición* (1st ed.). Instituto Colombiano de Antropología e Historia.

Sevilla-Guzmán, E., & Pérez Yruela, M. (1976). Para una definición sociológica del campesinado. *Agricultura y Sociedad*, (1), 15–39. https://www.mapa.gob.es/ministerio/pags/biblioteca/revistas/pdf_ays/a001_01.pdf

Shanin, T. (1979). Definiendo al campesinado: conceptualizaciones y desconceptualizaciones. Pasado y presente en un debate marxista. *Agricultura y Sociedad*, (11), 9–52. https://dialnet.unirioja.es/servlet/articulo?codigo=82399

Sierra Rivera, C. M. (2019). *El campesinado contemporáneo. Un estudio de caso para el municipio de Nobsa en Boyacá* [Undergraduate thesis, Universidad Externado de Colombia]. https://bdigital.uexternado.edu.co/server/api/core/bitstreams/a1080721-86f6-4e66-92a9-143a086de103/content

Stake, R. E. (1995). *Investigación con estudio de casos* (2nd ed.). Ediciones Morata.

Suárez Sánchez, A. (2015). *El campesinado en Colombia: la violencia en el mundo rural* [Doctoral dissertation, Universidad de Cantabria]. Repository UC. https://repositorio.unican.es/xmlui/handle/10902/7273

Tamayo Castro, J. A. (2012). La participación política del campesino en Colombia, entre los mecanismos institucionales y los procesos organizativos. El caso de las organizaciones campesinas del suroeste de Antioquia. *Cultura Investigativa*, (4), 97–120. https://biblat.unam.mx/hevila/CulturainvestigativaRevistavirtualsemillerosdederecho/2012/no4/9.pdf

Torres, L. E. (2015). Desarrollo rural sostenible: factores subyacentes en las mentalidades campesinas. *AiBi Revista de Investigación, Administración e Ingeniería*, *3*(2), 18–26. https://revistas.udes.edu.co/aibi/article/view/1748

Vallory, S. E. (2016). Las razones del programa Escola Nova 21, una alianza por un sistema educativo avanzado. *Aula de Innovación Educativa*, (255), 31–37. https://consejoescolar.educacion.navarra.es/web1/wp-content/uploads/2016/11/900.pdf

Van der Ploeg, J. D. (2016). *El campesinado y el arte de la agricultura. Un manifiesto chayanoviano.* Colección Perspectivas agroecológicas 13.

Vergara, W. (2020). *Derechos de propiedad agraria, concentración de la tierra y productividad agrícola en Colombia* [Doctoral dissertation, Universidad de La Salle]. https://ciencia.lasalle.edu.co/server/api/core/bitstreams/041149ee-bc7f-4fd7-9391-a32899859b6d/content

Villate Santander, G. (1998). Algunos rasgos de la agricultura de los muiscas. *Luna Azul*, *4*(1), 1–9. https://revistasojs.ucaldas.edu.co/index.php/lunazul/article/view/1490

SCIENTIFIC NOTE

Antifungal effect of limonene against different pathogenic *Fusarium* species

Efecto antifúngico del limoneno sobre diferentes especies patógenas de *Fusarium*

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ABSTRACT

Alternative control methods of fungal diseases have been studied with an emphasis on finding new compounds derived from plants, such as essential oils and extracts, which are considered safer for consumers and the environment. Limonene, a cyclic monoterpene widely found in nature, is the main component of essential oils obtained from the peels of citrus fruits such as grapefruit, lemon, lime, and particularly orange. Despite its prevalence and its use as an antifungal agent, especially against fungi that cause diseases in major crops worldwide, studies on its application in greenhouse assays have been limited. The aim of this research was to evaluate the antifungal activity of limonene against cereal-pathogenic *Fusarium* species and to assess its effectiveness in controlling Fusarium head blight through plant bioassays. Limonene inhibited mycelial growth *in vitro* for all tested species, showing effective fungistatic action on pathogens. Regarding plant bioassays, the most significant effect was observed when limonene was applied simultaneously with and after the pathogen, indicating that limonene is not acting as a defense-inducing agent in the plant but directly on the pathogen. When limonene was applied before the pathogen, no significant inhibition of incidence was detected. Further studies are necessary to explore the use of limonene in controlling Fusarium head blight in major crops such as *Triticum aestivum* L. This study presents promising results for controlling this disease using limonene.

Keywords: pathogen control, essential oil, Fusarium head blight, wheat.

RESUMEN

Se han estudiado métodos alternativos para el control de enfermedades, con énfasis en la búsqueda de nuevos compuestos derivados de plantas, como aceites esenciales y extractos, que se consideran más seguros para los consumidores y el medio ambiente. El limoneno, un monoterpeno cíclico ampliamente encontrado en la naturaleza, es el principal componente de los aceites esenciales obtenidos de las cáscaras de frutas cítricas como pomelo, limón, lima y, en particular, naranja. A pesar de su prevalencia, su uso como antifúngico, especialmente contra hongos que causan enfermedades en cultivos extensivos a nivel mundial, y los estudios sobre su aplicación en ensayos bajo invernadero han sido poco explorados. El objetivo de este trabajo fue evaluar la actividad antifúngica del limoneno contra especies de *Fusarium* patógenas de cereales y evaluar su eficacia mediante bioensayos en plantas. El limoneno inhibió el crecimiento micelial *in vitro* en todas las especies analizadas, mostrando su eficaz acción fungistática sobre los patógenos. El efecto más significativo se observó cuando el terpeno se aplicó simultáneamente con y después del patógeno. Esto indicaría que el limoneno no estaría ejerciendo una acción como agente inductor de defensa en la planta, sino que su acción es directa sobre el patógeno, dado que en el tratamiento en el que se aplicó limoneno antes del patógeno, no se detectó una inhibición significativa en su incidencia. Se requieren más estudios para explorar el uso del limoneno en el control de la fusariosis de la espiga en cultivos extensivos como *Triticum aestivum* L. Este trabajo presenta resultados prometedores para el control de esta enfermedad mediante el uso de limoneno.

Palabras clave: control de patógenos, aceite esencial, fusariosis de la espiga, trigo.

Introduction

The control of fungal diseases has been based for years on the use of phytosanitary products. However, alternative methods for disease control have been studied with an emphasis on finding new compounds derived from plants, such as essential oils and extracts, which are considered safer for consumers and the environment (Zaker, 2016). Most plant essential oils are chemically complex in their composition, which improves their efficacy due to synergy among their compounds (Dhifi *et al.*, 2016). Terpenes and their derivatives are the main components of essential oils and are of great importance due to their application in the agricultural, food, cosmetic, and pharmaceutical

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industries. Agronomic applications include their potential use as antifungals (Marei *et al*., 2012; Pawar & Thaker, 2007), insecticides (Huang *et al*., 2002), and nematicides (Duschatzky *et al*., 2004). Their action as fungicides involves reducing mycelial growth (by modifying the structure of cell membranes), inhibiting spore germination, and affecting the enzymatic activity of fungi (Marei *et al*., 2012; Zhou *et al*., 2014). There are also many other compounds applied as elicitors in plants, which act as inducers and trigger an immune response, promoting long-term inhibition of the pathogen's action. The use of fungicides and elicitors can be carried out together at different times of the vegetative cycle of the crops, triggering a favorable additive response (Jones & Dangi, 2006; Martinez *et al*., 2024; Thakur & Sohal, 2012). Limonene is the most widely distributed cyclic monoterpene in nature and is the major component of essential oils obtained from the peel of citrus fruits such as grapefruit, lemon, lime, and mainly orange (Pérez Mosquera *et al*., 2015). It is extensively used to produce fragrances and beverages, as a flavor additive in food, and as a biotransformation substrate. Nevertheless, limonene has been little explored as an antifungal agent in plants, mainly against those species that cause diseases in major crops worldwide (Gupta *et al*., 2021; Ravichandran *et al*., 2018; Ünal *et al*., 2012). *Fusarium* is a genus of fungi with a broad distribution that includes the causal agent of Fusarium head blight (FHB), one of the most relevant diseases of cereals, in humid and semi-humid wheat-growing areas (Bai & Shaner, 1994; Wiese, 1987). The importance of this disease lies in its impact on yield and grain quality as well as the production of mycotoxins that remain stable in barley malt, wheat flour, and even final products, thus adversely affecting human and animal health (Champeil *et al*., 2004; Xu, 2003). Considering that the use of phytosanitary products poses risks to human health and contributes to environmental contamination, as well as the well-documented negative effects caused by *Fusarium* spp. through crop losses and production of mycotoxins in grains. The aim of this research was to evaluate *in vitro* the antifungal activity of limonene against cereal-pathogenic *Fusarium* species as well as its effectiveness in controlling FHB in plant bioassays.

Materials and methods

In vitro antifungal activity

A mycelial growth inhibition test was performed for seven pathogenic species of *Fusarium*.: *F. avenaceum* (A3; MH362768), *F. cerealis* (Herrero 5; Castañares *et al*., 2013), *F. culmorum* (FC115; Toth *et al*., 2004), *F. poae* (25/5; Nogueira *et al*., 2018), *F. graminearum sensu stricto* (Ass5; Castañares *et al*., 2016), *F. pseudograminearum* (LPSC 1154; Castañares *et al*., 2012), and *F. subglutinans* (1.1; MG857113).

The stock solution of limonene, (R) - (+)- limonene 97%, Sigma-Aldrich (#183164) was prepared with absolute EtOH and Tween-20 (5% v/v). Different volumes of this solution were incorporated into 25 ml of potato dextrose agar (PDA) culture medium at 40-45°C, in 9 cm diameter Petri dishes, and the following treatments were defined: T1: 350 µl, T2: 400 µl, and T3: 450 µl of limonene. The controls were: Control 1, the pathogen without limonene and with 1 ml EtOH (solvent used to solubilize limonene) and Control 2, without limonene and without EtOH (Tsao & Zhou, 2000). For each *Fusarium* species, a disc of mycelium from an actively growing colony (7 d in a growth chamber under 20+2°C with alternating light/darkness for 12 h 3,500 lx) was inoculated in the center of the plate, which was then sealed with Parafilm to prevent the evaporation of limonene. Plates were incubated in a culture oven at 20±2°C with alternating light/darkness for 12 h (3,500 lx). Each treatment was conducted in triplicate. Colony diameter was measured every 24 h until the control treatment reached the edge of the plate (4-7 d). The percentage of mycelial inhibition was calculated for each concentration using the following formula (Marei *et al*., 2012):

$$\%\ Inhibition = \left(\frac{DC - DT}{DC}\right) \times 100\% \tag{1}$$

where:

DC=diameter of the control, DT=diameter of the treatment. This assay determined the minimum inhibitory concentration (MIC) to be used in the next assay.

Greenhouse assay and experimental design

The assay was carried out in the greenhouse of the Faculty of Agronomy (36°41'00" S, 59°48'00" W) at the National University of Central Buenos Aires Province (Argentina). The average temperature of the greenhouse was recorded every 24 h.

The experimental design was completely randomized, with three replicates per treatment. The test with *F. poae* was carried out using the minimum concentration of limonene that had demonstrated the greatest inhibitory effect *in vitro* (Li *et al*., 2015). The treatments were: T1, the limonene solution and the pathogens applied at the same time; T2, the limonene solution applied 24 h before the inoculation of the pathogens; T3, the limonene solution applied 24 h after inoculation of the pathogens. The controls were: C0, the pathogen inoculated with a solution without limonene and with ethanol; C1, pathogen inoculated with a solution without limonene and without Ethanol (only water); C2, only the pathogen inoculated and C3, only the limonene solution applied.

For treatments, in each replicate, 10 seeds of *Triticum aestivum* L. (hexaploid wheat) Apogee variety, reported as susceptible to FHB (Li *et al*., 2017), were sown in 20 L pots, containing clay loam soil without prior sterilization obtained from the farm of the Faculty of Agronomy of the National University of Central Buenos Aires Province. An aliquot of 350 µl of stock solution (MIC) of limonene (50% v/v), prepared with absolute ethanol and 5% Tween-20 (v/v) in 25 ml of sterile distilled water, was used. Inoculation of the pathogen and application of the monoterpene solution were done in Zadoks 6.0 (Zadoks *et al*., 1974) until dripping using sprinklers. They were left for 48 h with polyethylene bags to achieve a humid chamber effect. Humidity was maintained by irrigating twice a day and the fertilizer conditions were set according to Dinolfo *et al*. (2022). Symptom assessment was conducted by evaluating the severity percentage (number of symptomatic spikelets per spike) 21 d after inoculation (Martinez *et al*., 2020).

Inoculum production

Fusarium poae was grown in Petri dishes with PDA 2%. Cultures were incubated for 2 weeks in a culture oven at 20±2°C with alternating light/darkness for 12 h (3,500 lx). To obtain the spore suspension, 15 ml of sterile Tween 20® solution (0.85% NaCl, 0.1% Tween 20 in water) was added to each Petri dish, the mycelium was scraped with a previously flamed slide or scalpel, and filtered through sterile gauze. The resulting suspension was adjusted to $1x10^5$ conidia/ml using a Neubauer hemocytometer under a binocular optical microscope (Brennan *et al*., 2007; Dinolfo *et al*., 2022).

Statistical analysis

Mycelial inhibition (%) was evaluated using software R v.4.2.1 (R Core Team, 2022). A statistical analysis was carried out using a mixed model with repeated measures (species). Results are reported as the mean ± standard error of the mean (SEM) at a significance level of $\alpha = 0.05$.

To evaluate the antifungal efficacy of the treatment across different *Fusarium* species, a partitioned linear regression analysis was performed using Infostat software (Di Rienzo *et al*., 2015). Mycelial inhibition (%) was used as the dependent variable, while treatment concentration (in µl) served as the continuous explanatory variable. The model was stratified by species to account for interspecific variation in sensitivity. This approach allowed for the empirical estimation of species-specific dose–response trends and the identification of the minimum inhibitory concentration (MIC) associated with a 50% reduction in mycelial growth (MIC_{50}). The analysis provided insight into the differential effectiveness of the treatment across the *Fusarium* complex and facilitated quantitative comparisons among species (Li, 2015; Marei *et al*., 2012).

Disease severity (%) was evaluated using software R v.4.2.1 (R Core Team, 2022). The main factor was limonene treatment (L) nested within *F. poae* (F) treatments with two levels (presence or absence), with three blocks for each combination of treatments. Due to non-normal errors, comparisons were performed using a generalized linear mixed model (GLMM). The function glmer from the lme4 package was used for variance analysis (Bates *et al*., 2015). Data assumptions were verified graphically using plots of fitted values versus residuals for homogeneity of variances. Normal Q-Q plots and the Shapiro-Wilk test were used to check the normality of the residuals. Furthermore, fitted values versus residuals were graphically verified using plots to check variance homogeneity. The lsmeans function was used to test significant effects (emmeans package) (Lenth, 2018). The results are reported as the mean ± standard error of the mean (SEM), using a significance level of $\alpha = 0.05$.

Results

Inhibition of mycelial growth

The treatments showed a significant effect on the fungal growth of all pathogenic species of crops tested. Furthermore, significant growth was observed across the measurement days, with no interaction between the two variables (treatments and measurement days) (Tab. 1). All treatments significantly inhibited the mycelial growth of *Fusarium* species compared to the controls; however, no significant differences were observed among the treatments themselves (Tab. 2). Linear regression analyses were performed separately for each *Fusarium* species to assess the relationship between treatment concentration and the percentage of mycelial inhibition. The species that achieved at least 50% inhibition (using 350 µl of limonene in the Petri dishes) were *F. avenaceum, F. culmorum, F. cerealis, F. pseudograminearum, F. subglutinans, F. poae and F. graminearum*. However, only *F. avenaceum*, and *F. culmorum* showed significant difference at higher concentrations of limonene (Tab. 3). The minimum inhibitory concentration (MIC_{50}) was determined to be 350 µl of limonene solution.

TABLE 1. Analysis of the effect of limonene application on fungal mycelial growth of *Fusarium* spp. through time.

Source of variation	Df.	*P-value*
Treatments	8	$<2^{-16}$ ***
Day-measurement	1	$<2^{-16}$ ***
Treatment x day-measurement	8	0.4986 n.s.

Treatments: T1: 350 µl limonene, T2: 400 µl limonene, T3: 450 µl limonene, C1: the pathogen without organic compound and with 1 ml EtOH (solvent used to solubilize limonene), C2: without organic compound and without EtOH. Day of measurement: every 24 h until the control treatment reached the margin of the Petri dish (4-7 d). Statistical analyses were performed using a mixed model with repeated measures (species). df: degrees of freedom ***significant differences, ns: not significant at α-value$\leq$ 0.05.

TABLE 2. Effect of treatments with limonene on the inhibition of mycelial growth of *Fusarium* species.

Treatments	Means
T1 350 µl limonene	1.717146 a
T2 400 µl limonene	1.693551 a
T3 450 µl limonene	1.850651 a
C2-T1	2.903072 b
C2-T2	3.013036 bc
C2-T3	3.178050 bc
C1-T1	3.407806 c
C1-T2	3.421662 c
C1-T3	3.444731 c

Mean ± SEM comparisons were performed using a mixed model with repeated measures (species). Same letters are not significant at $P\leq$ 0.05. Treatments: T1: 350 µl limonene, T2: 400 µl limonene, T3: 450 µl limonene, C1: the pathogen without limonene and with 1 ml EtOH (solvent used to solubilize limonene), C2: without limonene and without EtOH. Day of measurement: every 24 h until the control treatment reached the margin of the Petri dish (4-7 d). Same letters are not significant at $P\leq$ 0.05.

TABLE 3. Summary of regression coefficients, and *P-values* for each *Fusarium* species.

Species	Treatment Coeff.	*P-value*
F. avenaceum	-0.28	0.002
F. cerealis	-0.07	0.2896
F. culmorum	0.22	0.0243
F. graminearum	0.19	0.1206
F. poae	0.12	0.1061
F. pseudograminearum	0.1	0.3248
F. subglutinans	0.18	0.3024

Statistical analyses were performed using partitioned linear regression. Significant *P-values* at $P<0.05$.

Greenhouse assay

The greenhouse assay carried out with wheat cultivar *T. aestivum* L. var. Apogee in pots in the greenhouse showed significant effects on the severity of *F. poae* and the antifungal action of limonene. Significant interaction between *F. poae* and limonene was observed for treatments T1 and T3, but not for treatment T2 (Tab. 4).

TABLE 4. Analysis of variance to detect the effect of limonene on *Fusarium* severity (%) under greenhouse conditions.

	T1		
Source of variation	**Chisq.**	**Df.**	**Pr (>Chisq.)**
F. poae (F)	42.6444	1	6.5650e-11*
Limonene (L)	12.8992	1	0.0003*
F x L	9.6913	1	0.0018*
	T2		
Source of variation	**Chisq.**	**Df.**	**Pr (>Chisq.)**
F. poae (F)	88.7826	1	<2e-16*
Limonene (L)	0.8796	1	0.3483
F x L	0.0296	1	0.8634
	T3		
Source of variation	**Chisq.**	**Df.**	**Pr (>Chisq.)**
F. poae (F)	41.341	1	1.278e-10*
Limonene (L)	11.877	1	0.0005*
F x L	10.283	1	0.0013*

Treatments. T1: *F. poae* and limonene applied at the same time. T2: Limonene applied 24 h before *F. poae*. T3: *F. poae* applied 24 h before treatment with limonene. Chisq: Wald type II Chi-square tests. Df: degrees of freedom*.

The severity of the disease caused by *F. poae* on wheat showed significant differences in T2 with respect to C0, C1, C3, and T1 and T3. Treatment T2 exhibited 60% pathogen severity, whereas C0, C1, and C3 showed values below 15% (7%, 12%, and 12%, respectively). Treatments T1 and T3 reflected values of 20% severity and had significant differences compared to C2, which had 60% severity (Figs. 1A-E).

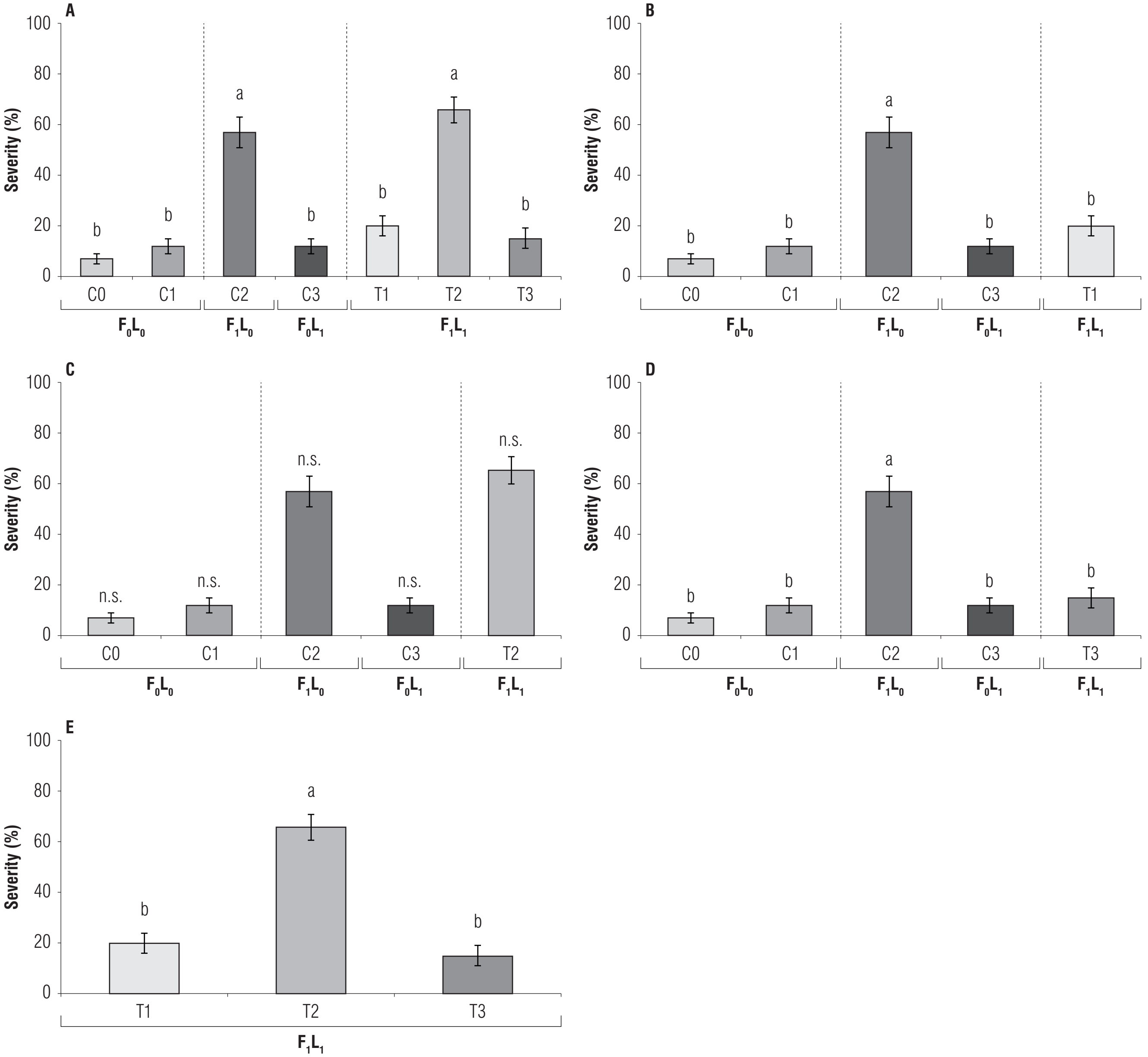

FIGURE 1. Disease severity (%) under greenhouse conditions. A) Disease severity (%) registered after different treatments on wheat spikes (cv. Apogee), B) comparison between the time of application of *F. poae* and limonene treatments (T1, T2, and T3). Treatments: T1, *F. poae* and limonene applied at the same time; T2, Limonene applied 24 h before *F. poae*; T3, *F. poae* applied 24 h before treatment with limonene. C) Comparison of T1, T2 (D), and T3 (E) regarding control treatments (C0-C1), *F. poae* treatment (C2), and limonene treatment (C3), respectively. Controls: C0, the pathogen inoculated with a solution without limonene and with ethanol; C1, the pathogen inoculated with a solution without limonene and without ethanol (only water); C2, only the pathogen inoculated; and C3, only the limonene solution applied. Values are means ± SEM, comparisons were performed using Tukey's test. Data with the same letters are not significantly different at $P \leq 0.05$.

Discussion

Cereal production worldwide is widely influenced by FHB. There are various strategies associated with its management, among which are agronomic practices that aim to reduce the concentration of the inoculum in the system. These include zero tillage system with crops that are not hosts of the pathogen (Shude *et al*., 2020), the use of wheat varieties that are less susceptible and the application of fungicides, primarily from the triazole group, which provide incomplete control of the fungus (Tini *et al*., 2020). Currently, essential oils are considered an alternative to chemically synthesized fungicides, as they are environmentally friendly and exhibit low toxicity (Taheri *et al*., 2023).

In this work, we used limonene, a well-studied terpene and the main component of essential oils derived from citrus fruits, which exerted *in vitro* inhibition of the mycelial growth of all *Fusarium* species tested, showing its effective fungistatic action on pathogens. The results obtained are consistent with findings of Achimón *et al.* (2022), who used essential oils from lemon, orange, and grapefruit peels, with limonene being the component responsible for the antifungal activity against *Rhizoctonia solani* and *Sclerotium rolfsii*. Similarly, Guédez *et al.* (2014) showed that the action of orange essential oil (*Citrus sinensis* L.) had inhibitory effects greater than 80% on postharvest fungi of *Carica papaya*. Recently, Jian *et al.* (2023) showed that the limonene formulation known as Wetcit® is a promising alternative to synthetic fungicides to control *F. graminearum* growth and deoxynivalenol (DON) production.

Few studies have been carried out using essential oils on extensive crops to control fungal pathogens, and there are no records of studies on the action of *F. poae*. The severity of *F. poae* on *T. aestivum* cultivar Apogee was decreased by 80% due to the action of limonene. Similar results were reported by Jian *et al.* (2023), who showed the effective action of limonene against *F. graminearum in vitro*. Likewise, Perczak *et al.* (2019) showed the antifungal activity of essential oils (derived from different plant materials used for cosmetic and research purposes), against *F. graminearum* and *F. culmorum,* and they were effective in reducing the production of toxins in seeds. The effect observed was mainly noted in treatments in which limonene was applied simultaneously with and after the pathogen.

Conclusion

Results presented here indicate that limonene was an effective antifungal against *Fusarium* sp in in vitro assays. Additionally, limonene showed potential to control FHB caused by *F. poae* in wheat plants. Further studies are necessary to explore the use of limonene in controlling FHB in extensive crops such as *Triticum aestivum* L. Nevertheless, this work presents promising results for the control of this disease using limonene or essential oils rich in limonene.

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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

SLB designed the experiments, performed statistical analysis, data interpretation, and writing. CM developed bioassays and assays, and data interpretation. FSF and CM did data interpretation. MVM designed the experiments and participated in data interpretation. All authors reviewed the final version of the manuscript.

Literature cited

Achimón, F., Leal, L. E., Pizzolitto, R. P., Brito, V. D., Alarcón, R., Omarini, A. B., & Zygadlo, J. A. (2022). Insecticidal and antifungal effects of lemon, orange, and grapefruit peel essential oils from Argentina. *AgriScientia, 39*, 71–82. https://doi.org/10.31047/1668.298x.v39.n1.33777

Bai, G., & Shaner, G. (1994). Scab of wheat: Prospects for control. *Plant Disease, 78*(8), 760–766. https://doi.org/10.1094/PD-78-0760

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*(1), 1–48. https://doi.org/10.18637/jss.v067.i01

Brennan, J. M., Leonard, G., Fagan, B., Cooke, B. M., Ritieni, A., Ferracane, R., Nicholson, P., Simpson, D., Thomsett, M., & Doohan, F. M. (2007). Comparison of commercial European wheat cultivars to *Fusarium* infection of head and seedling tissue. *Plant Pathology*, *56*(1), 55–64. https://doi.org/10.1111/j.1365-3059.2006.01458.x

Castañares, E., Dinolfo, M. I., Moreno, M. V., Berón, C., & Stenglein, S. A. (2013). *Fusarium cerealis* associated with barley seeds in Argentina. *Journal of Phytopathology, 161*(7-8), 586–589. https://doi.org/10.1111/jph.12097

Castañares, E., Dinolfo, M. I., Ponte, E. M., Pand, D., & Stenglein, S. A. (2016). Species composition and genetic structure of *Fusarium graminearum* species complex populations affecting the main barley growing regions of South America. *Plant Pathology*, *65*(6), 930–939. https://doi.org/10.1111/ppa.12470

Castañares, E., Wehrhahne, L., & Stenglein, S. A. (2012). *Fusarium pseudograminearum* associated with barley kernels in Argentina. *Plant Disease, 96*(5), 763–763. https://doi.org/10.1094/PDIS-01-12-0050-PDN

Champeil, A., Fourbel, J. F., Doré, T., & Rossignol, L. (2004). Influence of cropping system on *Fusarium* head blight and mycotoxin levels in winter wheat. *Crop Protection*, *23*(6), 531–537. https://doi.org/10.1016/j.cropro.2003.10.011

Dhifi, W., Bellili, S., Jazi, S., Bahloul, N., & Mnif, W. (2016). Essential oils' chemical characterization and investigation of some biological activities: A critical review. *Medicines*, *3(4)*, Article 25. https://doi.org/10.3390/medicines3040025

Dinolfo, M. I., Martínez, M., Castañares, E., Vanzetti, L. S., Rossi F., Stenglein S. A., & Arata A. F. (2022). Interaction of methyl-jasmonate and *Fusarium poae* in bread wheat. *Fungal Biology*, *126*(11-12), 786–792. https://doi.org/10.1016/j.funbio.2022.10.002

Di Rienzo J. A., Casanoves, F., Balzarini, M. G., Gonzalez, L., Tablada, M., & Robledo, C. W. (2015). Grupo Infostat, FCA, Universidad Nacional de Río Cuarto. https://www.infostat.com.ar

Duschatzky, C. B., Martinez, A. N., Almeida, N. V., & Bonivardo, S. L. (2004). Nematicidal activity of the essential oils of several Argentina plants against the root-knot nematode. *Journal of Essential Oil Research, 16*(6), 626–628. https://doi.org/10.1080/10412905.2004.9698812

Guédez, C., Cañizalez, L., Avendaño, L., Scorza, J., Castillo, C., Olivar, R., Méndez, Y., & Sánchez, L. (2014). Actividad antifúngica del aceite esencial de naranja (*Citrus sinensis* L.) sobre hongos postcosecha en frutos de lechosa (*Carica papaya* L.). *Revista de la Sociedad Venezolana de Microbiología*, *34*, 82–86. http://saber.ucv.ve/ojs/index.php/rev_vm/article/view/7998

Gupta, A., Jeyakumar, E., & Lawrence, R. (2021). Journey of limonene as an antimicrobial agent. *Journal of Pure and Applied Microbiology, 15*(3), 1094–1110. https://doi.org/10.22207/JPAM.15.3.01

Hartmans, K. J., Diepenhorst, P., Bakker, W., & Gorris, L. G. M. (1995). The use of carvone in agriculture: Sprout suppression of potatoes and antifungal activity against potato tuber and other plant diseases. *Industrial Crops and Products*, *4*(1), 3–13. https://doi.org/10.1016/0926-6690(95)00005-W

Huang, Y., Ho, S. H., Lee H. C., & Yap, Y. L. (2002). Insecticidal properties of eugenol, isoeugenol and methyleugenol and their effects on nutrition of *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae) and *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). *Journal of Stored Products Research*, *38*(5), 403–412. https://doi.org/10.1016/S0022-474X(01)00042-X

Jian, Y., Chen, X., Ma, H., Zhang, C., Luo, Y., Jiang, J., & Yin, Y. (2023). Limonene formulation exhibited potential application in the control of mycelial growth and deoxynivalenol production in *Fusarium graminearum*. *Frontiers in Microbiology, 14*, Article 1161244. https://doi.org/10.3389/fmicb.2023.1161244

Jones, J. D. G., & Dangl, J. L. (2006). The plant immune system. *Nature*, *444*, 323–329. https://doi.org/10.1038/nature05286

Lenth, R. V. (2018). Emmeans: Estimated marginal means, a.k.a. least-squares means, R package version 1.3.0. https://CRAN.R-project.org/package=emmeans

Li, G., Boontung, R., Powers, C., Belamkar, V., Huang, T., Miao, F., Baenziger, P. S., & Yan, L. (2017). Genetic basis of the very short life cycle of 'Apogee' wheat. *BMC Genomics*, *18*, Article 839. https://doi.org/10.1186/s12864-017-4239-8

Li, J. L., Liu, X. Y., Xie, J. T., Di, Y. L., & Zhu, F. X. (2015). A comparison of different estimation methods for fungicide EC50 and EC95 values. *Journal of Phytopathology*, *163*, 239–244. https://doi.org/10.1111/jph.12312

Marei, G. I. K., Rasoul, M. A. A., & Abdelgaleil, S. A. M. (2012). Comparative antifungal activities and biochemical effects of monoterpenes on plant pathogenic fungi. *Pesticide Biochemistry and Physiology, 103*(1), 56–61. https://doi.org/10.1016/j.pestbp.2012.03.004

Martínez, M., Arata, A., Dinolfo, M. I., Lázaro, L., Welin, B., & Stenglein, S. (2024). Evaluation of PSP1 biostimulant on *Fusarium graminearum*-wheat pathosystem: Impact on disease parameters, grain yield, and grain quality. *Pest Management Science*, *80*(7), 3578–3589. https://doi.org/10.1002/ps.8062

Nogueira, M. S., Decundo, J., Martinez, M., Dieguez, S. N., Moreyra, F., Moreno, M. V., & Stenglein, S. A. (2018). Natural contamination with mycotoxins produced by *Fusarium graminearum* and *Fusarium poae* in malting barley in Argentina. *Toxins, 10*(2), Article 78. https://doi.org/10.3390/toxins10020078

Pawar, V. C., & Thaker, V. S. (2007). Evaluation of the anti-*Fusarium oxysporum* f. sp. *cicer* and anti-*Alternaria porri* effects of some essential oils. *World Journal of Microbiology and Biotechnology*, *23*, 1099–1106. https://doi.org/10.1007/s11274-006-9339-6

Perczak, A., Gwiazdowska, D., Marchwińska, K., Juś, K., Gwiazdowski, R., & Waśkiewicz, A. (2019). Antifungal activity of selected essential oils against *Fusarium culmorum* and *F. graminearum* and their secondary metabolites in wheat seeds. *Archives of Microbiology, 201*, 1085–1097. https://doi.org/10.1007/s00203-019-01673-5

Pérez-Mosqueda, L. M., Trujillo-Cayado, L. A., Carrillo, F., Ramirez, P., & Muñoz, J. (2015). Formulation and optimization by experimental design of eco-friendly emulsions based on d-limonene. *Colloids and Surface B: Biointerface. 128*, 127–131. https://doi.org/10.1016/j.colsurfb.2015.02.030

Ravichandran, C., Badgujar, P. C., Gundev, P., & Upadhyay, A. (2018). Review of toxicological assessment of *d*-limonene, a food and cosmetics additive. *Food and Chemical Toxicology*, *120*, 668–680. https://doi.org/10.1016/j.fct.2018.07.052

R Core Team. (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org

Shude, S. P. N., Yobo, K. S., & Mbili, N. C. (2020). Progress in the management of Fusarium head blight of wheat: An overview. *South African Journal of Science*, *116*(11/12), 1–7. https://doi.org/10.17159/sajs.2020/7854

Taheri, P., Soweizy, M., & Tarighi, S. (2023). Application of essential oils to control some important fungi and bacteria pathogenic on cereals. *Journal of Natural Pesticide Research, 6*, Article 100052. https://doi.org/10.1016/j.napere.2023.100052

Thakur, M., & Sohal, B. S. (2012). Role of elicitors in inducing resistance in plants against pathogen infection: A review. *Biochemistry, 2013*, Article 762412. https://doi.org/10.1155/2013/762412

Tini, F., Beccari, G., Onofri, A., Ciavatta, E., Gardiner, D. M., & Covarelli, L. (2020). Fungicides may have differential efficacies towards the main causal agents of Fusarium head blight of wheat. *Pest Management Science, 76*(11), 3738–3748. https://doi.org/10.1002/ps.5923

Tsao, R., & Zhou, T. (2000). Antifungal activity of monoterpenoids against postharvest pathogens *Botrytis cinerea* and *Monilinia fructicola*. *Journal of Essential Oil Research*, *12*(1), 113–121. https://doi.org/10.1080/10412905.2000.9712057

Ünal, M. U., Ücan, F., Şener, A., & Dinçer, S., (2012). Research on antifungal and inhibitory effects of DL-limonene on some yeasts. *Turkish Journal of Agriculture and Forestry, 36*(5), 576–582. https://doi.org/10.3906/tar-1104-41

Wiese, M. V. (1987). *Compendium of wheat diseases* (2nd ed.). The American Phytopathological Society Press, St Paul. https://archive.org/search.php?query=external-identifier%3A%22urn%3Aoclc%3Arecord%3A742800815%22

Xu, X. (2003). Effects of environmental conditions on the development of *Fusarium* ear blight. *European Journal of Plant Pathology, 109*, 683–689. https://doi.org/10.1023/A:1026022223359

Zadoks, J. C., Chang, T. T., & Konzak, C. F. (1974). A decimal code for the growth stages of cereals. *Weed Research*, *14*(6), 415–421. https://doi.org/10.1111/j.1365-3180.1974.tb01084.x

Zaker, M. (2016). Natural plant products as eco-friendly fungicides for plant diseases control - a review. *The Agriculturists, 14*(1), 134–141. https://doi.org/10.3329/agric.v14i1.29111

Zhou, H., Tao, N., & Jia, L. (2014). Antifungal activity of citral, octanal and α-terpineol against *Geotrichum citri-aurantii*. *Food Control, 37*, 277–283. https://doi.org/10.1016/j.foodcont.2013.09.057

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Agronomía Colombiana has adopted the American Psychological Association (APA) standards (https://apastyle.apa.org/about-apa-style) to elaborate the final list of references cited in the text ("Literature cited" section). This standard will be required for new manuscripts received from March 1st, 2020 onwards.

Basic information about the use of APA for the list of references is available here: https://apastyle.apa.org/style-grammar-guidelines/references. In order to illustrate these standards, authors can check some examples about how to create each item of the list of references, keeping in mind the type of publication cited as follows (click on each option to open APA web information):

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Example: García-Arias, F., Sánchez-Betancourt, E., & Núñez, V. (2018). Fertility recovery of anther-derived haploid cape gooseberry (*Physalis peruviana* L.) plants. *Agronomía Colombiana*, *36*(3), 201–209. https://doi.org/10.15446/agron.colomb.v36n3.73108

Published dissertation or thesis references

Example: Franco, C. V. (2012). *Efecto de la colchicina sobre el número cromosómico, número de cloroplastos y características morfológicas del fruto en ecotipos de uchuva (*Physalis peruviana *L.) Colombia, Kenia y Perú* [Undergraduate thesis, Universidad Francisco de Paula Santander]. UFPS Library. http://alejandria.ufps.edu.co/descargas/tesis/1610259.pdf

Whole book

Example: Suescún, L., Sánchez, E., Gómez, M., García-Arias, F. L., & Núñez Zarantes, V. M. (2011). *Producción de plantas genéticamente puras de uchuva*. Editorial Kimpres Ltda.

Edited book chapter

Example: Ligarreto, G., Lobo, M., & Correa, A. (2005). Recursos genéticos del género *Physalis* en Colombia. In G. Fischer, D. Miranda, W. Piedrahita, & J. Romero. (Eds.), *Avances en cultivo, poscosecha y exportación de la uchuva* Physalis peruviana *L. en Colombia* (pp. 329–338). Universidad Nacional de Colombia.

For other types of references such as technical reports, conference presentations or proceedings, magazine articles or preprints see https://apastyle.apa.org/style-grammar-guidelines/references/examples. Archival documents, letters, collections and unpublished documents can be referenced using some standards users can find here: https://apastyle.apa.org/style-grammar-guidelines/references/archival. In this link (http://shorturl.at/xGIPZ) authors can find a summary of the APA style for references created by Mendeley.

Authors can also consider the use of some APA list of reference generators such as Reference Management (Mendeley, https://www.mendeley.com/reference-management/mendeley-desktop), Scribbr (https://www.scribbr.com/apa-citation-generator/#/) or MyBib (https://www.mybib.com/).

Whenever possible, please provide a valid DOI for each reference in the literature cited.

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