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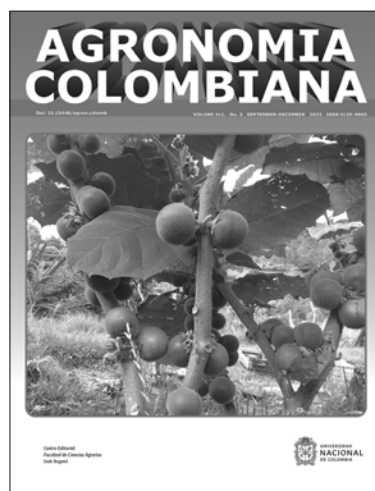
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Influence of the application of humic substances on the growth of watermelon and melon seedlings

Influencia de la aplicación de sustancias húmicas en el crecimiento de plántulas de sandía y melón

Hosana Aguiar Freitas de Andrade¹, Edson Dias de Oliveira Neto¹, Fernando Freitas Pinto Júnior^{2*}, Lídia Ferreira Moraes², Nitalo André Farias Machado³, and Raissa Rachel Salustriano da Silva-Matos²

ABSTRACT

Growing watermelon (*Citrullus lanatus*) and melon (*Cucumis melo*) is an important activity in the Brazilian Cerrado; however, many factors limit cultivation, including the difficulty of producing high-quality seedlings. In this study, the effect of humic substances (HS) on growth of 'Crimson Sweet' watermelon and 'Yellow' melon seedlings was evaluated. An experiment was carried out in a completely randomized design with five HS treatments applied to the soil: 0 (control), 5, 10, 15, and 20 g L⁻¹. The growth of watermelon and melon seedlings was influenced by the application of humic substances. Use of HS increased plant height, stem diameter, root length, root volume, shoot dry biomass, root dry biomass, and Dickson's quality index. HS boost plant growth, whose improved root system may have provided greater absorption and accumulation of mineral nutrients. Based on the quality of the seedlings (Dickson index), the application of 14 g L⁻¹ of HS is recommended for watermelon seedling production and 15 g L⁻¹ of HS for melon seedling production.

Key words: humic acids, *Citrullus lanatus* Schrad, *Cucumis melo* L., tropical horticulture, biostimulants, seedling quality.

RESUMEN

El cultivo de sandía (*Citrullus lanatus*) y melón (*Cucumis melo*) tiene gran importancia para los agricultores del Cerrado brasileño. Sin embargo, existen algunas limitaciones de producción, incluida la dificultad en la producción de plántulas de calidad. En este estudio evaluamos la influencia de sustancias húmicas (SH) en el crecimiento de plántulas de sandía 'Crimson Sweet' y melón 'Amarillo'. Para ello, el estudio se configuró en un diseño completamente aleatorio con cinco tratamientos, que consistieron en los siguientes tratamientos de SH aplicados al suelo: 0 (control), 5, 10, 15 y 20 g L⁻¹. El crecimiento de las plántulas de sandía y melón fue influenciado por la aplicación de sustancias húmicas. Hubo un aumento en la altura de las plantas, el diámetro del tallo, la longitud y el volumen de las raíces, así como la biomasa de brotes y raíces y el índice de calidad de Dickson. Las SH impulsaron el crecimiento de las plantas, cuya mejora del sistema radicular puede haber proporcionado una mayor absorción y acumulación de nutrientes. Según la calidad de la plántula (índice de Dickson), se recomienda aplicar 14 g L⁻¹ de SH para la producción de plántulas de sandía y 15 g L⁻¹ de SH para la producción de plántulas de melón.

Palabras clave: ácidos húmicos, *Citrullus lanatus* Schrad, *Cucumis melo* L., horticultura tropical, bioestimulantes, calidad de plántulas.

Introduction

Cucurbit crops, such as watermelon (*Citrullus lanatus*) and melon (*Cucumis melo*), are of worldwide importance, cultivated in over 3.5 and 1.2 million ha and yielding 101 and 33 million t, respectively (Ebert, 2019). Brazil is the fourth and eleventh largest producer of watermelon and melon, respectively (IBGE, 2020). The Brazilian state of Maranhão stands out, located on the last agricultural frontier of the Brazilian Cerrado. The state has a high fruit-producing

potential due to the considerable volume of annual rainfall, the well-distributed quality of the soils ranging from sandy to clayey, and proximity to ports. Therefore, Maranhão can become an important exporter (Caldas *et al.*, 2022).

The initial growth of cucurbits is one of the most important phases due to the plant's high nutritional and water demand and sensitivity to biotic and abiotic stresses (Nóbrega *et al.*, 2020; Ó *et al.*, 2020). Parameters evaluated at the beginning of the growing cycle indicate whether the plant will develop and establish satisfactorily in the field (Phani *et*

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al., 2021; Xanthopoulou *et al.*, 2022; Cáceres-Hernandez *et al.*, 2023). Good initial development depends on the use of technologies to support plants, especially in the Cerrado, with soils of low natural fertility and high acidity (Dias *et al.*, 2019; Lima *et al.*, 2019; Procópio & Barreto, 2021) and with a climate classified as humid tropical. In the tropics, finding a good and appropriate seed and a high-quality vegetable seedling has become one of the main key limitations. There is no doubt that the use of good horticultural practices such as a proper substrate and nutrient solution are key factors in achieving the production of vigorous seedlings and, subsequently, obtaining profitable yields (Ramírez-Guerrero *et al.*, 2015).

To overcome such limitations when seeking the production of high-quality seedlings, the use of substances with biostimulant properties is an interesting technology in sustainable agriculture to face problems related to fertilizer use in agricultural crops (Nardi *et al.*, 2021). Humic substances (HS) have been used to supply nutrients and/or stimulate the production of phytohormones that lead to root growth that the plant would otherwise not achieve (El-Hai *et al.*, 2019; Qin & Leskovar, 2020a; Soteriou *et al.*, 2021). Previous studies have demonstrated the effect of humic substances as seedling growth modulators, among other beneficial effects (Gomes Júnior *et al.*, 2019; Qin & Leskovar, 2020a; Silva *et al.*, 2022; Targino *et al.*, 2023).

However, there is much variation in the chemical composition of humic substances, in addition to different effects depending on the crop species (Asadi Aghbolaghi *et al.*, 2022; Rostami *et al.*, 2022; Sensoy *et al.*, 2022). Therefore, it is necessary to evaluate the application of humic substances in short-term cycle fruit species growing under the conditions of the Brazilian Cerrado. This study evaluated how and to what extent the growth of ‘Crimson Sweet’ watermelon and ‘Yellow’ melon seedlings can be affected by the application of humic substances at different concentrations.

Material and methods

Study location

The study on the production of cucurbit seedlings was carried out in a greenhouse with 75% shading, from August to September 2017, at the Center for Agricultural and Environmental Sciences (CCAA) of the Federal University of Maranhão (UFMA) (03°44’17” S, 43°20’29” W, altitude 107 m a.s.l.), located in the municipality of Chapadinha, state of Maranhão, Brazil. The region’s climate is classified as humid tropical (Aw) (Alvares *et al.*, 2013).

Experiment setup

To evaluate the effect of humic substances on watermelon and melon crops, the study was carried out in a completely randomized design with five treatments. Each treatment consisted of the application of a HS rate: 0 (control), 5, 10, 15, and 20 g L⁻¹, with five replicates. Each replicate consisted of five seedlings, totaling 125 seedlings.

The soil was classified as Dystrophic Yellow Latosol according to the Brazilian Soil Classification System (Santos *et al.*, 2018). This type of soil is typical in the tropical region of the Brazilian Cerrado. A soil sample was collected (0.0-0.20 m deep) for chemical and granulometric analyses (Teixeira *et al.*, 2017) (Tab. 1).

Polystyrene trays having 198 cells, with 18 cm³ cell volume, were filled with soil. The seeds were obtained from the company Feltrin (Brazil). Two seeds of watermelon cultivar ‘Crimson Sweet’ and melon cultivar ‘Yellow’ were sown in each cell at 1.5 cm deep from the soil surface. At 7 d after sowing, thinning was performed to keep one seedling per cell in the tray. The application of humic substances (HS) corresponded to 1 ml per cell of the respective treatments (0, 5, 10, 15, and 20 g L⁻¹) via soil before sowing the seeds. In control treatment (0 g L⁻¹), only water was applied so that the seedlings received the same amount of liquid. The source of HS was the organomineral compound Humitec

TABLE 1. Chemical and granulometric characterization of the soil.

pH (H ₂ O)	OM g kg ⁻¹	P mg kg ⁻¹	K ⁺	Ca ²⁺	Mg ²⁺	Na ⁺	Al ³⁺
5.06	15.4	13	0.07	0.80	0.30	0.31	1.50
S-SO ₄ ²⁻ cmolc kg ⁻¹	H+Al	BS	m	sand	fine sand	silt	clay
1.5	7.26	17	50	348	336	112	168

pH = potential of hydrogen; OM = soil organic matter; P = phosphorus; K⁺ = potassium; Ca²⁺ = calcium; Mg²⁺ = magnesium; Na⁺ = sodium; Al³⁺ = aluminum; H+Al = potential acidity; S-SO₄²⁻ = sulfur; BS = base saturation; and m = aluminum saturation.

WG® Tradecorp company (Brazil), consisting of 17% K₂O, 31% organic carbon, 68% total humic extract, 52% humic acids, and 16% fulvic acids. No fertilization other than HS was applied. Irrigation was performed daily to maintain soil moisture at 70% of soil water retention capacity. To ensure that there was no excess or shortage of water for the seedlings, the lysimeter weighing method was applied. Substrate humidity was monitored daily, and water lost through evapotranspiration was replaced with the aid of a beaker graduate.

Variable analyzed

Evaluations of the effects of HS on the production of seedlings were performed 20 d after sowing, which is the length of the production phase (growth and development) of the seedlings. Number of leaves (NL) was determined from direct count in each seedling; plant height (PH) was measured from the collar to the apex of the seedling using a millimeter ruler; stem diameter (SD) was obtained with a digital caliper (Digimess®); root length (RL) was measured using a ruler graduated in mm; root volume (RV) was determined by measuring the displacement of a water column in a graduated cylinder according to Harrington *et al.* (1994); shoot dry mass (SDM) and root dry mass (RDM) were determined by weighing on a scale with precision of 0.01 g the respective dry plant biomass. To obtain dry plant biomass, the plant material was dried in a forced-air oven at 65°C until constant weight. The quality of the seedlings was determined using the Dickson quality index (DQI) (Dickson *et al.*, 1960).

Statistical analysis

The data were subjected to analysis of variance (ANOVA) by the F test for detection of significant effect using the Infostat® software (Di Rienzo *et al.*, 2020) and the data explored by quantitative regression analysis when a significant effect was found ($P < 0.05$). Multivariate analysis was also carried out with principal component analysis

(PCA) using the SAS software (SAS® Inst. Inc., Cary, NC) to evaluate the relationships and determining factors.

Results

The growth of watermelon and melon seedlings was influenced by the application of humic substances (HS). Except for number of leaves (NL), the other variables of size, biomass, and quality of watermelon seedlings were influenced by the application of HS ($P < 0.05$). Similarly, there was no effect of HS application on NL of melon. On the other hand, there was influence ($P < 0.05$) of HS on the biometric and biomass responses of melon seedlings, including seedling quality index (Tab. 2).

Increasing HS rate in soil promoted a significant increase in watermelon seedling height of 25%. Height response of melon seedlings was consistent with a second-degree curve, in which there was a maximum increase of 6.9 cm in height at the HS rate of 9.6 g L⁻¹, followed by a decrease in height of the melon plants (Fig. 1A). There was a different response regarding stem diameter for each cucurbit species (Fig. 1B). Higher HS rate (20 g L⁻¹ of HS) was necessary for the melon plants to obtain a greater increase in stem diameter, while watermelon seedlings obtained the maximum stem (2.3 mm) increment with the application of only 11.2 g L⁻¹ of HS.

The HS rate of 18.2 g L⁻¹ promoted maximum root length of watermelon seedlings by increasing root growth by 31% (Fig. 1C), which validates how watermelon is responsive to HS in the initial growth phase. Despite the lack of response of root length in melon seedlings to HS application, root volume (development phase) of melon seedlings was strongly influenced by HS, with an increase of up to 0.5 cm³ at 11.8 g L⁻¹ of HS. This increase is 38% more than that of untreated plants (0 g L⁻¹ of HS). Watermelon root volume (development phase) was also strongly influenced

TABLE 2. Growth of watermelon and melon seedlings in response to doses of humic substances.

Watermelon	NL	PH	SD	RL	RV	SDM	RDM	DQI
P-value	0.17	<0.001	<0.001	0.001	0.008	<0.001	0.02	0.01
CV%	6.12	5.50	7.30	9.48	18.22	9.26	33.75	22.84
Melon	NL	PH	SD	RL	RV	SDM	RDM	DI
P-value	0.09	<0.001	<0.001	0.07	0.04	<0.001	<0.001	<0.001
CV%	3.09	8.72	10.13	9.27	27.97	22.15	22.75	20.76

NL: number of leaves, PH: plant height, SD: stem diameter, RL: root length, RV: root volume, SDM: shoot dry mass, RDM: root dry mass, and DQI: Dickson quality index. CV: coefficient of variation; P-value < 0.05: significant at the 0.05 level of probability according to the F-test.

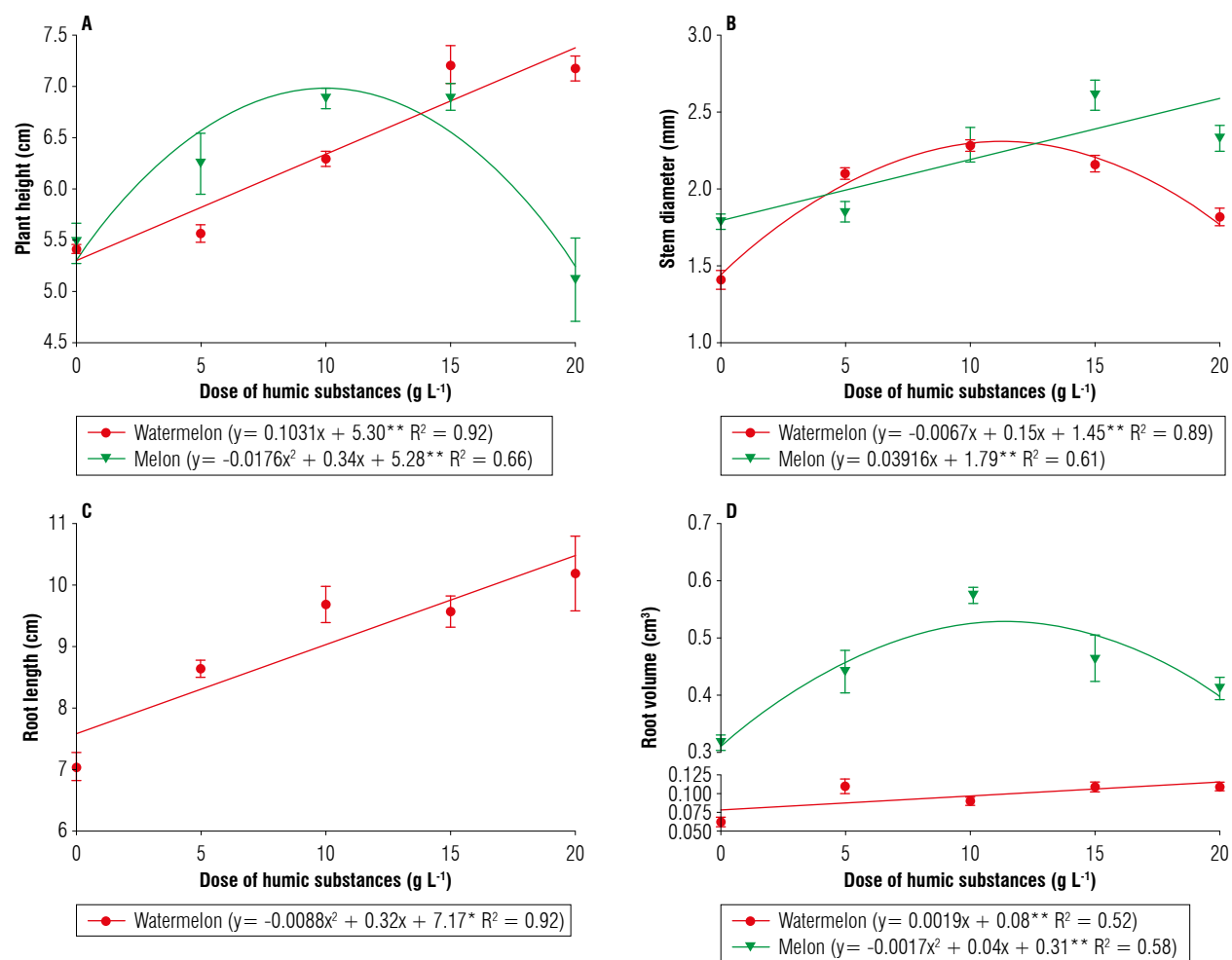


FIGURE 1. Responses of plant height (A), stem diameter (B), root length (C) and root volume (D) of watermelon and melon seedlings to application of humic substances. Mean (●▼) \pm standard error. * and ** are significant at the 0.05 and 0.01 level of probability, respectively.

by HS, with an increase of 43% for the maximum rate (20 g L⁻¹ of HS) (Fig. 1D).

The HS rate of 20 g L⁻¹ led to an increase in shoot dry mass of watermelon seedlings, with an increase of 42% compared to that of plants under 0 g L⁻¹ of HS. A lower HS rate (12 g L⁻¹) increased shoot dry mass of melon seedlings, with a maximum of 0.07 g of shoot biomass per seedling (Fig. 2A). Root dry mass of watermelon seedlings increased linearly, with an increase of 53% at 20 g L⁻¹ of HS when compared to the untreated treatment (0 g L⁻¹). Root dry mass of melon seedlings, however, fitted to a quadratic model, in which a maximum increase in root biomass was observed at a rate of 13 g L⁻¹ HS, when the seedlings had 0.05 g of root biomass (Fig. 2B).

Improved quality of cucurbit seedlings was observed with the use of HS. The Dickson quality index suggests that a

HS rate of 14 g L⁻¹ promoted better seedling quality with a value of 0.020, that is, 45% higher than that of the control treatment (0 g L⁻¹ of HS). Melon seedlings required a higher HS input (15 g L⁻¹ of HS) to obtain a better response regarding seedling quality, with a value of 0.024, which represents an increase of 57% in relation to that of the control treatment (Fig. 3).

Principal component analysis shows the correlation between rates (0, 5, 10, 15, and 20 g L⁻¹) of HS and growth and development responses, biomass production and seedling quality of watermelon (Fig. 4A) and melon (Fig. 4B). With regard to the production of watermelon seedlings, HS rates of 10 and 15 g L⁻¹ resulted in greater effects on SDM, PH, SD, and RL. Humic substances rates of 5 and 20 g L⁻¹ were highly correlated with the variables DQI, NL, RDM, and RV. The absence of HS (0 g L⁻¹ of HS) was associated with low values of watermelon seedling production variables.

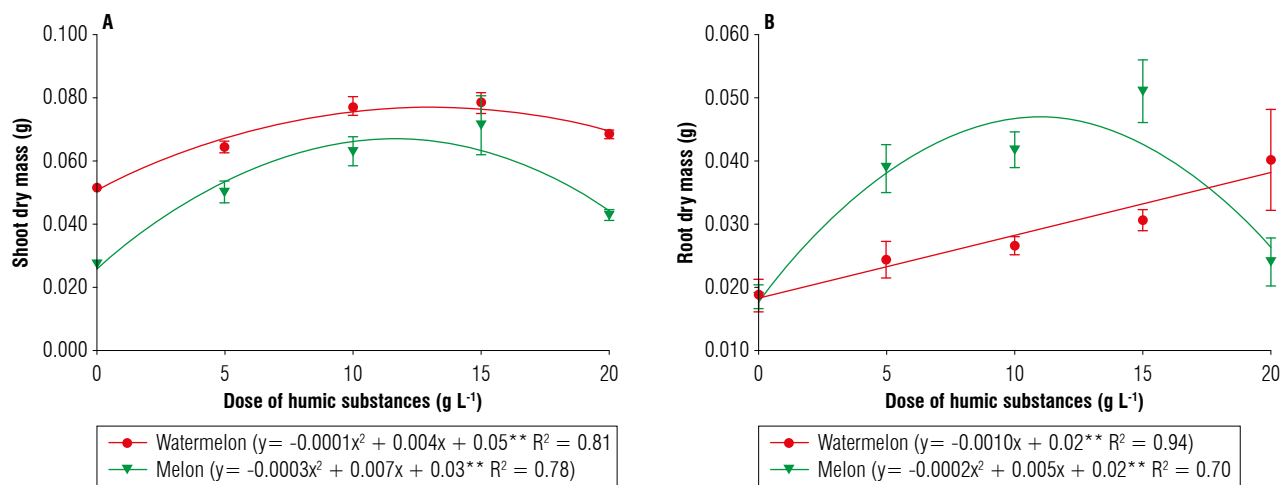


FIGURE 2. Responses of shoot dry mass (A) and root dry mass (B) of watermelon and melon seedlings to application of humic substances. Mean (●▼) ± standard error. * and ** are significant at the 0.05 and 0.01 level of probability, respectively.

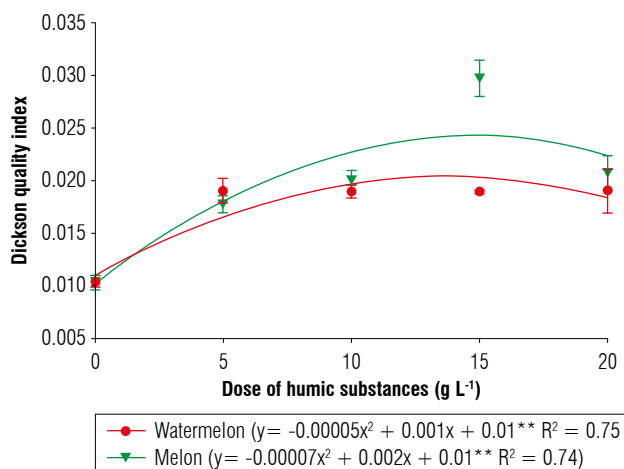


FIGURE 3. Responses of watermelon and melon seedlings to application of humic substances as measured by the Dickson quality index. Mean (●▼) ± standard error. * and ** are significant at the 0.05 and 0.01 level of probability, respectively.

In the production of melon seedlings, there was an overlap of the rate of 0 and 20 g L⁻¹ of HS, with a high mean value of PH, but low values of DQI, SDM, and SD. These variable responses are highly correlated with HS rates of 5 and 15 g L⁻¹. The HS rate of 10 g L⁻¹ is associated with high values of RL, NL, RDM, and RV.

Discussion

The use of humic substances in tropical Cerrado soil improves growth and development, biomass and quality of watermelon and melon seedlings. Reports have demonstrated that HS are able to regulate plant growth due to their biostimulation effects similar to auxins (Zandonadi *et al.*, 2007; Canellas & Olivares, 2014; Olaetxea *et al.*, 2015; Olivares *et al.*, 2017; Qin & Leskovar, 2020b), with structural and physiological changes in roots and shoots

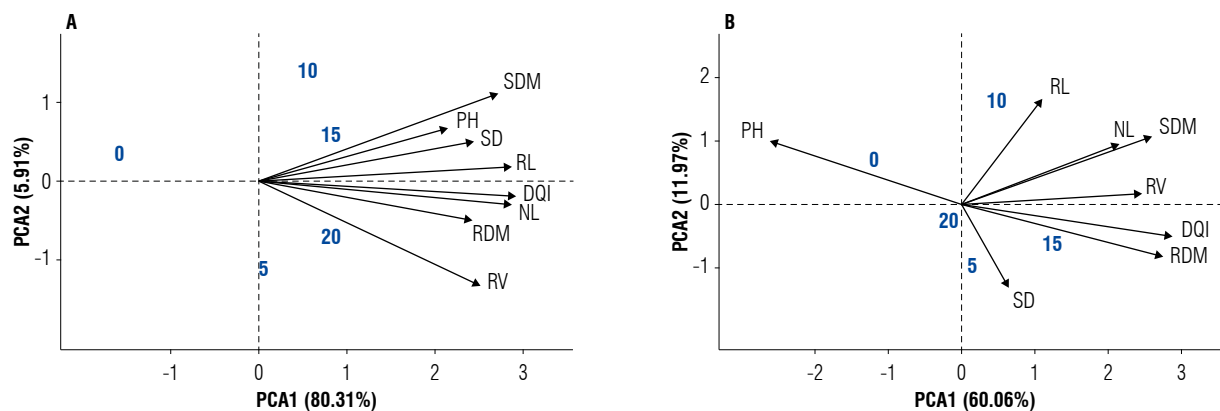


FIGURE 4. Principal component analysis of (A) watermelon and (B) melon seedling growth under application of humic substances. NL: number of leaves, PH: plant height, SD: stem diameter, RL: root length, RV: root volume, SDM: shoot dry mass, RDM: root dry mass, and DQI: Dickson quality index.

(Tarón Dunoyer *et al.*, 2022). This fact corroborates the results of increased plant height and stem diameter reported herein, in which the magnitude of the effects on seedling growth and development are induced by the contribution of HS. Several authors described that the HS-mediated auxin response was connected with the response in the H⁺-ATPase activity of the root plasma membrane (Ramos *et al.*, 2015; Olaetxea *et al.*, 2019). In our experiments, we did not measure this parameter, but it likely is one of the first responses in the HS-mediated action in plant growth.

The increase in plant biomass of melon and watermelon seedlings with the use of HS may be related to the effect of these substances in increasing the uptake and accumulation of nutrients. Seedlings assimilate the absorbed nutrients and use them for their metabolism and biomass production. Some changes induced by HS in the nutritional status of plants include the up-regulation of the gene expression of some of the nutrient transporters located in the plasma membrane, which promote the absorption of secondary ions and nutrients (Olaetxea *et al.*, 2019; Pizzeghello *et al.*, 2020), thereby increasing nutrient use efficiency (Tarón Dunoyer *et al.*, 2022). Jing *et al.* (2020) have also reported improved growth of maize seedlings with the use of HS. The authors reported that with the increase in HS, there was greater absorption of essential elements and, with this, stimulation of the growth and biomass of corn seedlings. But there was growth inhibition with a high dose of HS. Improved nutrient use efficiency with HS was also observed by Nardi *et al.* (2017). These authors, in turn, highlighted the fundamental function of HS in increasing the uptake and transport of nutrients by maize seedlings, as well as stimulating the solubilization of adsorbed or precipitated cations with the secretion of organic anions by the roots of seedlings.

A prominent effect of using HS is root development, as verified in this study. Jindo, Olivares *et al.* (2020) reported beneficial effects of HS on formation of lateral roots, which corroborates our observation of expressive increase in root volume of watermelon and melon seedlings. Conselvan *et al.* (2017) and Šerá and Novák (2022) have also found that HS stimulate elongation and proliferation of secondary roots in maize and poppy (*Papaver somniferum*) seedlings, respectively, which influences water and nutrient uptake, resulting in greater growth and development of the shoot in seedlings.

Increased growth and development in shoots and roots can also be related to greater nutrient availability due to increased production of exudates in the rhizosphere in

response to HS (Canellas *et al.*, 2019). Furthermore, HS stimulate auxin synthesis in roots, which has the function of stimulating lateral and adventitious root formation (Müller *et al.*, 1998). A well-developed root system during the seedling production phase is essential for seedling establishment in adverse field conditions. The results obtained here clearly demonstrate the importance of HS during early development of watermelon and melon seedlings.

Although using HS favors plant growth and development, dosage is an important criterion to be defined for each plant species (Jindo *et al.*, 2020). The results in this study indicate species-specific effects of HS on seedling growth. This is due to a relationship between HS-induced root exudation of organic and functional groups of these HS, which can cause different responses depending on the species (Rose *et al.*, 2014). Thus, it is important to define the best dose for a desirable response, such as greater growth of watermelon and melon seedlings. Such dose-response assessment is even more important when it is evident that crop species may play a major role in the dose-response relationship.

Humic substances normally do not impact growth linearly, with decline in plant growth rates at high HS concentrations (Pizzeghello *et al.*, 2020). These studies have also showed a decrease in SH, SD, RV, SDM, RDM, and DQI of melon seedlings as HS rates increased, and, for watermelon seedlings, a decline was observed in SD, RL, SDM, and DQI under high HS rates. This suggests watermelon seedlings are more responsive to HS than melon seedlings. Likewise, Jing *et al.* (2020) found a reduction in maize seedling growth with a high increase in HS concentration, while low concentrations resulted in the opposite effect on growth, thereby showing low HS concentrations are enough to obtain better production responses from melon seedlings.

Tropical Cerrado soils are predominantly sandy, meaning that a greater portion of nutrients and water added to soil are not used by crops in contrast to clayey soils. Most variables increased in value with the addition of HS, probably due to the central role that HS has in the formation and stabilization of soil aggregates (Swift, 1991; Mamedov *et al.*, 2014), causing more available water and nutrients to retain in soil.

Using HS in the initial growth and development phase of cucurbits is an interesting technology for farmers who lack technical information on melon and watermelon cultivation. The positive results obtained here when using HS are evident. Such results identify HS as a promising tool in the

rapid development of seedlings, subsequently contributing to increased yield in the field (Rodrigues *et al.*, 2017).

Conclusions

The use of humic substances is beneficial in the initial growth of watermelon and melon seedlings. It is an alternative for vegetable growers who cultivate in low-fertility tropical soils in the Cerrado. Based on seedling quality, 14 g L⁻¹ of HS is best for watermelon seedlings and 15 g L⁻¹ of HS for melon seedlings.

Conflict of interest statement

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

Author's contributions

HAFA and RRSSM designed the experiment and formulated the research goal. RRSSM developed the methodology and research activity planning. HAFA, EDON, FFPJ and LMM carried out the greenhouse experiment and the data collection. NAFM, HAFA and FFPJ contributed to the data analysis. HAFA wrote the initial draft. All authors reviewed the final version of the manuscript.

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Ablation reduces androgyny and improves flowering and bunch production in palms of Coarí x La Mé hybrid (*Elaeis oleifera* x *Elaeis guineensis*)

La ablación reduce la androginia y mejora la floración y producción de racimos en palmas del híbrido Coarí x La Mé (*Elaeis oleifera* x *Elaeis guineensis*)

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ABSTRACT

The first inflorescence in OxG hybrids after transplant to the field is often androgynous and should be removed to stimulate the initial vegetative growth of palms. The objective of this study was to evaluate the effects of early inflorescence ablation on later inflorescence emission and normal bunch development. The experimental design was completely randomized with two treatments (with early ablation and without ablation), four replicates, and 16 palms per replicate. The emission of female, male, and androgynous inflorescences, the number of leaflets, leaf area and dry weight of leaf 17, number of normal bunches formed, and annual production were estimated. The emission of normal female inflorescences increased with total ablation, while androgynous inflorescences increased without ablation. Ablated palms increased their leaf area, leaf dry weight, and annual bunch production.

Key words: flowering physiology, oil palm, reproductive development.

RESUMEN

La primera inflorescencia en los híbridos OxG después del trasplante al campo suele ser andrógina y debe eliminarse para estimular el crecimiento vegetativo inicial de las palmas. El objetivo fue evaluar los efectos de la ablación de inflorescencias tempranas sobre la emisión de inflorescencias posteriores y el desarrollo normal del racimo. El diseño experimental fue completamente al azar con dos tratamientos (con ablación temprana y sin ablación), cuatro repeticiones y 16 palmas por repetición. Se estimó la emisión de inflorescencias femeninas, masculinas y andróginas, número de folíolos, área foliar y peso seco de la hoja 17, número de racimos normales formados, y producción anual. La emisión de inflorescencias femeninas normales aumentó con la ablación total, mientras que las inflorescencias andróginas aumentaron sin ablación. Las palmas con ablación aumentaron el área foliar, el peso seco de la hoja y la producción anual de racimos.

Palabras clave: fisiología de la floración, palma de aceite, desarrollo reproductivo.

Introduction

Oil palm (*Elaeis guineensis* Jacq.) is a monocotyledonous and monoecious species of the Arecaceae family (Lin *et al.*, 2017) that produces unisexual male and female inflorescences that are temporarily separated in the same plant, where allogamous reproduction cannot have self-pollination (Adam *et al.*, 2011). OxG hybrids are the result of a cross between the American palm (*Elaeis oleifera* Kunth Cortes) and the African palm (*Elaeis guineensis* Jacq.) and are tolerant of bud rot (BR) disease (Genty & Ujueta, 2013). These hybrids have become the only economically viable alternative for the renewal of plantations in areas where BR is lethal (Chaves *et al.*, 2018).

Occasionally, young *E. guineensis* palms emit a particular type of inflorescence called androgynous or andromorphic; that is, it has the appearance of a male inflorescence, but male flowers are replaced by tiny individual female flowers (Corley & Tinker, 2016). Androgyny refers to an organism that has male and female characteristics, which, in the case of *E. guineensis* palms, is undesirable since the spikes of male inflorescences form female flowers, giving rise to the formation of small parthenocarpic fruits that does not contain oil (Rao & Donough, 1990). In some hybrid materials, the first inflorescence emitted by palms after transplant to the definitive site is often androgynous, later developing abnormal bunches with minute fruits that are generally parthenocarpic (Hartley, 1988; Hormaza *et al.*, 2012; Corley

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& Tinker, 2016) (Fig. 1). This anomaly can persist during the first years of growth or progressively decrease until it disappears, giving rise to normal inflorescences and significantly delaying normal harvests.



FIGURE 1. 'Indupalma' hybrid bunch with androgyny.

In young *E. guineensis* palms, the practice of ablation or castration is recommended, eliminating the first inflorescences, whether male or female, that palms emit at the end of the juvenile period (Prasad *et al.*, 2018) to stimulate vegetative growth and root development, especially in dry climates (Corley & Tinker, 2016). This channels nutrients to vegetative development, resulting in high yields at the beginning of harvest (Nazeeb *et al.*, 1988). This practice should be done at monthly intervals until the palms are 36 months old after transplanting to the final site (Chan & Mok, 1973; Chong Ming *et al.*, 2016) or, at most, for 6 months (Corley & Tinker, 2016). The physiological reason for ablation is that young palms begin to emit inflorescences in advance, which produce small bunches with little oil, making harvests inefficient (Nkongho *et al.*, 2014; Corley & Tinker, 2016), meaning it is more practical for palms to develop vegetatively before initiating normal reproductive development. Although ablation is recommended for OxG hybrids to reduce the emission of androgynous inflorescences and induce normal inflorescences (Zambrano, 2004), there are few studies on the effect of early ablation in planted hybrid materials. The objective of this study was to evaluate the effects of ablation on young palms on the subsequent emission of inflorescences (male, female, and androgynous), leaf area, dry weight, and bunch production in the 'Indupalma' OxG hybrid.

Materials and methods

The study was carried out on the Oleaginosas Las Brisas and Oleaginosas Monterrey plantations, both located in Puerto Wilches, Santander, Colombia (7°20'54" N, 73°53'54" W), at an altitude of 80 m a.s.l., with sandy loam soils, an average annual temperature of 29°C, 2,000 h year⁻¹ of sunshine, 75% relative humidity, and average rainfall of 2,800 mm year⁻¹. Two young, close and the same planting age (18 months after transplanting to the field) lots of the Indupalma hybrid (*E. oleifera* 'Coarí' x *E. guineensis* 'La Mé') were selected: a commercial lot in Oleaginosas Monterrey without ablation and a lot in Oleaginosas Las Brisas with ablation.

The experiment was carried out in a completely randomized design with two treatments, early ablation (EA) and no ablation (NA), with 4 replicates and 16 palms per replicate. The EA treatment began when the palms had elliptical-shaped inflorescences covered by a floral bract visible at stage 501 (Hormaza *et al.*, 2012), which were eliminated every week for 6 months; in the control treatment (NA), they were not removed. The ablation of the inflorescences was carried out with a 0.10 m wide flat chisel attached to a 1.5 m long wooden stick.

After opening the spathes—when the sex of the inflorescence was visible—the number of female, male, and androgynous inflorescences in each palm was recorded each month to estimate the monthly emission of inflorescences per hectare. Twelve months after the start of the trial, the final number of all inflorescences was recorded.

The leaf area of leaf 17 was determined with the statistical model developed by Contreras *et al.* (1999) for OxG hybrids: $AF17 = 0.639 \times C8$, where C is the average of 8 central leaflets multiplied by the total number of leaflets. The leaf dry weight (LDW) was calculated using the formula proposed by (Corley *et al.*, 1971).

The bunches were harvested 12 months after the start of the trial, when the first fruit fell off or the epicarp showed signs of cracking, recording the number and total weight of the bunches ha⁻¹.

The data of emitted inflorescences, leaf area (LA), leaf weight (LW), and bunch production (BP) as responses to ablation were subjected to analysis of variance (ANOVA)

using the statistical program SAS® 9.1 (SAS Institute Inc., Cary, NC, USA). To analyze the differences between the treatments, a T test was used ($P < 0.05$).

Results and discussion

Figure 2 shows the comparative evolution of the number of inflorescences ha^{-1} from the beginning of the experiment until 12 months after the ablation treatments. The number of androgynous inflorescences decreased significantly in the EA and NA palms during the 12 months after ablation (Fig. 2A), but their number per month was always lower in the EA palms. The young palms of some OxG hybrids tend to emit this particular type of inflorescence, an anomaly that seems to be associated with their 'Pisifera' parent since, in experimental plots in Costa Rica, the hybrids developed by crossing *E. oleifera* 'Taisha' mother palms with *E. guineensis* 'Yangambi', 'Ekona', and 'Ghana' showed excessive androgyny, which did not occur when the parents were Pisifera palms from the 'Compacta' population (Alvarado *et al.*, 2013). Williams and Thomas (1970) postulated that androgynous inflorescences of *E. guineensis* are produced during the change from the female to male flowering cycle; the mechanism by which this phenomenon occurs is still unknown.

The emission of female inflorescences (Fig. 2B) was significantly higher in palms with EA than in the NA control, and their number per ha remained constant during the subsequent 12 months but decreased monthly in the NA palms during this period. In contrast, the emission of male inflorescences (Fig. 2C) in the NA and EA palms was negligible during the 12 months after ablation. In the *E. guineensis* palms, the number of female inflorescences increased, and the number of male inflorescences decreased in young palms after early ablation (Corley & Breure, 1992; Legros *et al.*, 2009). The results indicated that ablation reversed the androgyny of these hybrids and preferentially induced the emission of normal female inflorescences. The early elimination of inflorescence sinks with ablation has an effect on sexual differentiation (Legros *et al.*, 2009), since sex determination in *E. guineensis* palms occurs about 24 months before anthesis (Corley, 1977; Dufour *et al.*, 1988) and depends on unfavorable conditions that can increase the frequency of male inflorescences (Corley, 1977); in OxG hybrids this condition is not yet known.

Figure 3 shows the effects of EA and control (NA) on the persistence of androgyny 12 months after the treatments were applied. The EA palms overcame the androgynous phenomenon and produced normal bunches (Fig. 3A),

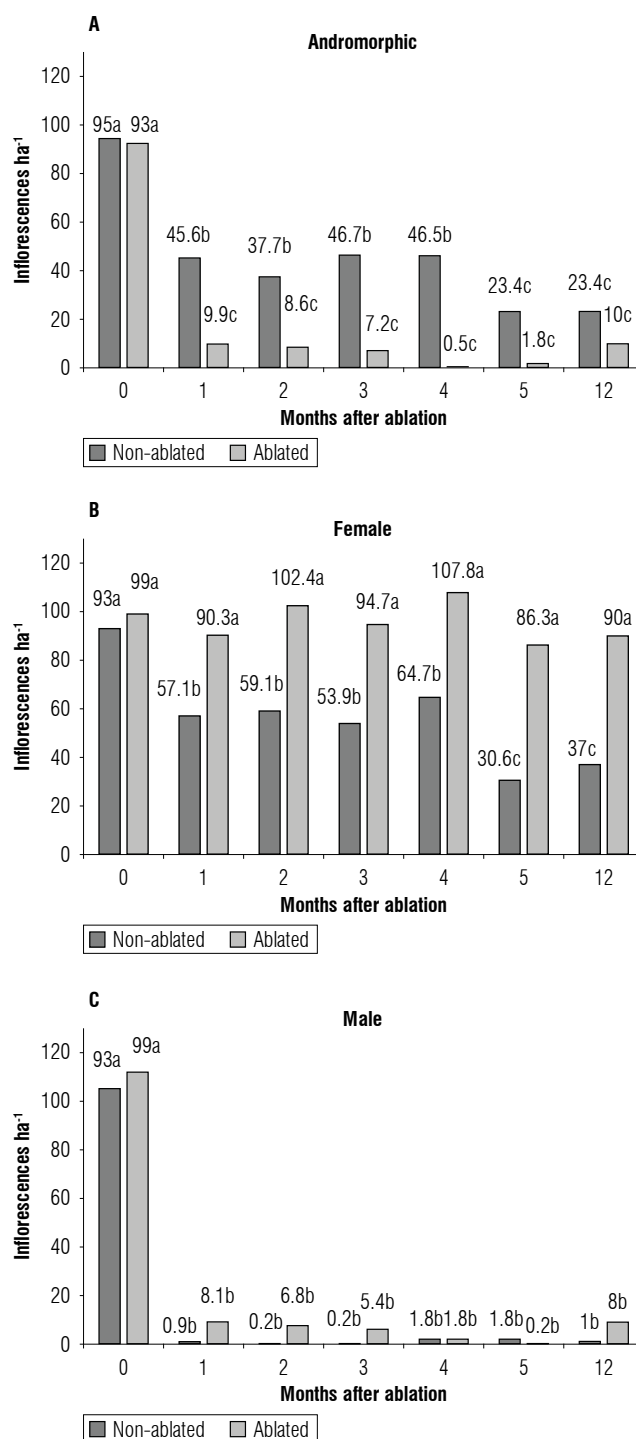


FIGURE 2. Effect of ablation on the subsequent emission of inflorescences in palms of OxG hybrid ('Coari' x 'La Mé'). Androgynous (A), female (B), and male (C). In each treatment, means with the same letters are not significantly different according to the T test ($P < 0.05$).

while the NA palms continued to emit androgynous bunches (Fig. 3B). With ablation, the preferential demand for inflorescences by assimilates is eliminated, favoring their distribution towards vegetative growth (Corley & Tinker,



FIGURE 3. Effect of early ablation on the phenomenon of androgyny in palms 12 months after applying the treatments. Plants with ablation (A) and control plants (B).

2016) because vegetative organs must be priority sinks for assimilates during the growth of young palms (Henson, 2007). In addition, the inflorescences and bunches of EA results in the adjustment and limitation of reproductive sinks and the availability of assimilates (Legros *et al.*, 2009), which favors reproductive development because surplus assimilates become available for bunch production (Squire & Corley, 1987; Breure, 1988).

The leaf area (LA) of the palms with ablation (EA) increased compared to the control (NA), while the dry weight (LDW) did not present differences between the EA and NA (Tab. 1). Several authors have observed that ablation carried out for long periods caused a slight increase in LA in young palms (Benard & Daniel, 1971; Corley & Breure, 1992) and that the number and total area of newly emitted leaves increased significantly with age in the EA inflorescences (Hew & Tam, 1971; Legros *et al.*, 2009).

TABLE 1. Bunch production, leaf area, and dry weight of leaf 17 in palms at 12 months after ablation.

Treatment	Leaf 17		Bunch production per year	
	Area (m ²)	Dry weight (kg)	Number	Weight (t ha ⁻¹)
No ablation	5.9 b	1.9	1,310 b	2.3 b
Ablation	6.7 a	2.0	1,382 a	4.9 a
Significance	*	Ns	*	*

*Significant differences according to the t-test ($P < 0.05$), ns non-significant differences according to the t-test ($P < 0.05$).

The number and weight of bunches were significantly increased by ablation (EA). This agrees with the results of an experiment in Indonesia in Ténara palms, in which bunch production was four times higher in palms with ablation

than in palms without ablation after six months (Pallas *et al.*, 2013). Corley and Breure (1992) also observed that mean bunch weight increased by 65% after early removal of 75% of inflorescences in Ténara palms, showing that bunch weight and the potential number of bunches increased with a greater intensity of ablation. The ablation of male and female inflorescences and small bunches lead more nutrients towards the initial vegetative development of palms instead of early reproductive development (Nazeeb *et al.*, 1988; Corley & Tinker, 2016).

Conclusion

Early ablation of the Indupalma hybrids reduced androgyny and increased later emission of normal female inflorescences and bunch production.

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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

DDRR: conceptualization, research. DGCS: conceptualization, writing, and supervision editing. ACB: conceptualization, visualization, writing, and editing. HRD: conceptualization, writing, and editing supervision. All authors have read and approved the final version of the manuscript.

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Physiological effects of *Acmella ciliata* essential oil on root development of *Nicotiana tabacum*

Efectos fisiológicos del aceite esencial de *Acmella ciliata* sobre el desarrollo radical de *Nicotiana tabacum*

Lizeth Daniela Méndez-Grateron¹, Luz Yineth Ortiz-Rojas¹, and Giovanni Chaves-Bedoya^{1*}

ABSTRACT

In this study, the essential oil of *Acmella ciliata*, characterized by the presence of significant amounts of spilanthol, α -phellandrene epoxide, and carvotanacetone, was assessed for its influence on the growth of *Nicotiana tabacum* cv. Xanthi, with the focus primarily on root hair density and primary root length. Following its extraction through microwave-assisted hydrodistillation, the oil was stored at 4°C in amber vials, distinguishable by its unique yellowish-reddish hue, with a refractive index of 1.3478 and a density of 0.847 g cm⁻³. Among the various dilutions evaluated, the undiluted oil (T3) and the dilution 1.5:0.5 (Oil:EtOH) (T6) demonstrated the most prominent effects. The T3 and T6 treatments markedly enhanced root hair numbers, with T6 additionally promoting root length compared to other treatments. Considering the presence of bioactive alkaloids such as spilanthol in the oil, these compounds may have contributed to the observed root growth modulation. When compared against the positive control, affinin, *Acmella ciliata* essential oil displayed a more pronounced effect on root hair proliferation, while affinin predominantly boosted primary root elongation. The findings highlight the differential effects of the essential oil on specific plant growth parameters.

Key words: alkaloids, root elongation, root hairs, bioactivity.

RESUMEN

En esta investigación, el aceite esencial de *Acmella ciliata*, caracterizado por la presencia de compuestos significativos como el espilantol, el epóxido de α -felandreno y la carvotanacetona, fue evaluado por su influencia en el crecimiento de *Nicotiana tabacum* cv. Xanthi, centrándose principalmente sobre la densidad de pelos radicales y la longitud de la raíz primaria. Luego de la extracción mediante hidrodestilación asistida por microondas, el aceite se almacenó a 4°C en viales de ámbar y se distinguió por su único matiz amarillo-rojizo, con un índice de refracción de 1.3478 y una densidad de 0.847 g cm⁻³. Entre las diversas diluciones evaluadas, el aceite sin diluir (T3) y la dilución 1.5:0.5 (Aceite:EtOH) (T6) demostraron los efectos más prominentes. Los tratamientos T3 y T6 aumentaron notablemente el número de pelos radicales, con T6 además promoviendo la longitud de la raíz de manera comparable a otros tratamientos. Considerando la presencia detectada de alcaloides bioactivos como el espilantol en el aceite esencial, es plausible que contribuyeran a la modulación observada del crecimiento de la raíz. En comparación con el control positivo, afinina, el aceite esencial de *Acmella ciliata* mostró un efecto más pronunciado en la proliferación de pelos radiculares, mientras que la afinina impulsó predominantemente la elongación de la raíz primaria. Los hallazgos destacan los efectos diferenciales del aceite esencial en parámetros específicos del crecimiento vegetal.

Palabras clave: alcaloides, elongación radical, pelos radicales, bioactividad.

Introduction

Roots are vital for a plant's nutrient and water uptake, as well as storage and plant anchorage. For plants to grow, they must uptake from the soil 14 essential mineral elements, as well as silicon beneficial for certain plants like rice, which shows yield improvement with increased doses of silicon (Tajima, 2021). Plant roots adapt for better acquisition of elements, influenced by agricultural practices aimed at enhancing yield and quality. Moreover, optimizing root systems can lessen environmental impacts by reducing

nutrient loss, thus preventing potential water pollution from substances like nitrate. Enhanced root development aids in efficient nutrient capture, linking root functionality directly to environmental and crop health (Tajima, 2021).

Seeking sustainable strategies to enhance agricultural productivity and crop resilience to environmental stresses is a critical challenge in modern agriculture. Among various approaches, the exploitation of natural compounds that stimulate root growth and development emerges as a promising pathway to optimize nutrient and water absorption,

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crucial for plant vigor and yield. In this context, *Acmella ciliata*, known for its rich composition of compounds such as alkamides, including spilanthol, stands out for its potential to positively influence root architecture. Previous studies have suggested that manipulating root biology with phytohormonal compounds can lead to significant improvements in crop yield by facilitating more efficient nutrient and water uptake (Bano *et al.*, 2022). However, significant gaps remain in our understanding of how specific natural compounds can be harnessed for this purpose.

Our grasp of plant roots, the unseen segment of plants, is notably limited compared to our understanding of their aerial counterparts. The roles roots play in supporting and nourishing the plants, alongside their complex communication mechanisms, are still shrouded in mystery. Efforts to enhance root traits are often overshadowed by the challenges in studying them within their natural soil habitat. However, the exploration of root biology is essential for advancing agricultural practices and environmental conservation (Ryan *et al.*, 2016). As we begin to unravel the complexities of root systems, we set the stage for breakthroughs in food security and sustainability. This involves tapping into the vast yet unexplored potential of roots to bolster plant health and crop yields. Therefore, it is important to understand the factors that affect root growth and development and how these factors can be manipulated to optimize nutrient and water absorption (Li *et al.*, 2016).

Phytohormones, as plant growth regulators, play a pivotal role in orchestrating the growth and development of plants by influencing root architecture and function. Plant hormones, including auxins, cytokinins, gibberellins, ethylene, abscisic acid and other compounds, mediate a wide array of physiological processes that directly or indirectly modulate root proliferation, elongation, and branching. In agriculture, harnessing the power of phytohormones presents a promising avenue for elevating crop productivity. Application of these hormones can stimulate root growth in a targeted manner, thereby optimizing the plant's ability to absorb essential nutrients and water from the soil. This enhanced root system not only supports the plant physical structure but also plays a critical role in the efficient utilization of soil resources, potentially leading to significant improvements in crop yield and quality (Mukherjee *et al.*, 2022)

Bridging the gap between the phytohormone effects on root development and the insights from plant extracts, like *Moringa oleifera*, which enhance root formation in *Arabidopsis thaliana* (Ortiz-Rojas *et al.*, 2017), unfolds a multifaceted

aspect of plant biology. This synergy reveals a wider lens through which the impact of both synthetic and natural compounds on plant growth is appreciated. The meticulous research into phytohormones elucidates the complex molecular and physiological frameworks facilitating root growth and stress adaptation (Wang & Komatsu, 2022).

Acmella ciliata (Kunth) Cassini (Fig. 1), also known as *Spilanthes ciliata* Kunth and belonging to the Asteraceae family, is a tropical herb native to South America. Studies conducted on this species identify the presence of various alkamides (Silveira *et al.*, 2016). Alkamides are a class of natural compounds found in a variety of plant species. These compounds, which are chemical messengers, have been studied for their potential role as plant growth regulators (Greger, 2016). *Acmella ciliata* is a plant species that is known for its production of spilanthol (Silveira *et al.*, 2016).



FIGURE 1. *Acmella ciliata* (Kunth) Cass.

In this study, we aimed to understand the impact of *Acmella ciliata* essential oil, obtained from the department Norte de Santander (Colombia), on root growth and development in *Nicotiana tabacum* cv. Xanthi. The decision to use *Nicotiana tabacum* cv. Xanthi was based on its well-established role as a versatile model organism in plant science (Kurotani *et al.*, 2023). This cultivar was specifically chosen for its consistent growth patterns and well-characterized genetic makeup, making it a robust subject for assessing the essential oil influence on root architecture. The use of cv. Xanthi facilitates a thorough exploration of the bioactive properties of *Acmella ciliata* essential oil and lays a robust foundation for applying these insights to broader

agricultural contexts, potentially enhancing the growth of other crops and plant species.

The findings obtained from this research may lay the groundwork for further exploration into the utilization of such natural bio-stimulants in agricultural environments to optimize nutrient and water absorption.

Materials and methods

Plant material

The plant material was collected manually from Vereda Iscala, Chinácota, department Norte de Santander, located at 7.46837° N and 72.55574° W at an altitude of 2547 m a.s.l. After collection, the material was placed in a paper envelope and stored in a -20°C freezer at the Fitobiomol Research Laboratory, Francisco de Paula Santander University, Cúcuta.

Extraction and chemical analysis of essential oil from *Acmella ciliata*

Microwave-assisted extraction of essential oil from *Acmella ciliata* was carried out starting with the plant material collected and dried for 7 d at room temperature. Subsequently, 300 g of the dried whole plant (stem, leaves, and flowers) was placed in a microwave reactor, mixed with 250 ml of distilled water, and heated at 75% power for 30 min, divided into three cycles of 10 min each. The resulting essential oil was collected in a Clevenger trap for subsequent analysis. Following extraction, water traces within the oil were efficiently removed using a Pasteur column filled with anhydrous sodium sulfate. Further purification was achieved through the use of a 0.45 µm silica gel filter, ensuring oil purity (Torres *et al.*, 2007).

The initial verification of alkalamides in the *Acmella* essential oil was conducted through thin-layer chromatography, using affinin as a standard. To gain a more comprehensive understanding of the oil's composition and to confirm the presence of specific compounds, the sample was subsequently subjected to gas chromatography-mass spectrometry (GC-MS) analysis.

The GC-MS analysis of the essential oil extracted from *Acmella ciliata* utilized an AT 6890 Series Plus gas chromatograph integrated with an MSD 5973 mass spectrometer (Agilent Technologies, Santa Clara, USA). The chromatographic separation was achieved on a DB-5MS column (5%-phenyl-poly(methylsiloxane)) with dimensions of 60 m x 0.23 mm x 0.25 µm. The sample introduction was performed using the Solid-Phase Microextraction

(SPME) method in Split mode (30:1). The mass spectral data obtained were compared with reference spectra from the Adams, Wiley, and NIST databases to identify the compounds present.

Organoleptic and physical characterization of the essential oil

Following extraction, the essential oil was analyzed for its organoleptic characteristics and physical properties. The estimation of color parameters of the *Acmella ciliata* essential oil was made according to the geometry of the CIELAB system, which is based on color saturation; color brightness (L = 0 means black; L = 100 means white); chroma (a*), where positive values correspond to red and negative values to green; and color tone (b*), where positive values correspond to yellow and negative values refer to blue (Dini *et al.*, 2019). Three replicates were performed per sample. The values obtained from a* and b* determined the hue angle, giving the red color attributes of each oil sample analyzed.

Physical parameters of oil, such as the refractive index and density, were measured using a digital Abbe refractometer and a pycnometer, respectively.

Seed germination and early growth conditions for *Nicotiana tabacum* L. cv. Xanthi

Nicotiana tabacum L. cv. Xanthi seeds were surface sterilized and germinated in peat. Fifteen days after germination the seedlings were passed to Petri dishes with MS medium to a plant growth chamber. The temperature in the growth chamber was maintained at 25 ± 2°C, the photoperiod cycle was 16/8 h of light/darkness, and the air humidity was 60% (Jalil *et al.*, 2019). The pH was adjusted to 5.7 after the addition of agar. The Petri dishes containing the germinating seeds were incubated vertically to promote root growth on the surface of the medium and measure the growth of the primary root.

Effect of *Acmella ciliata* essential oil on *Nicotiana tabacum* root growth

The bioactivity of *Acmella ciliata* essential oil on the root growth of *Nicotiana tabacum* seedlings was assessed through a set of treatments following a completely randomized design. The statistical analysis was conducted using ANOVA, with further multiple comparisons made using Tukey's method. All analyses were performed in the SAS software package, with a 95% confidence level maintained.

In the study, 10 *Nicotiana tabacum* plants, each 8 d post-germination, were exposed to various treatments, including

controls and various dilutions (v/v) of the essential oil, as detailed in Table 1. For each treatment, 60 µl of the solution was applied. The total study population comprised 180 plants. Parameters such as the growth of the primary root, development of lateral roots, and root hair density were meticulously recorded using a precision ruler for growth measurements in millimeters, and in-depth observations were made using a Stemi DV4 binocular stereomicroscope by Zeiss.

TABLE 1. Treatment descriptions.

Treatment ID	Description
T1	Positive control (affinin)
T2	Negative control (EtOH)
T3	Undiluted essential oil
T4	Dilution 0.5:1.5 (Oil:EtOH)
T5	Dilution 1:1 (Oil:EtOH)
T6	Dilution 1.5:0.5 (Oil:EtOH)

EtOH refers to ethanol. Oil:EtOH indicates v/v dilutions of *Acmella ciliata* essential oil and ethanol.

Results and discussion

Extraction and storage of essential oil

There are various methodologies for the extraction of essential oils, such as steam distillation, organic solvent extraction, microwave-assisted distillation, microwave hydrodiffusion and gravity, high-pressure solvent extraction, supercritical CO₂ extraction, ultrasonic extraction, and solvent-free microwave extraction (Okoh *et al.*, 2010). In this study, the microwave-assisted hydrodistillation method was utilized for the extraction of the essential oil from *Acmella ciliata*. This technique was chosen for its ability to provide an extraction free of organic solvents, with water being used as the extracting medium, ensuring a purer product without the residues that organic solvents might leave behind. Furthermore, this method offers enhanced preservation of natural compounds and carries additional environmental and safety benefits (Lucchesi *et al.*, 2007).

Following the extraction and purification process, a total volume of 12 ml of *Acmella ciliata* essential oil was obtained. To ensure the preservation of the oil metabolites and prevent their degradation, the oil was stored in amber vials at 4°C (Toplan *et al.*, 2022).

Organoleptic and physical characterization of the essential oil

The yellowish-reddish coloration of the *Acmella ciliata* essential oil, characterized using the CIELAB model (Luo, 2015), reflects a complex interaction of its chemical

components. The essential oil displayed a distinct yellowish-reddish hue. A hue* angle of 28°05' and a lightness of 33.3 were recorded (Tab. 2).

TABLE 2. Physicochemical colorimetry parameter of the essential oil from *Acmella ciliata* to determine its heterogeneity.

Essential oil	Average ± standard deviation
L (Lightness)	33.3 ± 0.165
a (green/red)	0.360 ± 0.175
b (blue/yellow)	0.220 ± 0.090
Hue angle*	28°05' ± 5.232
Saturation	345.667 ± 62.132

*Hue angle = arctan (b/a).

The primary compounds identified in the oil, α-phellandrene epoxide, carvotanacetone, and spilanthal, may play a significant role in this distinctive coloration. Although the direct impact of each compound on the oil's coloration may not be fully established, the literature suggests that variations in concentration and the presence of certain chemical compounds can influence the optical properties of essential oils (Khayyat, 2018). For instance, spilanthal, known for its bioactive properties, could contribute to the yellow color of the oil (Barbosa *et al.*, 2016).

While the exact relationship between these compounds and the essential oil coloration requires further studies, this observation aligns with previous studies indicating that the coloration of essential oils can be indicative of their chemical compositions (Sadgrove *et al.*, 2022).

In addition to color characterization, the refractive index of the essential oil was determined to be 1.3478. This is key information for a better understanding of the composition and characteristics of the oil (Tab. 3). The refractive index and density are critical metrics that can influence how the oil interacts in various applications (Ispiryan *et al.*, 2023).

TABLE 3. Physical characteristics of the essential oil from the leaves and flowers of *Acmella ciliata*.

Physical property	Average ± standard deviation
Refractive index	1.3478 ± 0.00
Density (g cm ⁻³)	0.847 ± 0.013

GC-MS profiling of *Acmella ciliata* essential oil

The essential oil from *Acmella ciliata* was profiled using gas chromatography-mass spectrometry (GC-MS). The GC-MS chromatogram indicated the presence and relative abundance of compounds in the form of peaks over time (Fig. 2).

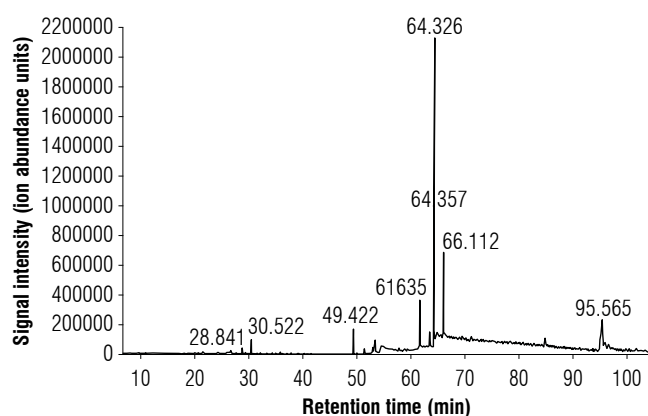


FIGURE 2. GC-MS chromatogram of *Acmella ciliata* essential oil. X-axis: retention time (min), indicating the elapsed time from sample injection to the elution of components through the chromatographic column, Y-axis: signal intensity (ion abundance units), reflecting the amount of each component detected by the mass spectrometer. Peaks represent the various compounds present in the sample, with their height proportional to the compound's concentration.

The profiling of *Acmella ciliata* essential oil via Gas Chromatography-Mass Spectrometry (GC-MS) provided a detailed snapshot of its chemical landscape, revealing α -phellandrene epoxide, carvotanacetone, and spilanthal as its major constituents (Tab. 4).

TABLE 4. Major compounds identified in the essential oil of *Acmella ciliata* by Gas Chromatography-Mass Spectrometry (GC-MS).

RT (min)	Tentative identification	Relative amount (%)
28.84	α -phellandrene epoxide	0.8
30.52	Carvotanacetone	1.9
49.42	Spilanthal	3.1

RT: retention time.

α -phellandrene epoxide and carvotanacetone, though present in smaller amounts than spilanthal, represented an intricate part of the oil's bioactivity spectrum. The chemical diversity observed in the chromatogram underscores the multifaceted role essential oils can play in modulating plant physiological processes. For instance, α -phellandrene epoxide's antimicrobial properties (Iscan *et al.*, 2012) could suggest a protective role for plants against pathogenic soil microorganisms, benefiting root health and development.

Spilanthal (Fig. 3), accounting for 3.1% of the *Acmella ciliata* essential oil and identified by its molecular formula $C_{14}H_{23}NO$ with a molar mass of 221.339, was notably prominent in the oil composition. This aligns with its well-documented bioactivity, underscoring spilanthal's pivotal role in the therapeutic and biological properties attributed

to the essential oil. Spilanthal has been shown to exert a variety of effects, including analgesic, neuroprotective, antioxidant, antimutagenic, anticancer, anti-inflammatory, antimicrobial, larvicidal, and insecticidal actions (Barbosa *et al.*, 2016). These diverse biological activities highlight spilanthal's potential influence beyond analgesic and anti-inflammatory effects in animals. While the exact mechanisms through which spilanthal might influence root development in plants are not yet clear, it is plausible to consider that, akin to its effects in animals, spilanthal could moderate stress responses in plants. This could lead to enhanced root growth under stress conditions, although further research is required to fully understand these effects in the context of root development in *Nicotiana tabacum*. The existing evidence, including the documented range of bioactivities of spilanthal, provides a strong basis for future studies to explore the role of spilanthal and similar compounds in modulating plant physiology.

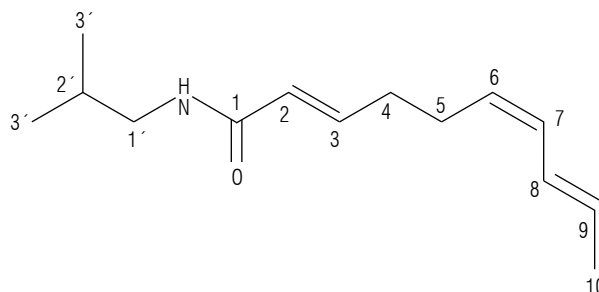


FIGURE 3. Chemical structure of spilanthal, a prominent alkamide found in *Acmella ciliata*.

Future research could explore the synergistic effects of these compounds, examining how combinations, rather than single constituents, influence plant development.

Effect of *Acmella ciliata* essential oil on the density of root hairs and length of the main root in *Nicotiana tabacum*

Prior literature findings have highlighted the presence of alkamides in *Acmella ciliata* (Rios-Chavez *et al.*, 2003; Greger, 2016; Silveira *et al.*, 2016). The identification of spilanthal offers a direct link between the observed bioactivity in the root growth of *Nicotiana tabacum* cv. Xanthi and the presence of this particular alkamide in the essential oil. Spilanthal has a range of properties, from providing oral pain relief to exhibiting antibacterial effects. In cosmetics, it is an ingredient in anti-wrinkle products, while culinary traditions include plants rich in spilanthal for their distinctive flavor (Barbosa *et al.*, 2016). There is also evidence pointing towards its potential in mosquito control, which can be vital in combating certain tropical

diseases. Furthermore, emerging studies hint at its possible anti-cancer properties (Barbosa *et al.*, 2016).

The essential oil from *Acmella ciliata* exerted a considerable influence on the number of root hairs and the primary root length of *Nicotiana tabacum* seedlings. The General Linear Model (GLM) analyses for the dependent variables number of hairs and root length in *Nicotiana tabacum* revealed statistically significant differences across treatments (Fig. S1). For the number of root hairs, the model yielded a highly significant p-value of less than 0.0001, indicating that substantial variability in root hair count can be attributed to the treatment effects. This is further corroborated by an F-value of 9.37, suggesting that at least one treatment is significantly different from the others in influencing hair count. The analysis for root length also showed statistical significance with a p-value of 0.0174, although with a smaller F-value of 3.07 (Fig. S1). This suggests that treatments have a discernible but less pronounced impact on root length as compared to root hair count. These findings

underscore the differential influence of the treatments on various aspects of root physiology in *Nicotiana tabacum*.

Treatments T6 and T3 resulted in the highest average number of root hairs (Fig. 4), with no statistical differences between them based on the Tukey's test (Fig. S2). The reported number of root hairs represents an average derived from the nine replicates. Although spilanthol is a component in this oil, the potential synergistic effects of all compounds in the oil cannot be overlooked. The observed effects are likely linked to the presence of these alkamides, as suggested by their documented bioactive properties in *Acmella* sp. and other plant species (Ramírez-Chávez *et al.*, 2004). This assertion is further reinforced by the documented bioactive properties of alkamides in *Acmella* sp. (Elufioye *et al.*, 2020).

For primary root length, the model is also significant (Fig. S1). The Tukey's Honestly Significant Difference (HSD) test reveals that, for the number of root hairs, treatment T6 had

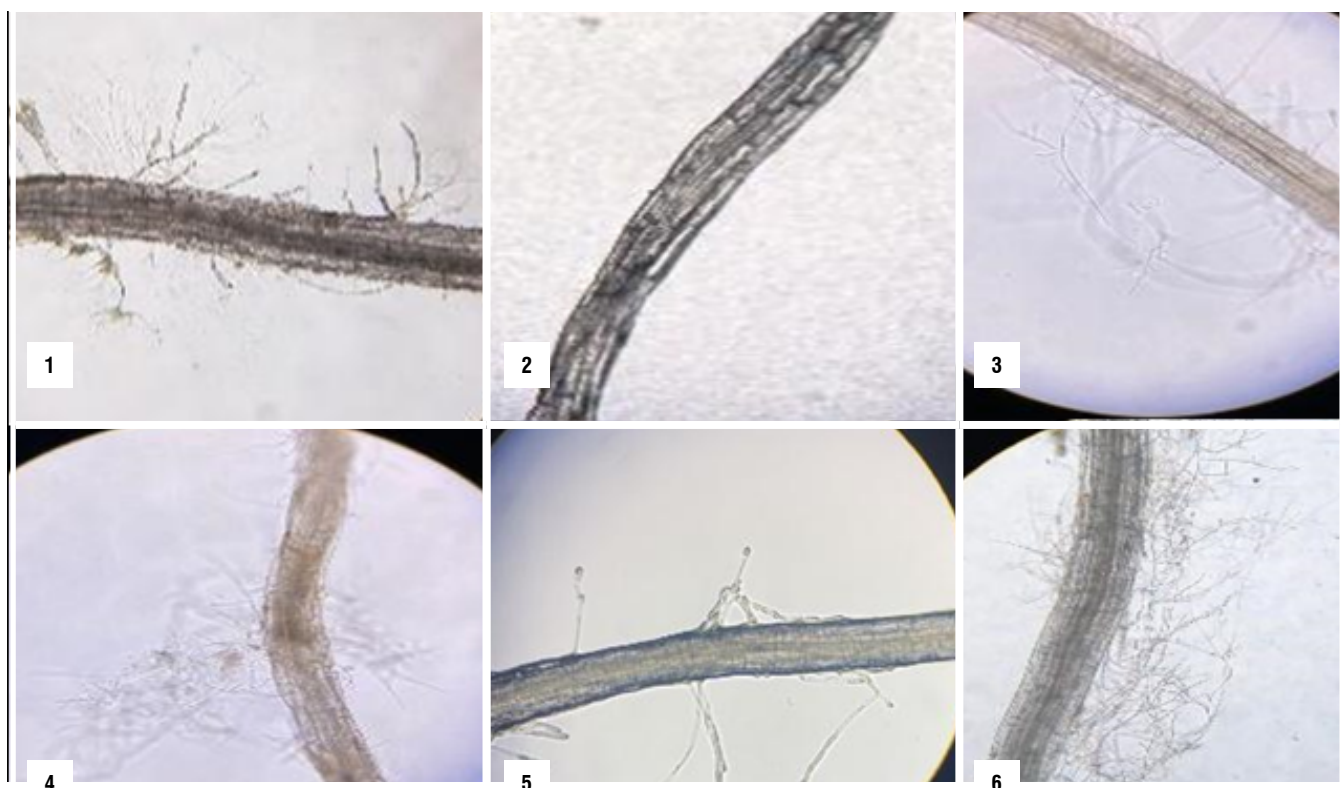


FIGURE 4. The effect of various treatments of essential oil extracted from *Acmella ciliata* on root hair density in *Nicotiana tabacum* plants. T1: Positive control (affinin), T2: Negative control (EtOH), T3: Undiluted essential oil, T4: Dilution 0.5:1.5 (Oil:EtOH), T5: Dilution 1:1 (Oil:EtOH), T6: Dilution 1.5:0.5 (Oil:EtOH). The number of root hairs is the average from nine replicates, counted within the visual field of a microscope using a 10X ocular and a 10X objective, resulting in a total magnification of 100X. EtOH refers to ethanol. Oil:EtOH indicates v/v dilutions of *Acmella ciliata* essential oil and ethanol.

the highest mean count, categorizing it in group A along with treatments T3 and T5, without statistically significant differences between these treatments (Fig. S2). Despite having the highest mean, the similarity with treatments T3 and T5 suggests that the essential oil concentration does not linearly increase the number of hairs. Regarding root length, treatment T6 resulted in the highest average root length. However, no significant differences were found between the treatments, indicating a more uniform response pattern in terms of primary root length among the different treatments (Fig. S2). Notably, although treatment T6 was the most effective for root hair growth, it was the least effective in increasing primary root length.

In the positive control group (treatment T1) using affinin, there was a notable increase of the average primary root length, while the effect on growth of root hairs was comparatively minimal. This suggests that alkamides like affinin could preferably increase primary root growth rather than the growth of root hairs. As bioactive compounds, alkamides have been recognized to influence plant growth and development (Ramírez-Chávez *et al.*, 2004). Comparing their effects with those of other treatments is important, offering a standard to evaluate the efficacy of various compounds or relative to these bioactive agents. Significantly, alkamides are natural plant products with a wide array of biological activities across multiple phyla. They exhibit properties with antifungal, antibacterial, antimalarial, and insecticidal effects, among others (Buitimea-Cantúa *et al.*, 2020). Furthermore, they play roles in plant growth promotion and defense gene induction, and even interact with intercellular signaling molecules in organisms ranging from humans to bacteria and plants (Buitimea-Cantúa *et al.*, 2020).

The results suggest that treatment effectiveness can vary depending on the plant growth characteristic being analyzed. For instance, a treatment that is effective in promoting one growth attribute might not be as effective for another. Given that treatment T6 was most effective for root hair growth but least effective for elongation of primary root, it is evident that the impacts of the treatments can be complex. This implies that when selecting treatments to enhance plant growth, it is essential to determine which growth features are most desired for the specific crop scenario. For example, in crops like lettuce where rapid nutrient absorption is essential due to their short growth cycle, treatment T6, which promotes root hair growth, might be more advantageous. Conversely, for deep-rooted plants like maize, which require deeper rooting for stability and water access from deeper soil

horizons, a treatment with affinin (T1: positive control), which increases primary root length, could be more appropriate. The findings from this research not only offer the basis for further exploration into the utilization of natural bio-stimulants to optimize nutrient and water absorption but also emphasize the potential of plants like *Acmella*, often considered weeds, as sources for biologically active compounds. Given the multifunctional nature of alkamides, their applications could extend beyond crop cultivation, including a broader spectrum of their application in botanical and agricultural research.

Conclusion

This study showed a significant effect of the essential oil extracted from *Acmella ciliata* on the density of root hairs and the length of the main root of *Nicotiana tabacum*. This effect suggests that the compounds present in the oil, potentially alkamides, play a crucial role in promoting root growth. The study establishes strong evidence that the alkamides present in the *Acmella ciliata* oil have bioactive effects on the growth of other plant species.

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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

LDMG: methodology, writing of original draft; LYOR: supervision, formal analysis, data curation. GCB: conceptualization, methodology, writing. All authors reviewed the final version of the manuscript.

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SUPPLEMENTARY FIGURE 1. Results of GLM analysis (in Spanish) for root growth of *Nicotiana tabacum* plants treated with *Acmella ciliata* essential oil. Top panel: number of root hairs, bottom panel: root length (cm).

Sistema SAS					
Procedimiento GLM					
Variable dependiente: pelos					
Fuente	DF	Suma de cuadrados	Cuadrado de la media	F-Valor	Pr > F
Modelo	5	12783.42593	2556.68519	9.37	<.0001
Error	48	13097.55556	272.86574		
Total correcto	53	25880.98148			
	R-cuadrado	Coef Var	Raiz MSE	pelos Media	
	0.493931	75.02162	16.51865	22.01852	
Fuente	DF	Tipo I SS	Cuadrado de la media	F-Valor	Pr > F
trat	5	12783.42593	2556.68519	9.37	<.0001
Fuente	DF	Tipo III SS	Cuadrado de la media	F-Valor	Pr > F
trat	5	12783.42593	2556.68519	9.37	<.0001

Procedimiento GLM					
Variable dependiente: long					
Fuente	DF	Suma de cuadrados	Cuadrado de la media	F-Valor	Pr > F
Modelo	5	18.14814815	3.62962963	3.07	0.0174
Error	48	56.66666667	1.18055556		
Total correcto	53	74.81481481			
	R-cuadrado	Coef Var	Raiz MSE	long Media	
	0.242574	34.51342	1.086534	3.148148	
Fuente	DF	Tipo I SS	Cuadrado de la media	F-Valor	Pr > F
trat	5	18.14814815	3.62962963	3.07	0.0174
Fuente	DF	Tipo III SS	Cuadrado de la media	F-Valor	Pr > F
trat	5	18.14814815	3.62962963	3.07	0.0174

SUPPLEMENTARY FIGURE 2. Results of the Tukey's HSD test (in Spanish) for root growth of *Nicotiana tabacum* plants treated with *Acmella ciliata* essential oil . Top panel: number of root hairs, bottom panel: root length (cm).

Sistema SAS

Procedimiento GLM

Prueba del rango estudentizado de Tukey (HSD) para pelos

NOTA: Este test controla el índice de error experimentwise de tipo I, pero normalmente tiene un índice de error de tipo II más elevado que REGWQ.

Alfa	0.05
Error de grados de libertad	48
Error de cuadrado medio	272.8657
Valor crítico del rango estudentizado	4.19724
Diferencia significativa mínima	23.111

Medias con la misma letra no son significativamente diferentes.

Tukey Agrupamiento	Media	N	trat
A	43.556	9	6
A			
B A	38.667	9	3
B A			
B A C	24.556	9	5
B C			
B C	17.000	9	4
C			
C	6.444	9	1
C			
C	1.889	9	2

Sistema SAS

Procedimiento GLM

Prueba del rango estudentizado de Tukey (HSD) para long

NOTA: Este test controla el índice de error experimentwise de tipo I, pero normalmente tiene un índice de error de tipo II más elevado que REGWQ.

Alfa	0.05
Error de grados de libertad	48
Error de cuadrado medio	1.180556
Valor crítico del rango estudentizado	4.19724
Diferencia significativa mínima	1.5201

Medias con la misma letra no son significativamente diferentes.

Tukey Agrupamiento	Media	N	trat
A	3.8889	9	5
A			
B A	3.5556	9	1
B A			
B A	3.4444	9	4
B A			
B A	3.1111	9	2
B A			
B A	2.7778	9	3
B			
B	2.1111	9	6

Sistema SAS

Procedimiento GLM

Prueba del rango estudentizado de Tukey (HSD) para long

NOTA: Este test controla el índice de error experimentwise de tipo I, pero normalmente tiene un índice de error de tipo II más elevado que REGWQ.

Alfa	0.05
Error de grados de libertad	48
Error de cuadrado medio	1.180556
Valor crítico del rango estudentizado	4.19724
Diferencia significativa mínima	1.5201

Medias con la misma letra no son significativamente diferentes.

Tukey Agrupamiento	Media	N	trat
A	3.8889	9	5
A			
B A	3.5556	9	1
B A			
B A	3.4444	9	4
B A			
B A	3.1111	9	2
B A			
B A	2.7778	9	3
B A			
B	2.1111	9	6

Application of herbicides in green cover crops to reduce *Meloidogyne javanica* inoculum in soybean plants

Aplicación de herbicidas en cultivos de cobertura verde para reducir el inóculo de *Meloidogyne javanica* en plantas de soja

Carolina Yumi Futigami¹, Angélica Calandrelli^{2*}, and Cláudia Regina Dias-Arieira¹

ABSTRACT

Nematodes are among the main plant parasites affecting Brazilian agriculture. Management practices involving the use of antagonistic or non-host plants are crucial for combating *Meloidogyne javanica* populations in the country. However, there is still limited information on the effects of herbicides on nematode populations. The aim of this research was to evaluate the impact of herbicides applied to crops on *M. javanica* populations in soybean and to examine the direct effect of herbicide products on the hatching of nematode juveniles. We conducted greenhouse and *in vitro* experiments. In the greenhouse experiment, soybean plants were grown in pots with sterile substrate, and a soybean seed was inoculated with 2000 nematodes per plant. The plants grew for 60 d, after which they were cut, and the following cover crops were planted: *Crotalaria spectabilis*, pigeon pea (*Cajanus cajan*), *Stylosanthes*, and buckwheat (*Fagopyrum esculentum*) for 60 d. These cover crops were desiccated with one of the following three herbicides: fomesafen, chlorimuron, or bentazone. Subsequently, soybeans were replanted and cultivated for additional 60 d. *In vitro* assays were used to determine the hatching percentage of *M. javanica* eggs exposed to herbicides. Both tests were repeated at different times of the year (Trials 1 and 2). *Meloidogyne javanica* reproduction was higher on soybean crops grown when buckwheat was a cover crop. Herbicide application reduced total nematode numbers in soybean grown in succession to buckwheat compared with the untreated control. The vegetative development of soybean crops was negatively influenced by herbicide treatment of cover crops, especially with the use of chlorimuron. Bentazone and fomesafen did not affect nematode hatching *in vitro*.

Key words: antagonistic plant, crop management, crop rotation, root-knot nematode.

RESUMEN

Los nematodos se encuentran entre los principales parásitos de las plantas que afectan la agricultura brasileña. Las prácticas de manejo que involucran el uso de plantas antagonistas o no hospederas son cruciales para el combate de las poblaciones de *Meloidogyne javanica* en el país. Todavía hay poca información sobre el efecto de los herbicidas en las poblaciones de nematodos. El objetivo de la presente investigación fue evaluar el efecto de los herbicidas aplicados a los cultivos de cobertura en las poblaciones de *M. javanica* en la soja y examinar el efecto *in vitro* de los herbicidas en la eclosión de los nematodos juveniles. Se realizaron experimentos de invernadero e *in vitro*. En el experimento de invernadero, las plantas de soja se cultivaron en macetas con sustrato estéril y luego se sembró una semilla de soja la cual se inoculó con 2000 nematodos por planta. Las plantas crecieron durante 60 d, luego se cortaron y se sembraron los siguientes cultivos de cobertura: *Crotalaria spectabilis*, guandú (*Cajanus cajan*), *Stylosanthes* y trigo sarraceno (*Fagopyrum esculentum*) durante 60 d y se desecaron con uno de los siguientes tres herbicidas: fomesafen, clorimuron o bentazon. Posteriormente, se volvió a sembrar soja y se cultivó durante 60 d adicionales. Se realizaron ensayos *in vitro* para determinar el porcentaje de eclosión de huevos de *M. javanica* expuestos a los herbicidas. Ambas pruebas se repitieron en diferentes épocas del año (ensayos 1 y 2). La reproducción de *M. javanica* fue mayor en los cultivos de soja que crecieron después del trigo sarraceno. La aplicación de herbicidas redujo el número total de nematodos en la soja cultivada en sucesión al trigo sarraceno en comparación con el control no tratado. El desarrollo vegetativo de los cultivos de soja fue influenciado negativamente por el tratamiento herbicida de los cultivos de cobertura, especialmente con el uso de clorimuron. Bentazon y fomesafen no afectaron la eclosión de nematodos *in vitro*.

Palabras clave: planta antagonista, manejo de cultivos, rotación de cultivos, nematodo del nudo radical.

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Introduction

High host diversity, management complexity, and damage potential of plant-parasitic nematodes place these pests among the major limiting factors affecting agricultural production worldwide. Nematode parasitism causes annual losses of 10-15% of soybean crops (*Glycine max* (L.) Merrill), amounting to more than US \$80 billion globally (Lima *et al.*, 2017). In Brazil, the world's largest producer of this oilseed, annual losses can reach US \$4.1 billion (Ralmi *et al.*, 2016; Lima *et al.*, 2017; Favoreto *et al.*, 2019).

Root-knot nematodes (*Meloidogyne* spp.) are obligate biotrophs. The most frequent and aggressive species associated with soybean crops is *Meloidogyne javanica* (Treub, 1885), widely distributed in areas where they are grown (Jones *et al.*, 2013; Mattos *et al.*, 2016; Favoreto *et al.*, 2019; Mazzetti *et al.*, 2019). Because of the complexity of interactions between root-knot nematodes and their hosts, managing these pathogens requires integrated strategies. Crop rotation with non-host, bad host, resistant, or antagonistic crops is highly recommended (Fileti *et al.*, 2011; Favoreto *et al.*, 2019). Furthermore, many plants used in crop rotation have the added benefits of adding organic matter to the soil, increasing soil microbial activity, and improving physical, chemical, and biological properties of the soil (Oka, 2010; Debiasi *et al.*, 2016; Franchini *et al.*, 2018).

For the success of pest management, it is necessary to carefully select the most adequate plant species for each crop rotation, as crop rotations can reduce nematode populations. However, in view of the polyphagous nature of *M. javanica*, it is important to bear in mind that some plant species may be suitable hosts for plant-parasitic nematodes. Another potential problem is that rotation crops may act as weeds for the next crop, serving as a nematode reservoir in the off-season (Braz *et al.*, 2016; Matias *et al.*, 2018; Favoreto *et al.*, 2019).

Herbicides may indirectly influence nematode management by controlling weeds or rotation species that serve as alternative hosts (Werle *et al.*, 2013; Matias *et al.*, 2018). Herbicide treatment may also have direct effects by reducing juvenile hatching (Wong *et al.*, 1993; Levene, 1995), soil populations (Castro-Carvajal *et al.*, 2015), and nematode development and reproduction (Nelson *et al.*, 2006; Riboldi *et al.*, 2013; Barbosa *et al.*, 2014; Kashiwaki *et al.*, 2020). Because root-knot nematodes depend on host plants, whether rotation, weed, or volunteer plants (Jones *et al.*, 2013; Rodiuc *et al.*, 2014; Ferraz & Brown, 2016), these parasites may die of starvation in case of rapid plant death and

degradation of root tissues. Despite this, information on interactions between herbicides, cover crops, and nematode management in crop rotations is virtually non-existent.

The aims of this study were to assess the effect of herbicide application to cover crops on *M. javanica* populations in soybean and evaluate the direct effect of herbicide products on the hatching of second-stage juveniles (J2) *in vitro*.

Materials and methods

Meloidogyne javanica reproduction and soybean development in succession to cover crops subjected to herbicide treatment

This study was conducted in a greenhouse (23°47'34.5" S; 53°15'22.1" W) according to a completely randomized design with a 4×4 (herbicide treatment × cover plant) factorial arrangement, seven replicates for each treatment, and two experimental trials. Trial 1 lasted from November 6, 2020 to May 20, 2021. The mean daily minimum and maximum temperatures during this period were 20°C and 32°C. Trial 2 was conducted from December 14, 2020 to June 27, 2021, when the mean daily minimum and maximum temperatures were 19°C and 29°C, respectively.

Each experimental unit consisted of a polystyrene cup containing 500 cm³ of substrate (soil (Tab. 1) and sand at a volumetric ratio of 2:1, autoclaved for 2 h at 120°C), and planted with one seed of soybean M6410 IPRO (seed provided by Cocamar, Maringá, Brazil).

Five days after emergence, each seedling was inoculated with 2000 eggs and J2 of *M. javanica* by pipetting a nematode suspension into a 2 cm deep hole in the soil close to the plant root. The nematode inoculum was extracted from pure populations maintained on soybean plants in a greenhouse. Nematodes were extracted from the root system by the method of Hussey and Barker (1973) as adapted by Bonetti and Ferraz (1981). The suspension was adjusted to 2000 eggs + J2 ml⁻¹ in a Peters chamber under an optical microscope. Plants were maintained in the greenhouse (12 h photoperiod) and irrigated daily as needed. Plants were fertilized once with 3 g of Osmocote® (15% N, 9% P₂O₅, 12% K₂O, 1% Mg, 2.3% S, 0.05% Cu, 0.45% Fe, 0.06% Mn, 0.02% Mo).

At 60 d after inoculation, the aerial part of the soybean plants was cut and discarded. Then, the following cover crop treatments were sown: (i) buckwheat (*Fagopyrum esculentum*) IPR-92 Altar (ii) pigeon pea (*Cajanus cajan*) IAPAR 43, (iii) *Stylosanthes* 'Campo Grande', and (iv)

TABLE 1. Chemical characteristics of soil samples collected in Umuarama, Paraná, Brazil, for use in the experiments.

pH CaCl ₂	P mg dm ⁻³	OM --%--	Ca	K	Mg -----cmolc dm ⁻³ -----	Al	CEC	BS --%--
5.04	2.46	0.27	1.50	0.09	0.25	0.18	3.59	51.27

OM - organic matter, CEC - cation-exchange capacity, BS - base saturation.

Crotalaria spectabilis. Cover crop plants were grown for 60 d under the same conditions as the soybeans. After this period, the following herbicide treatments were applied: (i) fomesafen (Flex®, Syngenta, 250 g active ingredient (a.i.) L⁻¹, rate of 1 L ha⁻¹, spray volume of 300 L ha⁻¹), (ii) chlorimuron-ethyl (Classic®, FMC, 250 g a.i. L⁻¹, rate of 80 g ha⁻¹, spray volume of 300 L ha⁻¹), and (iii) bentazone (Basagran® 600, BASF, 600 g a.i. L⁻¹, rate of 1.2 L ha⁻¹, spray volume of 250 L ha⁻¹). The herbicides were applied using a pressurized backpack sprayer with CO₂, equipped with flat fan nozzles 110.015, providing a spray volume of 120 L ha⁻¹. Plants without herbicide treatment were subjected to manual weeding and used as control.

After 15 d without irrigation, plant residues were weighed and deposited in the same pots from which they were cut. Pots were sown with soybean, and plants were cultivated for 60 d under the same conditions mentioned before. After this period, soybean plants were harvested and the root system was carefully separated from shoots, washed, and placed on absorbent paper to remove excess water. Root fresh weight was determined; and root samples were subjected to nematode extraction, as described above. Total nematode numbers were counted using a Peters chamber under an optical microscope. The number was divided by root weight to determine the nematode population density (nematodes g⁻¹ root).

Shoots were evaluated with a measuring tape for shoot height and fresh and dry weights were determined using a semi-analytical scale (Gehaka, 0.001 g, BG 440). For dry weight determination, shoots were dried in a forced-air

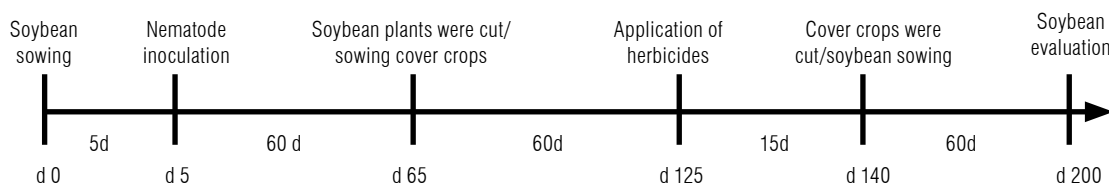
oven at 65°C for 72 h until constant weight was achieved. Figure 1 depicts a timeline schedule of experiments 1 and 2.

Effect of herbicides on *Meloigogyne javanica* hatching

The experiment was conducted in duplicate, following a completely randomized design with five treatments: 1) control - distilled water; 2) bentazone; 3) chlorimuron; 4) fomesafen, and 5) abamectin. The experimental unit consisted of a polypropylene Falcon tube to which 4 ml of herbicide (at the rate used under greenhouse conditions) and 1 ml of a suspension containing 100 eggs of *M. javanica* were added. Six replicates were used, meaning that each treatment was applied to six experimental units. Egg masses of *M. javanica* were collected from soybean roots, placed in a plastic tube containing 0.10% sodium hypochlorite, and shaken manually for 1 min to detach the eggs. The mixture was sieved through a 60-mesh sieve placed on top of a 500-mesh sieve under running water. The eggs were collected from the bottom sieve and the suspension was calibrated using an optical microscope. The control consisted of distilled water and an abamectin-based nematicide (Avicta® Completo, Syngenta, 500 g a.i. L⁻¹, rate of 60 ml 100 kg⁻¹ seeds and a spray volume of 200 L ha⁻¹). Tubes were incubated at 27°C in the dark for 10 d with manual shaking for 2 min three times a day. The hatching percentage was determined by counting the number of hatched juveniles and remaining eggs using a Peters chamber under an optical microscope.

Statistical analysis

The data were subjected to analysis of variance, and when significant, means were compared by the Scott-Knott test at $P < 0.05$ using SISVAR software (Ferreira, 2011).

**FIGURE 1.** Timeline schedule of 2 experimental trials, conducted in a greenhouse.

Results and discussion

Meloidogyne javanica reproduction and soybean development in succession to cover crops subjected to herbicide treatment

There was significant plant cover \times herbicide treatment effects on total nematode number and nematode population density in both trials (Tabs. 2-3). Soybean grown after buckwheat had higher nematode populations in both trials.

TABLE 2. Total numbers of *Meloidogyne javanica* in roots of soybean plants grown in succession to crop cover subjected to herbicide treatment after nematode inoculation.

Crop Cover	Control	Bentazone	Chlorimuron	Fomesafen
	Trial 1			
Buckwheat	51,774 ^{aA}	28,533 ^{aB}	3,473 ^{aC}	19,109 ^{aB}
Pigeon pea	3,772 ^{bA}	522 ^{bA}	1,000 ^{bA}	1,898 ^{bA}
Stylosanthes	957 ^{bA}	271 ^{bA}	221 ^{cA}	284 ^{bA}
Crotalaria	1,310 ^{bA}	758 ^{bA}	121 ^{cA}	104 ^{bA}
CV (%)	71.72			
Trial 2				
Buckwheat	15,750 ^{aA}	5,400 ^{aB}	325 ^{aC}	937 ^{aC}
Pigeon pea	450 ^{bA}	25 ^{bA}	225 ^{aA}	175 ^{aA}
Stylosanthes	180 ^{bA}	75 ^{bA}	175 ^{aA}	300 ^{aA}
Crotalaria	154 ^{bA}	125 ^{bA}	262 ^{aA}	175 ^{aA}
CV (%)	80.67			

For each treatment, means within columns followed by the same lowercase letter or means within rows followed by the same uppercase letter are not significantly different at $P < 0.05$ by the Scott-Knott test. CV – coefficient of variation. Original data were transformed to $\sqrt{x} + 0.5$ before analysis. The species correspond to buckwheat (*Fagopyrum esculentum*) IPR-92 Altar, pigeon pea (*Cajanus cajan*) IAPAR 43, *Stylosanthes* 'Campo Grande', and *Crotalaria spectabilis*.

In assessing differences between plant cover within each herbicide treatment, we observed that total nematode number was higher in soybeans grown after buckwheat in Trial 1, regardless of herbicide application (Tab. 2). In Trial 2, we observed no differences between the plant cover in treatments receiving either chlorimuron or fomesafen. Herbicide application led to a decrease in total nematode number in soybeans grown after buckwheat, compared with the control without herbicide, in Trials 1 and 2. The other treatments did not differ from the control. Therefore, in general, variations in nematode populations were only observed in soybeans grown after buckwheat, which allowed for higher populations of *M. javanica* in soybean.

The results of nematode population density (Tab. 3) were similar to those of total nematode number. We found that nematode population density was higher in soybeans grown in succession to buckwheat in Trial 1, regardless

of herbicide application. In Trial 2, there were no differences between plant cover treated with chlorimuron or fomesafen.

TABLE 3. Number of *Meloidogyne javanica* per gram of roots of soybean grown in succession to crop cover subjected to herbicide treatment after nematode inoculation.

Crop Cover	Control	Bentazone	Chlorimuron	Fomesafen
	Trial 1			
Buckwheat	8,481 ^{aA}	9,549 ^{aA}	1,728.8 ^{aB}	9,419.4 ^{aA}
Pigeon pea	907.8 ^{bA}	466.2 ^{bA}	966 ^{bA}	1,125.8 ^{bA}
Stylosanthes	235.7 ^{bA}	161 ^{bA}	84.1 ^{cA}	98.2 ^{cA}
Crotalaria	291 ^{bA}	342.8 ^{bA}	54.2 ^{cA}	36.2 ^{cA}
CV (%)	66.85			
Trial 2				
Buckwheat	6,064.1 ^{aA}	6,777.5 ^{aA}	754.4 ^{aB}	600.1 ^{aB}
Pigeon pea	387 ^{bA}	41.7 ^{bA}	750.2 ^{aA}	193 ^{aA}
Stylosanthes	96.8 ^{bA}	135.5 ^{bA}	388 ^{aA}	252.8 ^{aA}
Crotalaria	113.3 ^{bA}	273.7 ^{bA}	508.5 ^{aA}	219.5 ^{aA}
CV (%)	71.67			

For each treatment, means within columns followed by the same lowercase letter or means within rows followed by the same uppercase letter are not significantly different at $P < 0.05$ by the Scott-Knott test. CV – coefficient of variation. Original data were transformed to $\sqrt{x} + 0.5$ before analysis. The species correspond to buckwheat (*Fagopyrum esculentum*) IPR-92 Altar, pigeon pea (*Cajanus cajan*) IAPAR 43, *Stylosanthes* 'Campo Grande', and *Crotalaria spectabilis*.

Regarding the effects of herbicides within each plant cover treatment, differences were observed only in treatments with buckwheat. Chlorimuron led to a reduction in nematode population density in Trial 1 compared with the other treatments and chlorimuron and fomesafen led to reductions in the variable in Trial 2.

Overall, differences in *M. javanica* reproduction were only observed when buckwheat was used as a crop cover; nematode reproduction was higher in soybeans grown after buckwheat, although chlorimuron led to a reduction in pathogen multiplication. Buckwheat had varied responses to nematodes and are considered susceptible to *Meloidogyne* spp. (Melo *et al.*, 2022). In a study assessing the response of green manure to *M. javanica* populations, Chidichima *et al.* (2021) observed a reproduction factor (RF) of 2.94 to 53.46 on buckwheat, explaining the large nematode population in soybeans grown in succession to buckwheat.

Given the susceptibility of buckwheat to *M. javanica*, care must be taken when using this crop cover. However, buckwheat should not be disregarded completely, as it is an

important component for the maintenance of crop systems, acting in the cycling of macro- and micronutrients (Klein *et al.*, 2010; Gonçalves *et al.*, 2016), improving soil microbiota (Kautz *et al.*, 2004; Tejada *et al.*, 2008), and favoring the growth of other plant species with the production of soil organic matter (Yadav *et al.*, 2000; Gonçalves *et al.*, 2016). Furthermore, buckwheat extract was found to be rich in polyphenols, including rutin and quercetin, tannins, and aldehydes such as salicylaldehyde that may be responsible for the plant nematocidal activity against *Meloidogyne* spp. (Kalinova *et al.*, 2011; Aissani *et al.*, 2018). Use of this crop coverage on lands affected by root-knot nematodes requires planning and monitoring.

Green manure species may behave as weeds after successive crops, given their high contribution to the soil seed bank, leading to their classification as remnant plants (Soltani *et al.*, 2011). As such, cover crops may serve as alternative hosts to nematodes in the field (Braz *et al.*, 2016; Matias *et al.*, 2018).

The reduction in *M. javanica* reproduction on soybeans succeeding chlorimuron-treated buckwheat might be related to herbicidal effects. Chlorimuron is a systemic sulfonylurea capable of quickly inducing plant death (Oliveira Junior *et al.*, 2011). These and previous findings underscore the importance of managing invasive species as a delay. Lack of control of nematode-susceptible plants may promote an increase in the population of remaining nematodes (Nelson *et al.*, 2006; Werle *et al.*, 2013; Castro-Carvajal *et al.*, 2015; Kashiwaki *et al.*, 2016; Kashiwaki *et al.*, 2020).

Crotalaria spectabilis, *Stylosanthes*, and *Cajanus cajan* had the best nematode reduction potential. Studies have shown that *C. spectabilis* is effective in suppressing *M. javanica* reproduction (Inomoto *et al.*, 2008; Miamoto *et al.*, 2016; Soares *et al.*, 2022). *Crotalaria spectabilis* is known to be antagonistic and serve as a trap to nematodes (Warnke *et al.*, 2008; Curto *et al.*, 2015; Miamoto *et al.*, 2016). The plant accumulates secondary metabolites with nematocidal action in the roots, such as pyrrolizidine alkaloid (monocrotaline) (Colegate *et al.*, 2012).

In line with our results, Miamoto *et al.* (2016) observed low *M. javanica* penetration and reproduction in *Stylosanthes capitata* after 60 d of cultivation, indicating that the plant is not very attractive to the pathogen. In other studies, no galls were detected in the roots of *S. capitata*, with a reduction

of up to 98.4% of nematode populations compared with the control, and so is classified as immune to nematodes (Lenne, 1981; Sharma, 1984).

Cajanus cajan was shown to reduce *M. javanica* populations (Miamoto *et al.*, 2016) and is used as a nematode-resistant standard, with reproduction factor (RF) values of 0.11 to 0.13 (Araújo Filho *et al.*, 2010). Chidichima *et al.* (2021), however, observed RF values of 0.58-2.99 that varied according to *M. javanica* populations, suggesting a controversial antagonistic effect of *C. cajan* (Miamoto *et al.*, 2016) attributed to the different behaviors of cultivars (Araújo Filho *et al.*, 2010).

In the current study, there were significant interactive effects of crop coverage and herbicide treatment on soybean height in both trials (Tab. 4). In general, when there were significant differences between treatments, only chlorimuron negatively influenced soybean height compared with the other herbicides.

TABLE 4. Height (cm) of soybean plants grown in succession to cover crops subjected to herbicide treatment after nematode inoculation.

Cover crop	Control	Bentazone	Chlorimuron	Fomesafen
	Trial 1			
Buckwheat	34.1 ^{aA}	42.5 ^{aA}	21.5 ^{aB}	23.7 ^{bB}
Pigeon pea	34.7 ^{aA}	36.1 ^{aA}	25.1 ^{aB}	34.6 ^{aA}
Stylosanthes	39.4 ^{aA}	32.1 ^{aB}	27.1 ^{aB}	36.7 ^{aA}
Crotalaria	35.6 ^{aA}	35.2 ^{aA}	23.7 ^{aB}	40 ^{aA}
CV (%)	10.26			
	Trial 2			
	Control	Bentazone	Chlorimuron	Fomesafen
Buckwheat	33.8 ^{bB}	45.2 ^{aA}	26.7 ^{bB}	40.6 ^{aA}
Pigeon pea	45.7 ^{aA}	44.1 ^{aA}	27.1 ^{bB}	42.6 ^{aA}
Stylosanthes	46.8 ^{aA}	42.5 ^{aA}	34.7 ^{aB}	42.1 ^{aA}
Crotalaria	40.3 ^{bA}	41.7 ^{aA}	33.1 ^{aA}	35.5 ^{aA}
CV (%)	9.51			

For each treatment, means within columns followed by the same lowercase letter or means within rows followed by the same uppercase letter are not significantly different at $P < 0.05$ by the Scott-Knott test, CV – coefficient of variation. Original data were transformed to $\sqrt{x} + 0.5$ before analysis. Species correspond to buckwheat (*Fagopyrum esculentum*) IPR-92 Altar, pigeon pea (*Cajanus cajan*) IAPAR 43, *Stylosanthes* 'Campo Grande', and *Crotalaria spectabilis*.

Interaction effects were also exerted on root fresh weight in both trials (Tab. 5). In Trials 1 and 2, soybean plants grown after untreated buckwheat had higher root weight. In comparing herbicide treatments, we found that root weight was higher in soybean grown in succession to herbicide-free cover crops in both trials.

TABLE 5. Root fresh weight (g) of soybean plants grown in succession to crop covers subjected to herbicide treatment after nematode inoculation.

Cover crop	Control	Bentazone	Chlorimuron	Fomesafen
	Trial 1			
Buckwheat	8.4 ^{aA}	2.7 ^{aB}	2.0 ^{aB}	2.0 ^{aB}
Pigeon pea	4.5 ^{bA}	1.7 ^{aB}	1.4 ^{aB}	2.1 ^{aB}
Stylosanthes	4.1 ^{bA}	1.5 ^{aA}	2.3 ^{aA}	2.4 ^{aA}
Crotalaria	5.1 ^{bA}	2.0 ^{aB}	1.8 ^{aB}	1.9 ^{aB}
CV (%)	23.27			
Cover crop	Trial 2			
	Control	Bentazone	Chlorimuron	Fomesafen
Buckwheat	2.8 ^{aA}	0.7 ^{aC}	0.4 ^{aC}	1.6 ^{aB}
Pigeon pea	1.3 ^{bA}	0.7 ^{aB}	0.3 ^{aC}	1.0 ^{bA}
Stylosanthes	1.7 ^{bA}	0.6 ^{aC}	0.5 ^{aC}	1.2 ^{aB}
Crotalaria	1.4 ^{bA}	0.4 ^{aC}	0.4 ^{aC}	0.8 ^{bB}
CV (%)	18.65			

For each treatment, means within columns followed by the same lowercase letter or means within rows followed by the same uppercase letter are not significantly different at $P < 0.05$ by the Scott–Knott test, CV – coefficient of variation. Original data were transformed to $\sqrt{x} + 0.5$ before analysis. Species correspond to buckwheat (*Fagopyrum esculentum*) IPR-92 Altar, pigeon pea (*Cajanus cajan*) IAPAR 43, *Stylosanthes* 'Campo Grande', and *Crotalaria spectabilis*.

Shoot fresh weight was influenced by interaction effects in Trial 1 (Tab. 6), wherein lower means were observed in soybeans grown after chlorimuron-treated buckwheat and pigeon pea and fomesafen-treated buckwheat. In Trial 2, only the main effects of herbicide treatment were significant, with higher means in soybeans grown after untreated plants.

TABLE 6. Shoot fresh weight (g) of soybean plants grown in succession to crop coverages subjected to herbicide treatment after nematode inoculation.

Cover crop	Control	Bentazone	Chlorimuron	Fomesafen
	Trial 1			
Buckwheat	9.4 ^{aA}	7 ^{aA}	2.4 ^{bB}	2.3 ^{bB}
Pigeon pea	7.6 ^{aA}	5.3 ^{aA}	2.7 ^{bB}	5.8 ^{aA}
Stylosanthes	9.4 ^{aA}	5.8 ^{aB}	4.7 ^{aB}	6.8 ^{aB}
Crotalaria	10.1 ^{aA}	6.1 ^{aB}	3.7 ^{aB}	6.4 ^{aB}
CV (%)	21.09			
Mean	Trial 2			
	Control	Bentazone	Chlorimuron	Fomesafen
Mean	4.9 ^A	2.6 ^C	1.8 ^D	4.1 ^B
CV (%)	16.81			

For each treatment, means within columns followed by the same lowercase letter or means within rows followed by the same uppercase letter are not significantly different at $P < 0.05$ by the Scott–Knott test, CV – coefficient of variation. Original data were transformed to $\sqrt{x} + 0.5$ before analysis. Species correspond to buckwheat (*Fagopyrum esculentum*) IPR-92 Altar, pigeon pea (*Cajanus cajan*) IAPAR 43, *Stylosanthes* 'Campo Grande', and *Crotalaria spectabilis*.

Finally, for shoot dry weight, only the main effects of herbicide treatment were significant (Tab. 7). Shoot dry weight was higher in groups without herbicide application in both trials.

TABLE 7. Shoot dry weight (g) of soybean plants grown in succession to cover crops subjected to herbicide treatment after nematode inoculation.

Herbicide	Trial 1	Trial 2
Control	2.07 ^a	1.08 ^a
Bentazone	1.32 ^b	0.67 ^c
Chlorimuron	0.86 ^c	0.41 ^d
Fomesafen	1.25 ^b	0.81 ^b
CV (%)	21.29	14.50

Means within columns followed by the same letter are not significantly different at $P < 0.05$ by the Scott–Knott test, CV – coefficient of variation. Original data were transformed to $\sqrt{x} + 0.5$ before analysis. The control was distilled water.

Chlorimuron application reduced soybean plant height, shoot dry weight, and shoot fresh weight. Alencar *et al.* (2022), investigating the selectivity of different post-emergence herbicides on soybean, found that chlorimuron-ethyl at 17.5 g a.i. ha⁻¹ affected early plant development with a phytotoxicity of more than 30%. Alonso *et al.* (2013) reported that when combined glyphosate, chlorimuron-ethyl and fomesafen caused phytotoxicities of 65% and 40%. Chlorimuron is an inhibitor of acetolactate synthase with rapid absorption, high soil activity, and residual effects. Thus, it is suggested that the use of herbicides and their mixtures can affect phenological characteristics of soybean, such as plant height, pod number, and, consequently, yield (Oliveira Junior *et al.*, 2011; Alonso *et al.*, 2013).

Effect of herbicides on *Meloidogyne javanica* hatching

The nematicide abamectin was the most effective in reducing *M. javanica* hatching in both trials with hatching percentages of 0% and 3.15% (Tab. 8). Among the herbicides, bentazone did not affect *M. javanica* hatching in either trial, showing similar results to the control with distilled water. In Trial 1, fomesafen also had no effect on hatching compared with the control and did not differ from chlorimuron. In water, hatching reached 82.63% and 83.00% in Trials 1 and 2, respectively.

TABLE 8. *In vitro* hatching percentage of *Meloidogyne javanica* juveniles after 10 d of exposure of eggs to herbicide treatments.

Treatment	Trial 1	Trial 2
Control	82.63 a	83.00 a
Bentazone	66.55 a	73.38 a
Chlorimuron	24.57 b	52.32 b
Fomesafen	61.02 a	52.58 b
Abamectin	0.00 b	3.15 d
CV (%)	23.02	18.21

Means within columns followed by the same letter are not significantly different at $P < 0.05$ by the Scott–Knott test, CV – coefficient of variation. The control was distilled water.

Despite the direct effect of some herbicides on nematodes, it is possible that in agricultural lands their activity is related to the mode of absorption and translocation of the active ingredients in plants. Systemic herbicides are transported via the phloem and xylem (Oliveira Junior *et al.*, 2011), possibly reaching the feeding sites of sedentary endoparasites such as *Meloidogyne* in the plant roots. There are few data on the direct effects of herbicides on nematode hatching. Studies are limited to the soybean cyst nematode (*Heterodera glycines* Ichinohe) (Wong *et al.*, 1993; Levene *et al.*, 1998; Barbosa *et al.*, 2014).

There is little information on the interaction of cover crops and herbicides on the control of *M. javanica* in soybeans. Nevertheless, it is evident that there are specific relationships between these factors; and further research is needed to elucidate these topics, particularly under field conditions, including testing other active ingredients less harmful to the crop. Herbicides should not be used to control nematodes, but they can assist in the integrated management of pathogens by controlling host plants.

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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

CYF and CRDA designed the experiments, CYF carried out the field and laboratory experiments, AC contributed to the data analysis, CYF, AC, and CRDA wrote the article. All authors reviewed the final version of the manuscript.

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Characterization of *Trichoderma* species from agricultural soils of Paraguay

Caracterización de especies de *Trichoderma* de suelos agrícolas de Paraguay

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ABSTRACT

There is a growing interest in the development of sustainable alternatives to the use of chemical pesticides for pest management in agricultural systems. This research aimed to isolate and characterize native strains of *Trichoderma* spp. from different soils of Paraguay using morphological and molecular criteria. We processed plant and soil samples from eight commercial farms distributed in different departments of Paraguay and isolated 14 monospore isolates of *Trichoderma* spp., obtaining two isolates from the Department of Alto Paraná (FCQ36 and FCQ37), four isolates from Cordillera (FCQ42, FCQ43, FCQ44, and FCQ46), one isolate from Central (FCQ32), and seven isolates from Itapúa (FCQ13, FCQ16, FCQ18, FCQ19, FCQ21, FCQ23, and FCQ47). In addition, phylogenetic analyses using the ITS and *tefla* loci were carried out. A better resolution of the *tefla* gene than the ITS region was observed. Moreover, a third phylogenetic tree from the concatenated ITS and *tefla* sequences matrix was generated, obtaining the same topology with higher bootstrap support values. Through this approach, we reported for the first time the presence of *Trichoderma koningiopsis* (FCQ19, FCQ36, and FCQ37), *Trichoderma neokoningii* (FCQ13), and *Trichoderma asperellum* (FCQ42, FCQ43, FCQ44, and FCQ46), *Trichoderma brevicompactum* (FCQ18 and FCQ21), and *Trichoderma longibrachiatum* (FCQ 47) in Paraguay. The *Trichoderma* species identified in this study can be used to develop effective biocontrol products for agricultural and industrial purposes in Paraguay.

Key words: biological control, phylogenetics, fungi, taxonomy.

RESUMEN

Existe un creciente interés en el desarrollo de alternativas sostenibles al uso de plaguicidas químicos para el manejo de plagas en los sistemas agrícolas. El objetivo de este trabajo fue aislar y caracterizar por criterios morfológicos y moleculares cepas nativas de *Trichoderma* spp. de diferentes suelos de Paraguay. Se procesaron muestras de plantas y suelo de ocho fincas comerciales distribuidas en diferentes departamentos de Paraguay para el aislamiento de 14 aislados monospóricos de *Trichoderma* spp., obteniendo dos aislados del Departamento de Alto Paraná (FCQ36 y FCQ37), cuatro aislados de Cordillera (FCQ42, FCQ43, FCQ44 y FCQ46), un aislado de Central (FCQ32) y siete aislados de Itapúa (FCQ13, FCQ16, FCQ18, FCQ19, FCQ21, FCQ23 y FCQ47). Además, se realizaron análisis filogenéticos utilizando los loci ITS y *tefla*. Se observó una mejor resolución del gen *tefla* en comparación con la región ITS. Además, se generó un tercer árbol filogenético a partir de la matriz concatenada de ambas secuencias ITS y *tefla* obteniendo la misma topología con mayores valores de soporte bootstrap. A través de este enfoque, se reporta por primera vez la presencia de *Trichoderma koningiopsis* (FCQ19, FCQ36 y FCQ37), *Trichoderma neokoningii* (FCQ13) y *Trichoderma asperellum* (FCQ42, FCQ43, FCQ44 y FCQ46), *Trichoderma brevicompactum* (FCQ18 y FCQ21) y *Trichoderma longibrachiatum* (FCQ 47) en Paraguay. Las especies de *Trichoderma* identificadas en este trabajo pueden ser utilizadas en el desarrollo de productos de control biológico eficaces para fines agrícolas e industriales en Paraguay.

Palabras clave: control biológico, filogenética, hongos, taxonomía.

Introduction

The genus *Trichoderma* comprises more than 375 species of free-living fungi that are present mainly in the organic matter and as endophytes in plant roots colonizing their rhizosphere (Samuels, 2006; Pappu, 2018; Cai & Druzhinina, 2021). *Trichoderma* species are cosmopolitan and widely diverse in the tropics (Rivera-Méndez *et al.*, 2020).

Several studies have demonstrated that these fungi benefit plants due to their protection against plant pathogens and as plant growth promoters (Howell, 2003; López-Quintero *et al.*, 2013). Different species of *Trichoderma* produce different compounds that activate systemic plant defenses and regulate pathogen infections, significantly altering plant physiology (Li *et al.*, 2019; Patel *et al.*, 2019). Because of this, *Trichoderma* has been applied to crops as a plant growth

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promoter and inductor of resistance to abiotic stress and plant pathogens (Alkooranee *et al.*, 2019; Mayo-Prieto *et al.*, 2020; Moreno-Ruiz *et al.*, 2020).

Currently, commercial products of the fungus *Trichoderma* spp. are formulated for its application in agricultural systems (Fraceto *et al.*, 2018). These products do not always have the same efficiency because they depend on environmental conditions (Altintas & Bal, 2008; Nieto-Jacobo *et al.*, 2017; Di Lelio *et al.*, 2021). The biocontrol activities of *Trichoderma* species are significantly affected by the host plant and soil type as well as other microbial populations present in the same ecological niche (Lombardi *et al.*, 2018; Naseby *et al.*, 2000; Morán-Diez *et al.*, 2020). Therefore, *Trichoderma* species can produce highly specialized metabolites to interact with specific plant hosts and phytopathogens (Silva *et al.*, 2014; Contreras-Cornejo *et al.*, 2016; Kubicek *et al.*, 2019). Consequently, isolation and identification of effective native strains that are well adapted to a location's edaphoclimatic conditions in which they are to be deployed are necessary for effective biological control (Consolo *et al.*, 2012; Yendyo *et al.*, 2017; Ferreira *et al.*, 2020).

Numerous studies have been conducted in Latin America to identify *Trichoderma* isolates from different countries. These studies employ a polyphasic characterization approach that combines traditional phenotypic and physiological methods with modern molecular biology. Endophytic biological control agents such as *Trichoderma ovalisporum* have been isolated from the Peruvian Amazon's ecozones to manage cocoa diseases. These isolates are described morphologically, physiologically, and molecularly (Holmes *et al.*, 2004). Similarly, in Argentina research focused on studying the genetic diversity of *Trichoderma* species and their biocontrol mechanisms allow the registration of commercial products based on *Trichoderma*, which are used as biological control agents and plant growth promoters (Consolo *et al.*, 2012; Amerio *et al.*, 2020). Research on the use and application of *Trichoderma* in southern Brazil began in 1989, with the production and distribution of native strains performed by Empresa Brasileira de Pesquisa Agropecuária (Embrapa) (Bettiol & Morandi, 2009). *Trichoderma* species in Brazil, such as *Trichoderma harzianum*, *Trichoderma tomentosum*, *Trichoderma asperellum*, *Trichoderma ghanense*, *Trichoderma azevedoi*, *Trichoderma peberdyi*, *Trichoderma reesei*, and *Trichoderma atroviride*, are used for biocontrol of plant pathogens (Lopes *et al.*, 2012; Inglis *et al.*, 2020), enzyme production (Horta *et al.*, 2018), and secondary metabolite isolation (Brito *et al.*, 2014). In Paraguay, the isolation and

description of *Trichoderma* species has recently started compared to other countries in the region (Stauffer Bonzon, 1999; Ortellado Franco & Orrego Fuente, 2013; Sanabria Velázquez, 2020). Therefore, Paraguay remains an unexplored source of new *Trichoderma* species with potential biotechnological applications.

The first description of the Paraguayan species of *Trichoderma* at the molecular level was in 2017 when seven *Trichoderma* species from sesame soils were isolated from northeastern Paraguay. These *Trichoderma* spp. are characterized using only the Internal transcribed spacer (ITS) region as a DNA marker (Fernández Gamarra *et al.*, 2017). Despite this DNA marker being widely accepted as a tool for the preliminary taxonomic identification of fungi, it does not have enough resolution to differentiate between species of *Trichoderma* because of a high level of homoplasmy (Druzhinina *et al.*, 2005; Schoch *et al.*, 2012). Therefore, employing more genes with good resolution to describe these species is critical. A commonly used marker is the translation-elongation factor 1 α (*tef1 α*) since it is highly polymorphic and helps as an additional DNA marker for species delimitation of *Trichoderma* (Hermosa *et al.*, 2004; Chaverri *et al.*, 2015; Stielow *et al.*, 2015; Rivera-Méndez *et al.*, 2020).

The appropriate characterization of native species of *Trichoderma* is critical for developing commercial biocontrol products and to deploy these appropriately in the field (Chaverri *et al.*, 2015). Moreover, *Trichoderma* isolates can produce novel molecules of interest for biotechnological industries that require the correct identification and preservation of these selected isolates to register novel commercial products (Woo *et al.*, 2014; Błaszczyk *et al.*, 2016). Previous studies could not characterize *Trichoderma* fungi in Paraguay to the species level nor preserve them appropriately. Therefore, this research aimed to identify and describe the morphological, physiological, and molecular characteristics of the *Trichoderma* isolates. This information can be valuable for understanding the diversity and distribution of *Trichoderma* in the agricultural soils of Paraguay. To achieve this goal, we isolated native strains of *Trichoderma* spp. from different agricultural fields of Paraguay and characterized them morphologically using macro and microscopic morphometric observations and molecularly using ITS 1-4 and *tef1 α* gene regions. Since these isolates were obtained from commercial farms distributed in different parts of Paraguay, they have the potential to be used as biocontrol agents or for other biotechnological applications.

Materials and methods

Sampling and isolation

We collected soil, rhizosphere, and plant samples from different Paraguay crop production areas to isolate *Trichoderma* spp. Soil samples were collected using a soil sampler to a depth of approximately 20 cm. We labeled each sample and georeferenced them through a global positioning system (GPS) and transported them to the laboratory for analysis (Tab. 1). We prepared serial dilution of each sample following previously described methods (Samuels & Hebbbar, 2015). Two hundred μ l of soil suspension was poured on the surface of a PDA (Papa-Dextrose-Agar, Liofilchem®, Teramo, Italy) culture medium with oxytetracycline (TerramicinaLA®, Zoetis, AR).

In order to isolate potential endophytic *Trichoderma* spp. from the plants, we cut the tissues into pieces of approximately 0.5 cm, washed them with 70% ethanol for 30 s, then washed them with sodium hypochlorite 3% solution for another 30 s, and rinsed the material three times with sterile distilled water. We dried the disinfested samples and placed four pieces on the surface of the PDA medium with oxytetracycline. All plates were incubated at $28 \pm 2^\circ\text{C}$ for 5 d in complete darkness. Colonies with macroscopic characteristics of *Trichoderma* spp. were selected, and microscopic structures, conidiophores, and we analyzed conidiospores with appropriate identification keys (Barnett & Hunter, 1998). We transferred colonies identified as *Trichoderma* spp. to PDA medium to obtain

a pure culture. All plates were incubated at $28 \pm 2^\circ\text{C}$ for 5 d. Monosporic isolates of *Trichoderma* spp. were obtained to ensure genetic homogeneity.

We prepared serial dilutions of the suspension from the pure culture and placed 200 μ l on the surface PDA medium and incubated at $28 \pm 2^\circ\text{C}$ for 24 or 48 h. We transferred the first germinated spores to new PDA media. We identified cultures using sequential alphanumeric codes and stored them for posterior analyses in test tubes with PDA culture medium at 4°C and Eppendorf tubes with sterile glycerol at -20°C .

Morphology of the *Trichoderma* isolates

We grew fourteen Paraguayan monosporic isolates of *Trichoderma* spp. on PDA medium for 3 d at $28 \pm 2^\circ\text{C}$ and measured colony growth and color. To describe colony colors, we used the RGB color model available at ArtyClick Colors (ArtyClick Pty Ltd, Sydney, Australia, <https://colors.artyclick.com/color-name-finder/>). We measured the width and length of conidiophores, phialides, and conidia using a microscope with a digital camera (3MP, AMScope, USA), employing the software AMScope Version 4.7. We reported descriptive analyses of the measurements using Infostat (version 2017, Córdoba, AR), presenting means followed by standard deviation values. We used three biological replicates per isolate for macroscopic measurements, using 30 replicates per isolate for microscopic structural measurements.

TABLE 1. Description of the *Trichoderma* isolates obtained from different locations and crops from different production areas in Paraguay and their GenBank accession numbers.

Sample type	Crop	Isolate code	Specie	Genbank accession N°	
				ITS	<i>tef1α</i>
Soil	Tomato (<i>Solanum lycopersicum</i>)	FCQ13	<i>T. neokoningii</i>	MZ339274	MZ442668
Soil	Tomato (<i>S. lycopersicum</i>)	FCQ16	<i>T. harzianum</i>	MZ339256	MZ442661
Soil	Tomato (<i>S. lycopersicum</i>)	FCQ18	<i>T. brevicompactum</i>	MZ339234	MZ442659
Soil	Tomato (<i>S. lycopersicum</i>)	FCQ19	<i>T. koningiopsis</i>	MZ339263	MZ442663
Soil	Tomato (<i>S. lycopersicum</i>)	FCQ21	<i>T. brevicompactum</i>	MZ339235	MZ442660
Rhizosphere	Pepper (<i>Capsicum annuum</i>)	FCQ23	<i>T. harzianum</i>	MZ339258	MZ442662
Soil	Pepper (<i>C. annuum</i>)	FCQ32	<i>T. harzianum</i>	MZ339273	MZ442666
Soil	Corn (<i>Zea mays</i>)	FCQ36	<i>T. koningiopsis</i>	MZ339264	MZ442664
Soil	Soybean (<i>Glycine max</i>)	FCQ37	<i>T. koningiopsis</i>	MZ339265	MZ442665
Soil	Pepper (<i>C. annuum</i>)	FCQ42	<i>T. asperellum</i>	MZ339228	MZ442655
Soil	Pepper (<i>C. annuum</i>)	FCQ43	<i>T. asperellum</i>	MZ339229	MZ442656
Soil	Stevia (<i>Stevia rebaudiana</i>)	FCQ44	<i>T. asperellum</i>	MZ339230	MZ442657
Parasite of <i>Colletotrichum</i> spp.	Ornamentals (<i>Tagetes</i> spp.)	FCQ46	<i>T. asperellum</i>	MZ339231	MZ442658
Cortex	Lemon verbena (<i>Aloysia citrodora</i>)	FCQ47	<i>T. longibrachiatum</i>	MZ339223	MZ442667

DNA extraction and amplification

Fourteen monospore isolates of *Trichoderma* spp. were grown in PDB medium (Potato-Dextrose-Broth, Lio-filchem®, Teramo, IT) for 5 d at 25±2°C. We filtered the mycelium using a Büchner funnel, washed with sterile distilled water, and frozen until further processing. The modified hexadecyltrimethylammonium bromide (CTAB) method was used to extract the DNA (Murray & Thompson, 1980). Each frozen sample was briefly macerated with liquid nitrogen and incubated with 2×CTAB buffer at 65°C for 40 min. An equal volume of chloroform was added, and the phases were separated by centrifugation. The DNA in the supernatant was precipitated with isopropanol, washed with 70% ethanol, and resuspended in ultrapure water (Invitrogen, Carlsbad, CA). The ITS region was amplified by PCR using ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) and *tefla* locus using EF1-728F (CATCGAGAAGTTCGAGAAGG) and TEFIREV (GC-CATCCTTGGAGATACCAGC) primers (Hermosa *et al.*, 2004; Samuels & Hebbard, 2015). In both cases, 25 µl of mix containing 1 µl of a 1:10 dilution of the DNA sample, 2.5 µl of Buffer 10X TopTaq, 0.5 µl of 10 µM dNTPs, 1 µl of each primer 10 µM, and 0.25 µl of TopTaq polymerase (Qiagen, Mississauga, Ontario, Canada) were used. Amplifications were carried out in a thermal cycler (SimpliAmp™, ThermoFisher) with the following conditions: an initial denaturation cycle at 94°C for 5 min, followed by 35 cycles at 94°C for 1 min, 55°C for 1 min for ITS regions or 59°C during 1 min for the (*tefla*) gene, 72°C for 1 min with a

final extension of 72°C for 10 min. The PCR products were resolved through a 1% electrophoresis agarose gel and purified using PureLink™ Quick Gel Extraction Kit (Invitrogen, Carlsbad, USA). The PCR products were sequenced by MacroGen (Seoul, Korea).

Phylogenetic analysis

The sequencing files were first analyzed for quality and trimmed with BioEdit 7.0.5.3. We identified the sequences of the ITS and *tefla* regions with TrichoMARK2020 (<https://www.trichokey.com/index.php/trichomark>) that was formerly available on the homepage of the International Commission on *Trichoderma* (ICTT). We also performed a BLAST search to find related sequences in the NCBI database. We carried out sequence alignment using AliView 1.26 (Larsson 2014) with the MAFFT v. 7.450 binary (Katoh *et al.*, 2019), using local alignment parameters. The ICTT reference dataset ITS56 was first used to generate a local database (Cai & Druzhinina, 2021) (Tab. S1). After further primer removal, we analyzed the sequences to determine the best evolutionary model. We selected the K2+G model (10.1007/BF01731581) according to MEGA X Software 10.0.5 (Kumar *et al.*, 2018), and the Neighbor-Joining trees were assembled with 1000 bootstrap replications. We assembled the phylogenetic trees using previously reported *tefla* gene sequences (Tab. S1) to generate a local database. We removed the primer portions and aligned the sequences using the same parameters as previously described. We then joined together the processed ITS and *tefla* sequences for the concatenated tree.

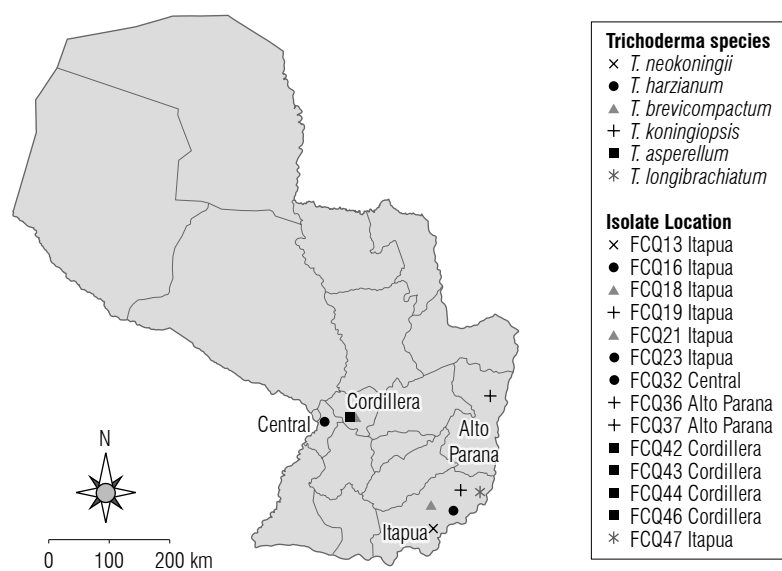


FIGURE 1. Map of Paraguay indicating sampled locations of *Trichoderma* spp. isolates obtained in 2016.

Results

Isolation and morphological characterization

We processed plant and soil samples from eight commercial farms distributed in different departments of Paraguay (Fig. 1) to isolate fourteen monosporic isolates of *Trichoderma* spp.

We obtained two isolates from departments of Alto Paraná (FCQ36 and FCQ37), four from Cordillera (FCQ42, FCQ43, FCQ44, and FCQ46), one isolate from Central (FCQ32), and seven isolates from Itapúa (FCQ13, FCQ16, FCQ18, FCQ19, FCQ21, FCQ23, and FCQ47). All isolates showed morphological characteristics of the genus *Trichoderma* with septate hyphae, branched conidiophores, and small conidia scattered or grouped (Fig. 2). Also, all isolates were able to cover completely the surface of the PDA medium on the 90 mm Petri plate after 3 d of incubation at $28 \pm 2^\circ\text{C}$, except for the isolate FCQ13 that presented the slowest growth with a rate of $13.60 \pm 1.10 \text{ mm d}^{-1}$ (Tab. 2).

The colonies of isolate FCQ13 were often white at first, forming greenish-grey conidiophores after one week of

incubation, with cylindrical phialides of $7.11 \pm 2.79 \times 2.59 \pm 0.37 \mu\text{m}$ of length and width. Conidia were ellipsoidal and measured $3.36 \pm 0.93 \times 2.47 \pm 0.51 \mu\text{m}$ on average (Tab. 2, Fig. 2A). Isolate FCQ16 produced light green and yellowish conidiophores that grew scattered throughout the plate with yellow pigment secreted into the agar (Fig. 2D). The 3-d-old monosporic cultures had ampulliform phialides of average length and width of $7.47 \pm 1.25 \times 3.46 \pm 0.29 \mu\text{m}$, and conidia were subglobose with measurements averaging $2.94 \pm 0.8 \times 2.64 \pm 0.45 \mu\text{m}$.

The isolate FCQ18 sporulated after 72 h, producing greenish-grey colonies with scant aerial mycelia, compact tufts, and without pigmentation on the reverse side of the plate. Microscopic examination of conidiophores revealed ampulliform to lageniform phialides of $6.67 \pm 1.61 \times 3.26 \pm 0.78 \mu\text{m}$ in length and width (Fig. 2C). The conidia were subglobose with a length and width of $2.69 \pm 0.47 \times 2.56 \pm 0.39 \mu\text{m}$. Although isolated from a similar soil and host (Tab. 1), the colonies of FCQ19 were slightly different from FCQ18, with a glade green color, and grew uniformly on PDA medium. Conidiophores of FCQ19 supported lageniform phialides of $8.22 \pm 1.9 \times 4.12 \pm 0.9 \mu\text{m}$ of length and

TABLE 2. Morphological characterization of 14 *Trichoderma* strains isolated from different locations and crops from different production areas in Paraguay.

Isolate	Colony			Conidia			Phialides		
	Growth ^a	Color	RGB color code	Length ^b	Width ^b	Characteristics	Length ^b	Width ^b	Characteristics
FCQ13	13.60 ± 1.10	Greenish Grey	#99AA99	3.36 ± 0.93	2.47 ± 0.51	Ellipsoidal	7.11 ± 2.79	2.59 ± 0.37	Cylindrical
FCQ16	28.47 ± 0.83	Greenish Yellow	#99A540	2.94 ± 0.8	2.64 ± 0.45	Subglobose	7.47 ± 1.25	3.46 ± 0.29	Ampulliform
FCQ18	27.88 ± 1.10	Greenish Grey	#99AA99	2.69 ± 0.47	2.56 ± 0.39	Subglobose	6.67 ± 1.61	3.26 ± 0.78	Ampulliform to lageniform
FCQ19	27.04 ± 1.91	Glade Green	#668066	3.86 ± 0.9	2.64 ± 0.35	Ellipsoidal	8.22 ± 1.9	4.12 ± 0.9	Lageniform
FCQ21	27.57 ± 0.91	Green Smoke	#99AA66	2.88 ± 0.71	2.73 ± 0.42	Subglobose to ovoidal	6.33 ± 2.44	3.71 ± 0.7	Ampulliform to lageniform
FCQ23	27.80 ± 1.29	Green Smoke	#99AA66	2.84 ± 0.67	2.78 ± 0.45	Subglobose	7.01 ± 1.56	3.77 ± 0.52	Ampulliform
FCQ32	28.26 ± 0.70	Glade Green	#668066	2.45 ± 0.44	2.4 ± 0.48	Subglobose	6.33 ± 1.34	3.5 ± 0.24	Ampulliform
FCQ36	28.36 ± 1.21	Greenish Grey	#99AA99	3.61 ± 0.75	2.56 ± 0.92	Ellipsoidal	8.55 ± 1.53	3.79 ± 1.01	Lageniform
FCQ37	28.07 ± 1.46	Glade Green	#698366	3.94 ± 0.98	2.96 ± 0.55	Ellipsoidal	7.9 ± 1.56	3.42 ± 0.9	Lageniform
FCQ44	28.15 ± 0.87	Glade Green	#698366	4.01 ± 0.75	3.66 ± 0.82	Subglobose to ovoidal	8.48 ± 1.73	3.65 ± 0.87	Lageniform
FCQ42	27.76 ± 1.37	Glade Green	#698366	3.62 ± 0.48	2.98 ± 0.46	Subglobose to ovoidal	8.46 ± 0.82	2.81 ± 0.72	Lageniform
FCQ43	28.35 ± 0.90	Glade Green	#698366	3.56 ± 0.55	2.88 ± 0.75	Subglobose to ovoidal	8.4 ± 1.58	3.55 ± 0.24	Lageniform
FCQ46	27.18 ± 1.36	Green Pine	#365536	3.79 ± 0.84	3.08 ± 1.18	Subglobose to ovoidal	9.82 ± 0.76	3.75 ± 0.97	Lageniform
FCQ47	28.54 ± 1.20	Glade Green	#668066	3.51 ± 0.6	3.03 ± 0.58	Ellipsoidal	9.83 ± 2.12	3.18 ± 0.84	Lageniform

^a Measured in mm d^{-1} . Values are means \pm standard deviation of three replicates per isolate.

^b Measured in μm . Values are means \pm standard deviation of 30 replicates.

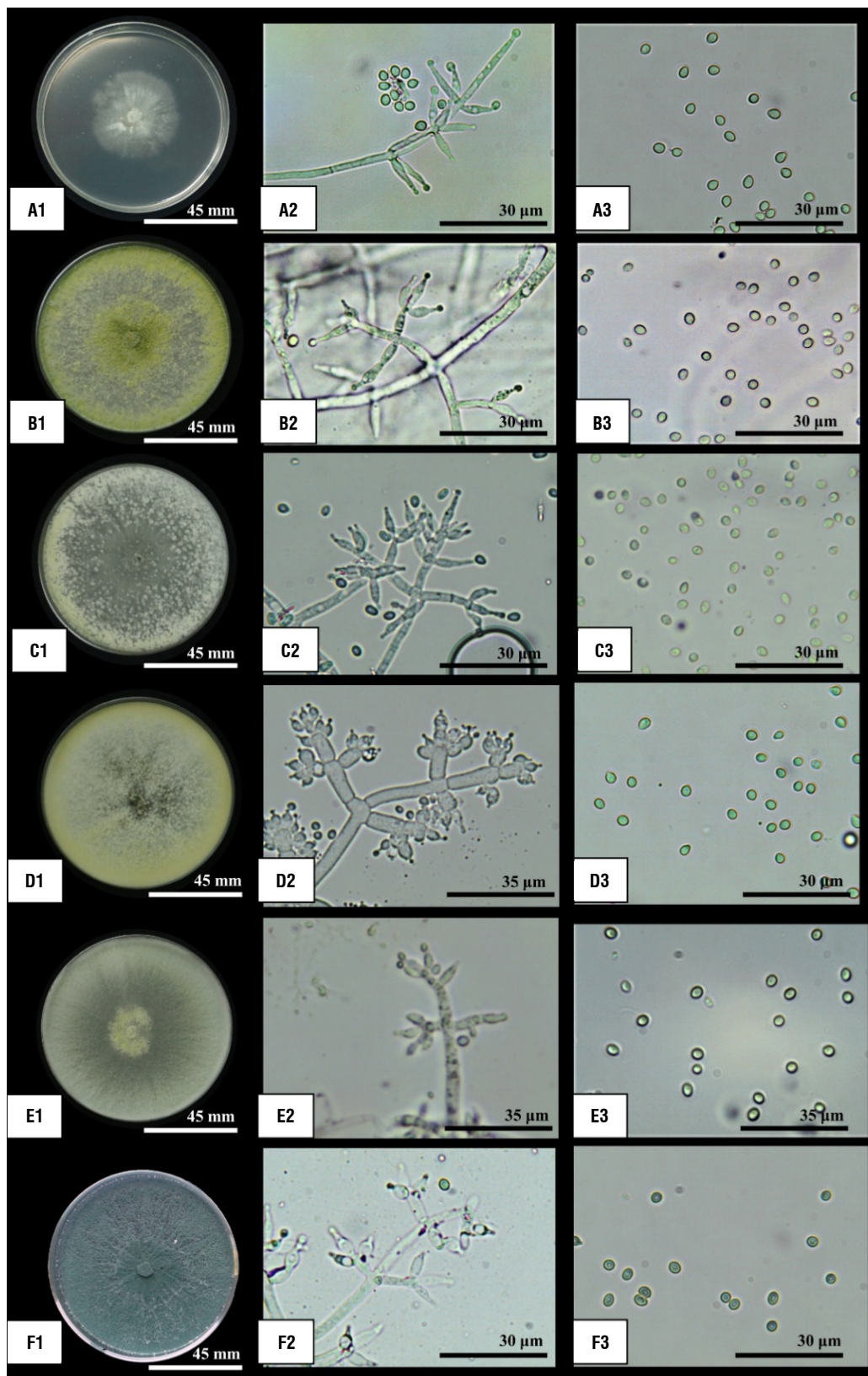


FIGURE 2. Representative cultures of each *Trichoderma* species: A) *T. neokoningii*, FCQ13; B) *T. asperellum*, FCQ42, FCQ43, FCQ44; FCQ46. C) *T. brevicompactum*, FCQ18, and FCQ21; D) *T. harzianum*, FCQ16, FCQ23, and FCQ32; E) *T. koningiopsis*, FCQ19, FCQ36, and FCQ37; and F) *T. longibrachiatum*, FCQ47. Each column represents the following: 1) potato-dextrose-agar (PDA) cultures after 3 d of incubation at 28°C; 2) Conidiophores; and 3) conidia of each *Trichoderma* species observed under a light microscope with 400 × magnification.

width with terminal ellipsoidal conidia measuring an average of $3.86 \pm 0.9 \times 2.64 \pm 0.35 \mu\text{m}$ (Tab. 2, Fig. 2E). The isolate FCQ21 produced green smoke colonies without pigmentation on the reverse side of the plate. All colonies sporulated on PDA with conidiophores that terminated in ampulliform to lageniform phialides of $6.33 \pm 2.44 \times 3.71 \pm 0.7 \mu\text{m}$ (Fig. 2C).

We obtained the isolates FCQ23 and FCQ32 from similar soil and host (Tab. 1) that yielded morphologically similar colonies. Monosporic colonies of FCQ23 had abundant mycelia, loose tufts, and filiform margins. The conidiophores had ampulliform phialides of $7.01 \pm 1.56 \times 3.77 \pm 0.52 \mu\text{m}$ in length and width. Conidia were subglobose and averaged a length and width of $2.84 \pm 0.67 \times 2.78 \pm 0.45 \mu\text{m}$ (Fig. 2D). Similarly, the isolate FCQ32 showed green colonies with loose tufts that were not uniformly distributed on the PDA medium. The conidiophores observed under the microscope presented opposite branches with shorter ampulliform phialides of $6.33 \pm 1.34 \times 3.5 \pm 0.24 \mu\text{m}$. The conidia were subglobose and yellowish-green in color with an average size of $2.45 \pm 0.44 \times 2.4 \pm 0.48 \mu\text{m}$ (Fig. 2D).

The isolates FCQ36 and FCQ37 were obtained from similar agricultural fields but different crops (Tab. 1). Still, the colonies of both isolates were morphologically similar and had greenish-grey color colonies that grew uniformly on PDA. For isolate FCQ36, conidiophores were long with lageniform phialides of $8.55 \pm 1.53 \times 3.79 \pm 1.01 \mu\text{m}$, while for isolate FCQ37 these were $7.9 \pm 1.56 \times 3.42 \pm 0.9 \mu\text{m}$. Likewise, the conidia of both isolates were ellipsoidal with FCQ36 measuring an average of $3.61 \pm 0.75 \times 2.56 \pm 0.92 \mu\text{m}$ and FCQ37 measuring $3.94 \pm 0.98 \times 2.96 \pm 0.55 \mu\text{m}$ (Fig. 2E).

The isolates FCQ42, FCQ43, and FCQ44 were obtained from different soils and fields (Tab. 1). Colonies were morphologically similar, with a glade green color, except for FCQ46 that had a darker green color (Tab. 2, Fig. 2B).

Closer examination revealed that conidiophores in these isolates were symmetrical, ending in three or more lageniform phialides and with subglobose or ovoid conidia (Fig. 2B). The isolate FCQ47 obtained from the plant tissue cortex (Tab. 1) was morphologically different from the previous isolates. Colonies were glade green with abundant mycelia, and loose tufts were uniformly distributed (Fig. 2F). Closer examination of microscopic structures confirmed septate hyphae and branched conidiophores with longer main branches and terminal lageniform ellipsoidal phialides $9.83 \pm 2.12 \times 3.18 \pm 0.84 \mu\text{m}$. Conidia were green

and ellipsoid with an average of $3.51 \pm 0.6 \times 3.03 \pm 0.58 \mu\text{m}$ of length and width (Tab. 2).

Phylogenetic analysis

Fourteen sequences of the ITS region of Paraguayan isolates of *Trichoderma* were obtained, with an amplification product size ranging from 540 to 599 base pairs. The pairwise similarity of the obtained sequences was compared phylogenetically with ITS reference sequences and assigned to the *Trichoderma* genus (Tab. S1). Paraguayan isolates clustered in different sections with three isolates grouped within section *Harzianum/Virens*: Isolates FCQ16, FCQ23, and FCQ32, grouping with AY605713 (*T. harzianum*) and NR144868 (*Trichoderma lentiforme*) (Fig. 3). Two isolates, FCQ18 and FCQ21, were grouped within the *Brevicompactum* clade alongside EU330941 (*T. brevicompactum*) with 100% bootstrap support. Moreover, eight isolates clustered within section *Trichoderma*: FCQ43, FCQ44, and FCQ46 grouped with MH021852 (*T. asperellum*) with 75% bootstrap support with FCQ42 clustering nearby this branch with 68% support. The isolate FCQ47 grouped within the unresolved section *Longibrachiatum*, close to *T. longibrachiatum*, *T. reesei*, and *Trichoderma parareesei*, and had 59% bootstrap support with NR120298 (*T. longibrachiatum*) (Fig. 3, Tab. S2).

Due to the difficulty of resolving the complex of *T. harzianum* and divide *Trichoderma* using only ITS region sequences, we obtained *tefla* sequences for each isolate by PCR, with amplicons ranging from 566 to 645 base pairs. Was observe longer distances for species grouping within the *Harzianum* clade, confirming a better resolution of the *tefla* gene than the ITS region (Fig. 4, Tab. S3).

Isolate FCQ32 grouped within the *Harzianum* clade, alongside FJ463310 (*T. lentiforme*), EU279992 (*T. harzianum*), and HM142375 (*T. amazonicum*) with 56% bootstrap support. Notably, despite FCQ16 and FCQ23 also clustering within the *Harzianum* clade with 83% bootstrap support, no *Trichoderma* reference sequence grouped near these two sequences (Fig. 4). As both isolates fell within the *T. harzianum* complex species, they either belong to *T. harzianum strictu sensu* or another species within this complex.

In the section *Trichoderma*, isolates FCQ43, FCQ44, and FCQ46 were grouped in the same clade as EU279961 (*T. asperellum*) with 55% bootstrap support and FCQ42 nearby this same clade with 99% support. In addition, isolates FCQ19, FCQ37, and FCQ36 grouped alongside EU279998 (*T. koningiopsis*) with 91% bootstrap support. The isolate FCQ13 clustered next to KJ665620.1 (*T. neokoningii*) with

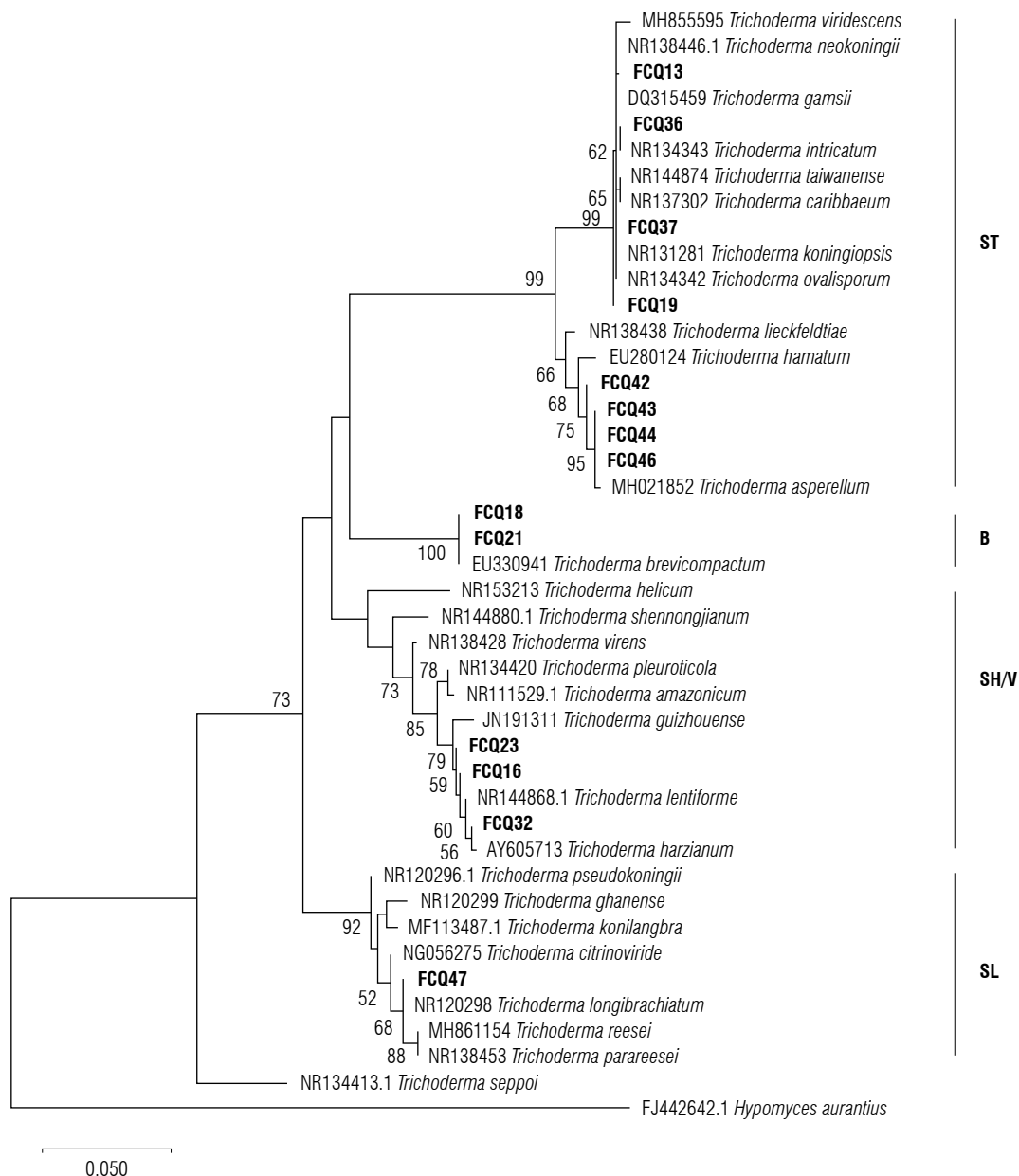


FIGURE 3. Phylogenetic tree of the Internal transcribed spacer (ITS) sequences from 14 *Trichoderma* isolates with *Hypomyces aurantius* as the out-group. The scale bar corresponds to the number of substitutions per site. ST - section *Trichoderma*, SH/V - section *Harzianum/Virens*, SL - section *Longibrachiatum*, and B - clade *Brevicompactum*. The phylogenetic tree was obtained using DNA distance-based and neighbor-joining analysis. Bootstrap percentages higher than 50% (1000 bootstraps) are indicated above the branches.

99% support (Fig. 4). Isolates FCQ18 and FCQ21 were grouped with EU280061 (*T. brevicompactum*) within the *Brevicompactum* clade with 99% bootstrap support. Finally, the isolate FCQ47 was grouped within section *Longibrachiatum*, alongside AY8656408 (*T. longibrachiatum*) with 99% bootstrap support (Fig. 4).

A third phylogenetic tree was generated from the concatenated matrix of both ITS and *tefla* sequences (Fig. 5).

Overall, we saw the same topology of the *tefla* tree with no changes in the clustering of Paraguayan *Trichoderma* isolates (Figs. 3-4). The differences between the concatenated ITS-*tefla* tree were mainly related to each node's branch length and bootstrap support values. For example, FCQ32 and *T. lentiforme* grouped with a bootstrap value of 76%, compared to 56% from the *tefla* gene alone (Fig. 4), while the value for FCQ16 and FCQ23 went from 83% support from the *tefla* tree (Fig. 4) to 95%. At the same

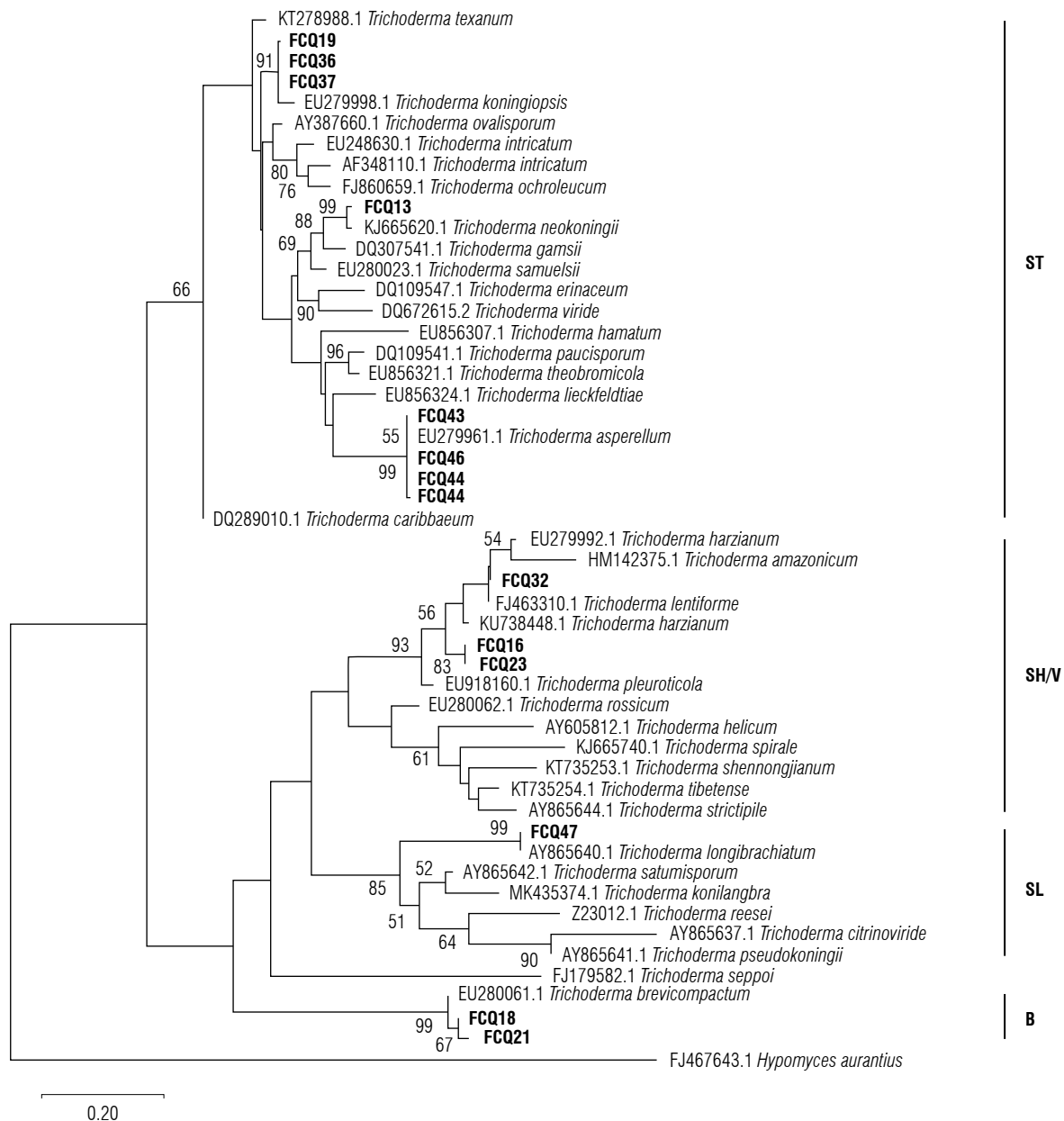


FIGURE 4. Phylogenetic tree of elongation factor gene 1 alpha (*tef1α*) sequences from 14 *Trichoderma* isolates with *Hypomyces aurantius* as the out-group. The scale bar corresponds to the number of substitutions per site. ST - section *Trichoderma*, SH/V - section *Harzianum/Virens*, SL - section *Longibrachiatum*, and B - clade *Brevicompactum*. The phylogenetic tree was obtained using DNA distance-based and neighbor-joining analysis. Bootstrap percentages higher than 50% (1000 bootstraps) are indicated above the branches.

time, the bootstrap value of the *T. harzianum* complex that includes the species cited so far, went from 93% (Fig. 4) to 98% (Fig. 5).

In section *Trichoderma*, the bootstrap support value of the clade with isolates FCQ19, FCQ36, FCQ37, and *T. koningiopsis* increased slightly to 98% (Fig. 5) from 91% in the *tef1α* tree (Fig. 4). Bootstrap support of the isolates FCQ43, FCQ44, FCQ46, and *T. asperellum* increased from

55% (Fig. 4) to 76% (Fig. 5). But, the bootstrap support for FCQ42 was slightly reduced from 99% in the previous *tef1α* tree to 95% bootstrap support in the concatenated phylogram (Fig. 5). Similarly, there was a slight reduction of support for FCQ13 and *T. neokoningii* from 99% support to 96% in the concatenated tree. The bootstrap values of the clades containing isolates FCQ18 and FCQ21 increased slightly from 99% support on the *tef1α* tree to 100% on the concatenated tree, similar to the clade

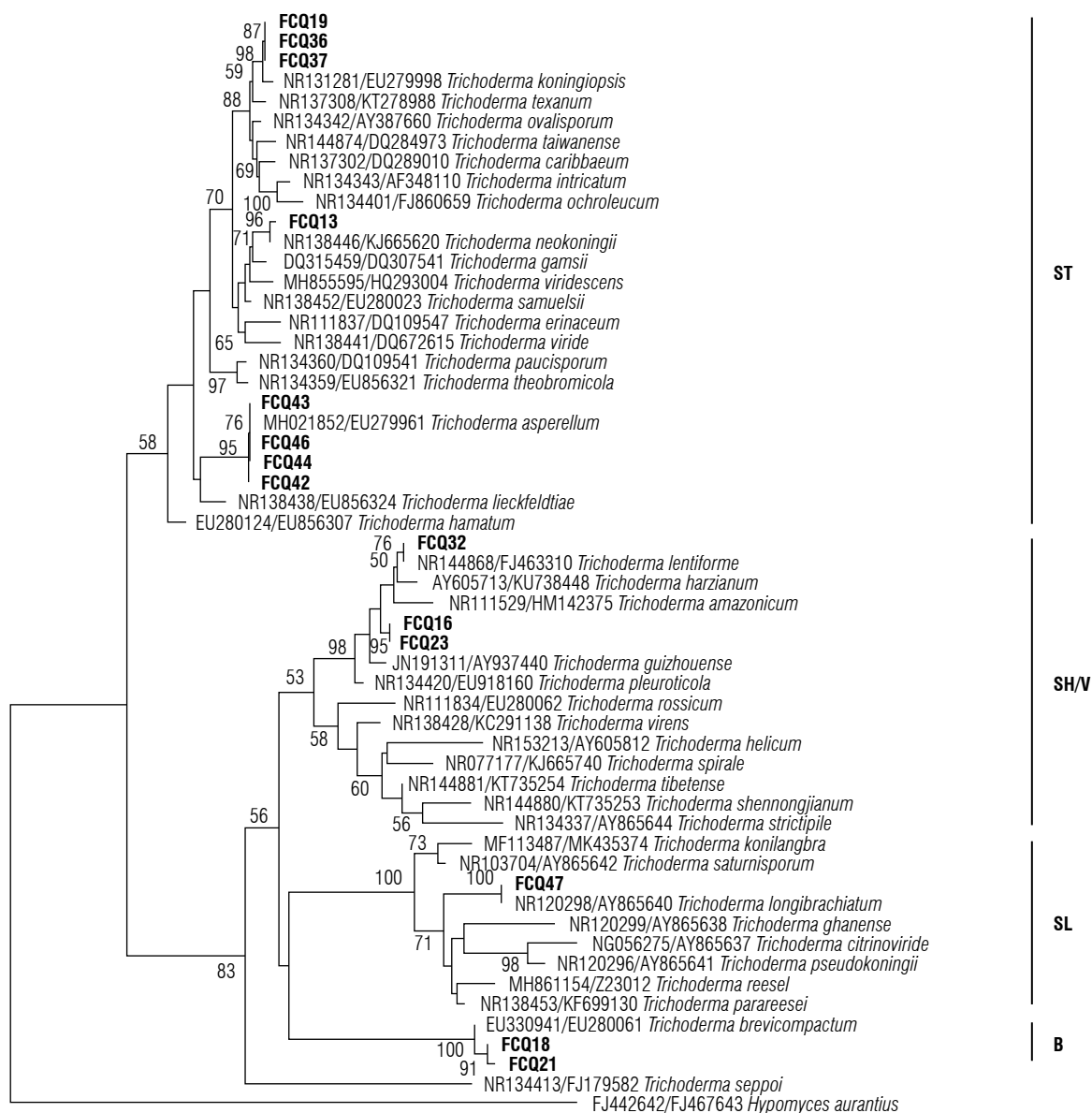


FIGURE 5. Phylogenetic tree of the concatenated ITS (left accession code) and *tef1α* (right accession code) sequences from 14 *Trichoderma* isolates with *Hypomyces aurantius* as the out-group. The scale bar corresponds to the number of substitutions per site. ST - section *Trichoderma*, SH/V - section *Harzianum/Virens*, SL - section *Longibrachiatum*, and B - clade *Brevicompectum*. The phylogenetic tree was obtained using DNA distance-based and neighbor-joining analysis. Bootstrap percentages higher than 50% (1000 bootstraps) are indicated above the branches.

containing isolate FCQ47 and the reference sequences for *T. longibrachiatum* (Fig. 5).

Discussion

Species of *Trichoderma* naturally inhabit agricultural soils, making them an interesting source of beneficial strains (Inglis *et al.*, 2020). In this research, a total of 14 isolates of *Trichoderma* were obtained from various agricultural production areas of Paraguay. In addition, *Trichoderma* isolates were characterized morphologically and molecularly using

ITS and *tef1α* markers, with species identified for the first time in Paraguay.

The morphology of colonies and conidiophores agreed with those commonly observed in *Trichoderma* spp. Morphological features between isolates were diverse, allowing their separation based on dissimilarities; however, these differences were not consistent given that, in some cases, isolates of the same species had different morphologies. For example, colonies FCQ18 and FCQ21 (*T. brevicompactum*) had slightly different morphologies; the conidia of the latter

tended to be ovoid, while the former were subglobose. In addition, it was difficult to differentiate between conidia of different species; this was the case for FCQ21 (*T. brevicompactum*) that was morphologically similar to FCQ23 (*T. harzianum*). Morphological characteristics can vary significantly depending on the incubation parameters, media, and even brands of reagents (Jaklitsch, 2011). Optimal incubation temperatures for *Trichoderma* range between 25-30°C in darkness with differences in growth rate between species not greater than 10 mm (Chaverri & Samuels, 2003; Samuels & Hebbar, 2015), which is supported by our results. The recommended media to conduct morphological characterization of *Trichoderma* species is PDA Difco (Becton, Dickinson and Company, Franklin Lakes, NJ, USA), as described in the reference work of Samuels and Hebbar (2015). Other commonly used media are Spezieller Nährstoffarmer agar (SNA), Cornmeal agar (CMA), and Malt Extract Agar (MEA) that allow better micromorphology characterization (Chaverri *et al.*, 2015; Samuels & Hebbar, 2015). However, in this work, we used only the PDA medium from Liofilchem®, which could have influenced the morphological characteristic of the isolates.

Initial characterization of *Trichoderma* isolates in Paraguay only used the morphological and antagonistic activity against plant pathogens as a method of characterization of *Trichoderma*. The first attempts to investigate the potential of native strains of *Trichoderma* as biological control agents were conducted by Stauffer (1999). Afterward, more studies focused on the isolation and description of native *Trichoderma* at the morphological level to develop alternative management products for sesame and soybean seed treatment against *Macrophomina phaseolina* (Garcete & Orrego, 2011; Ortellado Franco & Orrego Fuente, 2013). These studies encouraged the interest in the application of *Trichoderma* in other pathosystems aiming to protect other specialty crops such as macadamia (Sanabria Velázquez & Grabowski, 2016), stevia (Britos & Jongdae, 2016), and chia (Albrecht *et al.*, 2017). However, none of these previous studies employed molecular tools for species identification and were named only *Trichoderma* spp. Since phenotypic traits do not allow for species-level differentiation, they are complementary to the molecular characterization (Cai & Druzhinina, 2021).

The ITS region is considered the molecular barcode for fungal identification and is the most commonly used marker for diagnosing fungal species (Raja *et al.*, 2017). However, the ITS region does not have enough resolution to resolve the species complex of *T. harzianum* and divide groups of *Trichoderma*. This lack of resolution was confirmed by

multiple authors (Druzhinina & Kubicek, 2005; Samuels *et al.*, 2006; Chaverri *et al.*, 2015; Cai & Druzhinina, 2021). The ITS region proved useful for exploring the genetic diversity of *Trichoderma* in Brazil, resulting in more than 33% of the isolates being related to *T. harzianum* in the *Harzianum* complex. However, this region's analysis alone was insufficient to distinguish between species belonging to the same complex (Feitosa *et al.*, 2019).

The *tefla* sequences were analyzed to determine the species complex of *T. harzianum* and the section *Trichoderma*. This analysis showed better resolution of the *tefla* gene than the ITS gene. Two isolates, FCQ16 and FCQ23, clustered together and within the section *Harzianum/Virens*, yet these could not be properly identified as there was no close match for a reference sequence. Nonetheless, both isolates fell within the *T. harzianum* complex of species. *Trichoderma harzianum* is considered a complex of polyphyletic species that are morphologically indistinguishable. Similarly reported in previous works (Chaverri *et al.*, 2015), the analysis of *tefla* sequences provided a better-defined section *Trichoderma*. The intricate nature of the *T. harzianum* species complex has been previously reported by Druzhinina *et al.* (2010) and Kubicek *et al.* (2019).

The concatenation of ITS and *tefla* sequences improved statistical support for species identification. Similar to our results, previous recent reports using the combination of ITS and *tefla* have successfully characterized *Trichoderma* species (Lisboa *et al.*, 2017; Haouhach *et al.*, 2020). Other genes used for this purpose were *rpb2*, *ech42*, *cal1*, *act*, *acl1*, 18S rRNA, and 28S rRNA. However, as not all *Trichoderma* species were sequenced using these genes, for example old sequences from public databases, they have reduced utility for molecular identification at the species level. Cai and Druzhinina (2021) argue that using *tefla* and *rpb2* regions allows the correct identification of *Trichoderma* species based on the sequence similarities between the query strain and the reference strains. More complete evidence of different species within the complex could be achieved by considering sequence similarities and phylogenetic concordance.

We found the species *T. brevicompactum* (FCQ18 and FCQ21), which have been reported to produce high concentrations of trichodermin, an antifungal metabolite that inhibits protein synthesis and is deadly to living cells (Degenkolb *et al.*, 2008; Malmierca *et al.*, 2012; Barúa *et al.*, 2019). This compound has been reported to inhibit seed germination and reduce plant growth (Tijerino *et al.*, 2011). Also, when tomato seedlings were treated with *T.*

brevicompactum, the root length and plant size were significantly reduced. In addition, there were more necrotic lesions when treated with *T. brevicompactum* and inoculated with the plant pathogen *Botrytis cinerea*. For these reasons, the isolates FCQ18 and FCQ21 may potentially produce trichodermin in high concentrations, thus limiting their use as biological control agents. However, this capacity is an attribute of strains that cannot be attributed to the production of a specific metabolite or their endophytic capacity.

T. asperellum was frequently found in this study (FCQ42, FCQ43, FCQ44, and FCQ46). Similarly, isolates of *T. asperellum* were reported from agricultural soils of northern Paraguay but based only on morphological and ITS region analysis (Fernández Gamarra *et al.*, 2017). The species *T. asperellum* has been studied extensively because of its potential as a biological control agent for plant pathogens (Wu *et al.*, 2017). In the neighboring country, Brazil, *T. asperellum* application in soybean seeds significantly increased the yield (Chagas *et al.*, 2017) and suppressed the growth of the pathogen *Sclerotinia sclerotiorum* isolate to varying degrees (Macena *et al.*, 2020).

We reported one isolate of *T. longibrachiatum*, FCQ47, obtained from the cortex of lemon verbena (*Aloysia citrodora*) with the potential to act as an endophytic strain, though this was not confirmed in this study. Previous research reported the potential of endophytic strains of *T. longibrachiatum* isolated from *Dendrobium nobile* stem segments to increase plant metabolites and inhibit bacterial pathogens (Sarsaiya *et al.*, 2020), suggesting that this species has the potential to be employed as a biocontrol agent. This hypothesis was confirmed by Zhang *et al.* (2018), who reported that the strain of *T. longibrachiatum* T6 was effective against *Valsa mali*, the causal agent of apple tree valsa canker disease, and the primary mechanism of control of the pathogen, the production of secondary metabolites. However, some characteristics might limit the potential of *T. longibrachiatum* employed as a commercial biocontrol agent. This fungus produced trichokonin VI (TK VI), a peptaibol compound that significantly reduced the root growth of *Arabidopsis* (Shi *et al.*, 2016). Moreover, *T. longibrachiatum* was described as an opportunistic pathogen of immunocompromised humans in several publications (Kuhls *et al.*, 1999; Richter *et al.*, 1999; Akagi *et al.*, 2017).

The results of various studies show that the species composition of *Trichoderma* in agricultural soils in Latin America is diverse (Holmes *et al.*, 2004; Consolo *et al.*, 2012; Amerio *et al.*, 2020; Inglis *et al.*, 2020). This can be contrasted with the findings in Paraguay, considering that the sample of

isolates is relatively small; and, thus, the results should be interpreted with caution.

A large number of fungal species can act as biological control agents of agricultural pests (McSpadden Gardener & Fravel, 2002), whose antagonistic properties are based on the activation of multiple mechanisms such as competition for space and nutrients (Sempere Ferre & Santamarina, 2010), mycoparasitism (Atanasova *et al.*, 2013), and antibiosis with the production of enzymes (Wu *et al.*, 2017) and secondary metabolites such as antibiotics, mycotoxins, and phytotoxins that participate in the control of the pathogen (Tijerino *et al.*, 2011; Barúa *et al.*, 2019; Cubilla-Ríos *et al.*, 2019). Although many researchers focused their attention on the potential that these metabolites have for the biological control of phytopathogens, there is a diverse group of secondary metabolites that differ in chemical structure, and that exhibit multiple biological functions for applications in agriculture (McSpadden Gardener & Fravel, 2002), pharmacy (Daniel & Rodrigues Filho, 2007), and food processing (Galante *et al.*, 1998) that cannot be ignored. Therefore, this work of characterization of native *Trichoderma* isolates can help to raise interest in producing Paraguayan biotechnological products based on *Trichoderma* species as well as providing documentation of the biogeographic distribution of this economically significant group of microscopic fungi in this region of the world. In summary, we report *T. asperellum*, *T. brevicompactum*, *T. longibrachiatum*, *T. koningiopsis*, and *T. neokoningii* for the first time in Paraguay, based on morphological and molecular data from the ITS and *tefla* loci.

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Conflict of interest statement

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

Author's contributions

ADSV curated the data, conducted formal analysis, and wrote the original draft. MMFP conducted investigations, performed formal analysis, and contributed to the original

draft writing. LIA also conducted the experiments. MEFG conceptualized the project, wrote and edited reviews, and contributed to the writing. MCRR provided supervision, writing, and visualization. PHS developed the methodology, validated the findings, and reviewed and edited the project. JEBC conceptualized the project, provided resources, and edited the work. All authors reviewed the final version of the manuscript.

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SUPPLEMENTARY TABLE 1. *Trichoderma* spp. sequences used for the phylogenetic analyses.

Species	Genbank accession N°	
	ITS	<i>tef1α</i>
<i>Hypomyces aurantius</i>	FJ442642	FJ467643
<i>Trichoderma amazonicum</i>	NR111529	HM142375
<i>Trichoderma asperellum</i>	MH021852	EU279961
<i>Trichoderma brevicompactum</i>	EU330941	EU280061
<i>Trichoderma caribbaeum</i>	NR137302	DQ289010
<i>Trichoderma citrinoviride</i>	NG056275	AY865637
<i>Trichoderma erinaceum</i>	NR111837	DQ109547
<i>Trichoderma gamsii</i>	DQ315459	DQ307541
<i>Trichoderma ghanense</i>	NR120299	AY865638
<i>Trichoderma guizhouense</i>	JN191311	AY937440
<i>Trichoderma hamatum</i>	EU280124	EU856307
<i>Trichoderma harzianum</i>	AY605713	EU279992, KU738448
<i>Trichoderma helicum</i>	NR153213	AY605812
<i>Trichoderma intricatum</i>	NR134343	AF348110, EU248630
<i>Trichoderma konilangbra</i>	MF113487	MK435374
<i>Trichoderma koningiopsis</i>	NR131281	EU279998
<i>Trichoderma lentiforme</i>	NR144868	FJ463310
<i>Trichoderma lieckfeldtia</i>	NR138438	EU856324
<i>Trichoderma longibrachiatum</i>	NR120298	AY865640
<i>Trichoderma neokoningii</i>	NR138446	KJ665620
<i>Trichoderma ochroleucum</i>	NR134401	FJ860659
<i>Trichoderma ovalisporum</i>	NR134342	AY387660
<i>Trichoderma parareesei</i>	NR138453	KF699130
<i>Trichoderma paucisporum</i>	NR134360	DQ109541
<i>Trichoderma pleuroticola</i>	NR134420	EU918160
<i>Trichoderma pseudokoningii</i>	NR120296	AY865641
<i>Trichoderma reesei</i>	MH861154	Z23012
<i>Trichoderma rossicum</i>	NR111834	EU280062
<i>Trichoderma samuelsii</i>	NR138452	EU280023
<i>Trichoderma saturnisporum</i>	NR103704	AY865642
<i>Trichoderma seppoi</i>	NR134413	FJ179582
<i>Trichoderma shennongjianum</i>	NR144880	KT735253
<i>Trichoderma spirale</i>	NR077177	KJ665740
<i>Trichoderma strictipile</i>	NR134337	AY865644
<i>Trichoderma taiwanense</i>	NR144874	DQ284973
<i>Trichoderma texanum</i>	NR137308	KT278988
<i>Trichoderma theobromicola</i>	NR134359	EU856321
<i>Trichoderma tibetense</i>	NR144881	KT735254
<i>Trichoderma virens</i>	NR138428	KC291138
<i>Trichoderma viride</i>	NR138441	DQ672615
<i>Trichoderma viridescens</i>	MH855595	HQ293004

SUPPLEMENTARY TABLE 2. BLAST results for the ITS sequences of each *Trichoderma* species isolated in the study.

Isolate	Species	Identity (%)	Accession length	Matched accession
FCQ13	<i>T. koningiopsis</i>	100.00	613	MK791649.1
	<i>T. viride</i>	100.00	576	KC576682.1
	<i>T. sp.</i>	99.81	578	MF136557.1
FCQ16	<i>T. harzianum</i>	100.00	604	MN262484.1
	<i>T. rifaii</i>	100.00	585	OL757372.1
	<i>T. afarasin</i>	100.00	604	FJ442665.1
	<i>T. inhamatum</i>	99.81	545	MH861135.1
FCQ18	<i>T. turrialbense</i>	100.00	627	MT530012.1
	<i>T. sp.</i>	100.00	585	MN602854.1
	<i>T. brevicompactum</i>	100.00	579	MK253291.1
FCQ19	<i>T. sp.</i>	100.00	580	MW765133.1
	<i>T. sulphureum</i>	99.81	624	MT530250.1
	<i>T. koningiopsis</i>	99.81	565	MT520626.1
	<i>T. afroharzianum</i>	99.81	1131	MN644679.1
FCQ21	<i>T. turrialbense</i>	100.00	627	MT530012.1
	<i>T. sp.</i>	100.00	585	MN602854.1
	<i>T. brevicompactum</i>	100.00	579	MK253291.1
FCQ23	<i>T. harzianum</i>	100.00	619	MK738150.1
	<i>T. azevedoi</i>	100.00	603	MK714903.1
	<i>T. sp.</i>	100.00	582	MK808887.1
	<i>T. simmonsii</i>	100.00	560	MF078647.1
	<i>T. lixii</i>	100.00	592	OL741785.1
	<i>T. lentiforme</i>	99.81	603	FJ442251.1
FCQ32	<i>T. lentiforme</i>	100.00	600	MN262489.3
	<i>T. harzianum</i>	100.00	570	MK751758.1
	<i>T. breve</i>	100.00	640	MN400089.1
	<i>T. lixii</i>	100.00	653	KY315574.1
	<i>T. simmonsii</i>	100.00	598	MZ835628.1
	<i>Sordariomycetes sp.</i>	100.00	1106	MW529552.1
	<i>T. harzianum</i>	99.81	599	KX379172.1
FCQ36	<i>T. sulphureum</i>	99.81	624	MT530250.1
	<i>T. sp.</i>	99.81	565	MT520637.1
	<i>T. koningiopsis</i>	99.81	565	MT520626.1
	<i>T. afroharzianum</i>	99.81	1131	MN644679.1
FCQ37	<i>T. sulphureum</i>	100.00	624	MT530250.1
	<i>T. koningiopsis</i>	100.00	565	MT520626.1
	<i>T. afroharzianum</i>	100.00	1131	MN644679.1
	<i>T. atroviride</i>	100.00	602	MN341303.1
	<i>T. caribbaeum</i> var. <i>caribbaeum</i>	100.00	658	NR_166015.1
	<i>T. neokoningii</i>	100.00	581	MW269083.1
	<i>T. ovalisporum</i>	100.00	588	MW268857.1
	<i>T. ghanense</i>	100.00	862	MT892811.1

to be continued

Isolate	Species	Identity (%)	Accession length	Matched accession
FCQ42	<i>T. asperellum</i>	100.00	588	MT367901.1
	<i>T. sp.</i>	100.00	599	MT150599.1
	<i>T. hamatum</i>	100.00	898	MT111894.1
	<i>T. yunnanense</i>	100.00	597	MT102857.1
	<i>T. viride</i>	100.00	577	MT007532.1
	<i>Phytophthora cinnamomi</i>	100.00	603	MZ771300.1
	<i>T. pubescens</i>	100.00	548	MW280120.1
	<i>T. koningii</i>	100.00	548	MW265008.1
	<i>Fusarium oxysporum</i> f. sp. <i>Ricini</i>	100.00	609	MW074252.1
FCQ43	<i>T. harzianum</i>	100.00	583	MT995126.1
	<i>T. hamatum</i>	100.00	634	MT355443.1
	<i>T. asperellum</i>	100.00	595	MT341772.1
	<i>T. sp.</i>	100.00	557	MT133836.1
FCQ44	<i>T. harzianum</i>	100.00	543	MW965792.1
	<i>T. hamatum</i>	100.00	634	MT355443.1
	<i>T. asperellum</i>	100.00	595	MT341772.1
FCQ46	<i>T. sp.</i>	100.00	557	MT133836.1
	<i>T. hamatum</i>	100.00	634	MT355443.1
	<i>T. asperellum</i>	100.00	595	MT341772.1
	<i>T. sp.</i>	100.00	557	MT133836.1
FCQ47	<i>T. harzianum</i>	100.00	543	MW965792.1
	<i>T. longibrachiatum</i>	100.00	599	MT520646.1
	<i>T. sp.</i>	100.00	598	MT520642.1
	<i>Fusarium oxysporum</i>	100.00	604	MW775868.1

SUPPLEMENTARY TABLE 3. BLAST results for the *tef1* α sequences of each *Trichoderma* species isolated in the study.

Isolate	Species	Identity (%)	Accession length	Matched accession
FCQ13	<i>T. neokoningii</i>	98.43	818	KJ871265.1
	<i>T. neokoningii</i>	97.50	601	DQ841718.1
	<i>T. sp. vd1</i>	91.68	611	DQ841711.1
	<i>T. gamsii</i>	91.13	644	KR051477.1
	<i>T. paraviridescens</i>	91.12	629	MW791206.1
FCQ16	<i>T. harzianum</i>	100.00	516	KP890327.1
	<i>T. sp. VB-2019a</i>	100.00	531	MH352423.1
	<i>T. camerunense</i>	100.00	524	MG822709.1
	<i>T. azevedoi</i>	100.00	485	MN585285.1
	<i>T. rifaii</i>	98.00	555	MK644113.1
FCQ18	<i>T. brevicompactum</i>	98.72	631	MT058876.1
	<i>T. sp. VNB-2019b</i>	97.87	420	MT300493.1
	<i>T. turrialbense</i>	93.49	540	EU338282.1
	<i>T. arundinaceum</i>	89.63	623	MT058877.1
FCQ19	<i>T. koningiopsis</i>	99.22	959	EU279995.2
	<i>T. arenarium</i>	97.67	520	MT242306.3
FCQ21	<i>T. brevicompactum</i>	99.63	877	AB558910.1
	<i>T. arundinaceum</i>	90.00	623	MT058877.1
	<i>T. turrialbense</i>	93.70	540	EU338282.1
	<i>T. sp. VNB-2019b</i>	96.68	420	MT300493.1
FCQ23	<i>T. harzianum</i>	100.00	516	KP890327.1
	<i>T. sp. VB-2019a</i>	100.00	531	MH352423.1
	<i>T. camerunense</i>	100.00	524	MG822709.1
	<i>T. azevedoi</i>	100.00	485	MN585285.1
	<i>T. rifaii</i>	97.95	555	MK644113.1
	<i>T. pollinicola</i>	97.95	973	MF939619.1
	<i>T. lixii</i>	100.00	610	KJ855089.1
FCQ32	<i>T. lentiforme</i>	96.72	546	MK644114.1
	<i>T. harzianum</i>	96.32	888	AY605768.1
	<i>T. lixii</i>	95.88	601	JQ040449.1
	<i>T. guizhouense</i>	96.19	522	FJ463289.1
FCQ36	<i>T. koningiopsis</i>	100.00	959	EU279995.2
	<i>T. arenarium</i>	97.67	520	MT242306.3
FCQ37	<i>T. koningiopsis</i>	100.00	959	EU279995.2
	<i>T. arenarium</i>	97.67	520	MT242306.3
FCQ42	<i>T. asperellum</i>	99.62	535	GU198232.1
FCQ43	<i>T. asperellum</i>	99.81	892	MN307415.1
FCQ44	<i>T. asperellum</i>	100.00	892	MN307415.1
FCQ46	<i>T. asperellum</i>	100.00	892	MN307415.1
FCQ47	<i>T. longibrachiatum</i>	100.00	871	MH208265.1
	<i>T. bissetii</i>	100.00	817	HG931271.1

Physiology and biochemistry of naranjilla (*Solanum quitoense* Lam) fruit during postharvest and the main conservation strategies: A review

Fisiología y bioquímica del fruto de naranjilla (*Solanum quitoense* Lam) durante la poscosecha y principales estrategias de conservación: una revisión

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ABSTRACT

Naranjilla (lulo) is an Andean fruit that is very attractive for consumption due to its acid flavor and aroma, as well as its antioxidant, mineral, carbohydrate, and protein content. However, several aspects of the fruit's pre-harvest and ripening postharvest are unknown, which results in deficiencies during the postharvest handling and conservation. The aim of this review was to present and describe the naranjilla fruit's main physiological changes, such as respiration pattern, ethylene production, firmness reduction, and pigment variation, as well as the main preservation technologies implemented during the postharvest period. Naranjilla fruit has been cataloged as a climacteric fruit but its respiratory rate is lower than other fruits in this group. During ripening, there are changes such as a color evolution from green to yellow hue for the pulp and peel, an increase in the concentration of soluble sugars, ascorbic acid, and ethylene production, as well as a reduction in firmness and acidity. Given the nature of its ripening, naranjilla is considered a perishable fruit. To preserve the fruits throughout the postharvest period, different technologies involving cooling, packaging in modified atmospheres (MAP), use of 1-methylcyclopropene (1-MCP), UV-C and gamma radiation, and ozone application have been evaluated. From these technologies, refrigeration and MAP are the most often used commercially; they are efficient and relatively economical. More research is required to optimize the use of these technologies for naranjilla preservation.

Key words: respiration pattern, ethylene, antioxidant activity, senescence, lulo.

RESUMEN

La naranjilla (lulo) es una fruta andina muy atractiva para el consumo por su sabor y aroma ácido, así como por su contenido en antioxidantes, minerales, carbohidratos y proteínas. Sin embargo, se desconocen varios aspectos de la maduración del fruto precosecha y maduración poscosecha, lo que resulta en deficiencias durante el manejo y conservación poscosecha. El objetivo de esta revisión fue presentar y describir los principales cambios fisiológicos del fruto de naranjilla, como el patrón de la respiración, la producción de etileno, la reducción de la firmeza y la variación de pigmentos, así como las principales tecnologías de conservación implementadas durante el período de poscosecha. El fruto de la naranjilla ha sido catalogado como fruto climatérico pero su tasa respiratoria es menor que la de otras frutas de este grupo. Durante la maduración se presentan cambios como una evolución de la tonalidad del color, de verde a amarillo para la pulpa y la epidermis, un aumento en la concentración de azúcares solubles, ácido ascórbico y producción de etileno, así como una reducción de la firmeza y acidez. Dada la naturaleza de su maduración, la naranjilla se considera una fruta perecedera. Para conservar la fruta durante todo el periodo de poscosecha se han evaluado diferentes tecnologías que involucran enfriamiento, envasado en atmósferas modificadas (EAM), uso de 1-metilciclopropeno (1-MCP), radiación UV-C y gamma, y aplicación de ozono. De estas tecnologías, la refrigeración y el EAM son las más utilizadas comercialmente; son eficientes y relativamente económicas. Se requiere más investigación para optimizar el uso de estas tecnologías para la conservación de la naranjilla.

Palabras clave: patrón respiratorio, etileno, actividad antioxidante, senescencia, lulo.

Introduction

Naranjilla or lulo (*Solanum quitoense* Lam.) is a tropical fruit belonging to the Solanaceae family (González-Bonilla & Marín-Arroyo, 2022). Naranjilla is produced commercially in Colombia, Panama, Ecuador and Costa Rica (Ramírez *et al.*, 2021) at altitudes varying between 1,600

and 2,400 m a.s.l. (Fischer *et al.*, 2022). This fruit has strong acceptance in international markets such as the USA and Europe (Criollo-Escobar *et al.*, 2020; Jaime Guerrero *et al.*, 2022). The naranjilla has an oval or round shape, depending on the variety. The diameter of the fruit can vary from 4 to 9 cm (Gargiullo *et al.*, 2008). The surface of the fruit is covered with brown or black trichomes and the color of the

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epidermis changes from dark green to bright orange when it reaches physiological maturity. The pulp is translucent greenish yellow, the seeds are round and flat with a diameter of 2 mm to 3 mm, and each fruit can have between 800 to 1,200 seeds (Gargiullo *et al.*, 2008; Ramírez *et al.*, 2018).

As part of the group of tropical fruits considered exotic, the naranjilla is an attractive food due to its delicious acid flavor and aroma, whereas the peel has an attractive color due to the synthesis of carotenoids. This fruit has an important content of antioxidants that can improve health and it is a source of vitamins E, C, B1, B2, B3, B6, and provitamin A, minerals such as iron, calcium, phosphorus, potassium, and nitrogen, carbohydrates, and proteins (Gancel *et al.*, 2008; Pratt *et al.*, 2008; Ramírez *et al.*, 2018). During ripening, naranjilla fruits change color from green to yellow because of chlorophyll degradation (Forero *et al.*, 2014). In parallel to this process, a decrease in the pH and acidity of the fruits is generated due to the accumulation of organic acids and their subsequent degradation during respiration, while the concentration of total soluble solids and vitamin C increases until reaching maximum values during ripening (González Loaiza *et al.*, 2014; Andrade-Cuvi *et al.*, 2016). In ripening, naranjilla's firmness is reduced; this may be the result of starch hydrolysis and enzymatic degradation of pectins and hemicelluloses (Ochoa-Vargas *et al.*, 2016).

During postharvest, naranjilla fruit losses can be up to 50% in the stages of handling, transport, and storage (Forero *et al.*, 2014). For this reason, the development and application technologies for maintaining the product's quality and to reduce postharvest losses have become one of the most important aspects of research at the agricultural level (Escobar Hernández *et al.*, 2014). Several physical treatments can be applied so that the product delays its senescence and maintains the desired quality. Among these technologies are cold storage, modified atmosphere packaging (Fallik & Ilic, 2018), UV-C radiation (Zhang & Jiang, 2019), 1-methylcyclopropene (Balaguera-López, Salamanca-Gutiérrez *et al.*, 2014), UV-C and gamma radiation, and ozone application. The effectiveness of each technique will depend on the standards and conditions of their use and management to achieve the desired objective of increasing the postharvest life and reducing the losses of the naranjilla fruits.

This review is aimed to present the main physiological changes, such as respiration pattern, ethylene production, and biochemical processes related to the postharvest of naranjilla fruits, as well as the main technologies implemented to extend the fruit's shelf life and maintain its quality.

The pattern of respiration and ethylene production

The naranjilla is considered a climacteric fruit whose respiration rate is lower than that of other climacteric fruits, even when the typical climacteric peak is observed for the respiration rate vs. ripening stage curve (Acosta *et al.*, 2009). Its respiration rate has been recorded at around 13.9 mg CO₂ kg⁻¹ h⁻¹ at physiological maturity (Ochoa-Vargas *et al.*, 2016) and reaches values of 28.0 mg CO₂ kg⁻¹ h⁻¹ during the climacteric peak (Arango *et al.*, 1999). However, studies on the development of the climacteric pattern of the naranjilla are limited and could lead to a more rigorous evaluation of the climacteric pattern of the fruit.

Ethylene is a gaseous plant hormone that acts as a key regulator of many physiological processes in plants including fruit ripening; the gas has an even more marked effect on climacteric fruits (Bajguz & Piotrowska-Niczyporuk, 2023). Ethylene generates a stimulus for the expression of genes related to the ripening and senescence of fruit and vegetables and, consequently, regulates these processes in fruits such as naranjilla at the molecular, biochemical, and physiological levels (Balaguera-López, Salamanca-Gutiérrez *et al.*, 2014). Naranjilla fruits show changes in ethylene production rate during postharvest ranging from 1.6 to 11.8 mg C₂H₄ kg⁻¹ h⁻¹ over a period of 21 d after harvest (Andrade-Cuvi *et al.*, 2018). However, ethylene production rates are low compared to other climacteric fruits.

The respiration and ethylene production data available for naranjilla during postharvest are still scarce and few studies describe the behavior of the respiratory rate during the postharvest stage or its relationship with ethylene production during the same stage. Respiratory intensity does not have a direct relationship with storage time, type of packaging, or storage temperature in their research (Forero *et al.*, 2014). In naranjilla fruits treated with 1-MCP, there is a 27% reduction in the respiratory rate compared to untreated samples during the entire storage period (Andrade-Cuvi, 2018).

Changes in fruit firmness

One of the main quality factors of fruit and vegetable products during postharvest is firmness (Martínez-González *et al.*, 2017). Extreme softening reduces the fruit shelf life and is one of the symptoms associated with senescence (Yahia & Carrillo-López, 2019; Wang *et al.*, 2022). Textural changes in fruits during ripening and postharvest storage are mainly associated with middle lamella dissolution and primary cell wall disassembly that occur through the action

of pectin methylesterase (PME), polygalacturonase (PG), cellulase (Cel), and β -glucosidase (β -glu) (Lin *et al.*, 2020; Song *et al.*, 2022). Quiroga Alvarez & Murillo Caviedes (2012) show that, in naranjilla fruits, the activity of the enzyme β -xylosidase increases as a function of ripening. Likewise, the authors observe a reduction in firmness and color changes that is attributed to the hydrolysis of hemicellulose present in the primary wall of the rind because of the accumulation of cellulose microfibrils and the formation of a lax network that allows cell growth. Ospina Monsalve *et al.* (2007) evaluate the firmness of naranjilla fruits harvested at different maturity stages. As a result they show that unripe fruits have a longitudinal load resistance of 226 N and transverse of 84.8 N, while for ripe fruits the average fracture force in longitudinal position is 180 N and transverse 68.5 N. The maximum force measured in the exocarp showed a mean value of 14.4 and 15.7 N for unripe and ripe fruits.

Pigments and color change

Fruit color is one of the key quality characteristics for consumers and determines the acceptability of a product. Therefore, color evaluation is very relevant for estimating fruit quality (Ayustaningwarno *et al.*, 2021). González Loaiza (2014) describe the color change of the naranjilla fruit during ripening.

During ripening, the epidermis of the fruits gradually changes from dark green to light green and then shows yellow shades along the sides until it reaches a uniform yellow color characteristic of ripe fruit. According to Casierra-Posada *et al.* (2004), the naranjilla fruits have five maturity stages based on the percentage variation of color. These maturity stages are used to define the harvest time of the fruit and can be considered as a reference parameter of ripening. However, the Colombian Technical Standard (NTC 5093) classifies naranjilla fruits in six ripening stages (0-5) as follow: color 0, fruits with a dark green color, physiologically ripe; color 1, fruit with dark green and light green shades; color 2, green fruits with some orange shades; color 3, fruit with orange and green glimpses towards the center of the fruit; color 4, the fruit is orange but with a few green traces; and finally, color 5, entirely orange fruits (ICONTEC, 2002).

Mejía *et al.* (2012) analyze the color change of naranjilla fruits at six stages of ripening and find slight visual changes during the first stages of ripening (0-2), followed by a marked increase during stages of color break (3-4) and a

decrease towards the last stage of ripening. This behavior is caused by chlorophyll degradation and an increase in carotenoid pigments, processes that in turn are influenced by the activity of ethylene (Acosta *et al.*, 2009; Andrade-Cuvi *et al.*, 2015). Andrade-Cuvi *et al.* (2016) find similar results in three varieties of naranjilla, Inap-Quitoense (2009), Baeza and Agria, where the values of L^* color coordinate (lightness) and b^* chromatic coordinate (blue to yellow hue) increase by 20 and 30% at ripening stages and 3 to 5% in the three varieties evaluated. Likewise, Andrade-Cuvi *et al.* (2019) find an increase in the chromatic coordinate a^* (green to red hue) from 29.2 to 30.9 in the 21 d of sampling, attributed to the accumulation of compounds such as carotenoids replacing chlorophyll that is degraded by the action of chlorophyllase and chlorophyll oxidase (Arteaga Dalgo *et al.*, 2014; Yahia & Carrillo-López, 2019).

Carotenoids are important pigments that are synthesized during the ripening of many fruits, including naranjilla, whose content may vary according to the tissue evaluated in the fruits. Gancel *et al.* (2008) find that the total carotenoid contents in naranjilla fruits is 23.0 mg of β -carotene equivalents per 100 g (dry weight) in fruit skin compared to placenta tissues (5.0 mg) or pulp (7.4 mg). For their part, Acosta *et al.* (2009) report that carotenoid content is lower in naranjilla fruits from Costa Rica, Colombia, and Ecuador, compared to fruits such as grapefruit and nectarine with a higher concentration of $20 \mu\text{g g}^{-1}$. Acosta *et al.* (2009) identify β -carotene as the main carotenoid present in naranjilla fruits, of which all-trans- β -carotene and 13- cis- β -carotene stand out, as well as lutein. Similarly, they find that all-trans- β -carotene is the main carotenoid present in naranjilla fruit and can account for up to 45% of the total carotenoid content; it is found in greater proportion in the peel ($36.22 \mu\text{g g}^{-1}$ of fresh weight, FW), followed by the pulp ($3.80 \mu\text{g g}^{-1}$ FW) and placenta tissues ($2.08 \mu\text{g g}^{-1}$ FW). The above is consistent with the color changes observed during ripening, where the peel has an orange hue, the pulp a yellow coloration, and the placenta tissues a yellowish green color.

Although color is one of the most used parameters to define the ripening stages, even for commercial purposes, González-Bonilla & Marín-Arroyo (2022) recommend the consideration of other parameter for an objective determination of the ripening stages. They conclude, as a useful tool, it is possible to classify naranjilla fruits objectively through a limited number of non-destructive parameters from harvesting to consumption such as size and shape.

Carbohydrates

Among the metabolic changes involved in the sensory quality of fruits, the variation in the concentration of sugars and organic acids is one of the most important parameters since it affects the flavor of the product (Schulz *et al.*, 2021). The proportion of acids and sugars establishes the organoleptic quality of the fruit. Naranjilla fruits have a concentration of total soluble solids between 1.7 and 10% (w/w) and acidity between 2.2 and 4.7%, depending on the ripening stage, type of storage, and handling during postharvest (Andrade-Cuvi *et al.*, 2018; Torres Pintado, 2020; Escobar, 2022; Molano-Díaz *et al.*, 2022). The main soluble sugars are fructose, glucose, and sucrose and the main organic acids are malic, citric, and oxalic (Jawad *et al.*, 2020). In naranjilla fruits, the sugar content in the pulp of 24.57 µg glucose/g tissue is reported at maturity stage 5 (ripe); and in the fruit peel the values vary between 56.52-141.83 µg glucose/g tissue on a dry basis for maturity stages 1 and 5 (Andrade-Cuvi *et al.*, 2021). Acosta *et al.* (2009) report individual values for sucrose, glucose, and fructose content of 16.0, 6.8, and 7.0 mg g⁻¹ fresh weight, respectively.

Of the organic acids in the naranjilla fruit, citric acid is the most preponderant. Likewise, this fruit has significant levels of ascorbic acid, reporting an increase of up to 90% of this acid throughout the ripening process, from 0.023-0.312 mg g⁻¹ ml⁻¹ of filtrate (Acosta *et al.*, 2009; González Loaiza *et al.*, 2014; Andrade-Cuvi *et al.*, 2021).

Antioxidant activity

Antioxidants are compounds that provide benefits to human health since they protect the body from damage caused by free radicals (Mejía-Reyes *et al.*, 2022). Among the antioxidant compounds present in naranjilla are chlorogenic acids and their hexosides in the pulp and placental tissues and flavonol glycosides in the skin (Gancel *et al.*, 2008). It is also a source of vitamins E, C, B1, B2, B3, B6, and provitamin A, minerals such as iron, calcium, phosphorus, potassium, and nitrogen, carbohydrates, and proteins (Ramírez *et al.*, 2018).

Naranjilla fruit contains between 0.11-0.13 mg ascorbic acid/g fresh weight, while the total content of soluble phenolic compounds is 0.91 mg GAE/g, and its antioxidant capacity has a value of 3.2 µmol Trolox/g (Vasco *et al.*, 2008).

The epidermis of the naranjilla fruit has a total polyphenol content of 1.5 and 2.6 times more than the pulp and placental tissue and an ascorbic acid content of the fruit parts, placental tissue, pulp, and peel of 1.25, 0.69, and

0.20 mg/g dry weight, respectively (Acosta *et al.*, 2009). However, the antioxidant capacity of different parts of the naranjilla fruit cannot be attributed solely to ascorbic acid (Llerena *et al.*, 2020). The antioxidant activity of naranjilla fruit is the result of the interaction of its free water-soluble components (organic acids, vitamin C), some water-soluble phenolic compounds and carotenoids. Similarly, gallic acid levels range from 5.11 to 8.98 mg/g (Vasco *et al.*, 2008; Acosta *et al.*, 2009; Contreras-Calderón *et al.*, 2011; Llerena *et al.*, 2014).

Post-harvest technologies

Postharvest preservation technologies have been developed to maintain the quality of fruit and vegetable products for as long as possible, especially for those that are of greater interest to consumers due to their health benefits (Yahia & Carrillo-López, 2019). However, the selection, thoroughness, and application of these strategies are vital parameters to consider given that they affect the fruit physiology and the behavior of its quality characteristics. The following is a description of the technologies used in the preservation of naranjilla fruits as well as the results obtained in each study.

Temperature

High temperatures generate rapid fruit degradation by accelerating the rate of respiration and ethylene production, as well as the development of microbial deterioration (Yahia & Carrillo-López, 2019). Consequently, the use of low storage temperatures is considered the main technique for the preservation of agricultural products given its effectiveness in delaying senescence and decreasing microbial activity (Zhao *et al.*, 2022). The recommended storage temperature for naranjilla is 10°C with a relative humidity of 90%; under these conditions it can have a shelf life of up to 16 d (Galvis & Herrera, 1999). However, different storage temperatures have been evaluated in naranjilla fruits. Fruits preserved at room temperature (14°C), had the highest weight loss with a value of 11.3% and firmness loss of 42%, compared to the fruits in refrigeration at 2°C and 4°C, where the weight loss is of 4.9 and 5.8% and the firmness decrease is 30.6 and 27.3% (Molano-Díaz *et al.*, 2022). Naranjilla fruits of the INIAP Palora and the Puyo hybrid varieties maintain their firmness in refrigeration at 3°C during 1-2 weeks (Torres Pintado, 2020). Finally, Balaguera-López *et al.* (2014) record the highest weight loss with a value of 12.86% in naranjilla fruits at room temperature, while naranjilla fruits treated with CaCl₂ and refrigerated at 8°C had a higher firmness (56.53 N). Other results are presented in Table 1.

1-Methylcyclopropene

1-Methylcyclopropene (1-MCP) is a gaseous hydrocarbon used commercially to maintain postharvest product quality and extend shelf life (Blankenship & Dole, 2003; Li *et al.*, 2022). 1-MCP is an efficient inhibitor of ethylene whose mode of action focuses on blocking ethylene signaling by irreversibly binding to ethylene receptors (ETR1) with ten times higher affinity than ethylene, and consequently it abrogates ethylene-related responses (Blankenship & Dole, 2003). This delays the fruit ripening and senescence, in addition to regulating autocatalytic ethylene production (Blankenship & Dole, 2003; Shu *et al.*, 2020; Balaguera-López *et al.*, 2021). 1-MCP is mostly used on climacteric fruits and its method, application time, and dosage depend on the fruit and plant to be evaluated (Li *et al.*, 2022). During postharvest, the application of 1-MCP has shown benefits in terms of an inhibition of ethylene synthesis, preventing loss of fruit firmness, and reducing the presence of physiological disorders during storage (González *et al.*, 2021; Li *et al.*, 2022). In naranjilla fruits treated with 1-MCP, Andrade-Cuvi (2018) report that the respiration rate reduced by 27% compared to the untreated fruits (Tab. 2). Molano-Díaz *et al.* (2022) find that naranjilla fruits should be stored at 2°C with a 1-MCP dose of 560 µg L⁻¹.

Modified atmosphere packaging

Modified atmosphere packaging (MAP) is commonly used in the preservation of fruits and vegetables to increase their shelf life (Wood *et al.*, 2022). For products preserved without any processing, a balance between respiration and gas passage through the package must be created by decreasing the O₂ concentration and keeping the CO₂ concentration at the top of the package in a controlled manner (Castellanos *et al.*, 2016). To guarantee the balance in the MAP, the most used materials are polymeric films of different calibers and sizes, which can be flexible or semi-flexible with or without perforations (Castellanos & Herrera, 2017).

Among the benefits of MAP are the delay in product ripening, lower ethylene production, reduction of chilling injury, development of pathogenic diseases (Yahia & Carrillo-López, 2019). Despite the benefits, negative effects can also occur during storage when processes related to anaerobic metabolism occur, giving rise to alcohols and aldehydes that trigger odors and flavors that are unpleasant (De la Vega *et al.*, 2017). In naranjilla fruits under MAP conditions, Guevara (2017), Llive Flores (2018), and Escobar (2022) report respiratory rates of 580 cm³ kg⁻¹ d⁻¹ of O₂ consumed and 493.4 cm³ kg⁻¹ d⁻¹ of CO₂ produced, lower concentration of total flavonoids, and lower microbial affectation (Tab. 3).

UV-C radiation

In addition to the benefits obtained from the application of methods for the preservation of product quality and extension of shelf life, the worldwide trend of environmentally friendly management also is to apply postharvest technologies. Among the so-called “emerging technologies” for fruit and vegetable preservation is the application of UV radiation; this has gained momentum given its easy implementation and high effectiveness to counter microbial activity affecting the product and its low environmental impact (Andrade-Cuvi, 2018; Michailidis *et al.*, 2019).

The application of UV-B or UV-C radiation has a positive effect on the control of damage caused by pathogens and slows senescence, as well as color changes and the synthesis of antioxidants and phenolic compounds (Abdipour *et al.*, 2019). The UV-C radiation (200–280 nm), with 3–5 min exposure during the postharvest period is related to slowing down the softening process and increasing secondary metabolites of fleshy fruits, while inhibiting the firmness loss and the occurrence of browning (Andrade-Cuvi, 2018; Ma *et al.*, 2021). In naranjilla fruits, there is a reduced decay rate compared to untreated fruits, a lower firmness loss rate, and lower color changes in UV-C-treated fruits (5 kJ m⁻¹ radiation) (Andrade-Cuvi *et al.*, 2013).

Gamma radiation

Gamma radiation is non-thermal ionizing radiation emitted by cobalt (⁶⁰Co) or cesium (¹³⁷Cs) sources that generates an electromagnetic radiation of a very short wavelength, the same as occurs with X-rays, ultraviolet light, visible infrared, and microwaves (Andrade-Cuvi *et al.*, 2019). The use of gamma radiation is focused on pest and disease control during postharvest; it does not generate environmental damage, does not alter the quality of the fruit, and it extends its postharvest shelf life (Lizarazo-Peña *et al.*, 2022). An evaluation of the effectiveness of gamma rays shows that treated fruits have a mild deterioration index compared to control fruits that show a severe damage index (Andrade-Cuvi *et al.*, 2019). The shelf life of naranjilla treated with 500 Gy was increased by 7 d in relation to the fruits without treatment; however, the authors indicate that its use is limited due to the cost of application for practical purposes and access to this technology (Andrade-Cuvi *et al.*, 2019).

Ozone

Ozone (O₃) is considered an oxidizing agent for gaseous or aqueous use whose retarding effect on the loss of the physical characteristics of the naranjilla and other fruits as well as the reduction of damage by microorganisms (Oyom *et al.*, 2022). Among the characteristics that make

ozone commercially attractive for postharvest use is principally that it does not leave carcinogenic residues in treated products as occur with chemical treatments, so it has no negative effects on the health of consumers (Chen *et al.*, 2020). In naranjilla fruits, Andrade-Cuvi *et al.* (2018)

report that ethylene production for samples treated with gaseous ozone was half of that produced in control fruits and the antioxidant capacity of treated fruits is 21% higher compared to controls (Tab. 4).

TABLE 1. Results of the use of different temperatures in the preservation of naranjilla fruits.

Dose / Packing/ Days of evaluation	2°C, 4°C and 14°C/ 32 d	INIAP Palora and hybrid Puyo at 3°C, 7°C and 17°C/ 12 d	8°C and 18°C/ CaCl ₂ to 3% of Ca / (immersion time 0, 5, 10 and 15 min)
Respiration rate / Ethylene production	The lowest respiration values were obtained in fruits at 2°C, followed by those stored at 4°C and 14 °C	NA	NA
Loss of firmness	Fruits at 14°C = 42%, fruit at 2°C = 30.6% and fruit at 4°C = 27.3%	Temperatures of 3 °C and 17 °C showed the largest changes	15 min + 8°C: Increased firmness retention (56.53 N at 29 d)
Color	L*: 14°C = 57.2 ± 0.35 at 32 d, 2°C and 4°C descended at 12 and 28 d a* y b* no significant changes	NA	10 min + 8°C: Higher retention of green color
TSS/TTA/pH	TSS: no significant differences between temperatures TTA: 2°C > 4 and 14 °C at 8 d pH: Differences occurred at 8 and 16 d	TSS: 17°C = 7.63 °Brix, 3°C = 8.33 ° Brix 7°C = 9.00 °Brix, TTA: 3°C = 2.80%, 7°C = 2.76%, 17°C= 2.20% of citric acid at 12 d	NA
Weight loss	14°C = 11.3% at 20 d 2°C = 4.9% at 32 d 4°C= 5.8% at 32 d	Fruits at 3 °C showed the lowest percentage of weight loss	Control: 12.86% 10 min+8°C: < 10%
Deterioration index (DI)	NA	Overall, all treatments showed mild ID at day 3 and moderate ID at day 12	NA
Author	Molano-Díaz <i>et al.</i> (2022)	Torres Pintado (2020)	Balaguera-López, Ramírez <i>et al.</i> (2014)

TABLE 2. Results of the use of 1-MCP in the preservation of naranjilla fruits.

Dose / Packing/ Days of evaluation	0 µg L ⁻¹ , 280 µg L ⁻¹ and 560 µg L ⁻¹ / (immersion time 10 min)/32 d	0.5 µL L ⁻¹ de 1-MCP (immersion time 8, 12 and 24 h) 4°C/28 d
Respiration rate / Ethylene production	There were no significant differences. The climacteric peak was observed at 16 d of storage	1-MCP reduced the respiration rate by 27% compared to the control
Loss of firmness	32.4% on average over the 32 d with no differences between treatments	In control fruits it remained constant until day 7 (7.85 N) and decreased until day 21 (4.42 N). Treated fruits-maintained firmness until day 14 (8.86 N) and then decreased to a lesser extent at day 21 (6.07 N)
Color	Color index increased until day 16 in fruits with 1-MCP application, Control = decreased slowly and significantly	1-MCP contributed to maintaining better color characteristics, with Hue, L* and C* values 25, 35 and 34 % higher than the controls at the end of storage
TSS/TTA/pH	TSS: > Concentration 280 µg L ⁻¹ = 8.4 °Brix TTA: Control 9.1 y 21.1% more 280 and 560 µg L ⁻¹ of 1-MCP at 16 d	TSS: Control= increased °Brix up to day 7 decreased by 18% at 21 d and 1-MCP= increased °Brix up to day 14 and decreased 28% at 21 d. TTA: 1-MCP = 4.7% at 21 d. control = 3.9% at 21 d pH: remained stable throughout the storage period
Weight loss	No differences in weight loss for the 1-MCP factor or the control	1-MCP (0.5 µL L ⁻¹ /8h) significantly reduced weight loss during storage. After 21 d, the control fruits showed a weight loss of 7.7%, while the treated fruits showed a weight loss of 6.6%
Deterioration index (DI)	NA	The first symptoms of damage; in control fruit at 7 d and in treated fruit at 14 d. The maximum value was recorded on day 21 with DI = 3.2 and 1.8 for control and treated fruits, respectively
Microbiological	NA	The greatest effect was observed on molds and yeasts. In the control this population increased by 4.1 log units and in treated fruits by 1.1 log units with a final population of 8.1 log CFU/g (controls) and 5.1 log CFU/g (treated)
Biochemistry	NA	There was no statistical difference in total phenols content between control and treated fruit.
Author	Molano-Díaz <i>et al.</i> (2022)	Andrade-Cuvi (2018)

TABLE 3. Results of the use of MAP in the preservation of naranjilla fruits.

Dose / Packing/ Days of evaluation	10 d, Gas concentration: G1=O ₂ :2.5; CO ₂ :2.5 N ₂ :95. G2=O ₂ :2.5; CO ₂ :5 N ₂ :92.5; G3=O ₂ :80; CO ₂ :10 N ₂ :10; G4=O ₂ :80; CO ₂ :20 N ₂ :0 G5=O ₂ :90; CO ₂ :10 N ₂ :0	Polylactic acid (PLA): PLA, PLA + Rem 4 g and PLA + Rem 8 g a 5°C, 12°C and 23°C / 30 d
Respiration rate / Ethylene production	G2= no differences in O ₂ consumption. CO ₂ production= no differences between treatments	23°C = 580 and 493.4 cm ³ kg ⁻¹ d ⁻¹ of O ₂ consumed and CO ₂ produced, respectively, lower values than other climacteric fruits
Loss of firmness	The lowest loss of firmness occurred in fruits with the G1 mixture	NA
Color	There were no differences between treatments	A* = highest values in PLA + Rem 4 g L* = 47.1 – 61.3 and b* = 34.3 – 63.1 at 5 d after harvest and stable until the end of storage
TSS/TTA/pH	TSS: there were differences only in the fruits with the G3 mixture. TTA: All treatments showed differences except G3. pH= no difference	TSS = no significant differences. TTA: PLA had the highest percentage of acidity. pH= no difference
Weight loss	G1, G4, and G5 = weight loss between 14 and 32%	Control = 3% after 15 d of storage
Deterioration index (DI)	G1, G2, G4 = visually reduced the degree of wilting. G2= improved ripening uniformity. G1 and G5 were not affected and G3 and G4 were highly deteriorated	NA
Microbiological characteristics	G1: 133 CFU/g G4: 167 CFU/g. G5: > content of aerobic mesophilic aerobes. Growth of molds and yeasts was significant for G2, G4, and G5	NA
Biochemistry	G1 and G4 = significant differences in total polyphenols expressed in mg gallic acid/100 g ms. G1 and G5 < total flavonoid concentration G1, G2 and G4= significant differences in carotenoids. Total chlorophyll did not show differences between treatments	NA
Author	Guevara (2017), Lliva Flores (2018)	Escobar (2022)

TABLE 4. Results of the use of gaseous ozone in the preservation of naranjilla fruits.

Dose / Packing/ Days of evaluation	1.5 mg L ⁻¹ with an exposure time of 5 min and a manually controlled flow to 30%/21 d at 4°C
Respiration rate / Ethylene production	Control: 46.1 mg CO ₂ kg ⁻¹ h ⁻¹ and 1.6 mg C ₂ H ₄ kg ⁻¹ h ⁻¹ to 11.8 mg C ₂ H ₄ kg ⁻¹ h ⁻¹ . Treated: 48.1 mg CO ₂ kg ⁻¹ h ⁻¹ and 2.2 mg C ₂ H ₄ kg ⁻¹ h ⁻¹ to 5.5 mg C ₂ H ₄ kg ⁻¹ h ⁻¹
Loss of firmness	Control: 13.7 N Treated: 8.9 N
Color	Control: L* = 55 at 21 d and b* = 64 Treated: L* = 62 and b* = 65
TSS/TTA/pH	TSS: Control = increased on 2.8 °Brix. Treated = increased on 3.1 °Brix. TTA = a range of 3.2 and 3.6 was maintained for all fruits. pH = a range of 3.2 and 3.6 for all fruits was maintained
Weight loss	Control: 3.9% Treated: 5.9
Biochemistry	Control: At day 21 there was a 20% higher AsA concentration than at day 0. Treated: 52% higher AsA content at day 0 and on day 21 there was a 42% reduction of AsA concerning the initial values. The carotenoid content at the end of storage showed values of 8.2 and 28% higher at day 0, for control and treated fruits, respectively. By the end of storage, the control fruits showed 23% higher total phenol content. On day 21, the treated samples showed 21% higher antioxidant capacity than the controls
Author	Andrade-Cuvi <i>et al.</i> (2018)

Conclusions

The physiological characteristics of naranjilla fruits, such as their moderate-low respiration rate and low ethylene production, classify this product as perishable with a 2-3 week shelf life at room conditions. The main changes associated with ripening and deterioration are reduction in firmness, weight loss, yellowing of the peel and pulp, and fungal sporulation. For the specific case of naranjilla fruits and according to the available information, the storage at

low temperatures, individually or in combination with other technologies that contribute to the reduction of physiological processes such as MAP, can result in a significant increase in product shelf life (4-6 weeks).

The use of emerging treatments such as gamma and UV-C radiation has shown positive effects on the reduction of microbial activity, as well as on the preservation of the physiochemical, and biochemical characteristics of the fruit. The application of 1-MCP and gaseous ozone has

shown satisfactory results only in terms of the physical and biochemical characteristics of naranjilla fruits because its effect is only related to the control of ethylene activity.

It is advisable to carry out studies that allow a holistic understanding of the application of each technology and the possible combination between them and the effects on all aspects of product quality, such as chemical, physical, and biochemical characteristics, as well as microbial activity in naranjilla fruits to determine what technologies may be ideal for the preservation and storage of naranjilla fruit during post-harvest.

Conflict of interest statement

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

Author's contributions

AJRM: conceptualization, research, writing - original draft, visualization, writing, and editing. DACE: conceptualization, writing, supervision, and editing. HEBL: conceptualization, visualization, writing, and editing. All authors have read and approved the final version of the manuscript.

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Agronomic characteristics of carrot cultivars under water stress

Características agronómicas de cultivares de zanahoria bajo estrés hídrico

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ABSTRACT

The objective of the study was to evaluate the agronomic performance of carrot cultivars subjected to different levels of water supply. The experiment was conducted in a randomized block design, in a split-plot scheme with four replicates. Five irrigation depths were used in the plot, one to replace 100% of the crop's evapotranspiration (ETc), two in deficit (50 and 75% of ETc) and two in excess (125 and 150% of ETc). Four carrot cultivars were used in the subplots: Brasília, Alvorada, Esplanada, and Nantes. Two carrot cultivation cycles were carried out, the first lasting 121 d and the second lasting 103 d after sowing. The following variables were evaluated: total fresh mass of the plant, fresh carrot mass, carrot length, length of the aerial part, average carrot diameter, carrot volume, green shoulder, crop productivity, water productivity, and water potential of the plants. The Brasília carrot cultivar had better development and the Esplanada cultivar was less adapted to the studied environment. All carrot cultivars were affected by stress caused by excess and lack of water. Carrot irrigation must be carried out with a depth equal to 100% of the crop's evapotranspiration for the region and conditions similar to those of the present study.

Key words: *Daucus carota* L., protected cultivation, water deficit, water management.

RESUMEN

El objetivo del presente estudio fue evaluar el desempeño agronómico de cultivos de zanahoria sometidos a diferentes niveles de suministro de agua. El experimento se realizó en un diseño de bloques, en un esquema de parcelas divididas con cuatro repeticiones. En la parcela se utilizaron cinco profundidades de riego, una para reponer el 100% de la evapotranspiración (ETc) del cultivo, dos en déficit (50 y 75% de ETc) y dos en exceso (125 y 150% de ETc). En las subparcelas se utilizaron cuatro cultivares de zanahoria: Brasília, Alvorada, Esplanada y Nantes. Se realizaron dos ciclos de cultivo de zanahoria, el primero de 121 d y el segundo de 103 d después de la siembra. Se evaluaron las siguientes variables: masa fresca total de la planta, masa fresca de zanahoria, longitud de zanahoria, longitud de la parte aérea, diámetro promedio de zanahoria, volumen de zanahoria, hombro verde, rendimiento del cultivo, productividad del agua y potencial hídrico en la planta. El cultivo de zanahoria Brasília mostró mejor desarrollo y el cultivo Esplanada estuvo menos adaptado al ambiente estudiado. Todos los cultivos de zanahoria se vieron afectados por el estrés causado por el exceso y la falta de agua. El riego de zanahoria se debe realizar con una profundidad igual al 100% de la evapotranspiración del cultivo para la región y condiciones similares a las del presente estudio.

Palabras clave: *Daucus carota* L., cultivo protegido, déficit hídrico, gestión del agua.

Introduction

Carrot (*Daucus carota* subsp. *sativus*), belonging to the Apiaceae family, is a root vegetable of great economic importance in Brazil and the world (Alves *et al.*, 2020). Cultivated carrot is one of the most important vegetable plants in the world and favored by consumers for its typically sweet flavor (Schmid *et al.*, 2021). Carrots are cultivated on a large scale in the Southeast, Northeast and South regions of Brazil, with an estimated planted area of 26000 ha and root production of 780000 t (Carvalho *et al.*, 2017).

Scientific research into the carrot production process is necessary to meet this growing demand, reducing or eliminating deficiencies in the production sector. One potential solution to rectify these deficiencies involves selecting carrot varieties that are better suited to the climate and soil conditions of a particular region, facilitating enhanced crop productivity.

In conjunction with the selection of suitable genotypes tailored to the prevailing climate and soil conditions, the efficacy of carrot production hinges upon the implementation

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of irrigation practices to adequately fulfill or supplement the crop's water requirements. According to Guimarães *et al.* (2019) and Nasir *et al.* (2021), carrot is a crop susceptible to water imbalances, making its rational management essential to maximize production.

Water deficit represents one of the primary climatic adversities faced by agricultural crops worldwide, and carrot (*Daucus carota* subsp. *sativus*) cultivation is no exception (Cunha *et al.*, 2019; Mustafa *et al.*, 2022). Under conditions of water deficit, carrots manifest an array of physiological and biochemical reactions that adversely affect their growth and, consequently, yield potential. Water scarcity impairs nutrient uptake, reduces the rate of photosynthesis, disrupts nutrient transport, and hampers the growth of roots, which are the commercially valuable part of the plant (Zhao *et al.*, 2022; Rosińska *et al.*, 2023). Therefore, evaluating and understanding the effects of water deficit on carrot cultivation is of paramount importance. Research in this field is essential for developing proper management strategies, such as efficient irrigation and the development of drought-resistant varieties, to ensure food security and the sustainability of carrot production in an increasingly challenging climate change scenario (Abbas *et al.*, 2023; Kwiatkowski *et al.*, 2023). Excess water also causes problems for agricultural crops due to the reduction of free porosity and leaching of nutrients (Li *et al.*, 2018; Massa *et al.*, 2020; Santos & Silva, 2020). Therefore, research is important to verify the behavior of carrot crops in different water availability. This information is important for production estimates, economic analysis, and decision-making in commercial crops. In this way, the producer will be able to better plan for different water availability scenarios.

The use of irrigation enables successive crops throughout the year (Drysdale & Hendricks, 2018; Cunha *et al.*, 2019). Irrigation is necessary in a protected environment to meet the plant's water needs. The use of protected environments has become more frequent in recent years, as they protect the crop from climatic adversities, pests and diseases, providing an increase in productivity and product quality (Gómez *et al.*, 2019; Nikolaou *et al.*, 2019; Martínez-Gómez *et al.*, 2021; Filgueiras *et al.*, 2022).

Given the above, we tested the hypothesis that water stress, whether due to excess or deficit, affects different carrot cultivars. Thus, the objective of this study was to evaluate the agronomic performance of carrot cultivars subjected to different levels of water supply.

Materials and methods

Characterization of the area and experimental test

The research was conducted in a greenhouse at the Federal University of Viçosa (UFV, Brazil), in Viçosa-MG, at coordinates 20°45'14" S, 42°52'55" W, altitude of 648 m a.s.l. The climate, according to the Köppen classification, was type Cwb (Alvares *et al.*, 2013). The average annual temperature was 19.4°C and the annual rainfall was approximately 1,200 mm.

A greenhouse with a total area of 240 m² and dimensions of 8 m wide and 30 m long was used. The sides were covered with polyethylene yarn fabric, with the following characteristics: 100% polyethylene, 25 mesh - 1.0 x 1.0 mm opening, with 10 yarns per cm of fabric - and 25% shading. The ceiling was covered with blue plastic film, with the following characteristics: AV Blue, 120 µm, 78% and 67% light transmission and diffusion, respectively. The greenhouse was not controlled for air temperature, relative humidity or CO₂ concentration. Two carrot cultivation cycles were carried out. Cycle 1 lasted 121 d (18/01/2017 to 18/05/2017) and cycle 2 lasted 103 d (04/08/2017 to 14/11/2017) after sowing. In both carrot growth cycles, there was an accumulation of 1,500 growing degree days. To calculate the accumulation of growing degree days, air temperatures of 10°C were used as the lower base and 30°C as the upper base (Embrapa, 2004).

The experimental design was in randomized blocks (DBC), with four replicates, in a split-plot scheme, with five irrigation depths in the plots and four carrot cultivars in the subplots. The irrigation depths were used to replace 50, 75, 100, 125, and 150% of the crop's evapotranspiration (ET_c). The carrot cultivars were: Brasília, Embrapa (2004), summer cultivar; Alvorada, Embrapa (2003), summer cultivar; Esplanada, Embrapa (2005), spring-summer cultivar; and Nantes, Embrapa (2004), autumn-winter cultivar. The carrot varieties selected for the present study were those with the greatest potential and/or most cultivated in the state of Minas Gerais, which is the largest carrot producer in Brazil (Alves *et al.*, 2020). Each sampling unit (subplot) had an area of 1 m² (1 m long and 1 m wide), consisting of four rows of plants (0.25 m between rows and 0.06 m between plants), resulting in a planting density of 66 units per m². For the evaluations, four plants were sampled in the center of the center lines in each subplot.

Data from 80 observations (5 irrigation depths x 4 replicates x 4 carrot cultivars) were used for each characteristic analyzed for each cultivar and for each cycle.

Installation and conduction of research

A eutrophic Red-Yellow Oxisol was used, with the characteristics presented in Table 1. Soil sampling was carried out inside the protected environments to carry out physicochemical analysis (Tab. 1).

The soil preparation was done through plowing and harrowing, using a ridger. Liming and chemical fertilization were carried out based on the soil chemical analysis results and recommendations from Ribeiro *et al.* (1999). Before sowing, 5 t ha⁻¹ of cattle manure were incorporated into the soil. For mineral fertilization, 50 kg ha⁻¹ of P₂O₅, 75 kg ha⁻¹ of K₂O, and 40 kg ha⁻¹ of N were applied using simple superphosphate, potassium chloride, and urea, respectively. Fifteen kg ha⁻¹ of borax and 15 kg ha⁻¹ of zinc sulfate were also applied.

Carrots were sown directly in the beds (in small furrows spaced 0.25 m between rows) and subsequently, thinning was carried out (0.06 m spacing between plants) 28 d after sowing, similar to Silva *et al.* (2019). Weekly, manual weeding was carried out until the soil was naturally shaded by the aerial part of the plants. Incidences of pests and diseases capable of causing significant damage to carrot quality and productivity were not observed.

The drip irrigation system was used, with lateral lines made up of drip tapes (Amanco brand) 16 mm in diameter and 15 thousandths of an inch (2.54 cm) thick. The spacing between the drip tapes was 0.50 m, which made it possible to irrigate two rows of plants. The emitters (drippers), spaced 0.20 m apart, operated with a working pressure of 98 kPa (~10 mwc), applying an average flow of 1.8 L h⁻¹. The water for irrigation was stored in a 15 m³ reservoir and had

a pH of 6.7 and electrical conductivity of 57 µS cm⁻¹. The irrigation water also had a total hardness of 16 mg L⁻¹ and total dissolved solids of 15 mg L⁻¹.

Climate data

Meteorological data were obtained using the automatic meteorological station E 4000 (IRRIPLUS), installed centrally inside the greenhouse. Data on solar radiation (Rs), mean air temperature (T_a), and relative humidity (RH) were measured at 5-min intervals. The collected meteorological data were converted to a daily scale, and, subsequently, ETo values were calculated using the Penman-Monteith method (Allen *et al.*, 1998).

Due to the lack of rainfall inside the greenhouse, there was no need to collect this variable. This circumstance also influenced the application of treatments, as rain could have disrupted the imposition of treatments with different irrigation levels.

Irrigation management

The irrigation system was evaluated using the methodology proposed by Bernardo *et al.* (2019), which consists of collecting the dripper flow at eight points along the lateral line and at four lateral lines, along the derivation line. The distribution efficiency was 92.4%, according to the distribution uniformity coefficient, calculated using Equation 1:

$$DUC = \frac{Flo25\%}{FloM} 100 \quad (1)$$

where: DUC – distribution uniformity coefficient, %; Flo25% – average of the lowest quartile of flows, L h⁻¹; and FloM – average flow rate, L h⁻¹.

The actual irrigation required to treat 100% of ET_c was estimated as a function of climate and soil characteristics parameters, using Equation 2, adapted from the equation proposed by Bernardo *et al.* (2019):

TABLE 1. Physicochemical characteristics of soil at 0-0.20 m soil depths at the experiment site.

FC*	PWP*	BD	PD	Sp	Clay	Silt	Sand	Textural classification					
----- kg kg ⁻¹ -----		-- kg m ⁻³ --	-- kg m ⁻³ --	-- % --	----- % -----								
0.291	0.177	1,210	2,641	54.2	39	11	50	Sand clay					
pH	P	K	Ca ²⁺	Mg ²⁺	Al ³⁺	H+Al	SB	t	T	V	m	Prem	Ec
H ₂ O	---- mg dm ⁻³ ----		----- cmol _c dm ⁻³ -----				----- % -----					mg L ⁻¹	µS cm ⁻¹
6.1	328.4	196.0	5.4	1.2	0.0	2.6	7.2	7.2	9.8	73.3	0.0	51.5	145

FC*: field capacity (matric potential of -33 kPa); PWP*: permanent wilting point (matric potential of -1,500 kPa); *obtained from the soil water retention curve using the Richards extractor; BD: bulk density; PD: particle density; Sp: soil porosity; SB: sum of bases; t: effective cation-exchange capacity; T: cation-exchange capacity; V: base saturation; m: aluminum saturation; Prem: remaining phosphorus according to the methodology by Teixeira *et al.* (2017); Ec: electric conductivity at 25°C. Available P and K extracted with Mehlich I; exchangeable Ca, Mg and Al extracted with 1 mol L⁻¹ KCl; potential acidity at pH 7.0 extracted with 0.5 mol L⁻¹ calcium acetate.

$$AIR_{LOC} = \sum_{day 1}^i ETo K_C K_S K_L - C \quad (2)$$

where: AIR_{LOC} – actual irrigation required in localized systems, mm; ETo – reference evapotranspiration, mm d^{-1} ; K_C – crop coefficient, dimensionless; K_S – soil moisture coefficient, dimensionless; K_L – location coefficient, dimensionless; and C – constant referring to the elevation of the water table, mm.

The model used to estimate ETo was the Penman-Monteith FAO-56 (Allen *et al.*, 1998) according to Equation 3. Wind speed was considered equal to 0.2 m s^{-1} , as recommended for indoor protected environments (Guimarães *et al.*, 2019; Correia *et al.*, 2020).

$$ETo = \frac{0.408 \Delta (R_n - G) + \gamma \frac{37}{T_a + 273} U_2 (e_s - e_a)}{\Delta + \gamma (1 + 0.34 U_2)} \quad (3)$$

where: ETo – reference evapotranspiration, mm h^{-1} ; R_n – net radiation on the surface, $MJ \text{ m}^{-2} \text{ h}^{-1}$; G – soil heat flux density, $MJ \text{ m}^{-2} \text{ h}^{-1}$; T_a – average air temperature, $^{\circ}C$; U_2 – wind speed at 2 m height, $m \text{ s}^{-1}$; e_s – saturation vapor pressure, kPa; e_a – partial vapor pressure, kPa; Δ – slope of the saturation vapor pressure curve, $kPa \text{ }^{\circ}C^{-1}$; γ – psychrometric coefficient, $kPa \text{ }^{\circ}C^{-1}$.

Cultivation coefficient (K_C) values were used in accordance with the literature (Cunha *et al.*, 2016). The K_C used was determined according to the stage of crop development: phase I – initial growth; phase II – vegetative growth; phase III – root thickening; phase IV – pre-harvest. For phases I, II, III and IV, a K_C of 0.7, 0.9, 1.0, and 1.1 was adopted, respectively.

Soil moisture (K_S) and location (K_L) coefficients were calculated according to Bernardo *et al.* (2019):

$$K_S = \frac{\ln (CWD + 1)}{\ln (TWC + 1)} \quad (4)$$

$$K_L = \frac{P}{100} + 0.15 \left(1 - \frac{P}{100} \right) \quad (5)$$

where: K_S – soil water depletion coefficient, dimensionless; CWD – current water depth, mm; TWC – total water capacity, mm; K_L – location coefficient, dimensionless; P – highest value among percentage of wet or shaded area, %.

The AIR_{LOC} value was corrected depending on the irrigation efficiency, defining the total irrigation required for localized systems (TIR_{LOC}).

$$TIR_{LOC} = \frac{AIR_{LOC}}{Ie} \quad (6)$$

where: TIR_{LOC} – total irrigation required in localized systems, mm; AIR_{LOC} – actual irrigation required in localized systems, mm; Ie – irrigation efficiency, decimal.

Figure 1 shows the water balance for the treatment that received an irrigation sheet to replace 100% of ET_c . This figure shows the current soil moisture and the irrigation levels applied in the two carrot cultivation cycles. In cycle 1, soil moisture was below the critical point only twice (02/1/2017 and 05/08/2017). In cycle 2, this situation did not occur at any time. It can also be seen in Figure 1 that irrigations were more frequent at the beginning of the cultivation cycles since the root system was shallow and there was less water availability in the soil.

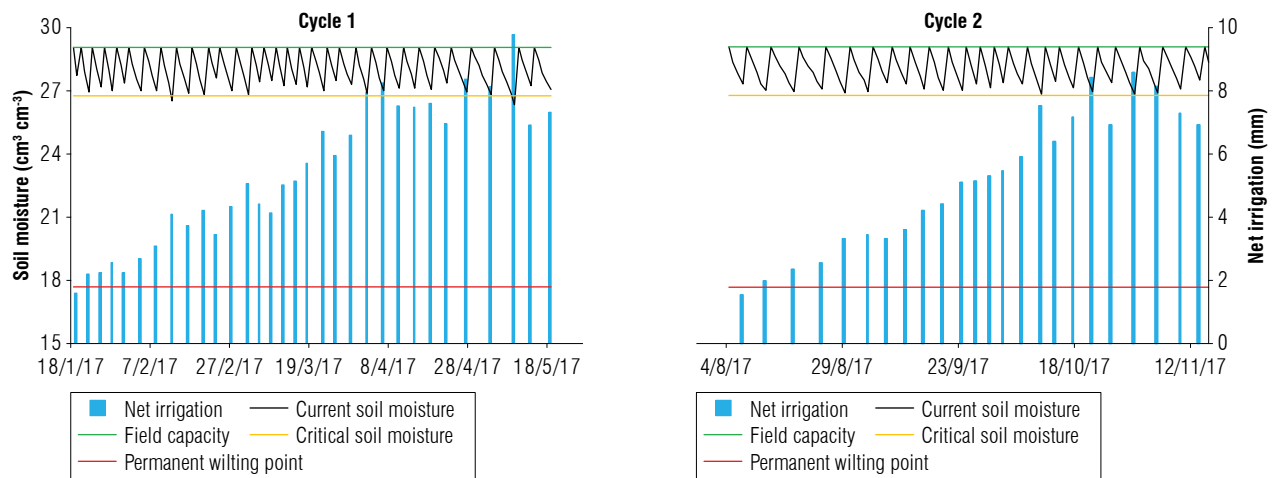


FIGURE 1. Water balance to meet 100% of crop evapotranspiration in two carrot cultivation cycles.

The application efficiency was 100% and the average distribution efficiency 92.4%. Thus, the irrigation efficiency was 92.4% for the two cultivation cycles.

For 5 d before the start of irrigation management via climate, the water content in the soil was monitored at a depth of 0-0.20 m, using tensiometers based on the soil water retention curve $\theta_{Azul} = 0.1813 + (0.3443 - 0.1813) / (1 + (0.01 |\Psi_m|)^2)^{0.49}$ and by the direct greenhouse method, in order to guarantee soil moisture at field capacity conditions. The differentiation of irrigation depths began 30 d after sowing.

To maintain pressure uniformity in the system during the irrigation time, two lateral lines were always kept open simultaneously: irrigation time = 20 min, L1=10 min (50% of ETc), L2=15 min (75% of ETc), L3=20 min (100% of ETc), L4=25 min (125% of ETc), and L5=30 min (150% of ETc).

The carrot crop was grown in two cycles lasting 121 and 103 d. The experiments were conducted in a randomized block design, in a split-plot scheme with four replicates. Four carrot cultivars were added to the subplots: Brasília, Alvorada, Esplanada, and Nantes.

Variables analyzed

Carrot harvests occurred when 1,500 growing degree-days were accumulated for all varieties. The variables analyzed in carrot cultivation are presented in Table 2.

Statistical analysis

The data were subjected to analysis of variance at 5% and 1% probability, using the F test. When significant at 5%, the effects of irrigations were subjected to regression analysis and the effects of cultivars were compared using the Tukey's test 5% probability.

For regression analysis, the linear, quadratic, cubic, square root, potential, exponential, hyperbolic, logarithmic, cubic-root, log-log, Ln-Ln and Exp (x) models were tested. To choose the best model, the following were considered: the significance of the F test for the regression equation at 5% probability, the coefficient of determination (R^2), and the representation of biological behavior by the equations.

Parameter data were subjected to principal component analysis (PCA). The GENES and SISVAR software were used for statistical analyses.

TABLE 2. The variables analyzed in carrot cultivation.

Description	Parameter	Unit	Method
Carrot length	CL	cm	measured with a ruler
Plant length	AL	cm	aboveground length measured with a ruler
Average carrot diameter	ACD	mm	average of three measurements (shoulder, middle and tip) using a caliper
Carrot volume	CV	cm ³	by displacement of water volume inside a millimeter beaker
Green shoulder	GS	cm	thickness of the greenish color in the "shoulder" of the carrot, measured with a caliper
Damaged carrots	DC	un 10 ⁻¹	number of unmarketable carrots due to damage, out of 10 carrots randomly collected in the useful plot
Fresh carrot mass	FCM	g	wet mass of whole carrots only
Dry carrot mass	DCM	g	whole carrot mass, dried in a forced ventilation oven at 70°C, until reaching a constant mass
Fresh foliage mass	FAM	g	wet mass of the aerial part of the plant
Dry foliage mass	DAM	g	mass of the aerial part dried in a forced ventilation oven at 70°C, until reaching a constant mass
Mass of 10 carrots	10CM	g	fresh mass of 10 carrots collected randomly from the useful plot
Crop yield	CY	m ⁻²	estimate of the ratio between the production of 10 carrots from the useful plot and the area occupied by them, extrapolating to an area of 1 ha, considering the use of access corridors of 0.40 m wide and maximum bed length of 50 m and using roots free from defects, such as cracks, bifurcations, nematodes and mechanical damage, with length and diameter greater than 5.0 and 1.0 cm, respectively, according to Soares <i>et al.</i> (2010)
Water productivity	WP	m ⁻³	obtained by the relationship between the mass of fresh matter of the plant (kg ha ⁻¹) and the amount of water applied (mm) for each treatment
Plant water potential	Ψ_p	kPa	estimated using the Scholander chamber methodology (Scholander <i>et al.</i> , 1964). Two measurements were taken per cycle (one at 55 d after sowing and another 2 d before harvest), following the recommendation of the methodology, with measurements obtained between 3:00 am and 6:00 am (before sunrise – predawn), when the water potential in the plant is maximum. The analysis was carried out on two leaves per plant, obtained from two different plants in the subplots

Results and discussion

Climate data

The average solar radiation (R_s) was $7.1 \text{ MJ m}^{-2} \text{ d}^{-1}$ in cycle 1 (ranging from 1.6 to $11.0 \text{ MJ m}^{-2} \text{ d}^{-1}$) and $6.2 \text{ MJ m}^{-2} \text{ d}^{-1}$ in cycle 2 (ranging from 1.8 to $9.1 \text{ MJ m}^{-2} \text{ d}^{-1}$). The daily average R_s decreased throughout cycle 1 and this behavior influenced the decrease in T_{mean} and E_{To} (Fig. 2). The average daily air temperature ranged from 16.5 to 26.4°C in cycle 1 (average of 22.4°C) and from 15.4 to 35.3°C in cycle 2 (average of 21.2°C). The daily average relative air humidity (RH) ranged from 61.1 to 87.6% (average of 77.7%) and from 58.6 to 93.5% (average of 77.4%) in cycles 1 and 2, respectively.

E_{To} presented daily average values equal to 1.8 mm d^{-1} (oscillation from 1.0 to 2.6 mm d^{-1}) in cycle 1 and equal to 1.7 mm d^{-1} (from 1.0 to 2.3 mm d^{-1}) in cycle 2. The E_{To} occurring throughout each carrot cultivation cycle was used to determine the actual irrigation required (AIR) and total irrigation required (TIR) for an irrigation depth of 100% of the E_{Tc} applied in the treatments.

Water consumption

Due to the absence of rainfall within the protected environment, a high frequency of irrigation (2-d irrigation shift) was required. The soil was maintained with moisture close to field capacity and with little water requirement to equal the total storage capacity at the time of each irrigation (Tab. 3).

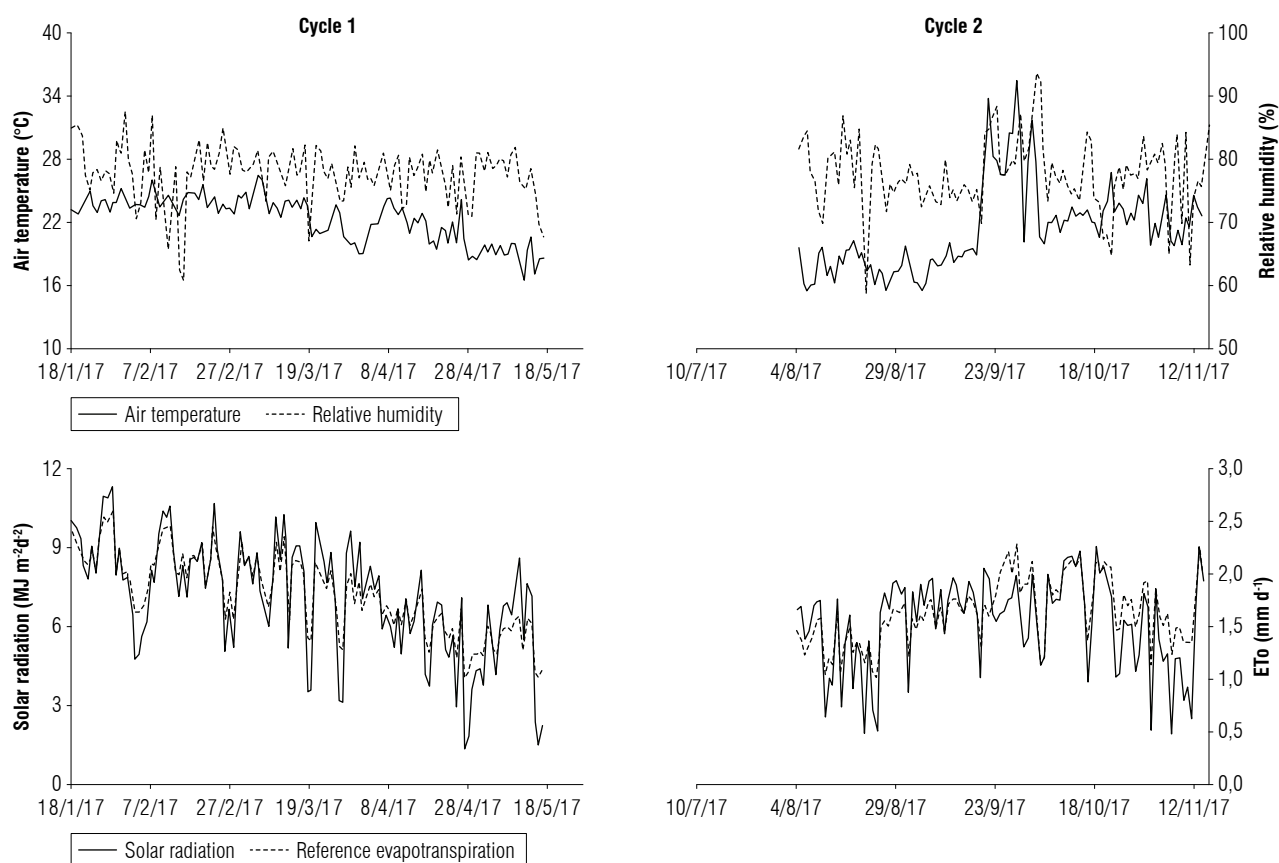


FIGURE 2. Daily variation in air temperature ($^\circ\text{C}$), relative air humidity (%), solar radiation ($\text{MJ m}^{-2} \text{ d}^{-1}$) and reference evapotranspiration ($E_{\text{To}} \text{ mm d}^{-1}$) for the two carrot cultivation cycles in 2017.

TABLE 3. Actual and total required irrigation applied in each carrot treatment and growing season. Viçosa, MG (Brazil), 2017.

Cycle	Parameter	Irrigation depths (% of E_{Tc})				
		50	75	100	125	150
1	Actual irrigation required (mm)	84.6	126.9	169.2	211.5	253.8
	Total irrigation required (mm)	91.6	137.3	183.1	228.9	274.7
2	Actual irrigation required (mm)	61.7	92.5	123.3	154.1	185.0
	Total irrigation required (mm)	66.7	100.1	133.4	166.8	200.2

Carrot cycle 1 showed higher water consumption due to meteorological elements that caused a higher average ETo in this cycle compared to the cultivation cycle 2. The drip irrigation system showed an efficiency of 92.4%.

Agronomic characteristics

There was no interaction between irrigation depths and carrot cultivars for any of the characteristics evaluated (Tabs. 4-5). This demonstrates that water supply affects carrots regardless of the variety being cultivated. Stress due to excess or deficit of water will have the same effect on carrot varieties, which confirms the hypothesis of the present

study. On the other hand, significance was observed, using the F test, for both the irrigation depth and carrot cultivars, independently. When there was no interaction between the factors (cultivar and environment) or significance in any of the factors evaluated.

Although crop yield did not vary significantly ($P>0.05$) between the cultivars, higher dry carrot mass and mass of 10 carrots values were observed for the Brasília cultivar, which tends to adapt better to the conditions of the protected environment studied. As the number of days until harvest

TABLE 4. Mean squares, of plant water potential (Ψ_p), aboveground length (AL), fresh aboveground mass (FAM), dry aboveground mass (DAM), carrot length (CL), average carrot diameter (ACD), carrot volume (CV), green shoulder (GS), damaged carrots (DC), fresh carrot mass (FCM), dry carrot mass (DCM), mass of 10 carrots (10CM), crop yield (CY) and water productivity (WP) as a function of different cultivars (Cult) and irrigation depths (ID) in two cultivation cycles.

Parameter	Cycle	Mean square		
		ID	Cult	ID x Cult
Ψ_p	1	2.0E+4 ^{ns}	4.9E+5**	6.2E+4 ^{ns}
(kPa)	2	8.2E+4 ^{ns}	1.2E+6**	4.1E+4 ^{ns}
AL	1	1.5E+2**	1.3E+2**	7.4E+1 ^{ns}
(cm)	2	9.0E+1**	2.1E+1 ^{ns}	9.9E+0 ^{ns}
FAM	1	3.2E+3**	2.5E+3 ^{ns}	1.8E+3 ^{ns}
(g)	2	7.0E+2**	9.4E+1 ^{ns}	9.4E+1 ^{ns}
DAM	1	6.6E+1*	6.9E+1 ^{ns}	3.0E+1 ^{ns}
(g)	2	6.3E+0 ^{ns}	3.1E-1 ^{ns}	1.9E+0 ^{ns}
CL	1	1.2E+1 ^{ns}	8.3E+1**	1.7E+1 ^{ns}
(cm)	2	9.5E+0 ^{ns}	4.7E+1**	3.9E+0 ^{ns}
ACD	1	7.6E+1 ^{ns}	1.1E+2**	2.8E+1 ^{ns}
(mm)	2	1.8E+1 ^{ns}	2.0E+1*	3.8E+0 ^{ns}
CV	1	8.8E+3*	5.9E+3 ^{ns}	4.7E+3 ^{ns}
(ml)	2	1.8E+3*	1.3E+3 ^{ns}	3.7E+2 ^{ns}
GS	1	2.4E+1 ^{ns}	7.0E+1 ^{ns}	2.5E+1 ^{ns}
(cm)	2	1.4E+1 ^{ns}	3.7E+0 ^{ns}	4.9E+0 ^{ns}
DC	1	2.3E+0 ^{ns}	3.5E+0 ^{ns}	1.6E+1 ^{ns}
(un/10)	2	7.7E-1 ^{ns}	3.8E-1 ^{ns}	2.0E-1 ^{ns}
FCM	1	6.9E+3 ^{ns}	8.9E+3 ^{ns}	4.5E+3 ^{ns}
(g)	2	2.4E+3*	1.6E+3 ^{ns}	4.0E+2 ^{ns}
DCM	1	7.1E+1 ^{ns}	3.0E+2*	1.0E+2 ^{ns}
(g)	2	1.2E+1 ^{ns}	7.0E+1*	1.6E+1 ^{ns}
10CM	1	5.0E+5*	3.7E+5*	2.0E+5 ^{ns}
(g)	2	1.9E+4 ^{ns}	4.9E+4**	6.1E+3 ^{ns}
CY	1	2.3E+8 ^{ns}	1.5E+9 ^{ns}	7.5E+8 ^{ns}
(kg m ⁻²)	2	4.0E+8*	2.7E+8 ^{ns}	6.6E+5 ^{ns}
WP	1	1.6E+4**	1.5E+3 ^{ns}	6.6E+2 ^{ns}
(kg m ⁻³)	2	3.2E+3**	2.4E+2 ^{ns}	4.8E+1 ^{ns}

ID x Cult: interaction between ID and Cult; ETC: crop evapotranspiration; * and **: significance at 5% and 1% probability, respectively.

TABLE 5. Significance of the F test and mean values of plant water potential (Ψ_p), aboveground length (AL), fresh aboveground mass (FAM), dry aboveground mass (DAM), carrot length (CL), average carrot diameter (ACD), carrot volume (CV), green shoulder (GS), damaged carrots (DC), fresh carrot mass (FCM), dry carrot mass (DCM), mass of 10 carrots (10CM), crop yield (CY) and water productivity (WP) as a function of different cultivars (Cult) and irrigation depths (ID) in two carrot cultivation cycles.

Parameter	Carrot cultivars			
	Brasília	Alvorada	Esplanada	Nantes
Ψ_p	730.9 b	755.0 b	730.8 b	1051.5 a
(kPa)	1261.7 a	1275.5 a	973.9 b	779.1 b
AL			$\bar{y} = 66.6$	
(cm)			$\bar{y} = 43.4$	
FAM			$\bar{y} = 83.4$	
(g)			$\bar{y} = 26.1$	
DAM			$\bar{y} = 13.1$	
(g)			$\bar{y} = 11.2$	
CL	21.3 ab	18.6 b	21.2 ab	23.6 a
(cm)	15 a	13.2 b	16.5 a	16.5 a
ACD	35.1 ab	36.8 a	31.8 b	32.5 b
(mm)	25.8 ab	25.4 ab	23.8 b	26.1 a
CV			$\bar{y} = 230.9$	
(ml)			$\bar{y} = 80.7$	
GS			$\bar{y} = 12.5$	
(cm)			$\bar{y} = 7.8$	
DC			$\bar{y} = 1.4$	
(un/10)			$\bar{y} = 0.83$	
FCM			$\bar{y} = 224.9$	
(g)			$\bar{y} = 89.5$	
DCM	40.5 a	34.2 ab	31.5 b	37.1 ab
(g)	15.3 a	12.6 ab	11.2 b	14.7 ab
10CM	1805.0 a	1511.4 b	1559.4 ab	1532.9 ab
(g)	708.7 a	637.9 ab	591.2 b	626.6 b
CY			$\bar{y} = 9.2$	
(kg m ⁻²)			$\bar{y} = 3.6$	
WP			$\bar{y} = 85.2$	
(kg m ⁻³)			$\bar{y} = 39.3$	

Means followed by the same letter in the line do not differ significantly according to the Tukey test ($P<0.05$).

was the same for all cultivars analyzed, the precocity of the Brasília cultivar probably favored it, allowing more time for root development in relation to the other cultivars. Due to its ability to adapt and prosper in the environment of the present study, the Brasília cultivar will present greater economic viability, improving the farmer's income. Larger carrots can offer advantages such as industrial processing efficiency, consumer appeal and practicality in certain culinary preparations.

In contrast, the Esplanada cultivar proved to be less adapted to the environment studied. Their responses to greenhouse conditions were less favorable, resulting in lower performance in several agronomic characteristics. The inferiority of Esplanada in relation to Brasília may have been caused by genetic differences. This indicates that although the Esplanada cultivar may be suitable for other growing conditions or environments, its performance in this specific environment was limited compared to the Brasília cultivar.

The Alvorada and Nantes cultivars were intermediate between the two cultivars. Although the Nantes cultivar is recommended for regions or seasons with milder temperatures (cold climate), in general, it presented intermediate development. The coverage with blue plastic film used in the study environment reduced the direct incidence of light on the plants. In the experiment, the irrigation levels were controlled and lower than the rainy periods that occur in the Brazilian summer. Furthermore, the experiment was carried out in a region far from carrot production centers, which contributes to the low inoculum pressure of foliar diseases that attack this crop in the summer. The use of plastic film compared to the external environment provided increases of 5.0% and 7.1% in air temperature for cultivation cycles 1 and 2, respectively.

The crop yield values in both cycles can be considered high, indicating that the experimental conditions provided the cultivars with adequate expression of their productive potential. It is worth highlighting that experiment averages were almost always higher than national production averages. The yields were higher than the world averages of 2.2 kg m^{-2} and 3.0 kg m^{-2} and higher than the national averages of 2.9 kg m^{-2} and 3.1 kg m^{-2} reported by Resende and Braga (2014) and by Carvalho *et al.* (2017), respectively.

The discrepant values of the crop yield between the two carrot cultivation cycles (Tab. 3), possibly, occurred due to the variation between the cycles of potential soil salinity caused by high salt contents accumulated in the soil used

in the present research. The maximum and minimum temperatures during the period may also have contributed. Resende and Cordeiro (2007) found a variation in carrot productivity from 3.7 to 8.1 kg m^{-2} (also higher than the world and national averages mentioned) depending on the quality of the irrigation water applied. The highest value of electrical conductivity (8.0 dS m^{-1}) promoted the lowest productivity (3.3 kg m^{-2}) and the lowest electrical conductivity (normal water at 0.1 dS m^{-1}) increased carrot productivity (8.1 kg m^{-2}).

The reduction in solar radiation (Fig. 2) within the protected environment, for cycle 2 compared to cycle 1, possibly, reduced the photosynthetic rate of the carrot crop in cycle 2, contributing to the difference in crop yield between cycles. However, there were no measures implemented in cycle 2 to compensate for this reduction in solar radiation, as no variations in performance were anticipated.

Luz *et al.* (2009) found carrot yield values of 3.8 , 3.6 , and 3.1 kg m^{-2} for the cultivars Brasília, Alvorada and Nantes, respectively, grown in open field. Resende and Braga (2014) found higher yield values for carrot crops: Brasília (9.6 kg m^{-2}), Alvorada (8.2 kg m^{-2}), Esplanada (6.5 kg m^{-2}), and Nantes (7.0 kg m^{-2}). Such crop yield values were close to those found in cycle 1 of the present study. This result reinforces that the study was well conducted in both cycles. It also reinforces that the results found were sufficient to elaborate the conclusions of the study.

The behavior of the agronomic characteristics of the studied carrot cultivars, depending on the different irrigation depths, is shown in Figure 3. The aboveground length (AL) and dry aboveground mass (DAM) of cycle 1 showed quadratic behavior depending on the irrigation depths. According to the regression equations, the irrigation depths that maximized AL and DAM were 143% of ETc and 128% of ETc, with values of 68.9 cm and 14.3 g , respectively. Water productivity (WP) in cultivation cycle 1 also showed quadratic behavior, with the irrigation depth of 145% of ETc minimizing this characteristic, with a value of 57.9 kg m^{-3} . The other agronomic characteristics suffered a linear effect, in which WP decreased as water supply increased and the others showed a positive effect.

The highest values of the agronomic characteristics that confer better plant development were obtained by applying the highest irrigation depth studied (150% of ETc), which is higher than 100% of the ETc replacement. This possibly occurred due to the overall efficiency of the system.

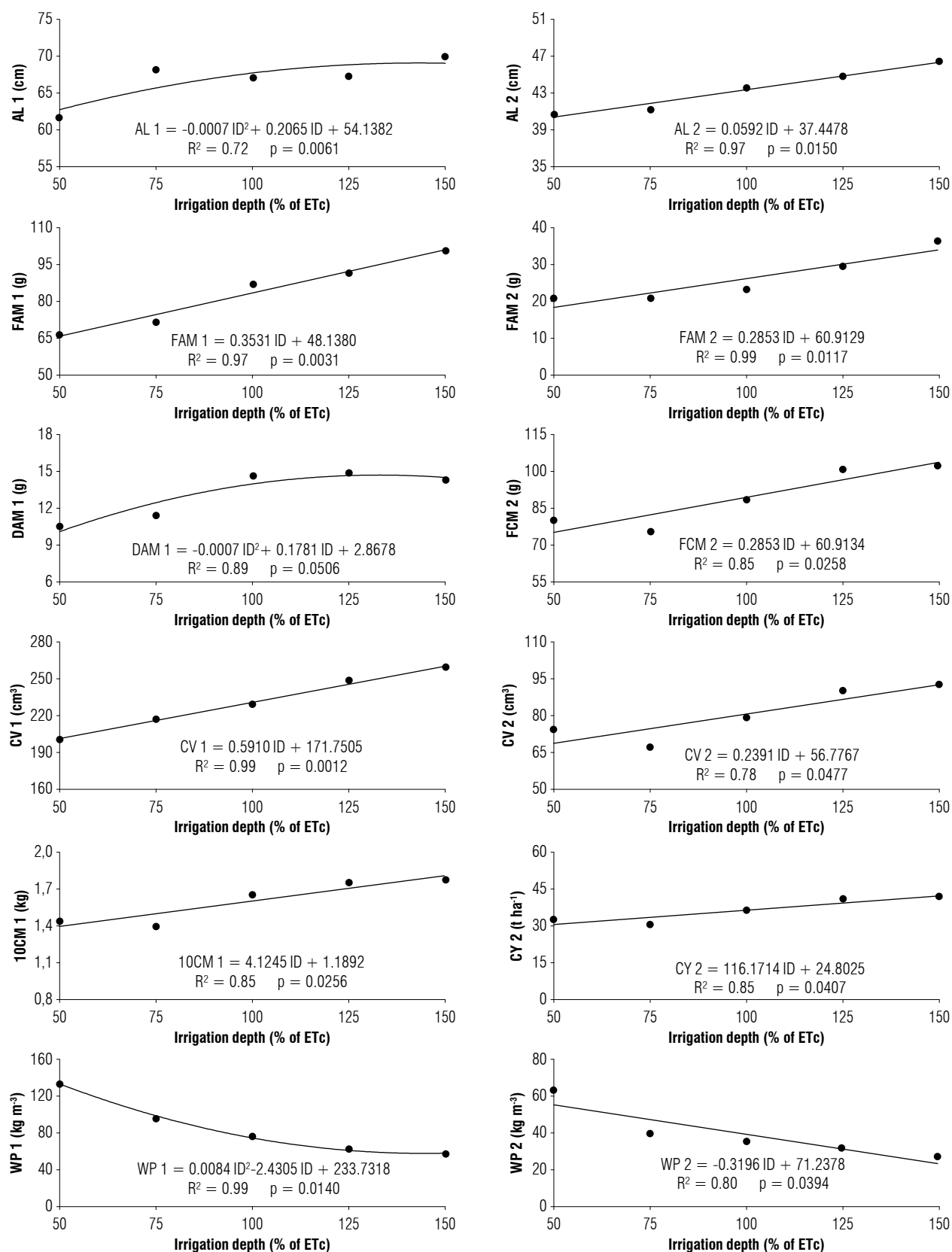


FIGURE 3. Average values of aboveground length (AL), fresh aboveground mass (FAM), dry aboveground mass (DAM), fresh carrot mass (FCM), carrot volume (CV), mass of 10 carrots (10CM), crop yield (CY), and water productivity (WP) for cultivation cycles 1 and 2 as a function of irrigation depths (ID).

Since there is no 100% water absorption efficiency — due to losses from percolation, water redistribution in the soil, and areas with water deficit (Sousa & Assunção, 2021) — the 100% irrigation depth may not have provided sufficient water for the plants to reach their maximum productive potential. Additionally, the electrical conductivity of the soil also influenced the results.

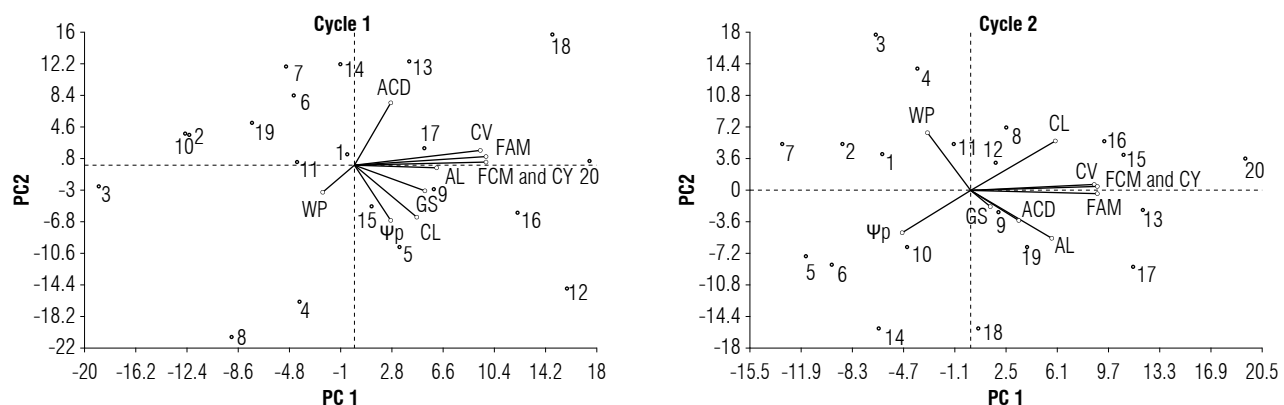
Although the highest water productivity was observed for the lowest irrigation depth used (50% of ETc), the highest carrot yield in cycle 2 was achieved with application of 150% of ETc (Fig. 3). Plants exposed to water stress (application of smaller irrigation depths) suffer a decline in leaf water potential, stomatal conductance and CO₂ flux, impacting the accumulation of photoassimilates and crop yield (Hussain *et al.*, 2020; Fang *et al.*, 2021). This fact can also be explained by the increase in the concentration of abscisic acid (ABA) in the xylem of some species (Lamarque *et al.*, 2020; Ramachandran *et al.*, 2021).

Identification of patterns and trends

Figure 4 presents the principal component analysis (PCA) of the present study. The intensity of the association between the evaluated agronomic characteristics is indicated

by the angle between the direction of their corresponding vectors, *i.e.*, the smaller the angle between the vectors, the greater the positive correlation between the represented characteristics. Thus, although there is a positive correlation, characteristics such as aboveground length, carrot volume, fresh aboveground mass and fresh carrot mass contributed more significantly to obtaining the high crop yield values in both cycle 1 and cycle 2 (Fig. 4).

In cycle 1, almost all characteristics evaluated had a positive association with crop yield, except water productivity, which displayed an inverse relationship with crop yield. This means that the highest crop yield value was achieved when water productivity was lower, and conversely, when water productivity was higher, crop yield decreased (Fig. 4). Most treatments that included irrigation lower than 100% of ETc (50 and 75%) were represented to the left of the central axis, with negative PC1 values (Fig. 4), showing a strong negative relationship with most of the variables of interest, demonstrating that these irrigation depths did not favor carrot yield. On the other hand, most treatments with water depths greater than 100% (125 and 150%) present positive PC values, indicating that higher irrigation depths provide higher crop yield values.



Treatment	Irrigation (% ETc)	Carrot cultivar	Treatment	Irrigation (% ETc)	Carrot cultivar	Treatment	Irrigation (% ETc)	Carrot cultivar	Treatment	Irrigation (% ETc)	Carrot cultivar
1	50	Brasília	6	75	Alvorada	11	100	Esplanada	16	125	Nantes
2	50	Alvorada	7	75	Esplanada	12	100	Nantes	17	150	Brasília
3	50	Esplanada	8	75	Nantes	13	125	Brasília	18	150	Alvorada
4	50	Nantes	9	100	Brasília	14	125	Alvorada	19	150	Esplanada
5	75	Brasília	10	100	Alvorada	15	125	Esplanada	20	150	Nantes

FIGURE 4. Principal component analysis (PCA) for carrot length (CL), aboveground length (AL), fresh aboveground mass (FAM), average carrot diameter (ACD), carrot volume (CV), green shoulder (GS), fresh carrot mass (FCM), crop yield (CY), water productivity (WP), and plant water potential (Ψ_p) as a function of different cultivars and irrigation depths in two cultivation cycles.

The preference for a 150% ETc level to obtain higher values of the studied characteristics is supported by Figure 3, which indicates that treatments 17, 18, 19, and 20 favored crop yield. This becomes even more evident in treatments 15, 16, and 17 and 20 as shown in Figure 4.

The irrigation depth of 150% ETc provided a higher carrot yield during the second cultivation. However, in practice, its use becomes unnecessary due to the lack of significant difference in cycle 1 (Tab. 3) and the marginal gain with 150% ETc replacement (Fig. 3). Therefore, a 100% ETc level is recommended for carrot cultivation under the conditions of this research.

Furthermore, although no statistical difference was observed between the cultivars based on the analysis of variance and mean test (Tab. 1), the principal component analysis (PCA) showed that almost all treatments containing the Brasília cultivar performed better, regardless of the irrigation depth, except for treatment 1 (Fig. 4), where the increased water stress mitigated the preference for this cultivar. This reveals that in conditions of reduced water supply, carrot varieties are unable to express their productive potential.

The observations in this study highlight the importance of choosing appropriate carrot cultivars for specific growing conditions, considering factors such as the protected environment, local climate and management practices. Furthermore, this study also offers valuable insights for farmers and agricultural professionals. With these results, producers can make more informed decisions when choosing the most suitable cultivars for their specific growing conditions, aiming to optimize performance and productivity. These findings have the potential to improve the efficiency and quality of carrot production, benefiting both producers and end consumers.

Conclusions

The Brasília carrot cultivar showed a tendency for better development in the protected environment studied. In contrast, the Esplanada cultivar proved to be less adapted to the environment, indicating the importance of choosing suitable cultivars for specific growing conditions.

All carrot cultivars were affected by stress caused by both excess and insufficient water. Irrigation depths greater than 100% of ETc provided better crop performance. However, the difference between 100% and 150% ETc was minimal,

making the 100% ETc a viable choice for growing carrots under conditions similar to those of the present study.

Based on the findings of the present study, producers should choose the most appropriate carrot cultivars for specific growing conditions, considering the protected environment and management practices. Careful selection of the irrigation depth is also crucial to optimize crop performance. Our results offer valuable guidance for farmers in making informed decisions and can improve the efficiency and quality of carrot production.

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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

CMG, FCS, DJHS, and FFC – conceptualization; CMG, FCS, and FFC – methodology; CMG, FCS, and FFC – formal analysis; EDA, ABFG, and CMG – research development; DJHS, and FFC – resources; CMG, FCS, JTO, and FFC – writing and original draft preparation; JTO and FFC – writing, review and editing; DJHS and FFC – funding acquisition. All authors have read and agreed with the final version of the manuscript.

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Correlations between soil properties and spectral index (healthy vegetation) in soybean crops

Correlaciones entre propiedades del suelo e índice espectral (vegetación saludable) en cultivos de soya

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ABSTRACT

Precision agricultural technologies, such as the use of spatial variability of soil properties, have been extensively studied for soybean cultivation. The objective of this study was to analyze the spatial variability of soil properties cultivated with soybean and to correlate the healthy vegetation (HV) spectral index with the bands B8A (classifying vegetation - 865 nm), B11 (measuring the moisture content of soil and vegetation - 1610 nm), B02 blue (useful for soil and vegetation discrimination - 490 nm). A sampling grid was installed for data collection in an area of 2,126.02 ha, with 270 regular points and 98 random points, totaling 368 points. For the soil, the contents of P (resin), K⁺, Ca²⁺, Mg²⁺, H⁺, Al³⁺, pH values, sum of bases (SB), cation exchange capacity (CEC), and base saturation were determined at a depth of 0.0 to 0.20 m. Most of the soil properties had exponential and spherical dependence. Clay percentages and Ca, Mg, and P contents had positive spatial correlation with the healthy vegetation spectral index (HV) while no spatial correlation was observed for pH, B, K, silt, sand, S, H+Al, Al, SB, and CEC. The sensor image used in this study in relation to HV showed good application for observing the spatial variability of the soil properties and soybean yield.

Key words: *Glycine max*, spatial variability, direct seeding system.

RESUMEN

Las tecnologías de agricultura de precisión, como el uso de la variabilidad espacial en las propiedades del suelo, han sido ampliamente estudiadas para el cultivo de soya. El objetivo de este estudio fue analizar la variabilidad espacial de las propiedades del suelo cultivado con soya y correlacionarla con el índice espectral de vegetación saludable (VS), con las bandas B8A (clasificación de la vegetación (865 nm)), B11 (medición del contenido de humedad del suelo y la vegetación (1610 nm)), B02 azul (útil para la discriminación del suelo y la vegetación (490 nm)). La malla de muestreo se instaló para la recolección de datos en un área de 2,126.02 ha, con 270 puntos regulares y 98 puntos aleatorios, totalizando 368 puntos. Se determinaron en suelo los contenidos de P (resina), K⁺, Ca²⁺, Mg²⁺, H⁺, Al³⁺, valores de pH, suma de bases (SB), capacidad de intercambio catiónico (CIC), y saturación de bases en una muestra del suelo de 0.0 a 0.20 m de profundidad. La mayoría de las propiedades del suelo tuvieron dependencia exponencial y esférica. El porcentaje de arcilla y los contenidos de Ca, Mg y P mostraron una correlación espacial positiva con el índice espectral de vegetación saludable (VS) mientras que no se observó correlación espacial con los valores de pH, B, K, limo, arena, S, H+Al, Al, SB y CIC. La imagen del sensor utilizada en este estudio en relación con la VS mostró una buena aplicación para observar la variabilidad espacial de las propiedades del suelo y la producción de soya.

Palabras clave: *Glycine max*, variabilidad espacial, sistema de siembra directa.

Introduction

In Brazil, the growing expansion of soybean cultivation highlights its importance and relevance on the national and global scene as one of the largest soybean producers in the world. The 2023/2024 national harvest reached 160 million t, 3.6% above the volume harvested in the previous season and an absolute production record until then (Conab, 2023).

Vegetation indices can be used as productivity indicators for a crop for different types of vegetation studies and vegetation monitoring. For example, the indices can be used to monitor vegetation activity and health, in addition to monitoring drought conditions and plant senescence (Semeraro *et al.*, 2019). In fact, these indices are capable of characterizing variations in the phenology and photosynthetic

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potential of crops that are useful for identifying cultivation and the growth cycle (Malladi & Sowlati, 2017).

Monitoring and mapping crops on a large scale are essential for assisting in management and decision-making for various crops, thus improving production efficiency more technologically (Chen *et al.*, 2023; Zhao *et al.*, 2023). However, remote sensing is an effective strategy, allowing better precision in agricultural monitoring. A more sophisticated imaging technique called hyperspectral imaging uses data collected in a wide spectral range with the aim of reconstructing a spatial representation of the plant under analysis through image processing procedures generating highly specialized healthy vegetation (HV) maps (Otone *et al.*, 2024).

The spatial analysis of soil chemical properties can indicate management alternatives, not only to reduce the effects of soil variability on crop (Rodrigues, Roque *et al.*, 2023) and plant production but also to increase the possibility of estimating responses of crops under certain management practices. Such techniques provide methods to quantify this spatial autocorrelation to incorporate it in the estimation of values in unobserved locations (Oliveira, Oliveira, Rojas Plazas, Andrade *et al.*, 2023).

Geostatistics have been shown to be the most effective method for analyzing spatial distribution features and patterns of variation of soil properties (Cortes *et al.*, 2023). The semiovariogram can be used to describe the spatial variability of soil properties (Oliveira, Oliveira, Rojas Plazas & Roque, 2023). The use of geostatistical techniques facilitates the interpretation of the behavior of soil properties for better decision-making in management. When analyzing a lot of information simultaneously, multivariate statistics become the best tool. Therefore, some studies have applied multivariate techniques to evaluate soil variables and they have found satisfactory results (Oliveira, Oliveira, Rojas Plazas, Andrade *et al.*, 2023; Rodrigues, Castro *et al.*, 2023).

The objective of this study was to analyze the spatial variability of soil attributes and correlate it with the healthy vegetation (HV) spectral vegetation index in a field cultivated with soybean.

Materials and methods

This study of the spatial variability of the reflectance index in soybean cultivation was carried out at Fazenda Emiliana in the municipality of Balsas (MA), Brazil, in 2019 (Fig. 1). The region's climate is tropical, type Aw according to

Köppen and Geiger, with an annual mean temperature of 26.4°C and a mean annual rainfall of 1190 mm.

We used an orbital image from the sentinel-2 satellite with a spatial resolution of 10 m, captured on January 8, 2019 with a healthy vegetation (HV) image, already processed and acquired by the earth observing system (EOS), adopting the WGS 84 datum and referencing the reproductive growth period of the R5 crop. Visual georeferencing was carried out using a polygon of the study region.

The soil was classified as a typical dystrophic red latosol with a very clayey to moderate dystrophic texture (Santos *et al.*, 2018). The soil had been cultivated with a succession of soybean/corn crops sown in the summer and off-season. The Monsoy soybean variety was sown on November 20, 2018. We sowed the crop using a 0.50 m space between rows with a density of 14 seeds m⁻¹ resulting in a density of 280,000 plants ha⁻¹. We covered the seeds with millet straw in a direct sowing system. Common cultivation practices such as phytosanitary and chemical treatments were carried out homogeneously throughout the experimental area.

We collected data from three plots totaling 2,126.02 ha where we determined soil and plant sample collection points using a regular mesh distributed randomly over the plots with 270 regular points and 98 random points totaling 368 points (Fig. 2).

The soil samples were collected on April 10, 2019 using a caneco auger at the 0.0-0.20 m depth with 4 simple subsamples collected to obtain a composite sample to determine soil properties. Analysis for soil chemical properties included the following: pH_(CaCl2), phosphorus content (P) (mg dm⁻³), potassium content (K) (mmolc dm⁻³), calcium (Ca) (mmolc dm⁻³), magnesium (Mg) (mmolc dm⁻³), aluminum (Al) (mmolc dm⁻³), potential acidity (H+A) (mmolc dm⁻³), boron (B) (mg dm⁻³) and sulfur (S) (mg dm⁻³) according to Teixeira *et al.* (2017).

Statistical analysis included an exploratory analysis in which a variable under study was characterized; consequently, its behavior and data distribution were identified and evaluated. The following descriptive statistics were generated: mean, median, minimum, maximum, standard deviation, and coefficient of variation. To classify the coefficient of variation (CV), the following classes and magnitudes were adopted: low (CV ≤ 10%), medium (10% < CV ≤ 20%), high (20% < CV ≤ 30%) and very high (CV > 30%) variability (Oliveira *et al.*, 2022).

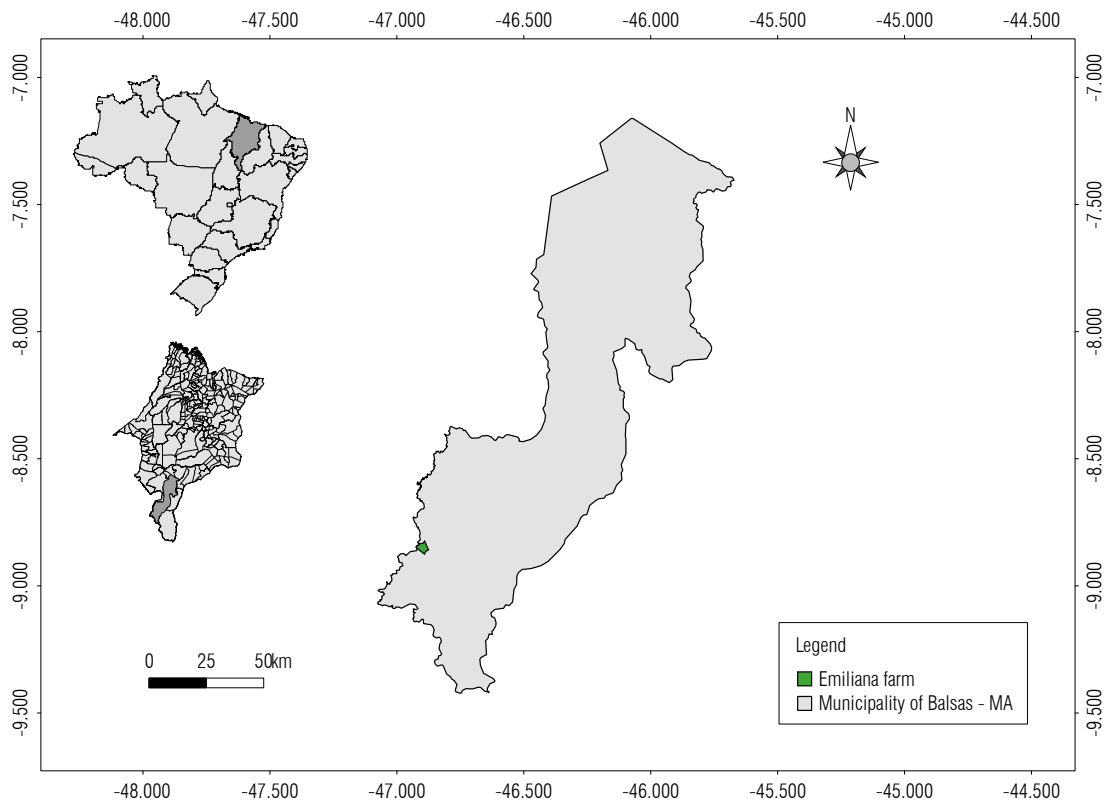


FIGURE 1. Location of the study area in Brazil.

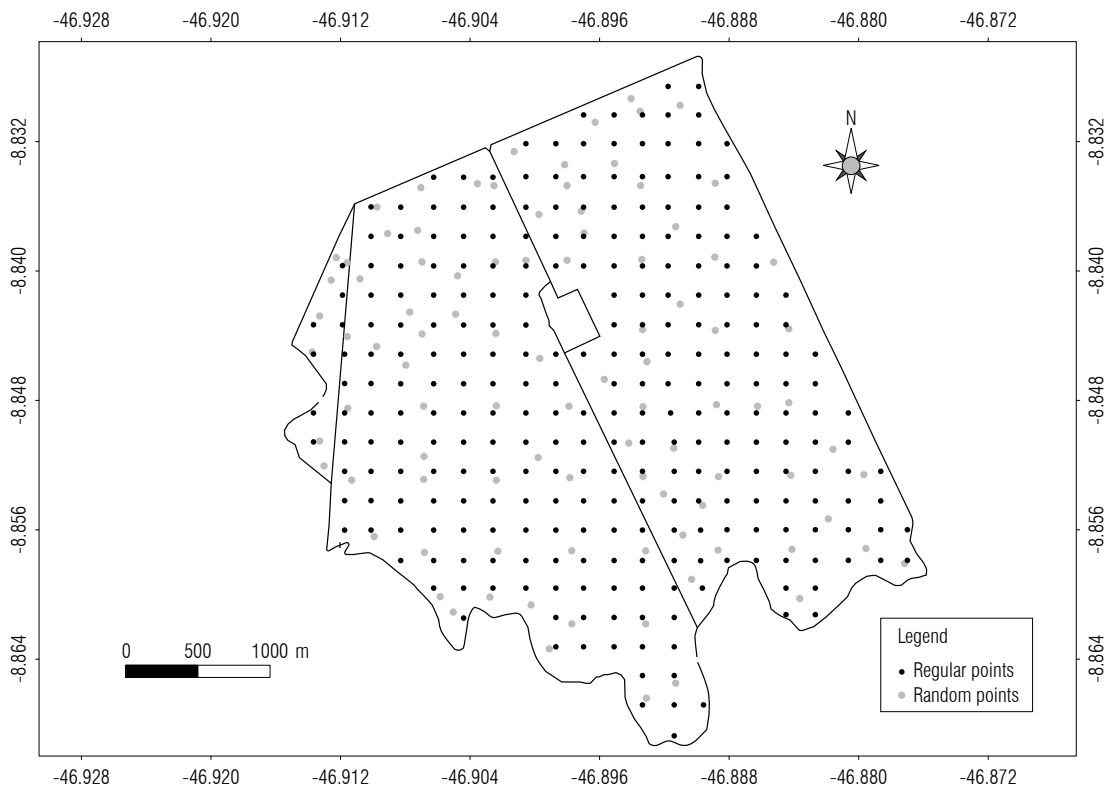


FIGURE 2. Sampling grid at Fazenda Emiliana located in the municipality of Balsas (MA), Brazil.

To evaluate the degree of relationship between the variables involved in the modeling process, a Pearson correlation analysis was carried out to calculate simple linear regressions for combinations, two by two, between all the soil properties studied. Therefore, linear correlations with higher values, that is, higher significance, were selected for regression modeling. A fundamental step that preceded geostatistical analysis was carried out as a careful exploratory analysis of the data.

The analysis of the spatial dependence of soil chemical attributes was carried out by calculating the semivariogram based on the stationarity assumptions of the intrinsic hypothesis. Adjustments to the simple semivariograms, depending on their models, were made primarily by initially selecting the following: a) the lowest sum of squared deviations (SSD), b) the highest coefficient of determination (r^2), and c) the highest evaluator of the degree of spatial dependence (DSD). The final decision of the model that represented the adjustment was made by cross validation as well as to define the size of the neighborhood that provided the best kriging mesh (Oliveira *et al.*, 2022).

For each soil property, the nugget effect (C_0) and the range (A_0) and threshold ($C_0 + C$) were related. The analysis of the dependence evaluator (DSD) was carried out according to Cambardella *et al.* (1994), modified by the GS+ Software according to Equation 1:

$$DSD = \left[\frac{C}{(C_0 + C)} \right] \times 100 \% \quad (1)$$

The proposed interpretation for the DSD was in accordance with Dalchiavon *et al.* (2012):

DSD < 20 % = very low dependence spatial variable (VL),

a) 20 % ≤ DSD < 40 % = low dependency (LO),

b) 40 % ≤ DSD < 60 % = medium dependence (ME),

c) 60 % ≤ DSD < 80 % = high dependence (HI), and

d) 80 % ≤ DSD < 100 % = very high dependency (VH).

In order to analyze the maps to define which had the best visual relationship with productivity, the relative deviation coefficient (RDC; Eq. 2) was proposed. It should be noted here that the RDC calculates the average difference in modulus of the interpolated values on a thematic map when compared with a map assumed as a reference. The justification is that the objective was to estimate departures from thematic maps using more friendly interpolators

other than kriging that is considered the best interpolator but which has difficulties in its implementation according to Equation 2.

$$RDC = \sum_{i=1}^n \frac{(P_{ijk} - P_{ipad})}{P_{ipad}} * \frac{100}{n} \quad (2)$$

where: n = number of interpolated points; P_{ipad} = reference search at point i; P_{ijk} = point test for sampling method.

Results and discussion

Analyzing the properties presented in Table 1, we found that H+Al (8.67%), silt (9.66%), clay (6.83%), yield (PROD) (6.81%), and pH (2.84%) showed low CV. In relation to the other attributes, the analyzes showed the following average CVs: Ca (15.20%), K (17.66%), Mg (19.93), sand (13.38%) and SB (15.99%) and very high CVs as follows: P (68.81%), B (79.47%), S (36.04%) and Al (51.68%). Analyzing the soil properties, we observed that the pH showed low variability in accordance with results obtained by Oliveira *et al.* (2020), whose study of the spatial variability of soil properties obtained results of 4.9% and 5.3% for depths of 0.00 to 0.10 m and 0.10 to 0.20 m.

The soil property of P content (68.81%) showed very high variability in line with Lima *et al.* (2013), who also found very high variability of 32.1% and 48.0% for phosphorus at both soil depths. The residual presence of fertilizers used in previous harvests in the study area may explain the change in phosphorus concentrations in the soil through the history of analyzes of the area (Oliveira *et al.*, 2021).

Dalchiavon *et al.* (2011) suggest that the P content in soil may be related to direct sowing by not disturbing the soil, reducing contact between colloids and the phosphate ion, softening adsorption reactions, especially due to the fact that organic P, originating from the decomposition of the remaining roots along the soil profile, constitutes an important reserve of labile P for plants in the deeper layers of the soil.

Potassium (K) (17.66%) showed medium variability, different from that obtained by Dalchiavon *et al.* (2011), where the authors found very high variability for K (38.5%). The authors describe the high variability for K due to the residual presence of fertilizers previously used in the predecessor crop (corn).

In Table 1, both Ca (15%) and Mg (19.93%) showed average variability, disagreeing with Alves *et al.* (2014), who obtain magnesium CV values in a direct planting system of around 35% and for conventional tillage of 49%. The

TABLE 1. Initial descriptive statistics of soybean productivity and some properties of a dystrophic red oxisol at Fazenda Emiliana located in the municipality of Balsas (MA), Brazil.

Property	Average	Median	Minimum	Maximum	CV (%)	Asymmetry	Kurtosis	Pr<W
Ca	2.70	2.57	2.54	2.72	15.20	-0.23	6.00	0.00
H+Al	2.60	2.65	1.90	3.80	8.67	1.07	7.57	0.00
K	0.09	0.08	0.06	0.17	17.66	3.29	4.09	0.00
Mg	1.21	1.12	1.16	1.21	19.93	-0.01	5.86	0.30
P	6.05	5.11	5.44	8.67	68.81	0.23	31.79	0.13
B	0.29	0.26	0.11	1.55	49.47	0.34	46.88	0.03
Silt	106.66	103.98	75.00	175.00	9.66	-0.12	10.49	0.00
Clay	580.55	574.30	529.88	632.19	6.83	-0.51	3.23	0.00
S	7.42	7.01	4.00	32.00	36.04	0.31	23.51	0.09
Sand	312.72	322.02	207.62	357.62	13.38	0.21	4.03	0.29
PROD	2996.25	3115.13	2662.6	3129.14	6.91	-1.97	-1.01	0.00
Al	0.01	0.00	0.00	0.60	51.68	-0.24	207.17	0.00
pH	5.59	5.52	5.20	6.10	2.84	1.08	0.84	0.00

P, pH, K, Ca, Mg, H+Al, Al, PROD, B, and S: phosphorus extracted by resin (mg dm⁻³), hydrogen potential (pH_{CaCl2}), potassium (mmolc dm⁻³), calcium (mmolc dm⁻³), magnesium (mmolc dm⁻³), potential acidity (H+Al), exchangeable aluminum (mmolc dm⁻³), soybean yield (PROD, kg ha⁻¹), boron (mg dm⁻³), sulfur (mg dm⁻³). CV: coefficient of variation; Pr<W: probability of the normality test.

non-conformity of the Ca and Mg levels can be explained by the failure to carry out liming in the last 3 years and that was only carried out in 2013. Bottega *et al.* (2013), evaluating the variability of Al, obtain very high CV values like those found in this research that can also be explained by the lack of liming.

The asymmetry and kurtosis coefficients were different from zero for all tested variables. The asymmetry was negative for properties Ca, Mg, silt, clay, PROD, Al, and SB. However, kurtosis showed negative values only for PROD. Similar results were found by Cambardella *et al.* (1994). Skewness represents the degree of deviation of a curve in the horizontal direction that can be positive with a greater concentration of high values or negative with a predominance of low values.

The study of Pearson's linear correlations was presented through a network of correlations where the green lines represent positive correlations, and the red lines represent negative correlations. The thickness of the line represents the degree of correlation between the variables. In other words, the thicker the line, the greater the correlation (Fig. 3). There were positive and significant correlations of PROD with Ca ($r = 0.78$), Mg ($r = 0.87$), P ($r = 0.63$), Clay ($r = 0.81$), and HV ($r = 0.89$). Oliveira *et al.* (2020) also obtain positive direct relationships between bean productivity and pH, Mg, and soil base saturation. In Figure 3, positive correlations

were also observed between Ca and Mg ($r = 0.94$), indicating significant correlations at 5% and demonstrating strong correlation.

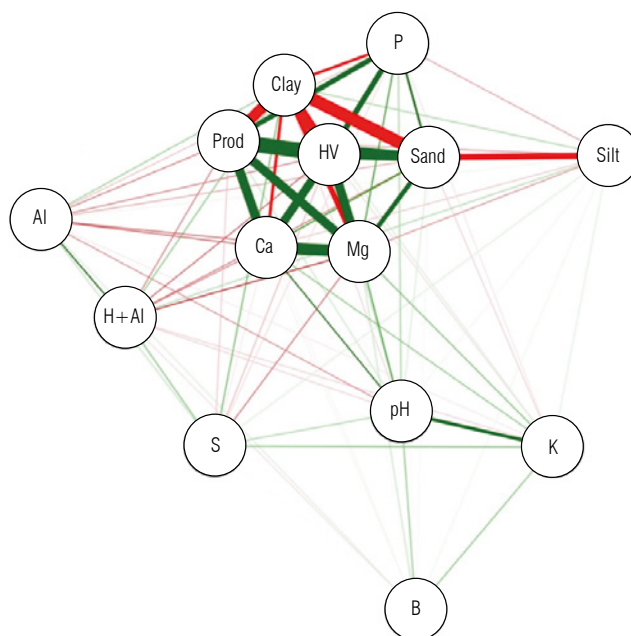


FIGURE 3. Network of correlations of soybean yield, HV index, and some properties of a dystrophic red oxisol at Fazenda Emiliana in the municipality of Balsas (MA), Brazil. P, pH, K, Ca, Mg, H+Al, Al, PROD, B, S, and HV are: phosphorus extracted by resin, hydrogen potential, potassium, calcium, magnesium, potential acidity, exchangeable aluminum, soybean yield, boron, sulfur, and healthy vegetation index, respectively.

Considering the vegetation spectral index HV as the main variable for analysis, it resulted in the same positive correlations as PROD with the chemical attributes of the soil (Fig. 3), that is, HV established positive and significant correlations with Ca ($r = 0.69$), Mg ($r = 0.79$), and Clay ($r = 0.71$).

Figure 4 presents the linear correlations of soil properties with the HV of soybean crops. The simple linear correlation coefficients were found for the soil properties, and also the linear correlation was obtained between HV and PROD. Therefore, to correlate HV with soil properties, the analysis of spatial dependence is justified to better understand the

pattern of occurrence of these properties in space that are very positive. In a study by Oliveira, Oliveira, Rojas Plazas, Andrade *et al.* (2023), the authors highlight the importance of the spatial autocorrelation of soil properties and soybean planting, presenting significance maps and correlation with crop productivity.

The geostatistical analysis (Tab. 2) showed that there was spatial dependence for the semivariogram of the Ca, Mg, and HV properties adjusted to the exponential model, while PROD and clay adjusted to the spherical model.

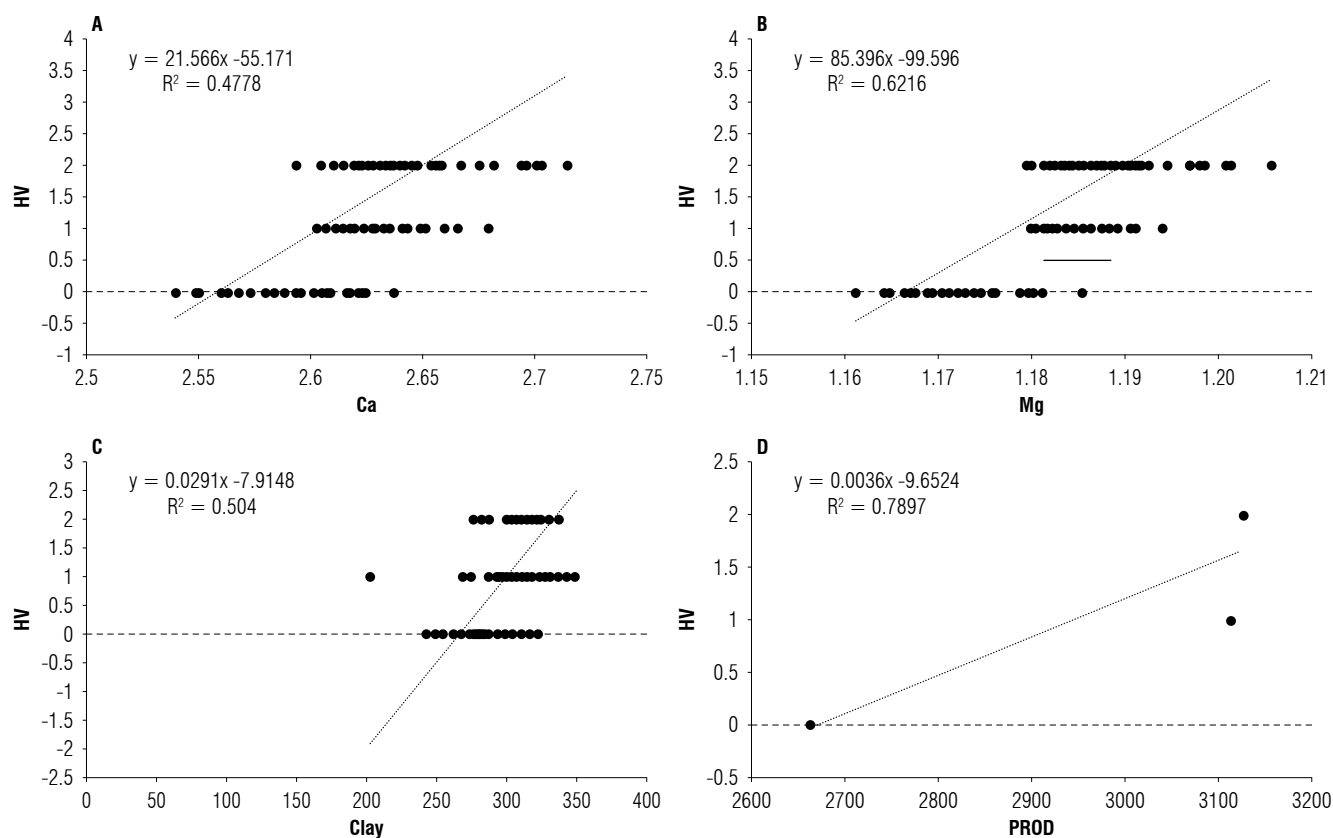


FIGURE 4. Regression equations of the HV – healthy vegetation spectral index of soybeans with all properties that demonstrated a positive relationship. A) Ca (mmolc dm^{-3}), B) Mg (mmolc dm^{-3}), C) Clay (g), and D) yield (PROD, kg ha^{-1}).

TABLE 2. Semivariogram parameters.

Property	Model	C_0	$C_0 + C$	$A_0(\text{m})$	R^2	DSD	
						%	Class
Ca	Exponential	0.00046	0.00092	222.3	0.677	50.10	ME
Mg	Exponential	0.00004	0.00007	1417.0	0.815	50.10	ME
PROD	Spherical	12750.00000	42970.00000	1273.0	0.982	70.30	HI
Clay	Spherical	127.10000	254.30000	1124.0	0.872	50.00	ME
HV	Exponential	0.09300	0.66300	67.0	0.473	86.00	VH

P, Ca, Mg, PROD; HV: phosphorus, calcium, magnesium, soybean yield, healthy vegetation index, respectively. ME: medium dependence; HI: high dependence; and VH: very high dependence; For each property, the nugget effect (C_0) and the range (A_0) and threshold ($C_0 + C$) and degree of spatial dependence (DSD) were related.

In this way, the variables Ca, Mg, PROD, Clay, and HV showed spatial dependence, showing that the mesh used was sufficient for the study of spatial variability. According to Cortes *et al.* (2023), one of the ways to evaluate the performance of semivariograms is their analysis based on their respective spatial determination coefficients (R^2), so that the behavior according to the decreasing relationship of the best fits was: 1) PROD (0.98), 2) clay (0.87), 3) Mg (0.82), 4) Ca (0.677), and 5) HV (0.47).

Range is a parameter provided by geostatistics that represents the spatial behavior of the studied variable, indicating the distance at which the variable has spatial continuity (Oliveira, Oliveira, Rojas Plazas, Andrade *et al.*, 2023). This

parameter becomes important for precision agriculture due to the use of geostatistical packages to feed agricultural machinery for the application of inputs at variable rates. Thus, in decreasing order of range: 1) Mg (1414.00 m), 2) PROD (1273.00 m), 3) clay (1124.00 m), 4) Ca (222.30 m), and 5) HV (67 m).

To analyze the degree of spatial dependence of the variables, Dalchiavon *et al.* (2012) was used, where semivariograms with a nugget effect (C_0/C_0+C1) were considered to have strong spatial dependence. Thus, Ca, Mg, clay, and P had a medium degree of spatial dependence and PROD and HV had high and very high dependence.

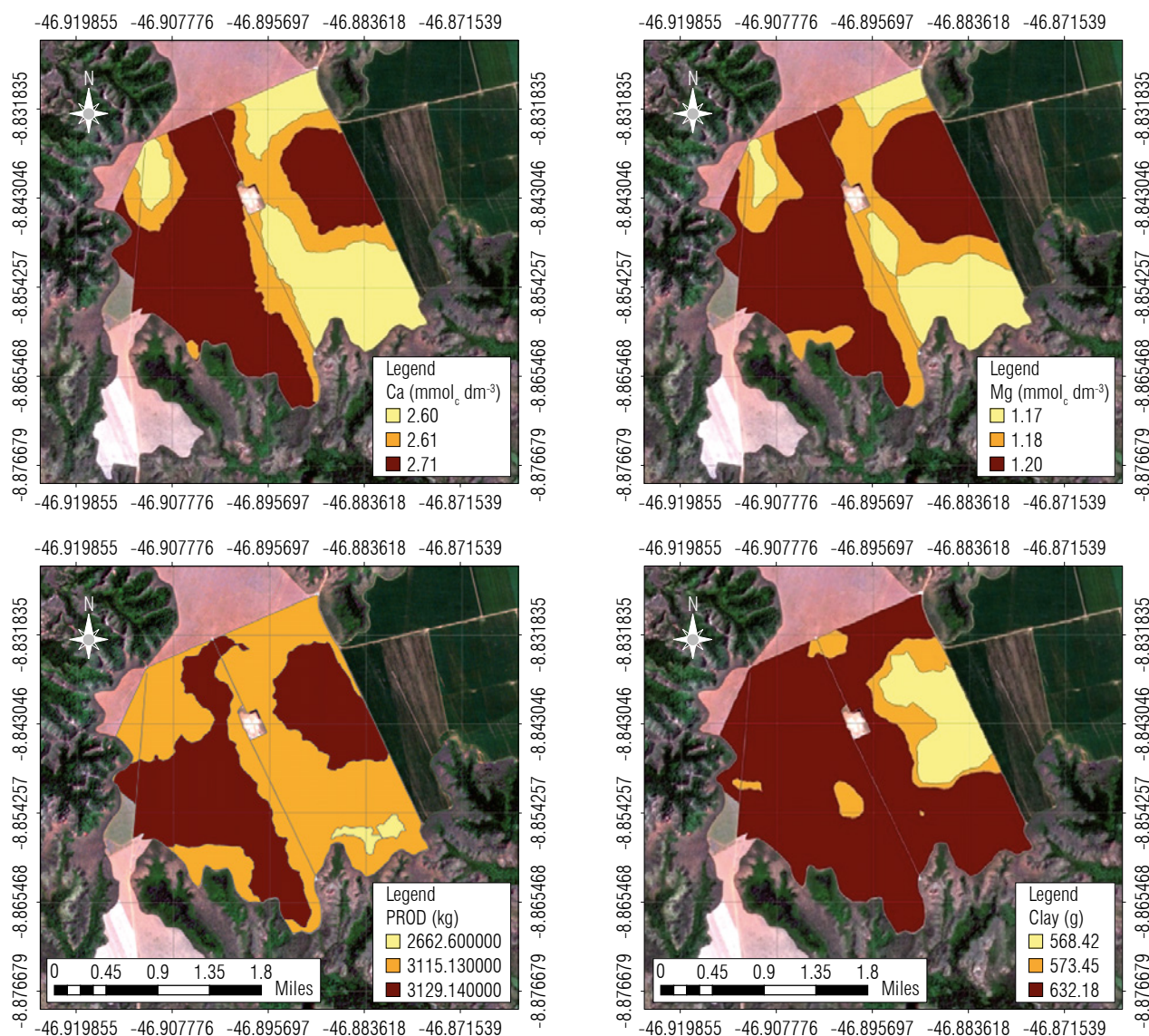


FIGURE 5. Kriging maps of the properties production, Ca, Mg, PROD, and Clay of attributes evaluated on the Emilian farm in the municipality of Balsas (MA), Brazil.

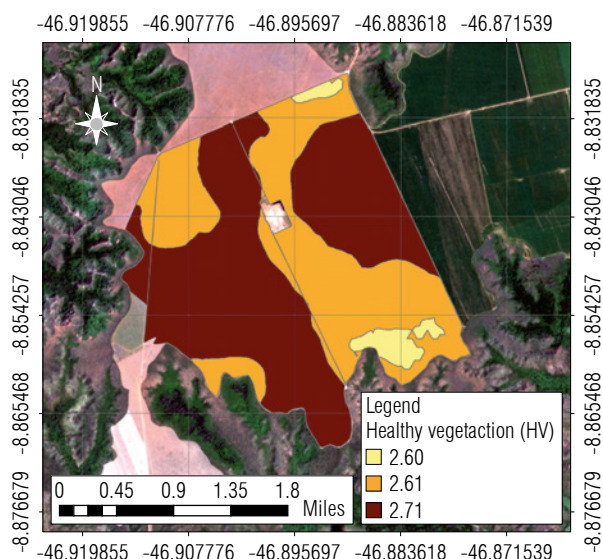


FIGURE 6. Kriging map of the HV evaluated on the Emiliana farm in the municipality of Balsas (MA), Brazil.

For soil properties, Kriging maps (Figs. 5-6) were prepared based on the semivariograms generated with the results presented in Table 2.

The spatial distribution maps for the variables provided an adequate diagnosis of the distribution of soil properties and yield components and showed sensitivity in identifying small variations (Figs. 5-6). A wide range could be seen for soil chemical properties, revealing problems that can occur when using average values for fertility management. For certain locations in the area of production, fertilizer application will not be necessary; in some locations, it will be consistent with requirements and in other locations excessive doses can be applied, compromising the productivity and quality of soybean seeds (Baio *et al.*, 2023).

Otone *et al.* (2024) highlight that the detection of possible problems using high-resolution images allows the acquisition of detailed information that reveals that their use, using machine learning and precision agricultural (geostatistics) techniques, can be effective in early detection and monitoring of possible problems in soybean cultivation, allowing rapid decision-making to control and prevent the loss of productivity.

Conclusions

The attributes studied that showed spatial correlation were the following: calcium, magnesium, soybean production, clay, and healthy vegetation.

The images from Sentinel-2 healthy vegetation (HV) with a spatial resolution of 10 m of the soybean crop presented

a good application for observing the spatial variability of the studied soil properties, since it presented an excellent linear correlation with the productivity of the crop.

Among the mathematical models of geostatistics, the ones that best explained the results were the spherical and exponential models.

Conflict of interest statement

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

Author's contributions

CGR: conceptualization, methodology, validation, formal analysis, investigation, writing - original draft; JTO: designed the experiment, analyzed the data, wrote, and edited the manuscript, final approval; FHRB: resources, writing, review and editing, final approval; OLG: validation, resources, writing - review and editing, final approval; FFC: validation, writing - review and editing, final approval. All authors reviewed the final version of the manuscript.

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Weeds and compacted soil in the establishment of an urban garden using the biointensive approach: Experiences and limitations

Malezas y suelo compactado en el establecimiento de una huerta urbana utilizando el enfoque biointensivo: experiencias y limitaciones

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ABSTRACT

The interest of people in consuming their own agricultural products is on the rise, leading to an increase in the number of urban gardens established in Bogotá over the past years. These gardens operated using the biointensive method as a model for urban agriculture present an environmentally sustainable alternative. However, this system comes with challenges and limitations that may hinder the establishment of such a project. To test this, an urban garden focused on biosystems with high levels of agricultural biodiversity was established within a greenhouse of the Universidad Nacional de Colombia, Bogotá campus. This was carried out in an area with a covered and an uncovered section. A weed germination trial was conducted in planting containers, assessing the relative representation of weeds in two random samplings taken from different containers over a two-month measurement period and a previous soil analysis was realized to evaluate the physical and chemical conditions of the soil. Consequently, 13 weed species were identified in the soil bank of weeds, with *Veronica* spp. being the most relatively represented in both samplings. However, within the established orchard, the predominant plants were those belonging to the Poaceae family, such as *Lolium temulentum* and *Cenchrus clandestinus*. Finally, through the biointensive method and the addition of organic materials such as biochar and regular topsoil, soil properties like structure, porosity, and friability were improved. This, in turn, enabled better root development and the successful establishment of various cultivars in the garden.

Key words: urban agriculture, diverse gardens, weed management, invasive grasses, clayey soil.

RESUMEN

El interés de las personas por consumir sus propios productos agrícolas va en aumento; esto ha conducido a un incremento del número de huertas urbanas establecidas en Bogotá durante los últimos años. Las huertas, explotadas mediante el método biointensivo como modelo de agricultura urbana, representan una alternativa sostenible desde el punto de vista medioambiental. Sin embargo, este sistema conlleva retos y limitaciones que pueden dificultar el establecimiento de un proyecto de este tipo. Para probarlo, se estableció un huerto urbano centrado en biosistemas con altos niveles de biodiversidad agrícola dentro de un invernadero de la Universidad Nacional de Colombia, sede Bogotá. Este fue llevado a cabo en un área con una sección cubierta y otra descubierta. Se preparó un ensayo de germinación de malezas en materas, evaluando la representación relativa de las malezas de dos muestreos aleatorios durante dos meses y se realizó un análisis de suelo previo para evaluar condiciones físicas y químicas del mismo. En consecuencia, se encontraron 13 especies de malezas en el banco de semillas de malezas del suelo, siendo *Veronica* spp. aquella con mayor representatividad relativa en ambos muestreos. Sin embargo, dentro de la huerta establecida, las plantas predominantes fueron aquellas pertenecientes a la familia Poaceae tales como *Lolium temulentum* y *Cenchrus clandestinus*. Finalmente, con el método biointensivo y adición de materia orgánica como biochar y tierra negra común, las propiedades como estructura, porosidad y friabilidad del suelo fueron mejoradas, permitiendo a su vez un mejor desarrollo radicular y el establecimiento de distintos cultivares en la huerta.

Palabras clave: agricultura urbana, huertas diversas, manejo de malezas, pastos invasores, suelo arcilloso.

Introduction

Urban agriculture is understood as the activity within or on the fringe of a city or metropolis where a variety of mainly food products are raised, cultivated, processed, and distributed (Degenhart, 2016). Worldwide, the largest proportion

of the population lives in cities, and around 25-30% of this population are involved in the agro-food sector (Orsini *et al.*, 2013). Urban agriculture is an activity that has spread due to its great impact on environmental sustainability. It is also characterized by a high level of variety and diversity, a more organic production, and the presence of new growers

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and other stakeholders who possess historical or traditional knowledge (Hernández, 2006).

The establishment of urban gardens gives rise to numerous positive effects for a more solid local economy. These benefits include food and employment for the most disadvantaged and sustainable urban development, which is related to the conservation of the landscape and rural heritage (López, 2014). Colombia has had similar results with cases of urban gardens in cities such as Popayán, Medellín, and Bogotá or municipalities such as Villamaría in Caldas, strengthening food security and taking advantage of organic waste (Gómez, 2014; Martínez *et al.*, 2022).

There are different methodologies and processes to establish an urban garden, among which is the biointensive agriculture methodology. This method consists of a production system based on the use of local inputs, with no machinery or commercial inputs implemented to avoid damage to the environment, animal and human health, and ecosystems (Ruiz, 2013).

Despite the various advantages of urban agriculture, it requires dedication and constant labor for its management and maintenance. Some of these practices include weed control, optimal fertilization, and the maintenance of soil moisture for the development of the plants of interest. In this regard, some limitations can interrupt the social fabric, affecting the continuity and presence of people in the gardens. An example of such limitations is the COVID-19 pandemic, which occurred when the garden of Universidad Nacional de Colombia was established. This pandemic limited the development of activities and triggered two problems that were then identified and that remain until now: the presence of difficult-to-control weeds and soil conditions that limit plant growth. Therefore, we proceeded to conduct a study of these two problems to identify possible solutions.

In this context, the objective of this research was to determine the main limitations associated with the implementation of an urban garden using the biointensive method, considering the influence of the soil characteristics and the bank of weeds in the soil on greenhouses at the Universidad Nacional de Colombia.

Materials and methods

Study area

The garden studied was called “Huerta UNAL” (UNAL Garden) within the framework of the Agrobiodiverse

Biosystems project funded by “Convocatoria Nacional de Alianzas Interdisciplinarias” (National Call for Interdisciplinary Alliances). The garden was located in a plastic-covered greenhouse of the Faculty of Agricultural Sciences of the Universidad Nacional de Colombia - Bogotá campus (4°38'8.9" N, 74°05'21.7" W) at 2640 m a.s.l., with an area of 280 m². The average maximum temperatures external and internal to the greenhouse fluctuated between 18°C and 20°C and 22°C and 24°C, respectively. At dawn, the minimum external temperature was between 8°C and 10°C, and the average total annual rainfall in the area is 797 mm, distributed in two dry seasons and two rainy seasons. The characteristics of the soil are shown in Tables 2 and 3.

For the physical-chemical analysis of the soil, samples were collected from the first 30 cm of soil depth; the first 2 cm of soil was removed, and the sample was extracted, taking about 10 sub-samples throughout the length and width of the area, which were then mixed in the bucket to form one soil sample. Following the IGAC (2021) methodology, a zigzag pattern was formed throughout the area, as it is the most appropriate and simple method to recollect the 10 soil sub-samples. The samples were sent to the Agrosoil laboratory (Bogotá) for analysis. The soil texture was determined according to the Bouyoucos (1936) method, the soil organic matter content was quantified according to the Walkley and Black (1934), the bulk density was determined with the paraffin-coated clod method according to Blake and Hartge (1986), and the real density was taken from FAO (2022). Furthermore, the description of soil structure was based on characteristics defined by Jaramillo (2002), in which the soil structure is classified by shape, kind and evolution grade.

Soil preparation

The soil was initially very compacted and degraded and had high contents of clay (52%) and a low content of organic matter (3.1%) and nitrogen (0.21%) (Tab. 2). The interpretation of compaction was made by taking the value of bulk density of the studied soil of 1.3 g cm⁻³ and comparing it with the reference values presented by Jaramillo (2002), who describes that those predominant soils of fine texture greater than 1.3 g cm⁻³ indicate soil compaction. Potassium (0.88 cmol kg⁻¹) and calcium (6.38 cmol kg⁻¹) were in excess, unbalancing the ionic relations in the soil (Tab. 3). To prepare the soil, intensive amendments with organic matter were applied 15 d before transplanting, using products from commercial brands Lombritenjo® granulated worm humus (Vermiculture of Tenjo, Tenjo, Colombia, 2 kg m⁻¹), Solidblend® organic fertilizer with biochar (Biodiversal, Bogotá, Colombia, 2 kg m⁻¹) or

material from other greenhouses which was transported on wheelbarrows to be evenly incorporated into the soil of the study area using a hoe.

Before transplanting, the double digging method was used and weeds were removed manually based on the Las Cañadas Agroecological Center (Centro Agroecológico las Cañadas, 2009) methodology, using a biointensive approach (Jeavons, 2001) to ensure that the crop management was in line with agroecological principles. After building the sections, 1 kg m⁻² of commercial biochar (Pentón Fernández *et al.*, 2021) was added to the soil 15 d before transplanting, with beneficial microorganisms such as phosphorus-solubilizing fungi *Penicillium janthinellum* (Fosfobiol®, Biocultivos, Ibagué, Colombia - 5 ml L⁻¹ - soil drench application) and plant-pathogenic fungi controllers such as *Trichoderma* spp. (Fitotripen®, Natural Control, La Ceja, Antioquia, Colombia - 2 g L⁻¹ - soil drench application) applied 15 d after transplanting.

Garden design and establishment

The 280 m² area was divided into two half-greenhouses measuring 14 m x 20 m. The first half was covered with a plastic roof, and the second was left uncovered. Inside each part of the greenhouse, six beds of 6 m x 3 m were spaced 0.4 m apart (Fig. 1); this was done to observe the emergence of the distinct kinds of weeds in the garden where several species were planted at a conventional density for each species (Tab. 1) since production was for self-consumption. The plant species established were vegetables, aromatic herbs, fruit trees, tubers, legumes, and cereals, with the largest number of species in the first two categories. Inside the main cultivated species were tomato, corn, potato, vegetables such as lettuce, carrot, radish, and herbs such as basil and oregano. These species were planted with the biointensive cultivation method, with the distances between plants and rows reduced to achieve a higher density of plants per 1 m² and boost yield (Pérez & Hernández, 2022).

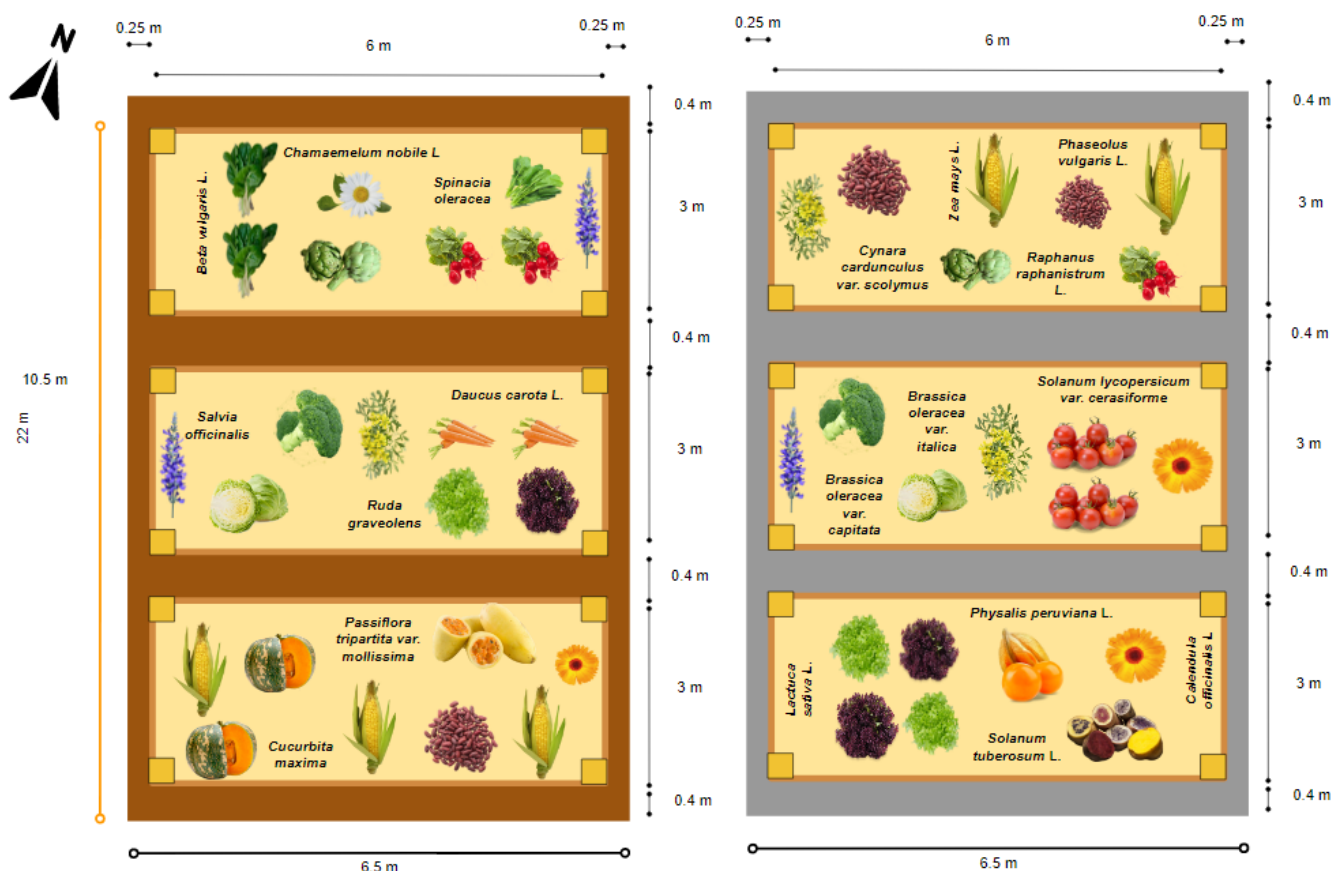


FIGURE 1. Greenhouse sections layout. The 280 m² area was divided into two half-greenhouses measuring 14 m x 20 m. The first part of the greenhouse was covered with a plastic roof (gray background), and the second was an open space (brown background). Some of the species cultivated are shown.

TABLE 1. Productive species grown in the garden and sowing distance.

Species	Days to harvest	Sowing distance (cm between plants x cm between rows)	Number of plants per section (18 m ²)
<i>Solanum tuberosum</i> L.	90-120	25 x 90	20
<i>Phaseolus vulgaris</i> L.	90-120	20 x 100	45
<i>Physalis peruviana</i> L.	90+	200 x 200	2
<i>Lactuca sativa</i> L.	80-90	25 x 25	150
<i>Spinacia oleracea</i>	49-64	15 x 15	200
<i>Daucus carota</i> L.	105-120	8 x 40	140
<i>Cucurbita máxima</i>	210	100 x 300	3
<i>Zea mays</i> L.	150-160	40 x 100	25
<i>Passiflora tripartita</i> var. <i>mollissima</i>	250-270	400 x 200	2
<i>Beta vulgaris</i> L.	75-90	35 x 40	65
<i>Cynara cardunculus</i> var. <i>scolymus</i>	120-180	80 x 80	15
<i>Solanum lycopersicum</i> var. <i>cerasiforme</i>	120	35 x 80	35
<i>Raphanus raphanistrum</i> L.	35	10 x 15	350
<i>Brassica oleracea</i> var. <i>italica</i>	90-110	40 x 40	50
<i>Brassica oleracea</i> var. <i>capitata</i>	90-110	40 x 40	50

Source: Ruiz (2013).

The seedlings were germinated and cared for before transplanting to the seedling production area of the Universidad Nacional de Colombia. Their planting and distribution were previously designed considering some relationships between plant species; for example, plants of the family Lamiaceae, such as *Mentha spicata*, that exhibit allelopathy (Islam *et al.*, 2022) that would facilitate management and reduction of phytosanitary problems, such as pests, through its insecticidal, antimicrobial, and antifungal capacity inside the garden.

For the transplanting, maintenance, and harvesting processes, collaborative workdays (*mingas*, in Spanish) were promoted through different means to invite university students to participate. As a result, the activity would strengthen learning about the establishment and care of an urban garden among people unfamiliar with the basic processes of agricultural production.

Biological products were used for pest and disease management. For pest management, some of these products were applied at different times of the day, including *Bacillus thuringiensis* (Subticip®), Bio-Crop, Palmira, Colombia - 2 ml L⁻¹ - soil drench application), *Beauveria bassiana*, *Lecanicillium lecanii*, *Metarhizium anisopliae* and *Bacillus thuringiensis* (Sáfermix WP®, Sáfer, Medellín, Colombia - 2 g L⁻¹ - soil drench application), potassium soap (Bonfyton®, Biorracionales de Colombia, Bogotá, Colombia - 2 ml L⁻¹ - foliar application), and for disease management

Trichoderma harzianum (Fitotripen®, Natural Control, La Ceja, Antioquia - 2 g L⁻¹ - soil drench application), and copper sulfate (Antrasin®, Sáfer, Medellín, Colombia - 2 g L⁻¹ - foliar application) were used.

Finally, crop fertilization was based on the application of products such as Actiphyl Kfruto® and Nutriponic® (Ingeplant, Colombia) and compost obtained from biodigester bales located in a greenhouse near the garden, prepared openly and in piles, as schematized by FAO for its preparation in Latin America (Román *et al.*, 2013).

Soil seed bank behavior

The germination conditions mirrored those of the garden location, with a temperature of 22°C±4°C, relative humidity ranging from 40 to 90%, and a standard photoperiod of 12 h. The analysis was conducted under conditions similar to those of the garden to gain insight into the species that germinate more rapidly and predominate in the garden's soil.

The weed characterization was made to identify invasive and competitive species in the garden soil. This information was used to plan the planting of species in the next planting cycle, selecting those that could outcompete the rapidly emerging weeds. Additionally, it allowed implementation of a preventive weed management strategy.

For the analysis of weed behavior, soil samples were collected in the uncovered area of the garden, after three

months without any intervention and prior to the next planting cycle, on March 14, 2022. The samples were gathered from the first 30 cm at four points in the garden (Cardenal Rubio *et al.*, 2016) and placed in 20 pots of 10 cm in diameter. Irrigation was carried out daily to allow the evaluation of the seed bank of weeds in the soil with a maximum temperature of 24°C and a minimum of 8°C. Maximum and minimum humidity of soil were 73% and 32%, respectively. The weed species were then observed, identified, and quantified. A first destructive sampling (S1) was carried out using 10 pots when achieving 100% coverage to identify species that stopped growing due to competition with others until 30 d after seeding (DAS). Subsequently, all weeds were removed from the pots. A second destructive sampling (S2) was carried out 60 DAS in the same pots to observe the species of the bank that were more competitive and established based on the Cardenal Rubio *et al.* (2016) methodology. Finally, Equation 1 for Relative representation (Rr) was utilized to calculate the importance of each weed species in the ecosystem, where the Species value represents the assessment of a species, and Total density of all species is the total number of species found in the sample (Moreno-Preciado *et al.*, 2021):

$$(Rr) \text{ Relative representation} = \frac{\text{Species value}}{\text{Total density of all species}} \times 100 \quad (1)$$

Finally, the weed identification process was carried out through morphological characterization of seedlings both in a controlled environment and in the field. We used the reference materials “Seedlings of common weed species in the central zone of Colombia” (Fuentes *et al.*, 2006) and the “Illustrated guide to weedy plants of the Marengo agricultural center” (Gámez *et al.*, 2018).

Results and discussion

Limitations associated with soil poor structure and texture

The clay content was predominantly higher in the studied soil. These clays can be defined as low-activity clays based on the observations in the field that evidenced deficiencies as a result of the lack of exchangeable elements available for the plant (Jaramillo, 2002). Additionally, the absence of soil structure prevents the breakdown of inorganic materials, which in turn inhibits the production of elements that plants can metabolize (Tab. 2).

Also, the soil exhibited small pores related to fine textures and poor structure without sand soil. Jaramillo (2002) attributed these soil features to compaction. The soil had clayey clods of moderate resistance and larger than 80 mm throughout the intervened area. This is the product of anthropogenic actions associated with the material in the area, which is poor in weatherable materials and sometimes contains rubble of different sizes and plastic debris linked to the low soil evolution degree. Del Valle Neder *et al.* (2010) describe the anthropic effect as a cause of loss of productivity in soils, stating that human intervention acts as a modifying factor of the environment, forming soils of anthropogenic origin. This garden is a clear example of such an effect.

These characteristics make the establishment of cultivated plants even more difficult, affecting the optimum physical properties of the soil for the root growth, such as pore space, air capacity, moisture retention, actual and bulk density, and resistance to compaction (Antúnez *et al.*, 2015).

Therefore, soils with anthropic influence that are typical of urban spaces must be renovated and conditioned to be

TABLE 2. Physical and chemical properties of the soil studied.

Physical properties						Chemical properties					
Clay	Loam	Sand	Textural class	BD	RD	pH	EC	ECEC	BS	OM	OC
%				g cm ⁻³			dS m ⁻¹	cmol ⁽⁺⁾ kg ⁻¹		%	
52	28	20	Ar	1.3	2.65	5.15	9.69	9.69	8.85	5.35	3.1

BD – bulk density, RD – real density, EC – electrical conductivity, ECEC – effective cation exchange capacity, BS – base saturation, OM – organic matter, and OC – organic carbon (Agrosoil laboratory, Bogotá). RD taken from FAO (2022).

TABLE 3. Chemical composition of the soil.

N-Total	N-Available	P-Available	K ⁺	Ca ²⁺	Mg ²⁺	Na ⁺	Al ³⁺	S ⁻²	Fe ²⁺	Mn ²⁺	Cu ⁺	Zn ²⁺	B ³⁺
%	mg kg ⁻¹		cmol(+) kg ⁻¹										
0.27	34.78	73.51	0.88	6.38	1.49	0.10	0.84	11.27	366.13	11.48	2.44	6.03	0.42

used in urban gardens. It is important to define the ideal soil practices that can help to transform low-fertility soils into areas with the desired fertility and the ability to sustain plant growth. An ideal scenario is described by Torres Sanabria and Cuartas Ricaurte (2013), who mention that the pre-Columbian cultures of the Amazon transformed low-fertility soils into highly productive anthropic soils called *Tierras prietas*. One of the numerous examples of soil conditioning is the use of aromatic plants that improve soil conditions such as pH and organic carbon availability (Dikr, 2022).

Alternatives to soil limitations

While the addition of organic matter to the studied soil improved its structural characteristics, such as porosity, texture and friability, various authors propose alternatives that expand the options for soil enrichment and which may be considered in the future. For instance, Pozza *et al.* (2020) state that the use of cover crops and the incorporation of organic matter not only maintain soil structure and prevent soil exposure, but also promote diversity within the system. They also suggest that the addition of organic matter to soil should go hand in hand with the cultivation of resilient crop varieties and the incorporation of nitrogen-fixing legumes to reduce the use of fertilizers.

The product Lombritenjo® was applied using vermicomposting, in which earthworms convert organic materials into humus. It enhances soil fertility, provides more nutrients to plants, and adds more porosity, density, aeration, and water retention to the soil (Lim *et al.*, 2015). The biochar Solidblend® improves the structure of compacted soil. Allohverdi *et al.* (2021) mentioned that compost mixed with biochar produces higher biomass degradation that provides nutrients to plants rapidly. Thus, using biochar's porosity improves soil aeration and increases porosity.

Fosfobiol®, which contains *Penicillium janthinellum* isolates as part of the *Penicillium* species, was also used. Hao *et al.* (2020) identified its capacity to solubilize inorganic P, making it available for the plant. Fitotripen®, which contains *Trichoderma* spp., was used to increase the soil health. Zin and Badaluddin (2020) stated that it works as a plant growth promoter and increases the decomposition of organic matter to enhance the availability of nutrients in the soil.

Finally, the utilization of arbuscular mycorrhizae frequently establishes beneficial associations that enhance nutrient uptake by plants through a symbiotic relationship, ultimately ameliorating the fitness and growth of the plants integrated within the system (Alyokhin *et al.*, 2020).

Limitations associated with the presence and abundance of invasive species

Thirteen weed species were found in the soil of the UNAL Garden (Tab. 4). These species corresponded to 10 families that were recorded throughout the experiment, but only 8 remained until the end. Of these weed species, 84.6% were broad-leaved and 15.4% were narrow-leaved (Tab. 4). The most represented species calculated with the Equation 1 of relative representation (Rr) in the first sampling was *Veronica* spp. (43.6%) (Fig. 3E), followed by *Lolium temulentum* (21.6%) and *Oxalis corniculata* (11.7%) (Figs. 2 and 3G).

TABLE 4. Main weeds found in the soil of the UNAL Garden.

Species	Family	Leaf type
<i>Cenchrus clandestinus</i>	Poaceae	Narrow
<i>Veronica</i> spp.	Scrophulariaceae	Broad
<i>Oxalis corniculata</i>	Oxalidaceae	Broad
<i>Polygonum segetum</i>	Polygonaceae	Broad
<i>Trifolium</i> spp.	Fabaceae	Broad
<i>Lolium temulentum</i>	Poaceae	Narrow
<i>Spergula arvensis</i>	Caryophyllaceae	Broad
<i>Galinsoga quadriradiata</i>	Asteraceae	Broad
<i>Sonchus oleraceus</i>	Asteraceae	Broad
<i>Euphorbia peplus</i>	Euphorbiaceae	Broad
<i>Chenopodium petiolare</i>	Amaranthaceae	Broad
<i>Cardamine hirsuta</i>	Brassicaceae	Broad
<i>Medicago</i> spp.	Fabaceae	Broad

In the second sampling, the most relative representative species was also *Veronica* spp. (33.3%), followed by *Lolium temulentum* (21.6%) and *Cenchrus clandestinus* (17.7%). All the species representations tended to decrease, except *Spergula arvensis* (Fig. 3D) and *C. clandestinus* (Fig. 3H). In addition, in the first sampling, *C. clandestinus* represented only 2.7% of the number of individuals found in the garden but was the most difficult to manage. Although the other species were more represented in the samplings, they were easily controlled manually (Fig. 3A).

The characteristics of *Veronica* spp. as a highly relative representation are explained by Bond *et al.* (2007) who observed that this species grew on calcareous and acid soils. These features were found in the soil during the experiment, which could explain the predominance in the relative representation. The authors claimed that *Veronica* spp. makes up a persistent seed bank which could explain its major predominance in the soil bank of weeds.

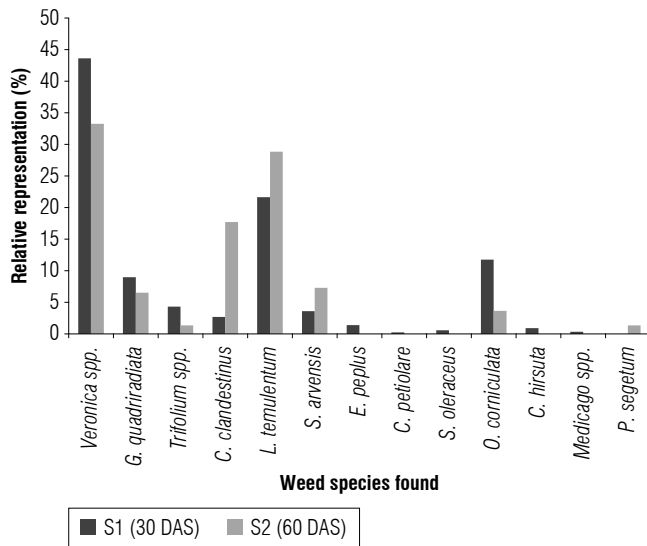


FIGURE 2. Weed species found in the UNAL Garden (Bogota). Samples "S1" and "S2" were collected up to 30 and 60 d after seeding (DAS), respectively. The x-axis shows the different weed species and the y-axis shows the number of individuals per number of analyzed samples, with $n = 10$.

The results of the bank of weeds in the soil test contrasted with what was observed in the study area. During the establishment of the garden, the weed *C. clandestinus* (Kikuyu grass) became a major problem since it was rooted up to 30 cm deep and showed an abundance of rhizomes (Fig. 3H) (Cordero Rodríguez *et al.*, 2021). These characteristics make *C. clandestinus* a species with excellent reproductive potential which, added to its ecology (a perennial species with a wide range of adaptability to different environments), results in a very invasive grass species. It reproduces by rhizomes and stolons, allowing it to cover the entire area where it is found (Contexto Ganadero, 2022). This behavior negatively affects the biosystem and diversity which is of utmost importance for the maintenance and conservation of agroecosystems (Camacho-Ballesteros, 2018; Rodríguez, 2021).

Although competition and existing allelopathy were considered for the cultivable species, an in-depth diagnosis of the bank of weeds in the soil was not performed, which would have prevented competition and drastic invasion

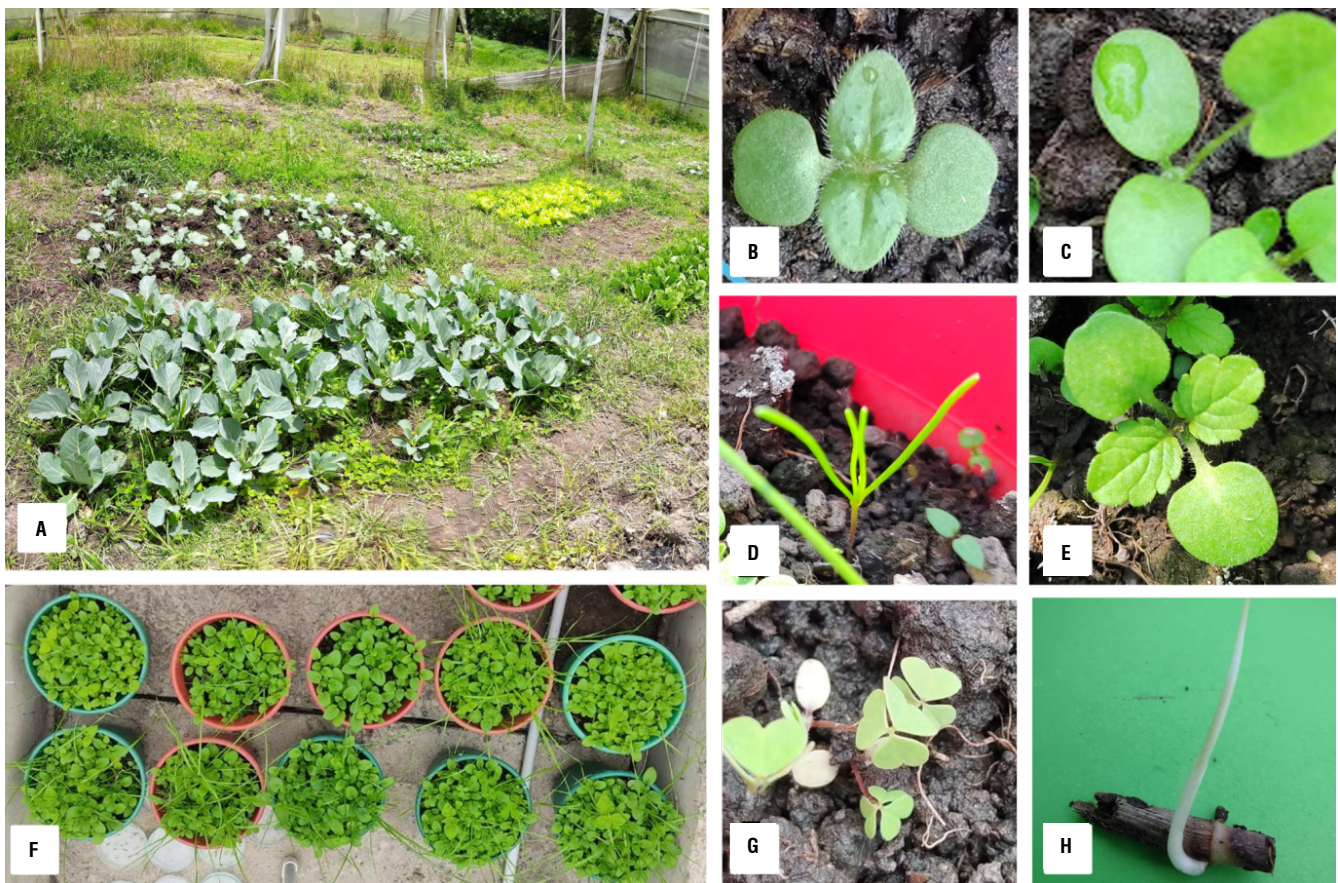


FIGURE 3. Photographic record of the presence of weeds in the UNAL Garden. A) *Cenchrus clandestinus* growing around cabbage, chard and lettuce sections in the UNAL Garden, B) *Galinsoga quadriradiata*, C) *Trifolium* spp., D) *Spergula arvensis*, E) *Veronica* spp., F) pots of the soil bank of weeds, G) *Oxalis corniculata*, and H) *Cenchrus clandestinus* rhizome found during the trial.

by *C. clandestinus* (Fig. 3A). Camacho-Ballesteros (2018) pointed out that this species of grass competes with cultivated plants for water, light, and space. The use of water resources is the most critical problem, not only because of competition but also because this species prevents water infiltration, seriously affecting water availability in reservoirs and the soil.

The relative representation by *Veronica* spp. during the experiment does not relate with the establishment of the different species of crops over the field. Since the biology of *C. clandestinus* depends on the competition of water and nutrients, it might have a higher impact on the production system. The opposite occurred with *Veronica* spp., which had a low persistence in the field, although it was the most representative in the experiment in pots. This shows the importance of knowing the biology and weather response of these different weeds in the production system.

Although the problems of managing and maintaining a biointensive garden can affect the continuity of urban agriculture projects, there are relevant factors that are rarely considered. For instance, compacted soil can be improved by adding various materials, such as organic matter from diverse sources, to enhance the soil structural integrity and, in some instances, supply essential mineral elements for plant growth.

Additionally, soil conditioning techniques can be implemented and the bank of weeds managed by selecting beneficial weed species, creating the possibility to use the qualities of distinct plants in a way that allows soil improvement and the establishment of different crops. Finally, plant growth-promoting rhizobacteria can be used along with the addition of vegetal material and mycorrhizae to create a symbiosis with plants.

Conclusions

Despite the limitations imposed by poor soil conditions, this project was able to establish a successful garden with improved soil quality, which supported the growth of various vegetables, even when the soil presented many challenges. This project demonstrated the potential of urban gardens as a sustainable and viable solution to address food insecurity and promote healthy eating habits in communities, even in areas with suboptimal soil conditions, allowing them to be replicated in different scenarios of the city and accomplish their mission.

Adding root type and growth form characteristics to the methodology of weed evaluation will improve future

studies by providing a better understanding of the behavior of weeds and their competitive ability. This will allow for more accurate predictions of their impact on crops, aid in the selection of appropriate control measures, and promote sustainable agriculture while reducing negative impacts on the urban gardens.

Finally, knowing the relative representation and the biology characteristic of the weeds allows control and study of different weeds during the first crop stages, when they still have a low relative representation, to avoid losing the garden profit to competition.

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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

MPQ, JPC, and CC formulated the research goals. MPQ supervised and directed the planning and execution of the research activities. KPG, JPC, and CC developed the methodology and carried out the research and investigation process with the collaboration of AB, JS, IN, and ODG, particularly in the field trials and data collection process. KPG, CC, IN, and JPC applied statistical techniques, discussed the results, and performed a critical review of the manuscript. MPQ revised the initial version of the manuscript and translated the initial draft. All authors reviewed the final version of the manuscript.

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Assessing the impact of emerging technologies on sustainable fruit production: A systematic review of the literature

Evaluación del impacto de las tecnologías emergentes en la producción frutícola sostenible: una revisión sistemática de literatura

Angélica María Pardo-Pardo^{1*} and William Javier Cuervo-Bejarano¹

ABSTRACT

Agriculture 4.0 refers to innovations in technological tools used in agriculture to achieve different objectives, such as adapting the supply chain to avoid waste, increasing productivity and collecting mass data through ICT (Information and Communication Technologies) to meet the growing food demand of the population. The objective of this study is to conduct a systematic literature review to evaluate the impact of emerging technologies on sustainable fruit production. Initially, a bibliographic search was conducted on the technologies currently implemented in agriculture; the Bibliometrix library of the R Studio software was used, and then an analysis of relevant scientific publications published in the last ten years was carried out through the VOSviewer® software, which allowed the construction and visualization of bibliometric networks. The results show Europe and China as the leading regions in technological development, while developing countries face economic and research limitations; in Colombia, the use of Agriculture 4.0 is focused on the implementation of satellite images for monitoring agro-climatic conditions. In summary, Agriculture 4.0 aims to achieve economic, social, and environmental sustainability in the agri-food sector through data-generating technologies to improve production, reduce costs, and ensure food safety and quality. However, there is a technology gap between developed and developing countries that affects the adoption of these innovations. More support is therefore needed from governments, academia, and the private sector to drive innovation, training, and adoption of these technologies, which can contribute to the economic, social, and environmental development of the country.

Key words: productivity, machine learning, artificial intelligence, fruits, yield, bibliometrics.

RESUMEN

La Agricultura 4.0 se refiere a las innovaciones en las herramientas tecnológicas utilizadas en la agricultura para lograr diferentes objetivos, como la adaptación de la cadena de suministro para evitar el desperdicio, el aumento de la productividad y la recolección masiva de datos a través de las TIC (Tecnologías de la Información y la Comunicación), para satisfacer la creciente demanda de alimentos de la población. El objetivo de este trabajo es realizar una revisión bibliográfica sistemática para evaluar el impacto de las tecnologías emergentes en la producción frutícola sostenible. Inicialmente, se realizó una búsqueda bibliográfica sobre las tecnologías actualmente implementadas en la agricultura; se utilizó la biblioteca Bibliometrix del software R Studio, y luego se llevó a cabo un análisis de las publicaciones científicas relevantes publicadas en los últimos diez años, a través del software VOSviewer® que permitió la construcción y visualización de redes bibliométricas. Los resultados muestran a Europa y China como las regiones líderes en desarrollo tecnológico, mientras que los países en desarrollo enfrentan limitaciones económicas y de investigación; en Colombia, el uso de la Agricultura 4.0 se centra en la implementación de imágenes satelitales para el monitoreo de las condiciones agroclimáticas. En resumen, la Agricultura 4.0 pretende lograr la sostenibilidad económica, social y ambiental del sector agroalimentario a través de tecnologías generadoras de datos para mejorar la producción, reducir costes y garantizar la seguridad y calidad de los alimentos. Sin embargo, existe una brecha tecnológica entre los países desarrollados y los países en desarrollo que afecta a la adopción de estas innovaciones. Por lo tanto, se necesita más apoyo de los gobiernos, el mundo académico y el sector privado para impulsar la innovación, la formación y la adopción de estas tecnologías, que pueden contribuir al desarrollo económico, social y medioambiental del país.

Palabras clave: productividad, aprendizaje automático, inteligencia artificial, frutos, rendimiento, bibliometría.

Introduction

Emerging technologies (ET) integrate advanced technologies like Internet of Things (IoT), Artificial Intelligence

(AI), and Big Data to establish smart agriculture, aiming to address global food demands, enhance crop yield, reduce water consumption, optimize pesticide use, and improve crop quality (Xu *et al.*, 2018). Drones, IoT sensors, and

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machine learning are key technologies employed in Agriculture 4.0, primarily in open-field farms (Saha *et al.*, 2018; Shafi *et al.*, 2020). The use of ET in greenhouse crop production has revolutionized agriculture in controlled environments and advanced climate control systems to optimize temperature, radiation, relative humidity, and CO₂ levels, while sensor-based fertigation increases water and nutrient use efficiency (Putra & Yuliando, 2015; Khan, 2018; Pennisi *et al.*, 2019). Vertical farming and hydroponics maximize production capacity and resource efficiency through automation, data analytics, and robotics; these technologies enhance precision in nutrient delivery, lighting, and crop maintenance (Hemming *et al.*, 2019). The integration of ET in greenhouses offers great potential for sustainable food production, with precise control of environmental conditions and increased crop yields.

Sensors play a crucial role in the success of IoT in agriculture, facilitating map-based and sensor-based approaches to manage spatial and temporal changes in agricultural production to improve yields and sustainability (Zhang *et al.*, 2014; Ferrández-Pastor *et al.*, 2016; Tzounis *et al.*, 2017). Remote sensing, enabled by Wireless Sensor Networks (WSN), finds diverse applications in agriculture, including vegetation health evaluation and crop stress detection. Integration of AI models with remote sensing data enables crop yield prediction, while real-time monitoring of soil and atmospheric conditions through IoT sensors enhances agricultural practices, optimizing efficiency, productivity, and profitability while minimizing waste and environmental impact (Mulla, 2013; Rajak *et al.*, 2023).

Efficient and sustainable crop production requires accurate meteorological data collection and monitoring of greenhouse gas emissions. Various technologies, such as weather stations, WSN, drones, and intelligent recognition systems, are utilized for these purposes (Malaver *et al.*, 2015; Ojha *et al.*, 2015; Johnson *et al.*, 2016; Shafi *et al.*, 2019). AI-based recognition systems assist early detection and classification of crop diseases, pests, and weeds, using remote sensing and multispectral cameras (Sun *et al.*, 2018). Remote monitoring and optimization of irrigation and fertilization systems using IoT sensors, AI-based data analysis, and decision support systems are emerging soil management trends in Agriculture 4.0 (Agudelo Cano *et al.*, 2023). Intelligent water quality monitoring systems with IoT sensors are deployed to measure various parameters for effective water management.

Fruit crops can be established in both open-field and protected conditions. IoT applications in fruit agriculture

facilitate enhanced monitoring and management of soil moisture, temperature, humidity, and light intensity. IoT sensors placed in orchards and fields collect real-time data, allowing farmers to make data-driven decisions regarding irrigation, fertilization, and pest control (Disraelly *et al.*, 2011). This leads to optimized resource utilization, improved crop yield, and increased quality of fruits (Ebrahimi *et al.*, 2017; Kamilaris *et al.*, 2017; Abbasi *et al.*, 2022). In Latin America, where agriculture plays an important role in the economy, the adoption of IoT in fruit crops has been accelerating (Aker, 2011; Puntel *et al.*, 2022; Strong *et al.*, 2022). Colombia has recognized the potential of IoT to improve productivity and sustainability in the agricultural sector. Initiatives have been launched to adopt IoT sensors in fruit orchards, enabling continuous monitoring of environmental conditions and facilitating precision farming practices (Pineda, Pérez *et al.*, 2022; Pineda, Tinoco *et al.*, 2022; Agudelo Cano *et al.*, 2023). These sensors provide farmers with valuable insights and enable early detection of issues such as diseases or pests for prompt and targeted interventions (Ramírez Alberto *et al.*, 2023).

The use of IoT in fruit agriculture in Colombia offers several advantages, including increased efficiency, reduced costs, and minimized environmental impact (Rodríguez *et al.*, 2021; Arrubla-Hoyos *et al.*, 2022). By leveraging IoT technologies, Colombian fruit farmers can optimize resource management, conserve water through precise irrigation techniques, and minimize the use of agrochemicals (Ziesche *et al.*, 2023). Additionally, IoT-enabled systems can improve traceability and transparency in the supply chain, ensuring the delivery of safe and high-quality fruits to consumers (De la Peña & Granados, 2023). The aim of this article was to conduct a systematic literature review to assess the impact of emerging technologies on sustainable fruit production, identifying and describing the current status of sustainable fruit production as well as the challenges and opportunities presented by the incorporation of emerging technologies.

Materials and methods

Scopus databases were used to search for open access articles published in the last decade. The search was limited to specific topics, and the 2,000 most relevant were analyzed using the terminology of title, abstract and keywords (Tab. 1). The review of the evolution over time of research conducted in the world on the use of ET in agriculture and the map of the main countries producing scientific research in the fruit sector were carried out using the Bibliometrix library from the R software. The VOSviewer® software

was used to visualize bibliometric networks based on the connection and linkage between the keywords of the documents.

Results and discussion

The Scopus search yielded a total of 47,319 articles in Group A, 1,050 in Group B, 6,878 in Group C, and 172 in Group D. Figure 1 depicts the use of WSN in agriculture, enabling real-time monitoring of crops, soil, and weather conditions to improve food crop yields and alleviate the

TABLE 1. Search groups in Scopus.

GROUP	Search string
A	("Internet of Things" OR "Artificial Intelligence" OR "Machine Learning" OR "Data science" OR "Robotic") AND ("Agriculture" OR "Smart Farm" OR "Precision Farm")
B	("Internet of Things" OR "Artificial Intelligence" OR "Machine Learning" OR "Data science" OR "Robotic") AND ("Agriculture" OR "Smart Farm" OR "Precision Farm") AND ("Colombia")
C	("Internet of Things" OR "Artificial Intelligence" OR "Machine Learning" OR "Data science" OR "Robotic") AND ("Agriculture" OR "Smart Farm" OR "Precision Farm") AND ("fruit" OR "fruit growing")
D	("Internet of Things" OR "Artificial Intelligence" OR "Machine Learning" OR "Data science" OR "Robotic") AND ("Agriculture" OR "Smart Farm" OR "Precision Farm") AND ("fruit" OR "fruit growing") AND ("Colombia")

workload on farmers (Ojha *et al.*, 2015). However, energy efficiency poses a challenge for WSN, especially in remote areas with limited access to energy. Recent research focuses on developing energy-efficient WSN that can operate using renewable energy sources (Zhang & Meng, 2021).

Machine learning algorithms can analyze data collected by WSN, providing information on crop growth patterns and early identification of potential problems. This information helps to make decisions in agriculture, such as determining optimal planting and harvesting times (Tan *et al.*, 2021). In Colombia, there are diverse edaphoclimatic conditions that favor the production of fruit species; differential monitoring according to species is needed to improve sustainability. Monitoring involves aspects such as physiology, pests, postharvest, costs and labor. Most crops in Colombia, including fruit trees, are located in remote areas where power grids are often not available and where the availability of communication networks is low. Therefore, the use of WSN in agriculture, specifically in crop monitoring, can be a valuable tool to improve crop sustainability (Hernández Leal, 2016).

An important increase in scientific article production was identified between 2014 and 2022, reaching 711 articles (Fig. 2). This growth is attributed possibly to increased research

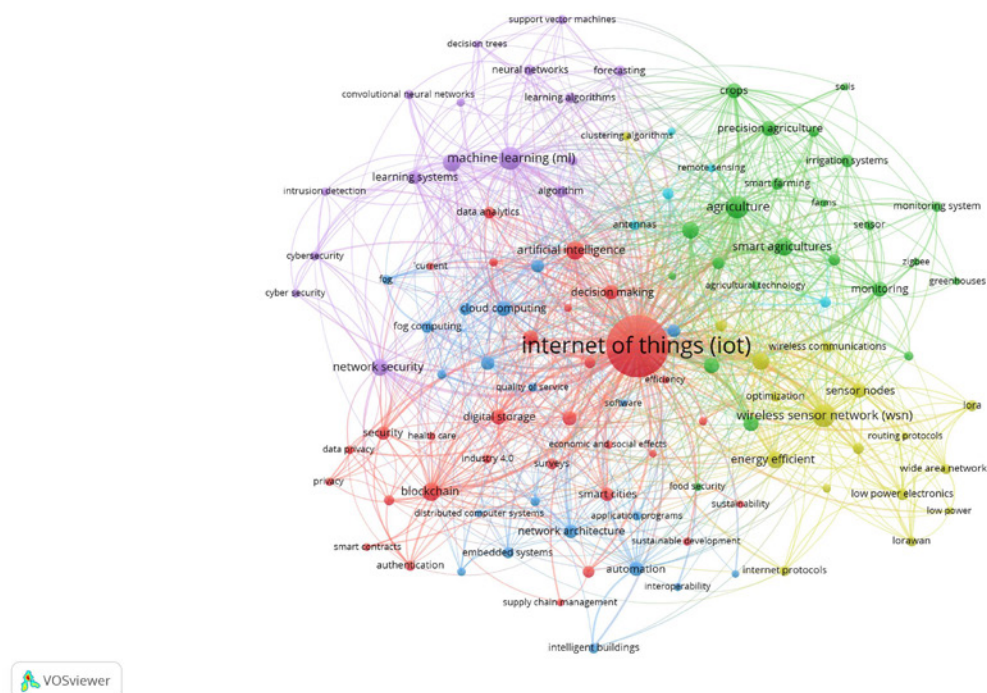


FIGURE 1. Correlation between the most used keywords (“Internet of Things” OR “Artificial Intelligence” OR “Machine Learning” OR “Data science” OR “Robotic”) AND (“Agriculture” OR “Smart Farm” OR “Precision Farm”) in the search for GROUP A. The map was generated by VOSviewer®.

on IoT, particularly in the areas of security, surveillance, health, architecture, construction, and precision agriculture (Kusek, 2018).

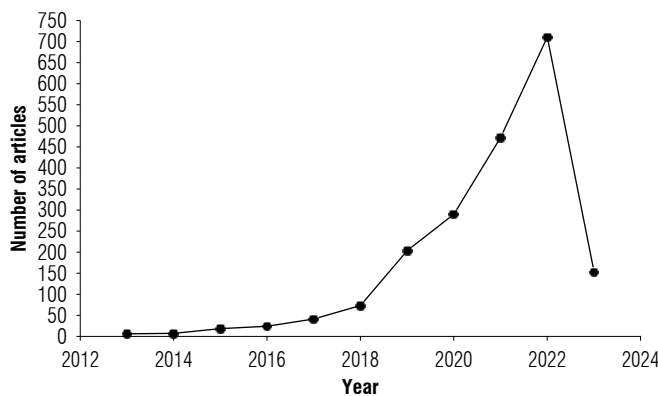


FIGURE 2. Evolution of articles produced globally in the last decade related to research on ET used in agriculture. The graph was generated by Bibliometrix R package.

According to the results of group B (Fig. 3), the use of remote sensing in biodiversity conservation predominates in Colombia. Remote sensing allows non-invasive data

collection, making it possible to monitor changes in habitats and ecosystems, critical for the protection of threatened species and the identification of conservation areas. It also facilitates the study of land cover, essential for land use planning, natural resource management and climate change mitigation. Colombian agriculture faces challenges from climate change, particularly from El Niño and La Niña phenomena (CIAT, 2013; Instituto Interamericano de Cooperación para la Agricultura, 2016; Melo León *et al.*, 2017), which affects agroecosystems and the economy. To estimate risk and manage soil, water, and crops, modeling and prediction tools with remote sensing and AI can be used. These tools collect data on crops and the environment and use machine learning algorithms to predict and detect problems. Thus, farmers can make data-driven decisions that improve yields, reduce resource wastage, and increase profitability (Akhter & Ahmad, 2022).

Models incorporating data on weather patterns, soil characteristics, and crop-specific parameters are useful to forecast yields and growth rates. They provide valuable information on managing the impact of these factors on agricultural productivity, contributing to global food security (Candelaria Martínez *et al.*, 2011; Benos *et al.*, 2021). Colombia

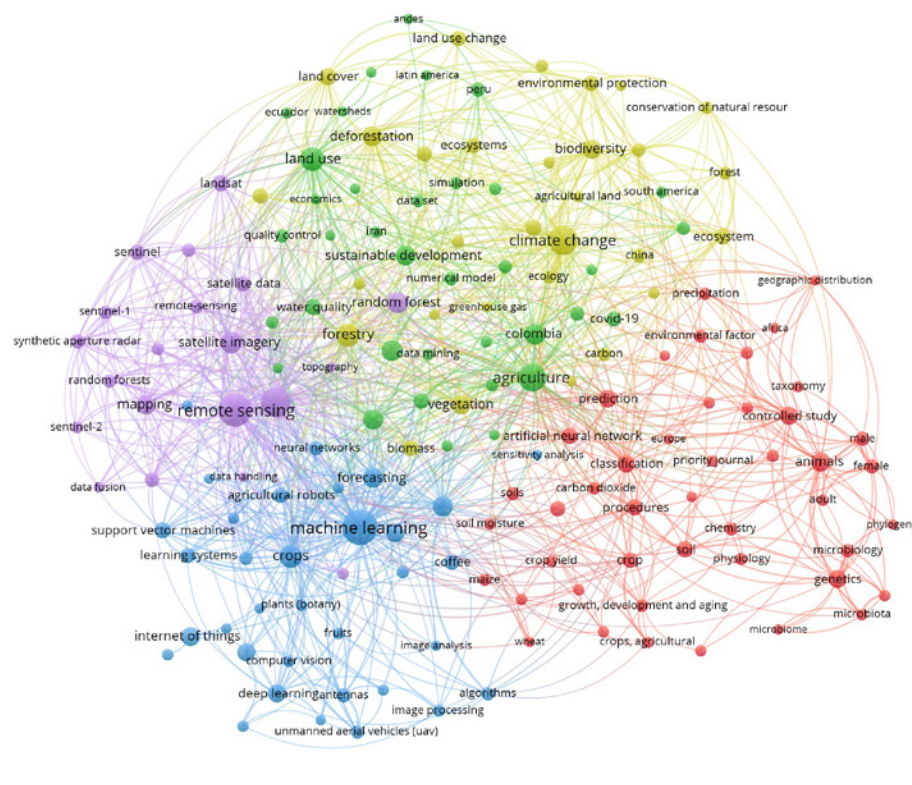


FIGURE 3. Correlation between the most used keywords ("Internet of Things" OR "Artificial Intelligence" OR "Machine Learning" OR "Data science" OR "Robotic") AND ("Agriculture" OR "Smart Farm" OR "Precision Farm") AND ("Colombia") in the search for GROUP B. The map was generated by VOSviewer®.

uses predictive models to anticipate the effects of biotic and abiotic factors on agriculture, including crop growth and ecological models. Some examples of these models are the DSSAT model (Decision Support System for Agrotechnology Transfer), which simulates the development of different crops under different climatic, management and fertilization conditions, and which has been applied in Colombia to assess the impact of climate change and climate variability on crops such as maize, rice, beans and potatoes (Sarkar, 2012); another example is the AquaCrop model, which estimates the water consumption and biomass production of crops as a function of water availability in the soil and atmosphere (Dercas *et al.*, 2022). Finally, the CROPGRO (Crop Growth) model, which simulates the growth and development of leguminous crops such as beans, peanuts and soybeans, considering the effects of biotic factors such as insects, diseases and weeds, has been used in Colombia to optimize integrated pest and disease management practices (Van Loon *et al.*, 2018). However, there is little implementation of phenological models in crops that allow scheduling of cultivation and harvesting activities. Machine learning, remote sensing and satellite imagery are increasingly important tools in the study of the Colombian environment and natural resources (Murad & Pearse, 2018).

These applications can provide valuable information to support sustainable land use planning and management, including classifying land cover types, monitoring vegetation health, and detecting changes in land use over time. For example, researchers have studied the feasibility of early prediction of yield per coffee tree based on multispectral aerial imagery in Colombia (Sousa *et al.*, 2020; Giang *et al.*, 2022; Jato-Espino & Mayor-Vitoria, 2023). This type of classification can be useful for determining areas with an agricultural vocation to expand crop areas according to the specific soil and climate needs of the crops.

The results of groups A, B, and C (Fig. 4) demonstrate the prevalence of “machine learning” and “deep learning” techniques in the global fruit industry. “Deep learning” is particularly effective for image analysis and has been successfully applied in tomato crop monitoring using

FIGURE 4. Correlation between the most used keywords (“Internet of Things” OR “Artificial Intelligence” OR “Machine Learning” OR “Data science” OR “Robotic”) AND (“Agriculture” OR “Smart Farm” OR “Precision Farm”) AND (“fruit” OR “fruit growing”) in the search for GROUP C. The map was generated by VOSviewer®.

drones. Tomato is one of the highest volume production vegetables worldwide, with approximately 182 million t (Caruso *et al.*, 2022). Deep learning-based algorithms are valuable for pest and disease monitoring in fruits as well as post-harvest sorting, by analyzing image datasets and identifying patterns that are difficult to detect (Dokic *et al.*, 2020; Bouguettaya *et al.*, 2022). Deep learning algorithms detect specific patterns and facilitate fast and accurate responses to problems. They also improve the efficiency and accuracy of agricultural processes. Machine learning is linked with remote sensing and IoT to promote sustainable practices. Remote sensing technologies, such as unmanned aerial vehicles (UAV), provide valuable data for precision agriculture, while IoT devices, such as WSN, collect crucial data on temperature, humidity, and soil moisture to make informed crop management decisions (Ding & Xie, 2023; Yousaf *et al.*, 2023). Thus, the fusion of machine learning, remote sensing and IoT has the potential to revolutionize sustainable agriculture (Cadenas *et al.*, 2023).

Figure 5 highlights China and the United States as leading producers of scientific research in the fruit sector. These countries, along with East Asia, South Asia, South America, Southeast Asia, and Southern Europe, are significant regions for fruit and vegetable production (FAO, 2021). Globally, over 50% of fruits are cultivated on agricultural land of less than 20 ha, mainly owned by families. In developing nations, these small farms produce most horticultural products, with over 80% in Asia, sub-Saharan Africa, and China (Frelat *et al.*, 2016).

In Colombia, group D findings (Fig. 6) indicate the utilization of machine learning for decision-making in coffee cultivation, along with precision agriculture tools and remote sensing technologies. Machine learning algorithms accurately estimate coffee production and classify crops (Suarez-Peña *et al.*, 2020). Implementing these technologies in coffee cultivation enhances efficiency, productivity, and profitability, important because the agricultural global gross domestic product of the country heavily relies on coffee, flowers, bananas, sugar, rice, and potatoes (Delgado-Delgado *et al.*, 2021).

During postharvest, there could be considerable losses of fruits due to poor handling, grading and distribution practices (Bantayehu *et al.*, 2017; Singh *et al.*, 2022; Knott *et al.*, 2023). Machine learning and deep learning algorithms are currently being used for fruit transport and storage as well as fruit sorting and distribution after harvest by analyzing images and sensor and historical data (Knott *et al.*, 2023). For example, machine learning algorithms can be used to detect defects, diseases and damage to fruit, as well as to estimate fruit quality, maturity and shelf life. In this way, fruits can be assigned to different categories or destinations according to their commercial value (Meshram *et al.*, 2021). Deep learning algorithms can also be used to recognize complex patterns in fruit images, such as shape, color or texture. These algorithms can learn automatically from the data without prior programming. This can improve the accuracy and speed of fruit sorting as well as reduce costs and human error (Piedad *et al.*, 2018).

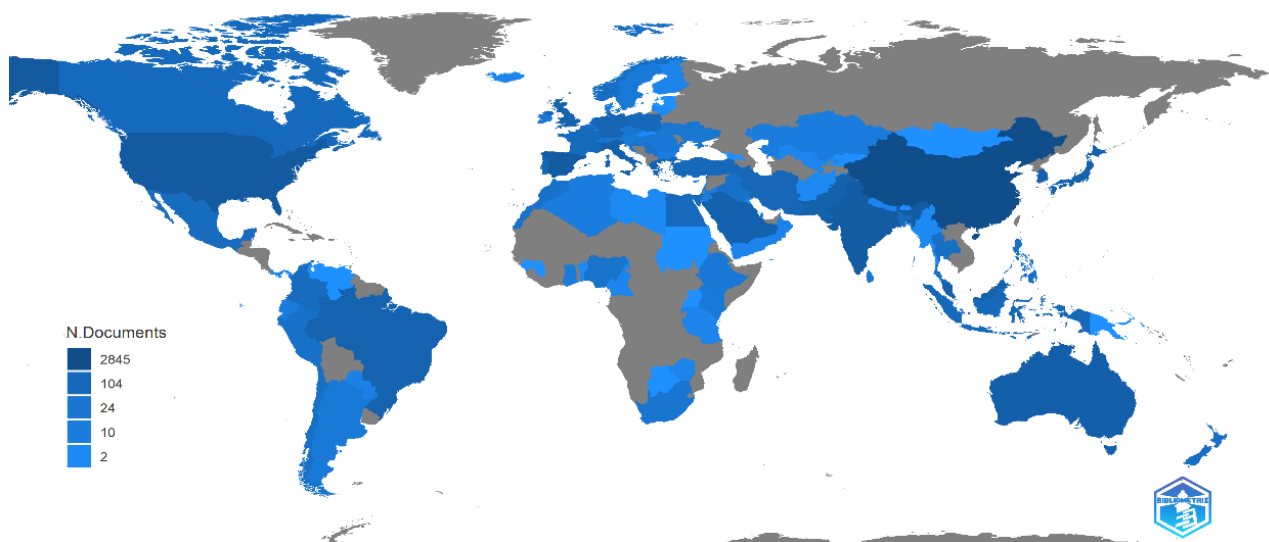


FIGURE 5. Map of scientific output generated by searching for (“Internet of Things” OR “Artificial Intelligence” OR “Machine Learning” OR “Data Science” OR “Robotics”) AND (“Agriculture” OR “Smart Farm” OR “Farm precision”) AND (“fruit” OR “fruit growing”) GROUP C.

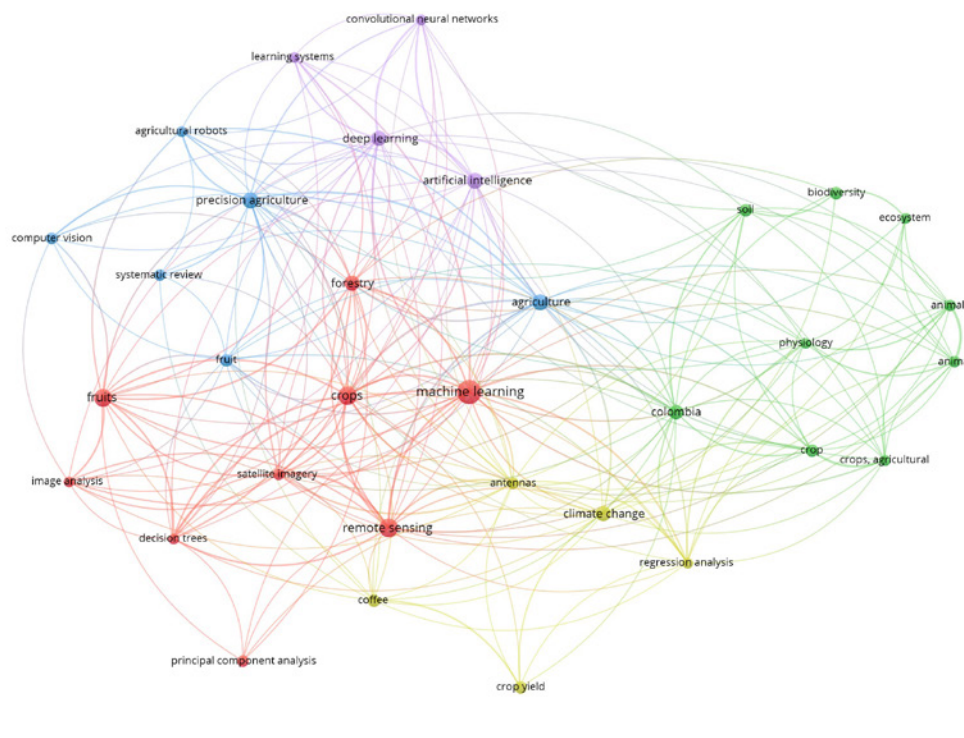


FIGURE 6. Correlation between the most used keywords (“Internet of Things” OR “Artificial Intelligence” OR “Machine Learning” OR “Data science” OR “Robotic”) AND (“Agriculture” OR “Smart Farm” OR “Precision Farm”) AND (“fruit” OR “fruit growing”) AND (“Colombia”) in the search for GROUP D. The map was generated by VOSviewer®.

Conclusions

The use of Wireless Sensor Networks (WSN) in agriculture, as evidenced in the scientific literature, has proven to be a valuable tool for real-time monitoring of crops, soil and weather conditions, contributing to improved food crop yields and easing the workload of farmers. However, the challenge of energy efficiency in remote areas with limited access to energy represents a significant obstacle to WSN implementation in agriculture. Recent research has focused on the development of energy-efficient WSNs that can operate using renewable energy sources. In addition, machine learning algorithms can analyze the data collected by WSNs, providing information on crop growth patterns and early identification of potential problems, which helps to make decisions in agriculture, such as determining optimal planting and harvesting times. In the Colombian context, where diverse soil and climatic conditions favor the production of fruit species, differential crop monitoring is crucial to improve crop sustainability, especially in remote areas with limited availability of electricity and communication networks. Therefore, the use of WSN in agriculture, specifically in crop monitoring, can be a valuable tool to improve crop sustainability. Colombian agriculture faces challenges from climate change, particularly from the “El Niño” and

“La Niña” phenomena, which affect agro-ecosystems and the economy. Remote sensing and artificial intelligence modeling and prediction tools can be used to estimate risk and manage soil, water and crops. These tools collect data on crops and the environment and use machine learning algorithms to predict and detect problems. As a result, farmers can make data-driven decisions that improve yields, reduce resource waste and increase profitability.

Finally, the use of “machine learning” and “deep learning” techniques in the global fruit industry has proven to be prevalent and effective. Specifically, “deep learning” has been successfully applied in the monitoring of tomato crops using drones, tomatoes being one of the most widely produced vegetables worldwide. “Deep learning” based algorithms are valuable for fruit pest and disease monitoring and post-harvest sorting by analyzing image datasets and identifying patterns that are difficult for humans to detect. These algorithms enable fast and accurate responses to problems, improving the efficiency and accuracy of agricultural processes. In addition, ‘machine learning’ is linked with remote sensing and IoT to promote sustainable practices, where remote sensing technologies, such as unmanned aerial vehicles (UAVs), provide valuable data for precision agriculture, and IoT devices, such as WSNs,

collect crucial data on temperature, humidity and soil moisture to make informed crop management decisions. The fusion of machine learning, remote sensing and IoT has the potential to revolutionize sustainable agriculture.

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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

AMPP performed the bibliometric search, article writing, and bibliometric analysis and conducted the research process by leading the article review. WJCB contributed to the bibliometric analysis and performed the critical revision of the manuscript and its translation. All authors reviewed the final version of the manuscript.

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Factors associated with the adoption of technologies for avocado production systems

Factores asociados a la adopción tecnológica en sistemas de producción de aguacate

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ABSTRACT

The growth of avocado crops has led to an increase in technological needs and research to satisfy the demands of the value chain. There is a wide range of technologies applicable for this fruit crop, and there are challenges for transferring and adopting these processes. The objective of this work was to explore the determining factors in the adoption of technologies for avocado production systems and the perception of producers about these factors. For this, we carried out a socioeconomic characterization of avocado producers in Colombia including the recognition of the perception regarding technological adoption variables and an exploratory factorial analysis to evaluate the adoption factors based on the perception and technological level (TL). We found that some socioeconomic variables are related to the TL of the production systems. Meanwhile, perceptions regarding the adoption variables varied depending on the TL of the producers. Low TL presented a greater number of determinant variables in adoption decision-making. In contrast, for the medium and high levels of TL, adoption of technology was based on economic analysis. This research provides evidence for the effect of socioeconomic factors on the adoption of technologies in avocado production systems and shows how the perception of producers regarding these adoptions involves determinants associated with TL.

Key words: technological changes, multivariate analysis, *Persea americana*, technological level.

RESUMEN

El crecimiento de los cultivos de aguacate ha provocado un aumento de las necesidades tecnológicas y de investigación para satisfacer las demandas de la cadena de valor. Existe una amplia gama de tecnologías aplicables a este frutal, y existen desafíos para transferir y adoptar estos procesos. El objetivo de este trabajo fue explorar los factores determinantes en la adopción de tecnologías para los sistemas de producción de aguacate y la percepción de los productores sobre estos factores. Para esto, realizamos una caracterización socioeconómica de los productores de aguacate en Colombia incluyendo el reconocimiento de la percepción sobre las variables de adopción tecnológica y un análisis factorial exploratorio para evaluar los factores de adopción en función de la percepción y el nivel tecnológico (NT). Encontramos que algunas variables socioeconómicas están relacionadas con el NT de los sistemas de producción. Mientras tanto, las percepciones sobre las variables de adopción variaron dependiendo del NT de los productores. El NT bajo presentó mayor número de variables determinantes en la toma de decisiones de adopción. En contraste, para los niveles medio y alto de NT, la adopción de tecnología se basó en el análisis económico. Esta investigación proporciona evidencia del efecto de los factores socioeconómicos en la adopción de tecnologías en los sistemas de producción de aguacate y muestra cómo la percepción de los productores respecto a estas adopciones involucra determinantes asociados al NT.

Palabras clave: cambios tecnológicos, análisis multivariado, *Persea americana*, nivel tecnológico.

Introduction

Worldwide avocado (*Persea americana* Mill.) cultivation increased to 86% between 2012 and 2021. The continents with the largest increase of harvest were America (66%) and Africa (16%) (FAO, 2023). In Africa, the country with the largest harvest in 2021 was Ethiopia, with 27,946 ha. However, Zimbabwe showed greater growth, increasing

from 230 to 1,059 ha between 2012 and 2021. In the case of America, the country with the largest avocado crop in 2021 was Mexico, with 226,534 ha, and the country with the highest increase for this period was Colombia (27,705-94,110 ha) (FAO, 2023).

By 2022, Colombia ranked second place in planted areas and sixth in volume of avocado exports with 98,595 t,

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3.3% of the total volume imported in the world (ITC, 2023). Colombia has garnered global significance in the cultivation of avocado, symbolizing a remarkable growth trajectory and an increasingly influential role on the world stage (Cáceres-Zambrano Ramírez-Gil *et al.*, 2022). The country's ascent in avocado production is underscored by its favourable climatic conditions, diverse ecosystems, and strategic geographic positioning (Ramírez-Gil *et al.*, 2019). Colombia's prominence is not merely quantitative but extends to the quality of its avocado varieties that meet international standards. This newfound importance is shaping the nation into a relevant player in the global avocado market (Ramírez-Gil *et al.*, 2019). The socio-economic implications are profound, as the avocado industry becomes a key driver of economic growth and a source of international recognition for Colombia.

As this crop has grown in importance, its production has increased considerably (Cáceres-Zambrano Jiménez-Hernández *et al.*, 2022). However, while new forms of technology may have arisen, this does not mean that they are widely used. In this respect, issues relating to technology transfer, fruit quality, and crop productivity are significant (Ramírez-Gil *et al.*, 2019). Even though research on the cultivation of this fruit tree has led to a wide range of products, there remain challenges in the production system in terms of real and viable solutions to the problems of producers, with a positive benefit-cost ratio (Cáceres-Zambrano Jiménez-Hernández *et al.*, 2022). Additionally, technology transfer must be strengthened and made more strategic, given that regional links between producers facilitate transfer processes through leaders of territorial opinion (Varshney *et al.*, 2022).

The adoption of a new form of technology consists of accepting an innovation and integrating it into the productive context of the adopter (Straub, 2009). This process has been modelled and addressed by various authors. For example, Rogers (1962) proposes an innovation adoption curve, in which adopters are classified according to the time of adoption, focusing on the transfer of innovation. Other authors propose modelling the adoption of technologies according to the behaviour of the adopters (Ajzen, 1985). The Technology Acceptance Model (TAM), which incorporates the factors of perceived ease of use (Davis, 1986) in the utilization of technology (Venkatesh & Bala, 2008). Subsequently, Venkatesh *et al.* (2003) proposes the Unified Theory of Acceptance and Use of Technology (UTAUT) that utilizes various models as a basis and considers four direct factors in its adoption: performance expectation, effort expectation, social influence and facilitating conditions.

The technological classification of producers depends on the practices to be adopted, and transfer processes are constantly changing according to innovation and the characteristics of the social system (Ayisi *et al.*, 2022). In this regard, Curry *et al.* (2021) state that the sociocultural environment should be considered when developing new technology. When the proposed technologies go against the values and traditions of the community, adoption becomes less likely. At the regional level, the technology and knowledge associated with a given crop should be a cross-cutting tool in everyday and educational processes, involving producers and the family nucleus in crop management and thereby promoting local innovation (Gutiérrez García *et al.*, 2020).

The factors involved in the process of adoption of a technology in agriculture include social and demographic determinants, access to technology (Oyetunde-Usman *et al.*, 2021), risks and uncertainties of application, and user perception of benefits (The World Bank, 2007). Šūmane *et al.* (2018) state that a technology is adopted by a producer if it has been previously used and approved by its neighbours. Morris *et al.* (2017) find that technology can support business models in different ways and the active or passive adoption by producers depends on access to information.

Despite the existence of several technologies for the avocado production system, a gap is evident in its adoption (Cáceres-Zambrano Ramírez-Gil *et al.*, 2022). The adoption of technology in avocado production systems encounters multifaceted challenges rooted in a complex web of factors (Cáceres-Zambrano Ramírez-Gil *et al.*, 2022). These obstacles, largely undefined and interconnected, span social, economic, cultural, and market dimensions. The lack of well-established causative relationships hampers effective problem-solving (Cáceres-Zambrano Ramírez-Gil *et al.*, 2022). Understanding these issues is important for devising strategies that enhance the evaluation and subsequent adoption of relevant technologies by producers. By comprehensively unraveling the intricate tapestry of influences, stakeholders can formulate more effective approaches. This, in turn, promises to ameliorate economic, financial, and productivity indicators, fostering sustainability in avocado production systems. The confluence of diverse factors necessitates a nuanced understanding for the development of targeted and impactful interventions.

Elucidating the factors involved in the adoption of technologies enables efforts to be directed towards the generation and transfer of knowledge, thereby improving the

technological level and efficiency of production systems. Given the lack of knowledge relating to the determinants of technological adoption in the avocado agribusinesses in the neotropical country of Colombia, the objective of this research was to explore the determinant factors in the adoption of technologies in avocado production systems and the perception of producers regarding these factors. Based on preceding elements, our research hypothesis posits that the adoption of technology in avocado cultivation within tropical conditions, particularly in the context of Colombia, is multifaceted, context-specific, and contingent upon various social, cultural, economic, and productive considerations inherent to each agricultural system.

Materials and methods

Information collection

Information used in our study was collected from 125 avocado producers located in five departments of Colombia representing the most area planted and greatest volume of production. The departments and their respective municipalities were: Cundinamarca (municipalities of Silvania and Anolaima), Caldas (Belalcazar, Aguadas, Anserma, Manzanares, Manizales, Marquetalia, La Merced, Pácora, Pensilvania, Riosucio, Risaralda, Salamina, San José, Victoria and Villamaría), Antioquia (Sonsón, Abejorral and San Vicente Ferrer), Risaralda (Guática and Quinchía) and Tolima (Ibagué). For this purpose, a semi-structured survey was used to collect information on socioeconomic factors, frequency of use of technologies, and perception of adoption factors. The survey was carried out in person on each producer, during events organised by institutions, unions, associations, or trading houses.

The selection and quantification of producers deviated from conventional sampling techniques due to the absence of consolidated estimates and data within the sector. Ensuring the randomness of everyone posed technical challenges and incurred substantial costs. Consequently, an alternative approach was adopted, wherein individuals were randomly chosen from a finite population linked to the participation of producers in the events. This method was chosen acknowledging the practical constraints and logistical intricacies associated with achieving true randomness in individual selection within the given context. In this sense, the reliability of each instrument was evaluated with the alpha (α) criterion of Cronbach (Cronbach, 1951).

Characteristics of the adopters and the production system

As previously mentioned, the ability to adopt new technology is affected by the characteristics of the production system and of the adopters. Therefore, we characterized the producers and their production systems according to the following: gender, age, academic training, distance to the municipal capital, type of land tenure, source of income, use of credit, availability of internet and computer equipment in the production center, production area, number of trees planted, crop age, workforce, social security payment to workers, technical assistance, certifications, use of technical records, marketing channel, and producer participation in organisations. Subsequently, connections between variables were explored through multiple correspondence analysis (MCA). The objective of this was to infer whether, in the avocado agribusiness in Colombia, the technological level (TL) is linked to the characteristics of the adopters and the production system.

Technological adoption level

To compare the socioeconomic and adoption information collected with the TL, we used the system of Cáceres-Zambrano Ramírez-Gil *et al.* (2022), where by we characterized the TL based on the frequency of use of 82 technologies in the following links of the avocado agribusiness value chain: (i) propagation of plant material and nursery (16), (ii) production (43) and (iii) postharvest (23). The frequency of use was evaluated according to the Likert scale (1932), with five options for each response: 5 = always, 4 = almost always, 3 = sometimes, 2 = almost never, and 1 = never. We previously evaluated the technologies considered in each group, and we used the results of the factorial loads as a weight for the calculation of the TL. We used the TL results generated in the study to find relationships between it and the characteristics of the adopters, the production system, and the perception of adoption factors.

Perception of adoption factors

We consulted producers regarding the perceived importance of a group of preselected technology adoption factors following the UTAUT theory proposed by Venkatesh *et al.* (2003) (Tab. 1). We used a Likert scale (1932) based on the perception of importance, with five response options: 5 = very important, 4 = important, 3 = moderately important, 2 = of low importance and 1 = not important. We grouped the results according to the TL, to elucidate the relationship between it and the adoption factors.

TABLE 1. Technological adoption factors in avocado production systems under neotropical conditions, Colombia.

Factor	Variable	Source
Performance expectation	Increase in cultivation output	(Xie & Huang, 2021)
	Contribution to fruit quality	(Barrios <i>et al.</i> , 2020)
	Contribution to avocado export process	(Xie & Huang, 2021)
	Reduction of production costs	(Ruzzante & Bilton, 2021)
	Improvement in employee productivity	(Tami-Barrera, 2021)
Effort expectation	Market availability of technology	(Doss, 2003)
	Experiences of technology use	(Barrios <i>et al.</i> , 2020)
Social influence	Adoption by other producers	(Ruzzante & Bilton, 2021)
	Gaining recognition in the region	(Barrios <i>et al.</i> , 2020)
	Value of the farm	(Xie & Huang, 2021)
	Environmental impact of technology	(Liu <i>et al.</i> , 2018)
	Buyer requirements	(Foster & Rosenzweig, 2010)
	Acceptance by business partners or family	(Ruzzante & Bilton, 2021)
Facilitating conditions	Ease of use of technology	(Taherdoost, 2018)
	Access to credit	(Ruzzante & Bilton, 2021)
	Technical assistance	(Tami-Barrera, 2021)
	Internet availability on the farm	(Tami-Barrera, 2021)
	Technology price	(Ruzzante & Bilton, 2021)
	Support by technology provider	(Tami-Barrera, 2021)

Data analysis

We used the free software R Project v4 (R Core Team, 2022) to perform the statistical analyses. The characterization data were explored with MCA. Adoption factor data were analysed using principal component analysis (PCA) with the aim of carrying out an initial exploration of the data and reducing the number of dimensions, facilitating the observation of relationships between variables. Perception data on adoption factors were used with 125 producers and 19 variables (Tab. 1). A PCA was used prior to the factorial analysis, to verify that the groupings between variables matched what is in the literature and what was proposed in this study (Tab. 1). For this analysis, the libraries *factoMineR* (Lê *et al.*, 2008) and *factoextra* (Kassambara & Mundt, 2020) were used.

Having found that the groups of factors evaluated did not show a grouping like what was proposed, we decided to use an exploratory factor analysis (EFA). We used the EFA to find the distribution according to the perception of importance on the part of the surveyed producers (Lloret-Segura *et al.*, 2014). For this, we used 17 of the 19 variables, since for the other two, the perception of importance was unanimous among the producers. The analysis was carried out for the group of producers in general and by TL (high, medium, and low), with the objective of inferring whether

the grouping and loads of the variables changed depending on the TL of the producers. To carry out the EFA, we considered four assumptions. Firstly, for the number of factors to extract, we selected the number with the greatest number of supporting methods (Lloret-Segura *et al.*, 2014). Secondly, for type of rotation to be used, we chose a varimax orthogonal type, considering the absence of relationships between the items evaluated. Thirdly, for the estimation method, we used the weighted least squares (WLS), as this is a robust method used in the analysis of data that does not meet the assumption of multivariate normality, as is the case for the data analysed in the present work (Lloret-Segura *et al.*, 2014). Finally, for the matrix used for estimation, we selected a polychoric type, due to the five-option scale used in the questionnaire based on the Likert scale. For the EFA, the fit of the proposed model was evaluated using the Comparative Fit Index, the Tucker-Lewis index ($TLI > 0.90$), the relative measure for model comparison, and the Bayesian information criterion (BIC) (West *et al.*, 2012; Cavanaugh & Neath, 2019). The library used for the factorial analysis was *nFactors* (Raiche & Magis, 2020).

Subsequently, and considering that there were producers from each of the three TLs, we used a model for each level to verify whether the fit of the model could be improved for each TL. We also used cross-sectional libraries for the

psych analysis (Revelle, 2021), psycho analysis (Makowski, 2018) and plotly analysis (Sievert, 2020) for descriptive analysis and generation of visualization tools.

Results

Characteristics of the adopters and of the production systems

Among the surveyed avocado producers, men (88.8%) predominated over women (11.2%) with non-binaries unrepresented. Ages were between 30 and 60 years (67.2%). For the gender or age, a distribution related to the TL was not found. Predominant education levels were primary (52.8%), followed by secondary (24%), technical or technological education (8.8%), professional (8.8%) and postgraduate (5.6%). Producers with primary and secondary academic

training included the three levels of TL (high, medium, and low); the medium and high TL included producers with technical or higher education not found in the low TL. The characteristics of the adopters were grouped according to the TL (Fig. 1).

The departments of Caldas (48.0%) and Antioquia (36.8%) led the distribution of the surveyed producers followed by Cundinamarca (8.8%), Risaralda (4.8%) and Tolima (1.6 %). Producers in the departments with the largest number of people surveyed were mainly in the medium and high TL. The majority of the production systems studied were located less than 10 km from the municipal capitals (62.4%) and 95.2% of the producers surveyed and they carried out the activities on their own properties. The main economic activity was agriculture (84.8%), while 6.4% received income from sources other than production.

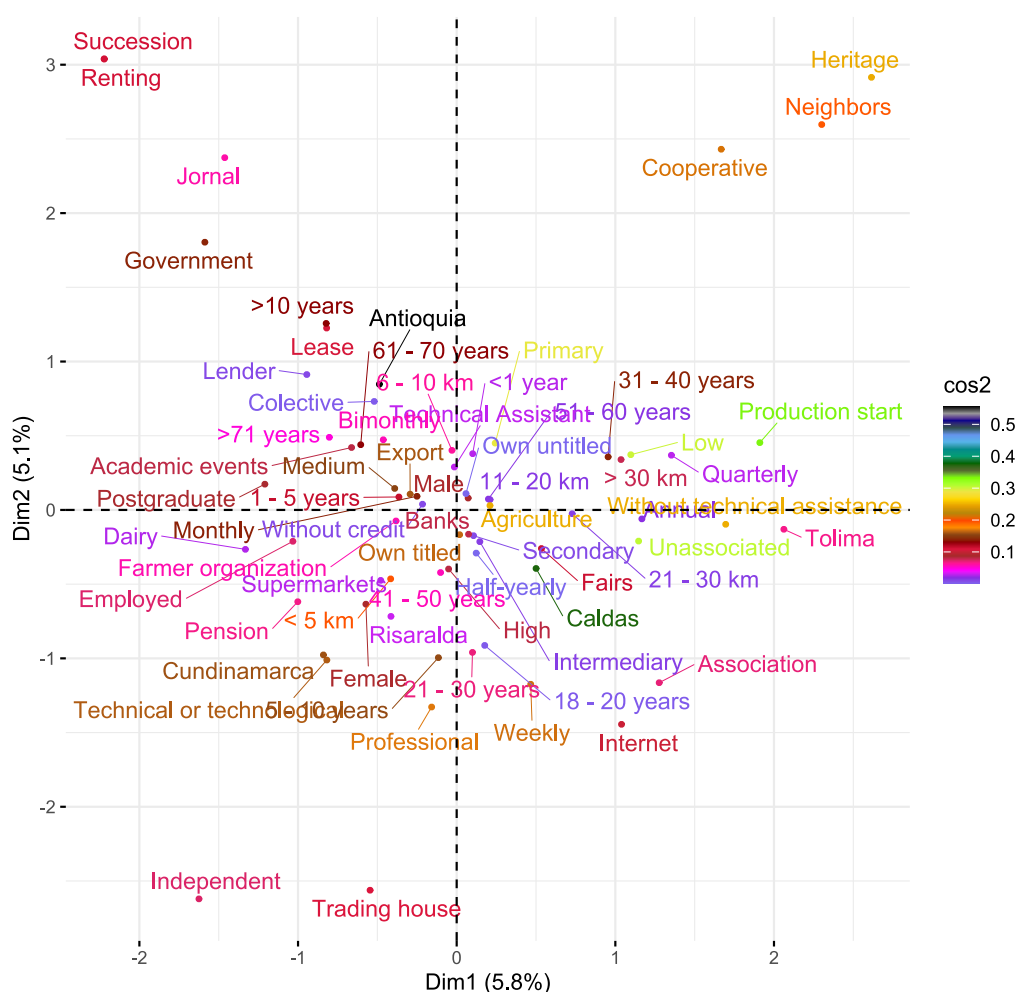


FIGURE 1. Characteristics of the producers and avocado production systems according to the technological level using the multiple correspondence analysis.

Some 77.6% of the interviewers stated that they had used credit to finance their productive activity, and banks were the main source of leverage. Regarding connectivity and use of computer equipment, 65.6% (connectivity) and 68% (computer equipment) of the interviewers did not have internet and computer equipment at the production site. The highest percentage of these characteristics (*i.e.*, credit, possession of computer equipment and access to the Internet) occurred in the high TL.

The area of the production systems and the number of trees were the parameters with high variation in each TL. We observed that with a decrease of the TL the upper limit of the range also decreased (greater number of plants and planted areas). However, we noted a small area of the production systems in the upper TL. Regarding the age characteristics of the cultivation, we found no distribution related to technological level and age characteristics.

The production systems used family labour in 50.4% of the cases. Producers with high TL had a greater use of hired labour, while the medium and low TL producers used a greater proportion of family labour. Of the productive units that used family labour, 46% did pay for this work. We found a low proportion of social security payments. Access to technical assistance by the surveyed producers was high (92%) and was mostly on a contractual basis with a frequency of visits of once per month.

The distribution of GAP global certification had no relationship with the TL. However, the medium and high TL producers were more frequently registered exporting properties. We found that 83.2% of the surveyed producers kept some kind of record of their activities; this was a characteristic that was not related to the technological levels. Meanwhile, the predominant marketing channel was export (62.4%), followed by intermediaries (20.8%). There was a high percentage of producers linked to a trade union or local association (82.4%).

Perception by avocado producers of technological adoption factors

The survey showed average reliability based on a criterion of Cronbach ($\alpha = 0.60$; $P < 0.05$; average $r = 0.08$), with items usually un-associated with each other. In general, the surveyed avocado producers perceived the adoption factors evaluated as being very important. Of the variables, increase in crop yield and ease of use of the technology stand out, since they are considered very important by 100% of the producers (Fig. 2). The variable considered of least

importance was adoption by other producers. Facilitating conditions and social influence factors were rated as less important than those of performance expectation and effort expectation.

By grouping the producers by TL and verifying the frequency in the perception of importance of the selected variables, we found some overlap in the importance rating among producers with different TLs (Fig. 3). The medium and high TL producers share variables with each other and, to a lesser extent, with the low TL producers. However, the producers located in the low TL valued a greater number of variables as “very important” compared with the other two levels. The producers located in the lower LT unanimously rated as very important the variables of value of the farm, adoption by partners and family, and market availability of technology. Additionally, we found that variables that presented high variability in the importance rated independently of the technological level.

The variables analysed were reduced to three dimensions that explained 70.1% of the variance of the data in which buyer requirements, adoption by other producers, and the price of the technology were the variables with the greatest contribution (Fig. 4). Producers usually have little technical independence in decision-making, meaning that they usually ask for advice, not only from technical consultants, but also from neighbours or other experienced producers. Decisions are made according to observable economic results that will allow better income. However, producers also consider aspects related to new markets or the possibility of providing a superior product. On the other hand, the support of state or financial institutions decreases uncertainty when adopting a new technology.

The original model evaluated showed a good fit ($P < 0.05$; BIC = 15.33; TLI = -0.27) that was improved through the elimination of those variables with a lower proportion of explained variance ($P < 0.05$; BIC = 10.38; TLI = -0.57). Two factors were found, namely “decreased uncertainty and market entry” that linked four variables related to the market, “quality and financing”; and “information and financial advantages” that linked six variables related to technical aspects, social influence, and technological aspects (Fig. 5).

We found that the importance assigned to the proposed variables was different among the producers from the different TLs (Tab. 2). Similarly, the grouping, factorial loads and excluded variables were specific to each TL. High TL

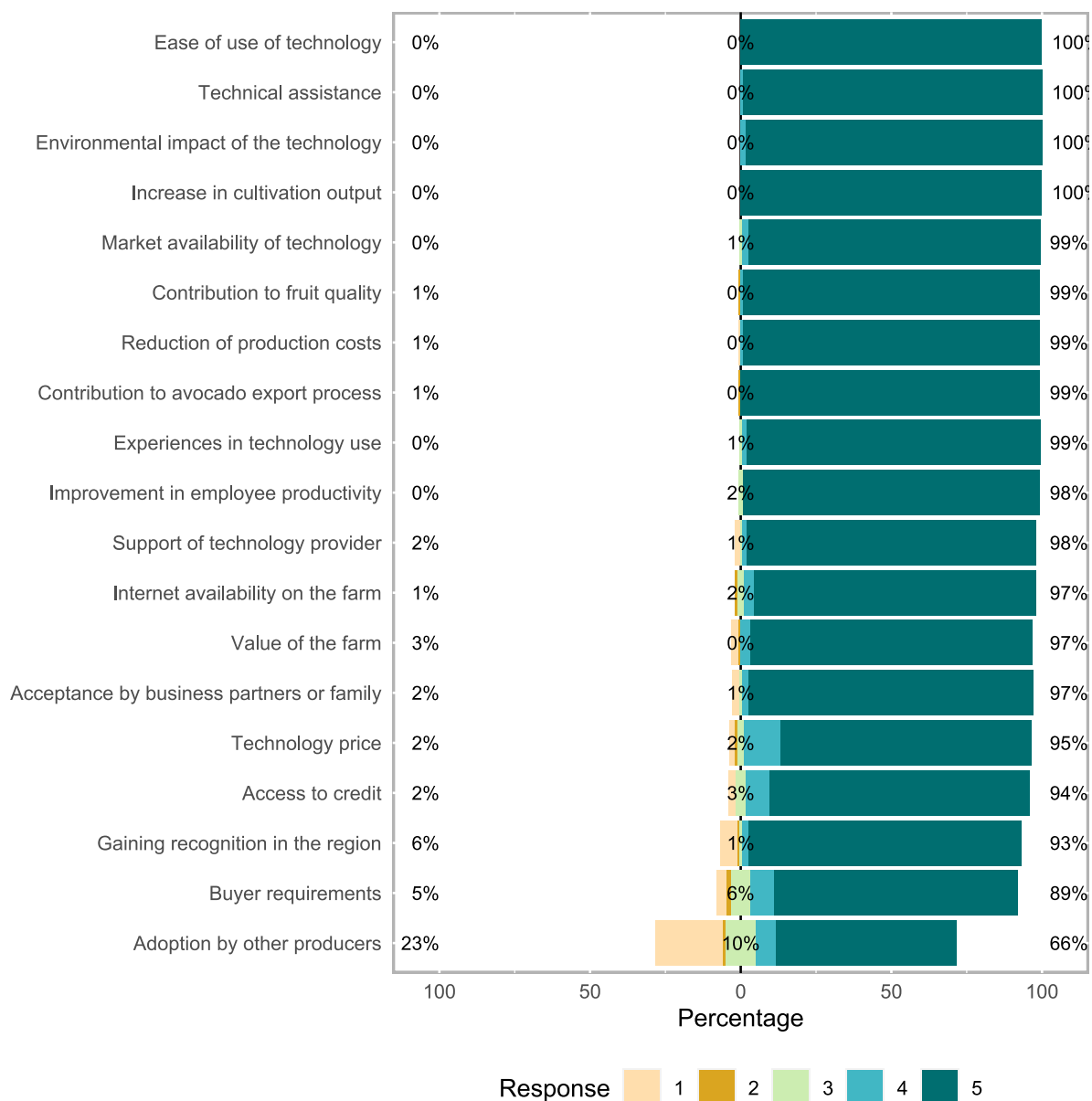


FIGURE 2. Perception of the importance of the variables and factors of technological adoption by avocado producers based on Likert scale analysis.

producers downplayed variables related to finance and to adoption of technology by other producers ($P < 0.05$; BIC = 1632; TLI = -2.60). At this level, the experience of using the technology was more important than adoption by other producers; this reflects the fact that the thinking behind the production system goes beyond the regional or national environment.

The middle TL producers considered three factors with associated variables, excluding the reduction of costs, adoption by other producers, value of the farm, demand by

the customer, contribution to the quality of the fruit, and price of the technology ($P < 0.05$; BIC = 3335; TLI = -1.78). The low TL model was initially built with seven variables since a high degree of importance was given to the other variables by all the producers. Based on these variables, we constructed a model in which the variables of internet presence on the farm and support from the technology provider were excluded ($P < 0.05$; BIC = 372; TLI = -7.9). In this research, we regrouped the initial groups based on the perception of the producers, to find a factor that decreased uncertainty.

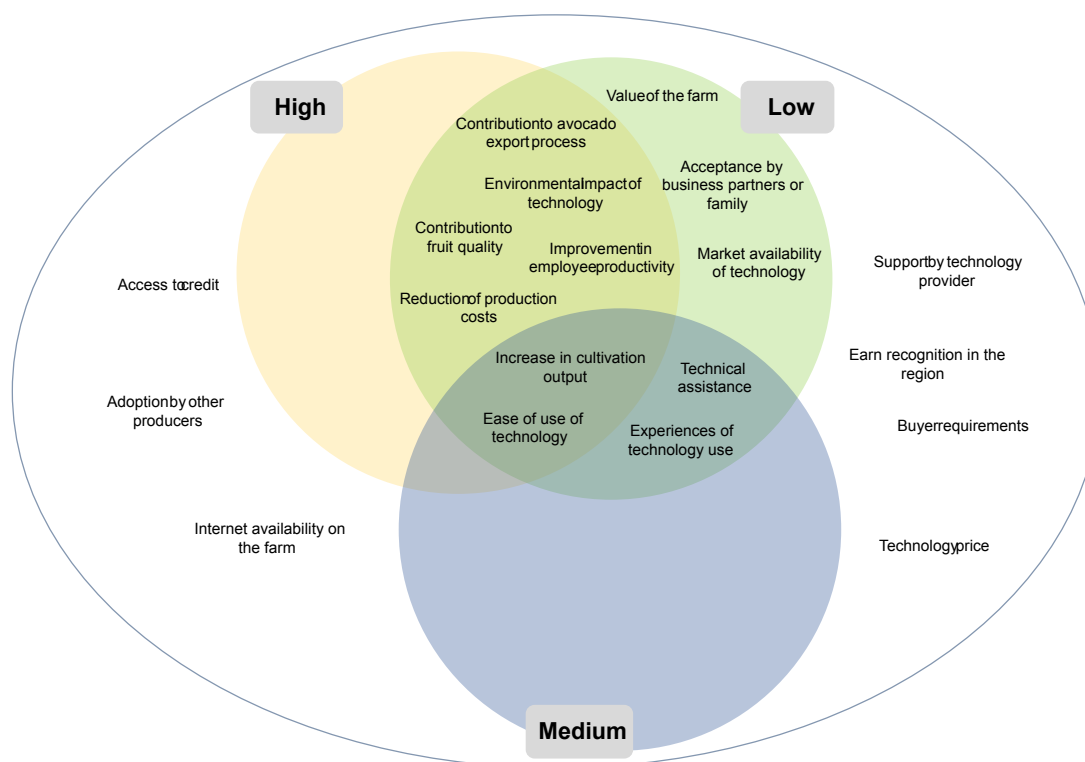


FIGURE 3. Perception of importance of adoption variables according to technological level of avocado production systems. The variables inside the coloured circles were rated as “very important” by 100% of producers in the corresponding technological level. The variables outside the coloured circles are those that were considered less important by the surveyed producers in all the TLs.

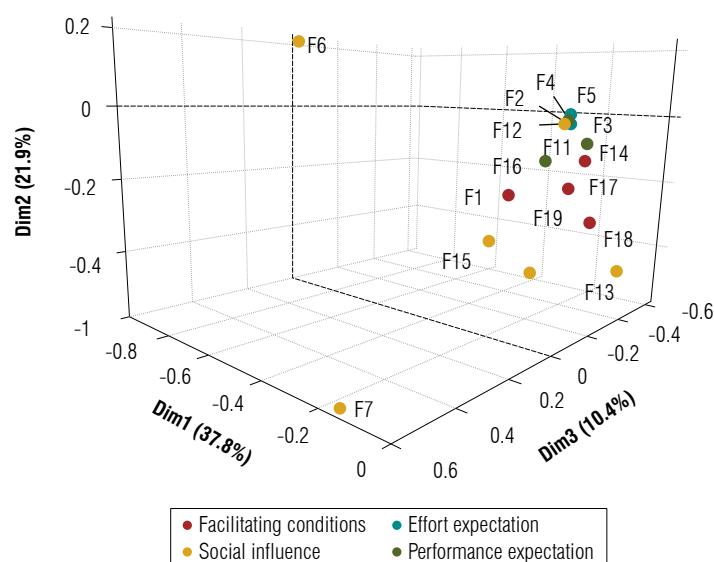


FIGURE 4. Principal component analysis as a tool for exploring the distribution of factors according to the variance of the data. F1 = access to credit; F2 = technical assistance; F3 = contribution to the avocado export process; F4 = reduction of production costs; F5 = availability of the technology in the market; F6 = adoption by other producers; F7 = gaining recognition in the region; F8 = value of the property; F9 = increase in crop yield; F10 = ease of use of technology; F11 = experiences in the use of technology; F12 = environmental impact of technology; F13 = buyer requirements; F14 = contribution to the quality of the fruit; F15 = acceptance by partners or family; F16 = improvement in employee productivity; F17 = internet presence on the farm; F18 = price of technology; and F19 = support from the technology provider.

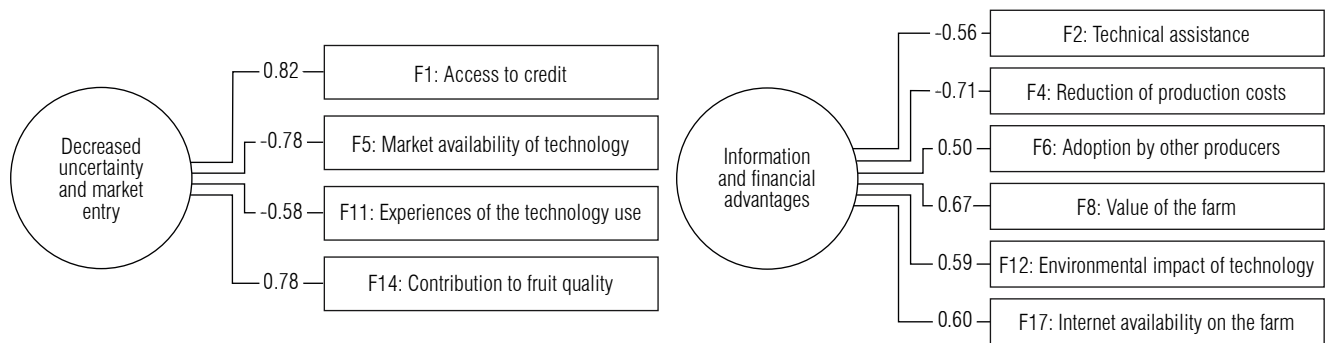


FIGURE 5. Perception factors related to the adoption of technologies in avocado production systems.

TABLE 2. Technological adoption factors and factorial loads in avocado production systems according to technological level.

High technological level				
Facilitating conditions and social influence	Technical assistance	Acceptance by business partners or family	Market availability of technology	Internet availability on the farm
	0.95	0.84	-0.49	-0.46
Reduction of uncertainty	Support from technology provider	Experiences in the use of technology		
	-0.89	0.87		
Medium technological level				
Facilitating conditions	Market availability of technology	Access to credit	Environmental impact of technology	Support from technology provider
	-0.87	0.81	0.66	0.64
Social influence	Acceptance by business partners or family	Contribution to avocado export process	Gaining recognition in the region	
	0.88	-0.78	0.76	
Performance expectation	Improvement in employee productivity	Internet availability on the farm		
	-0.89	0.58		
Low technological level				
Market	Buyer requirements	Access to credit	Technology price	
	0.82	-0.74	0.74	
Social influence	Adoption by business partners or family	Gaining recognition in the region		
	0.69	-0.93		

Note: The numbers in the table correspond to the factorial loads for the structural equation model at each technology level.

Discussion

We found a gender gap that corresponded to what is generally found in agriculture: women showed low participation in decision-making spaces (The Consultative Group on International Agricultural Research (CGIAR) & Leveraging Evidence for Access and Development (LEAD), 2021). However, it should be emphasised that the participation of women in agricultural production systems allows the strengthening of value chains and their resilience (Huyer,

2016). As such, the value chain would benefit from improving the gender gap.

The most frequent age range was between 30 and 60 years, while the least frequent was between 18 and 30. Parra and Knobloch (2022) find that the experience favours good decision-making in this regard. Liu *et al.* (2018) claim that greater experience leads to lower receptiveness to new technologies. This could be related to a decreased transfer of agricultural businesses between generations and to the low

proportion of young people dedicated to agricultural work. This is an important aspect given that age is considered a relevant factor for the adoption of technologies.

We found that producers with higher academic training tended to have higher technological levels. Academic training favours the adoption of new technology (Liu *et al.* 2018). However, Curry *et al.* (2021) state that technology adoption and related factors are not limited to academic, financial, or technical aspects, and that a comprehensive vision, considering the way in which technologies, habits and traditions of adopters are communicated, is necessary.

Avocado cultivation, especially of cv. Hass, has grown with greater intensity in particular areas of Colombia. This has led some producers to join forces and manage resources for training in productive and commercial aspects. This growth is reflected in the distribution of producers with respect to technological levels in certain regions, with those areas with the highest growth becoming stronger (Quintero-Ramirez *et al.*, 2019). The producers showed high associativity percentages at all technological levels, a characteristic that has a positive effect on innovation and technology networks by favouring interaction with other actors in the chain. Krishnan *et al.* (2021) find that collaborative work and producer organisations promote the implementation of innovations.

Other factors such as type of land tenure, source of income and access to credit are related to the technological level (Kassie *et al.*, 2013). However, the data collected in this study did not find such a relationship. We observed that the technological level was high in the production systems with larger areas, matching the findings of previous reports (Doss, 2003). On the other hand, the use of technological and communication tools by production systems allows access to markets via the internet and to technological information related to management practices (Morris *et al.*, 2017). Similarly, formality in recruitment processes is decisive in technological and innovation processes, since it allows the evaluation of potential employees and therefore the optimization of processes (Juma, 2015).

Registration as an exporting farm was more frequent than global GAP certification, even though the latter is an international certification requirement to export to certain destinations (Castrillón Correa, 2020). This behaviour can be explained by the fact that registering as an exporting farm is a mandatory national standard for marketing fruit abroad (ICA, 2016). Lippe and Grote (2017) find that the

global GAP certification process requires investment that is sometimes not reflected in a monetary incentive for producers; this is why this certification is usually adopted only if it is financed or supported through public-private partnerships.

Tiruneh *et al.* (2015) state that the adoption of a new technology depends on the ability of producers to perceive the advantages of this in the existing socioeconomic conditions. In this research we found that the factors of social influence and facilitating conditions were perceived as less important than expectations of effort and performance. However, social influence has a role in adoption decisions (Tiruneh *et al.*, 2015).

The producer perception of the variables evaluated depended on their technological level. Producers with lower TL placed greater importance on those variables that represented financial benefits and ease of access, such as farm value, acceptance by partners and family, and availability of technology in the market. Porteous (2020) finds that the adoption of technologies is linked to commercial and financial incentives for producers that improves their technological level. But, producers face risks when adopting new technology; therefore, reducing the associated uncertainty facilitates decision-making regarding technological matters (Liu *et al.*, 2018). The hypothesis of this research was corroborated, since the decision to adopt a technology is more complex and is crossed by aspects of uncertainty and technical analysis in addition to income alone.

The results of this study supported the assertion that the size of the farm and the characteristics of the farmers do not directly influence technological adoption, but rather they influence the perception of producers in decisions regarding the adoption of technology (Tiruneh *et al.*, 2015). The need to collect information regarding other variables and dimensions is evident. In this regard, Maertens and Barrett (2013) state that the collection of data on social networks, information flows, and other unobservable variables are relevant for modelling technological adoption. This would involve an analysis of the entire adoption process and not just an evaluation of the technological level and perception at a given moment.

Our results indicated that the adoption of technology among avocado producers is influenced by a great number of factors: age, gender, educational attainment, cultural considerations, risk perception, investment capacity,

opportunity cost, technology availability, applicability, among others. Recognizing the unique dynamics of each productive unit, we abstained from prescribing a universal judgment on the efficacy of specific technologies, emphasizing the need for nuanced assessments tailored to local contexts and individual circumstances. This approach enables a comprehensive understanding of the intricate interplay between multifaceted factors influencing technological adoption in the avocado production domain, but not associated with which technology is more adequate or not adequate by the production systems evaluated.

It is imperative to acknowledge the limitations inherent in our study. The representativeness of our sample posed a constraint, as it may not fully encapsulate the broader population perspectives on crucial factors in technological adoption in the avocado production systems. Moreover, the absence of validation with non-surveyed individuals limited the generalizability of our findings. Moving forward, a comprehensive exploration involving a balanced representation of all potential determinants is warranted. Future research should delve into strategies aimed at improving technological adoption, elucidating how this enhancement can catalyse into production efficiency, competitiveness, and sustainability within the avocado cultivation sector. This avenue holds promise for advancing both academic understanding and practical outcomes in agricultural technology adoption.

Conclusion

This study allowed us to explore the relationship between social factors and the perceptions of the producers on the determinants of technological adoption in the avocado value chain. The area of the productive unit, the level of academic training of the producers, and the type of marketing channel were variables related to the technological level of the production systems. Additionally, there was a relationship between the technological level and the perception of adoption factors. Producers with a low technological level valued a greater number of variables as determinants for the adoption of decision-making, while at medium and high technological levels fewer variables were considered, and these were related to commercial, technical, and financial benefits. The results of this research showed the need to study aspects of academic training in the rural sector, given that this is a determining variable in the adoption of technologies. In addition, it was necessary to focus the extension of technology and transfer efforts on the financial benefits of technological adoption, as well as addressing the perception of associated risks.

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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

JCZ, JGRG and DB: conceptualization, methodology, validation, data curation. JGRG and DB: review and editing, supervision. JCZ and JGRG: software. DB: project administration. JCZ: formal analysis, investigation, resources, original draft preparation, visualization, funding acquisition. All authors have read and approved the final version of the manuscript.

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Exploring adoption factors of innovations in arracacha crop: A case study in Cajamarca, Colombia

Explorando los factores de adopción de innovaciones en el cultivo de arracacha:
un estudio de caso en Cajamarca, Colombia

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ABSTRACT

Currently, the demand for new arracacha (*Arracacia xanthorrhiza* Bancr.) varieties has increased in Colombia; however, yields are still below their potential. This is because farmers in the country still find it challenging to adopt innovations that allow them to improve both productivity and their income. Furthermore, there is limited information and scientific documentation on arracacha cultivation. Therefore, the aim of this study was to analyze the factors influencing the adoption of innovations for cultivating arracacha in the municipality of Cajamarca, Colombia, through a multivariate analysis. The research was carried out with 104 farmers, and surveys containing variables such as the productive activity dynamics and the profile of the farmer were applied. Two conglomerates (clusters) of adopters were created, and the innovation adoption index (INAI) was analyzed in eight categories, including 28 technologies. The factors that were most differentiated and significant were those related to the farm, such as management, organization and health indexes, as well as factors related to farmer characteristics, such as level of schooling.

Key words: *Arracacia xanthorrhiza*, multivariate analysis, rural development strategies, technology adoption, farmers.

RESUMEN

Actualmente, la demanda por nuevas variedades de arracacha (*Arracacia xanthorrhiza* Bancr.) se ha incrementado en Colombia; no obstante, los rendimientos aún están por debajo de su potencial. Esto porque a los agricultores del país todavía se les dificulta adoptar innovaciones que permitan mejorar tanto la productividad como sus ingresos. Además, existe limitada información y documentación científica sobre este tema para el cultivo de la arracacha. Por lo tanto, el objetivo de este estudio fue analizar los factores que influyen en la adopción de innovaciones para el cultivo de la arracacha en el municipio de Cajamarca, Colombia, a través de un análisis multivariado. La investigación se llevó a cabo con 104 agricultores y se aplicaron encuestas que contenían variables de la dinámica de la actividad productiva y el perfil del agricultor. Se crearon dos conglomerados (clústeres) de adoptantes y se analizó el índice de adopción de innovaciones (INAI) en ocho categorías, incluyendo 28 tecnologías. Los factores que más se diferenciaron y tuvieron significancia fueron los grupos de aspectos relacionados con la finca, como los índices de administración, organización y sanidad, además de factores relacionados con rasgos del agricultor como el grado de escolaridad.

Palabras clave: *Arracacia xanthorrhiza*, análisis multivariado, estrategias de desarrollo rural, adopción de tecnología, agricultores.

Introduction

The municipality of Cajamarca is located in the western part of the department of Tolima, 35 km from Ibagué on the Pan-American Highway. It has a total area of 520 km², of which 0.2% is urban and 99.8% rural, with 42 districts (*veredas*, in Spanish) and a township called Anaime; Cajamarca is recognized as Colombia's agricultural pantry (Alcaldía Municipal de Cajamarca, 2020). In Cajamarca, the rural area is predominantly extensive, which favors

diverse intensive agriculture, with production systems that include arracacha (*Arracacia xanthorrhiza* Bancr.), with average plantings of 5,000 ha per year, making it the municipality with the highest production at the national level (Garnica *et al.*, 2021).

Because arracacha is a typical crop of the inter-Andean valleys, it requires a medium level of technification, which makes it a very efficient option for small and community farmers, with a commercial approach provided by

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intermediaries (Garnica *et al.*, 2021). It should be emphasized that it is a productive system deficient in innovations, based exclusively on an increase in the area planted. Dividends could only be increased if constant innovations are promoted at the local level to achieve better profitability, looking at the system as a whole (Köhler & González, 2014).

Innovation is defined as the introduction of a new or improved product or production process; this definition is based on the concept that innovation is the creation and adoption of new ideas or solutions that improve the efficiency and effectiveness of existing products, services and processes (Sotomayor *et al.*, 2011). Additionally, the concept of adoption plays an important role in this study. The first definition of adoption was postulated in 1962 by Everett Rogers, who proposed it as a mental process through which an individual goes from hearing about an innovation to its final adoption. Adopters of the innovation tend to explore the new technology and experience how effectively it would work in their areas before accepting or rejecting those technologies (Rogers *et al.*, 2019).

In fact, the adoption of innovations by farmers through knowledge transfer models is a challenge in the agricultural sector. Even today, there are still doubts about the technology transfer model currently used in Colombia. This model, which dates from the 1980s, follows a linear approach and considers the farmer as the final recipient of the research results (Rogers *et al.*, 2019). The above implies that the farmers initial direct participation with the institutions that carry out the research process is not considered, resulting in low levels of adoption of innovations (Mercado *et al.*, 2019).

Adoption is analyzed to understand how and why farmers use certain technologies and the impact of their implementation, which is essential for researchers and policymakers (Martínez & Pachón, 2021). In this sense, adoption depends on a wide variety of personal, social, and cultural characteristics, economic factors, and innovation aspects (Liu *et al.*, 2018; Yue *et al.*, 2023). The literature includes reports related to the evaluation of these indicators for adoption analysis. One example is that farmers who have a market insurance contract have higher adoption levels, at 66%, while those who did not have a contract had a 34% adoption rate (Chelang'a *et al.*, 2023).

Low productivity is related to limited adoption by farmers. Therefore, analysis frameworks have been created to better understand this rural phenomenon (Ramírez-Gómez *et al.*, 2020; Ramírez-Gómez & Rodríguez Espinosa,

2022; Torres-Avila *et al.*, 2022). Various studies highlight the influence of non-economic factors on the decisions made by farmers regarding the adoption of innovations, with six categories proposed to categorize these factors: i) farmer and household traits, ii) general aspects of the farm, iii) financial and administration details of the farm, iv) external factors, v) characteristics of the practice, and vi) psychological elements (Liu *et al.*, 2018; Foguesatto & Machado, 2022).

According to the previous context and due to the limited information available on this topic, the aim of this study was to analyze the factors that contribute to the adoption of innovations for the cultivation of arracacha in the Municipality of Cajamarca, Colombia. The factors to be analyzed in the study were selected based on research conducted by Muñoz *et al.* (2007) and Aguilar-Ávila *et al.* (2020), where factors specific to the farmer, the farm and its production process are taken into account, with the objective of increasing productivity by incorporating new technologies or practices.

Materials and methods

Study area

This research was carried out in 2022 in the 27 largest arracacha-producing areas of the municipality of Cajamarca, Department of Tolima, Colombia (Fig. 1). The data collection instrument included three sections: the first was producer attributes with open questions, the second was dynamics of the productive activity, and the third was innovation dynamics, where it was necessary to indicate whether or not the innovation or practice was used. In this sense, information collection and analysis were done with previous authorization by the farmers and under informed consent.

Population and sampling

Our study population includes arracacha farmers in the municipality based on the census held by Corporación Colombiana de Investigación Agropecuaria – AGROSAVIA of 304 farmers. A simple random sampling design was used. The sample size calculation was carried out for a known population (Eq. 1) (Aguilar-Ávila *et al.*, 2020), establishing a sample size of 104 farmers:

$$n = \frac{Npq}{\left(\frac{N-1}{Z^2}\right) d^2 p^2 + pq} \quad (1)$$

where, n is the number of individuals to survey, N is the total number of individuals in the population (304), p is the

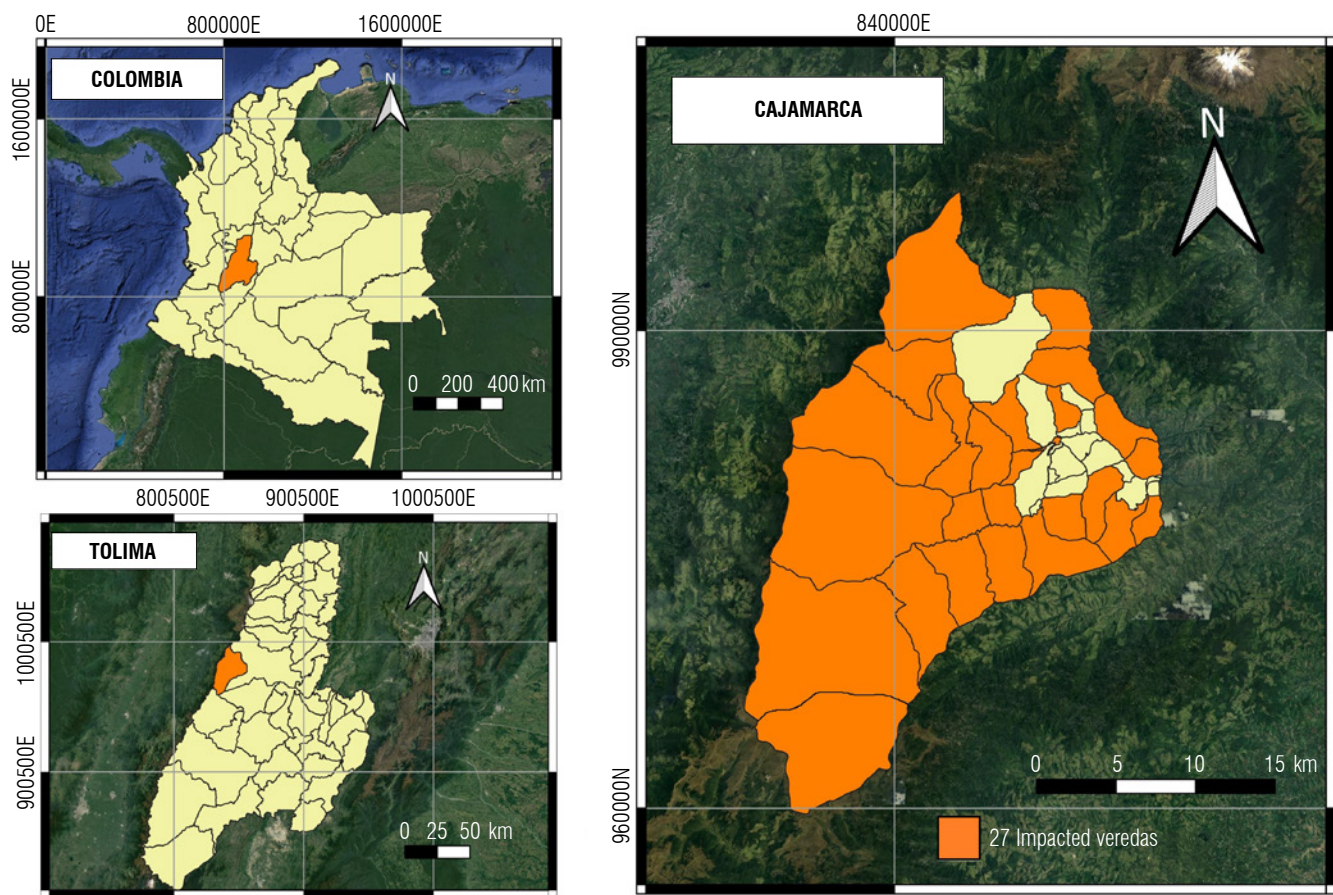


FIGURE 1. Study area in Cajamarca, Tolima, Colombia. The districts (*veredas*, in Spanish) where farmers were surveyed are highlighted in orange.

probability that the event under study will occur, *i.e.*, that the information will be successfully obtained. Garnica *et al.* (2021) determined that approximately 4 out of 5 (80%) producers are small or medium-sized, which would make it easier to obtain the information (0.8); q is the p differential: $(1-p) = 0.2$. Z is the reliability, but for populations with more than 10, it was estimated at 90% = 1.64, and d is the precision (10% = 0.1).

Innovation adoption analysis

For this diagnosis, the methodology postulated by Muñoz *et al.* (2007) and Aguilar-Ávila *et al.* (2020) was used to analyze innovation processes in the agri-food and rural sectors. In this methodology, a baseline of the innovation analysis in arracacha was obtained through a catalog of innovations for this crop, considering categories such as plant nutrition, plant health, sustainable resources management, plantation management, administration, organization, harvest, and plant propagation (Aguilar-Ávila *et al.*, 2020), by means of a workshop of professionals and researchers (seven people) knowledgeable about the crop, thanks to their knowledge and experience in research and

dissemination processes, which they have developed since 1990. Accordingly, the identification and validation of this catalog of innovations and practices for the cultivation of arracacha was based on the productive model of arracacha (Garnica *et al.*, 2021) published for this municipality, which consolidates and recommends appropriate management practices for this crop, which are often not known by the rural stakeholders themselves.

A survey identified the indicators for the analysis. One is the innovation adoption index (INAI), which shows the relationship of the degree of adoption of farmers based on a number of innovations of the technology that must be adopted for the good development of their productive system (Aguilar-Ávila *et al.*, 2020); the second, characteristics of arracacha farmers, included adoption factors such as age, sex, education, demographic, socioeconomic, institutional, and plot characteristics (Sileshi *et al.*, 2019).

The innovation adoption index (INAI) is an indicator (Eq. 2) that measures the degree of adoption of a series of innovations or practices (Aguilar Ávila *et al.*, 2020):

$$INAI_{ik} = \frac{\sum_{j=1}^n Innov_{jk}}{n} \quad (2)$$

where, $INAI_{ik}$ is the innovation adoption rate of the i^{th} farmer in the k^{th} category, $Innov_{jk}$ is the presence of the j^{th} innovation in the k^{th} category, and n is the total number of innovations in the k^{th} category.

The Microsoft Excel program was used to calculate the innovation indicators according to the methodological guide for analyzing innovation processes in the agri-food and rural sectors (Aguilar-Ávila *et al.*, 2020). Twenty-eight innovations were evaluated to calculate the indicators of innovation dynamics and were divided into eight categories. A statistical analysis of the different variables identified the categories of innovations that most favor the adoption of innovations, in order to prioritize these categories in regional intervention processes aimed at producers.

To obtain the indicators of the farmer knowledge network related to the input and output grades, the survey included the question: Whom do you consult when you have a technical question about the cultivation of arracacha? The answers with the names were coded to obtain the indicators for this network, using the UCINET program (Borgatti *et al.*, 2002).

Statistical analysis

A multivariate data analysis (Cluster) was performed to classify arracacha farmers following the methodology of Aguilar-Gallegos *et al.* (2015) and Ramírez-Gómez *et al.* (2023). First, the squared Euclidean distance was calculated with the data of the 28 innovations coded as a dichotomous variable (1=adopted, 0=not adopted). Then, four basic units of characterization were established (Núñez-Colín & Escobedo-López, 2011). These were the basis for the cluster analysis and indicated how similar or dissimilar the arracacha farmers are (Tab. 1). The INAI is usually used to establish adoption through a global indicator. Binary variables were used to calculate similarity and dissimilarity coefficients to propose a method for grouping producers based on the combination of practices validated in

different studies (Cheetham & Hazel, 1969; Núñez-Colín & Escobedo-López, 2011; Aguilar-Gallegos *et al.*, 2015; Sánchez-Cañizares *et al.*, 2022; Ramírez-Gómez *et al.*, 2023; Zeleke *et al.*, 2023). The method used for grouping the basic characterization units was Ward's minimum variance method. It is based on reducing the residual variance by grouping observations, which is carried out by testing all possible pairs (Pérez, 2004). The number of groups was defined based on the following indices: cubic grouping criterion (CGC) (Sarle, 1983), ball (Ball & Hall, 1965), cindex (Hubert & Levin, 1976), CH (Calinski & Harabasz, 1974), DB (Davies & Bouldin, 1979), Hartigan (Hartigan & Wong, 1979), and Silhouette (Rousseeuw, 1987).

Seventeen variables were compared between the groups formed to validate the ones formed in the cluster analysis. These corresponded to characteristics of arracacha farmers, productive units, and variables calculated in different adoption level categories. Once two groups of producers were selected according to the above-mentioned indices, the groups were compared using a t-test for independent samples. Normality was evaluated with the Shapiro-Wilks test. The non-parametric Wilcoxon rank test was used if this assumption was not met. The established criteria were as follows. The null hypothesis (H_0) states no difference in variable x between the two groups evaluated; the alternative hypothesis (H_1) states a difference in variable x between the two groups assessed. In the case of qualitative variables, the chi-square test was used. Additionally, the Spearman correlation coefficient was used between the global INAI and the different variables evaluated. The analyses were carried out in the statistical program R version 4.2.1 (R Core Team, 2022). Based on this analysis, each cluster was described, detailing the factors that have a direct and significant influence on the innovation adoption process.

Results and discussion

Catalog of innovations in arracacha cultivation

The validation with experts carried out in the research process yielded the catalog of innovations shown in Table 1.

TABLE 1. Catalog of innovations in arracacha crop.

Category	Innovation or practice
Crop mineral nutrition	Use of soil analysis to determine fertilization doses.
	Application of fertilizers to the soil in two or more applications, buried or in a drench.
	Use of lime or amendments
Crop health	Monitoring pests and diseases to apply agrochemicals.
	Elimination of atypical plants.
	Selection of suckers (asexual seed) for planting.
	Disinfection of suckers for planting
Sustainable resources management	Biological control of pests and diseases.
	Production and/or use of organic fertilizers.
	Application of irrigation
Establishment and management of the plantation	Sowing based on planting density, slope, light, and plot history.
	Considers planting height.
	Hole digging.
	Removal of flowering stems.
	Carries out more than three manual weed controls.
	Carries out more than two chemical weed controls
Administration	Includes a schedule of activities.
	Record of work carried out in the crop.
	Record of income and expenses.
	Technical assistance
Organization	Belongs to an association or cooperative.
	Sales of arracacha in grower associations.
	Participation in training
Harvest	Harvest according to the plant age.
	Packing in bags other than the traditional of 62.5 kg
Plant reproduction and breeding	Use of genetically improved varieties.
	For sucker planting, medium-sized plants with good production of tuber roots, leaves, yellow suckers without spots, and healthy varieties are used.
	Discarding the central suckers of plants during planting

Relationship of the Global INAI and the INAI in categories and producer characteristics

Figure 2 shows the correlation of the global INAI with other categories and characteristics of arracacha producers. The administration, health, and organization INAIs showed direct positive correlations of 0.70, 0.69, and 0.55, respectively. Age presented an inverse correlation of -0.25 with the global INAI.

Group of arracacha producers based on the adoption of 28 innovations

Figure 3 shows a dendrogram using Ward method and a squared Euclidean distance. Two groups of farmers were identified from 28 innovations with the INAI. Cluster 1, with lower adoption, covers 59% of the producers; Cluster 2,

with the highest adoption, covers 41%. These groups vary in farmer profile and adoption rates (Tab. 1).

Arracacha farmers with low levels of adoption of innovations (C1)

This first cluster included less innovative farmers, with an average value of the overall INAI of 37.9%, which represents a low value according to other studies, where they refer to low indicators with values below 50% (Aguilar-Gallegos *et al.*, 2015; Ramírez-Gómez *et al.*, 2023). Cluster 1 shows low yields compared to Cluster 2 with $18.775 \pm 7.909 \text{ t ha}^{-1}$; however, farmers have a higher total farm area ($12.577 \pm 12.271 \text{ ha}$), and men represent 96.43% of the total participants. This cluster has an average yield of $19.77 \pm 7.909 \text{ t ha}^{-1}$. This cluster has the lowest indicator in the administration category, with 13.4%, followed by the organization

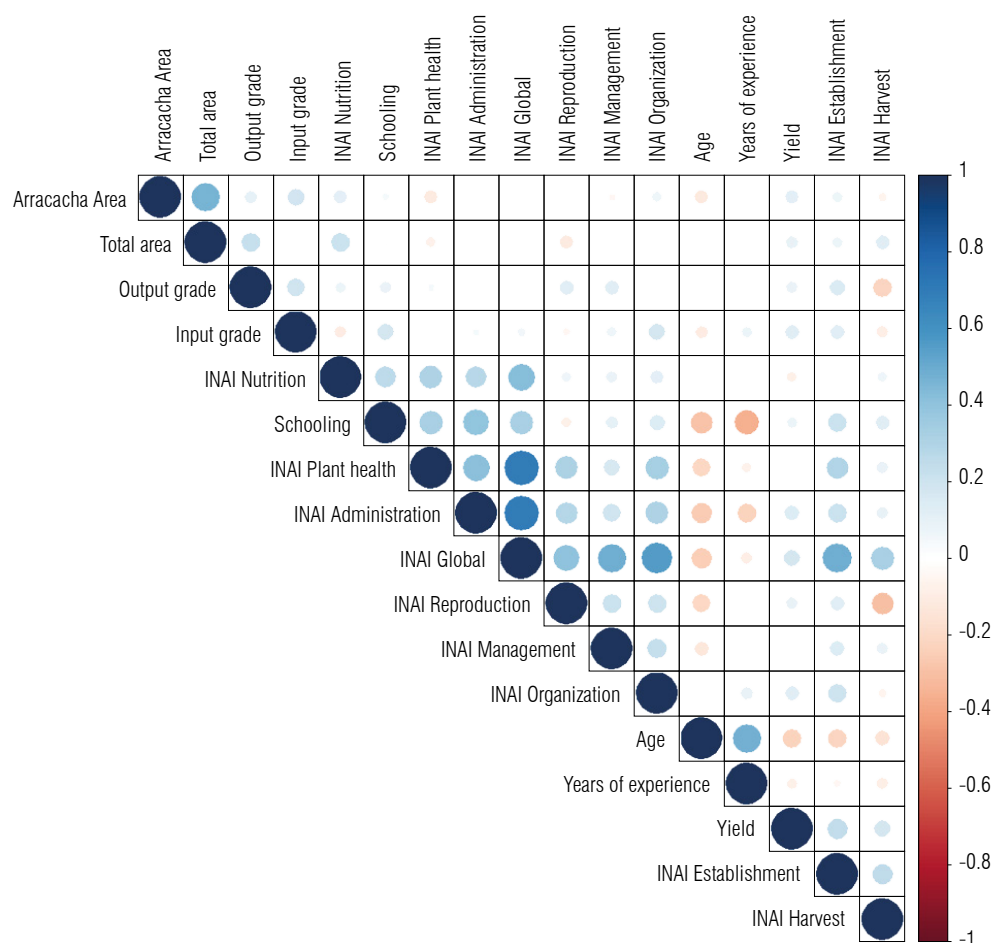


FIGURE 2. Correlation analysis between the characteristics of arracacha producers, production units, and the adoption rate.

TABLE 2. Characteristics of arracacha farmers and productive units by cluster.

Variable	Cluster 1 (59%)	Standard deviation	Cluster 2 (41%)	Standard deviation
Years of experience	26.10 ^a	13.52	24.12 ^a	12.26
Age (years) °	54.01 ^a	10.66	50.84 ^a	11.52
Schooling °	4.28 ^a	3.10	6.71 ^b	3.41
Hectares occupied with the arracacha variety La 22 °	3.25 ^a	4.49	2.51 ^a	2.23
Yield of the arracacha variety La 22 (t ha ⁻¹) °	18.77 ^a	7.90	19.37 ^b	4.84
Total area (ha) °	12.57 ^a	12.27	10.14 ^b	8.42
Gender (% of men) *	96.43 ^a		84.62 ^b	
Credit (% approved) *	64.27 ^a		61.54 ^a	

Means with different letters in the same row are significantly different ($P < 0.05$) according to Student t or Wilcoxon's test (°). According to the chi-square test, the cases marked with * are statistically different ($P < 0.05$).

category, with 18.5%. This means that this group develops fewer practices, such as keeping farm records, technical assistance services, as well as issues related to associativity. Likewise, this group recorded a statistically significant

lower INAI ($P < 0.05$) in plantation establishment and management, nutrition, and health, with values of 56.5%, 33.9%, and 45.5%, respectively (Tab. 3).

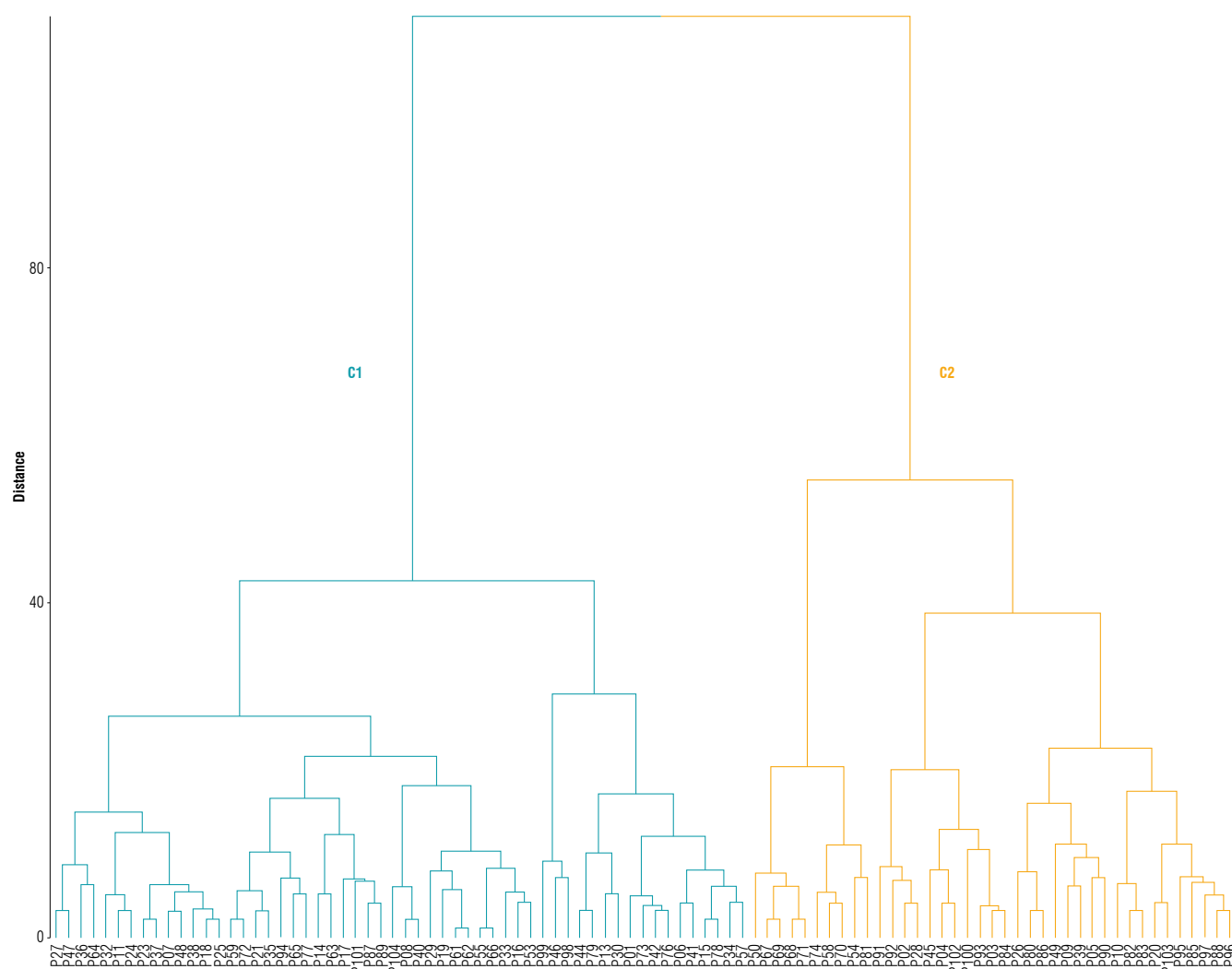


FIGURE 3. Dendrogram of 104 arracacha farmers based on innovation adoption. C1: cluster 1, C2: cluster 2.

TABLE 3. Adoption levels of arracacha farmers per cluster.

Variable	Cluster 1	SD	Cluster 2	SD
INAI administration °	0.13 ^a	0.20	0.48 ^b	0.38
INAI harvest °	0.49 ^a	0.26	0.56 ^a	0.32
INAI establishment and management of the plantation °	0.56 ^a	0.18	0.73 ^b	0.16
INAI sustainable resources management °	0.20 ^a	0.25	0.21 ^a	0.29
INAI mineral nutrition °	0.33 ^a	0.20	0.56 ^b	0.24
INAI organization °	0.18 ^a	0.25	0.35 ^b	0.27
INAI plant reproduction and breeding °	0.66 ^a	0.22	0.75 ^a	0.29
INAI plant health °	0.45 ^a	0.22	0.75 ^b	0.24
Input degree °	0.188 ^a	0.396	0.587 ^a	2.416
Output degree °	1.355 ^a	0.747	1.127 ^a	0.651
INAI global	0.379 ^a	0.103	0.553 ^b	0.126

Means with different letters in a row indicate a significant difference ($P < 0.05$) according to Student t or Wilcoxon's test (°). SD – standard deviation. INAI: Innovation adoption index.

Arracacha farmers with high levels of adoption of innovations (C2)

This group has a higher adoption rate than cluster 1, with 55.3%; it has a higher level of schooling of 6.718 years, compared to C1 which has 4.286 years of schooling. It obtains a higher yield ($19.37 \pm 4.85 \text{ t ha}^{-1}$) than cluster 1 and has a smaller farm area ($10.141 \pm 8.424 \text{ ha}$). In this group, 15.38% of the farmers are women compared to 3.57% for cluster 1. When this group of producers presents a smaller total farm area (C1: 12.557 ha, C2: 10.141 ha), it has a higher adoption rate, which is evidenced in studies of transitory crops such as wheat, corn, sunflower, soybean, and sugar beet, where the smaller the farm size, the higher the adoption of rural innovations (Despotović *et al.*, 2019).

This group of farmers incorporated up to 55% of innovations in seven categories (Tab. 2). It stands out in management of plant health (75.0%), plantation management (73.9%), mineral crop nutrition (56.4%), and administration (48.1%). This last category includes the practices of programming, recording of activities and technical assistance. Furthermore, in the 2022 knowledge network, this group of farmers showed a higher index of input degrees, indicating more sources of information, but the statistical analysis found no significant difference, so it is recommended to make an analysis of the indicators in this type of methodologies and evaluate the impact of the knowledge networks on the arracacha farmers.

The difference between the clusters is clear. Farmers in C1 are less dynamic in technical work, such as practices related to the establishment and management of the plantation, mineral nutrition of the crop, such as the use of soil analysis and fertilizer application, as well as plant health management; they had a quality seed from the beginning of the production process (Van Oorschot *et al.*, 2018). Producers with higher education and with a lower percentage of men adopt more innovations, which is directly related to higher yields, as reflected in similar studies (Ullah *et al.*, 2023), which evaluated the importance of schooling and gender in innovation adoption processes (Piñeiro *et al.*, 2020). In contrast, in the case of transient crops such as rice, it was found that where men predominate in 70% there is a higher adoption (Li *et al.*, 2021).

Rogers (2019) states that adoption is positively related to the degree to which technical assistance or extension programs are compatible with the needs of clients or actors. For example, C1 producers adopt basic innovations or local technologies related to plantation establishment, management, and harvesting. On the other hand, C2 producers are

better organized, have better technical assistance, a higher level of associativity, attend more training processes in the territory, carry out better nutrition and crop management practices related to plant health, and manage their farms using activity records to a greater extent.

Adoption is directly influenced by the characteristics of the farmer; the one that stands out the most is the level of schooling. This premise is in line with other studies where the average schooling of farmers with a lower level of adoption was 3.6 years attending mostly elementary school, compared to the more innovative farmers, who had an average educational level of 6.7 years (Chhom *et al.*, 2023). Years of experience do not limit the adoption of innovations for this study. Factors such as education favor the adoption of practices that improve productivity and income. Different studies have reported a positive correlation ($P < 0.01$) between schooling and technology adoption, highlighting that a producer with higher educational level accesses or seeks more technologies or practices for the farm (Nikitha *et al.*, 2018; Vásquez Pérez *et al.*, 2022).

According to the level of schooling of farmers in the adoption processes, in the agricultural sector, this study coincides with the assumptions that this factor is a crucial variable, since years of schooling are greater than 6.71, while for the case of rice there are 9.6 and 9 years of schooling in agricultural families in Mexico (Dhakal & Kumar Rai, 2020; Sánchez-Sánchez *et al.*, 2020). This is reflected in Colombia, where for the agricultural sector, education is very low; education must be reinforced, as it has a positive effect on farmer income by 25% (Tenjo & Jaimes, 2018).

An essential aspect of the research that should be studied further is the gender factor in adoption processes. Cluster 2, with a lower percentage of men, had a higher adoption rate. This result is similar to other studies which show that gender difference in the rural sector plays an important role in adoption processes (Kuivanen *et al.*, 2016; Ifie *et al.*, 2022). However, some studies show that men and women play the same role in the adoption process, thus eliminating gender inequality difficulties (Serote *et al.*, 2021).

A category that increases INAI is crop administration. The higher correlation of the INAI in administration concerning the global INAI evidences this. This category includes practices such as activity scheduling, work registration, income/expense registration, and technical assistance during cropping, which are practices related to good agricultural practices (GAP). Different studies show the importance of their use for the increase of innovations in rural farms

(Sennuga *et al.*, 2020; Tudela *et al.*, 2021). In relation to the importance of this category, some studies evidence that farmers do not use administrative tools or accounting records that can support decision-making on the farm (Tudela *et al.*, 2021). The second category includes innovations in plant health, monitoring practices, elimination of atypical plants, and selection and disinfection of suckers, since the low adoption of practices is defined by producer decision-making for these technical factors (Vargas-De la Mora *et al.*, 2021). The third category includes innovations related to nutrition, fertilization and liming, together with the use of soil analysis, to obtain higher production levels, where the implementation of these innovations with the use of good production practices is evidenced (Abiola *et al.*, 2020).

Another important category is related to organizational practices, which are essential to obtain high adoption rates, as evidenced in the studies of Vásquez *et al.* (2022), where large producers are organized and obtain high rates of innovation, since they have easy access to knowledge and information. Concerning this, the importance of developing associative practices has been highlighted. Thus, knowledge dissemination processes can be generated among farmers, from social interaction among peers in the territories, as evidenced in the studies of Tu *et al.* (2018), Mathios Flores *et al.* (2019), Li *et al.* (2021), and Yue *et al.* (2023). Participation in training in the cultivation of arracacha, with learning-by-doing methodologies such as the new field school approaches generates processes of dialogue of knowledge and knowledge for the adoption of practices and innovations (Khumairoh *et al.*, 2019; Navarro-Niño *et al.*, 2022).

It is important to note that in the municipality of Cajamarca, a process of research and technological diffusion has been developed by the Colombian Research Corporation - AGROSAVIA, where the impact of new planting varieties such as Agrosavia La 22, which by the year 2022 was planted on 2000 ha, leads to an update of management recommendations, given the characteristics of this variety for this agroecological zone (AGROSAVIA, 2023).

Conclusions

This research shows which factors influence the adoption of innovations. The main influence are factors of administration and organization, where producers had an average adoption rate of 55.3%, which is categorized as high, according to the comparison with other studies. In addition, there are the factors related to the plot in plant health

issues. In the same way, factors related to characteristics of the producer, such as schooling, are relevant at the time of adopting technologies in the cultivation of arracacha. These factors are essential and need to be prioritized in institutional intervention processes, in technology transfer and rural extension activities, as well as in future research processes of public or private entities.

Finally, it is recommended that future research should carry out an exhaustive evaluation of the influence of market prices, the products used in the production system and costs, in the context of the phenomena that interact mutually in the process of innovation adoption.

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Conflicts of interest

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

Author's contributions

DANN formulated the overall goals and objectives of the research; CIJB contributed to the statistical analyzes; DANN carried out the research and the research process; FME coordinated the planning and execution of the research activity; CIJB contributed to the writing of the manuscript; JEV and JPM reviewed the manuscript. All authors reviewed the final version of the manuscript.

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Characterization of the peasant economy in two small villages of Cundinamarca (Colombia): Case studies

Caracterización de la economía campesina en dos veredas de Cundinamarca (Colombia): estudios de caso

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ABSTRACT

The peasantry is an important social conglomerate in Colombia due to its large participation in the production and supply of food for the cities. Its permanence and transformation are strongly linked to the influence of the economic and social development of its environment. This study aimed to analyze the economic characteristics of the peasants of the Roble Hueco and Rincón Santo villages in the municipalities of Bojacá and Zipacón, both located near the city of Bogotá in the department of Cundinamarca. The source of empirical information was 16 peasant families from both villages. The sampling was non-probabilistic and based on convenience. Information was collected through surveys, interviews (5), and observation. The results show that the participation of agricultural and agro industrial activities in the generation of family income was less than 50%. This also means that self-consumption had a lower percentage in monetary value. The small size (2.5-3.0 ha) of the productive units and families (2.25-2.75 persons) contributed significantly to this result. In the Rincón Santo Village (Zipacón), there was a higher income, but also a higher expenditure. In both villages, the peasant units generated a small surplus of monetary reserves.

Key words: family income, self-consumption, profitability, economic organization, state support.

RESUMEN

El campesinado es un conglomerado social importante en Colombia por su gran participación en la producción y abastecimiento de alimentos a las ciudades. Su permanencia y transformación están fuertemente ligadas a la influencia del desarrollo económico y social de su entorno. Este trabajo se propone analizar las características económicas de los campesinos de las veredas Roble Hueco del municipio Bojacá y Rincón Santo del municipio Zipacón, ambas ubicadas cerca de la ciudad de Bogotá, en el departamento de Cundinamarca. Las fuentes de información empírica fueron 16 familias campesinas de ambas veredas. El muestreo fue no probabilístico y por conveniencia. La recolección de información se realizó a través de encuestas, entrevistas (5) y observación. Los resultados muestran que la participación de las actividades agropecuarias y agroindustriales en la generación de ingresos de la familia fue menor al 50%. Eso determina también que el autoconsumo tenga un menor porcentaje en valor monetario. El tamaño pequeño (2.5-3.0 ha) de las unidades productivas y de las familias (2.25-2.75 personas) contribuyeron significativamente a este resultado. En la vereda Rincón Santo (Zipacón) se vio un mayor ingreso, pero también un mayor gasto. En ambas veredas las unidades campesinas generaron un pequeño excedente de reserva monetaria.

Palabras clave: renta familiar, autoconsumo, rentabilidad, organización económica, ayuda estatal.

Introduction

The concept of a peasant

Throughout history, the concept of a peasant has evolved and has been interpreted in different ways. During the Middle Ages in Europe, the word “paysan” had negative connotations, signaling the submission of the rural population to feudal lords (Edelman, 2022). However, over time, this notion changed. Chayanov (1966) defined it as an autonomous way of life, based on subsistence and non-market exchange. Marxist-Leninist ideology considered

it an intermediate state between the proletariat and the rural petty bourgeoisie (Heynig, 1982; Hernández, 1994). With the arrival of the 20th century, peasants became a source of labor, losing their autonomy. Today, they are defined as agricultural and livestock family units with complex social relations, marked by inequalities in political, cultural and economic power with respect to the rest of society (Sevilla-Guzmán & Pérez Yruela, 1976).

With the changes that have occurred in the rural areas of Latin American countries, the concept of peasant has

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acquired special political and social force. The rural peasant population is seen as a survivor of the colonial era and the origin of the resistance for the autonomy and freedom of each country. Currently, based on some definitions of classic and modern authors (Blanco-Ibarra & Shanin, 1979; González & Fernández, 1992; Hernández, 1994; Saade, 2020; Edelman, 2022), the peasant is defined here as “a politically active intercultural subject, with complex community relations and love for the land and the activities generated there. Peasant dynamics are complementary and parallel to the current capitalist model, as suppliers of a large percentage of food and raw materials in the commerce of large cities at the local and global level (thanks to globalization and international markets). Nevertheless, farmers maintain economic dynamics of non-accumulation of capital represented in self-consumption activities, sustainable use of resources and the use of unpaid family and community labor. Peasant logics are not rigid nor do they follow a law or rule, they are complex and heterogeneous depending on the context in which they live, their relationship with the territory, with society and with the local community, thanks to this they have a deep-rooted sense of territoriality that surpasses any legality in the tenure of their land”.

The peasants in Colombia

The Colombian peasantry's historical evolution has been influenced by various social forces (Saade, 2020). Its origins trace back to pre-Columbian times, notably with the *Muisca* communities, who excelled in diversified agricultural and livestock practices, utilizing multi-layered polycultures for efficient, agroecological production (Villate, 1998; Sierra, 2019). However, the arrival of the Europeans led to a shift from these practices to monoculture systems, disrupting indigenous production (Chonchol, 1994). Feudal slave systems then emerged, utilizing indigenous, afro, and mestizo labor (Beltrán, 2006). Concurrently, a group of Creoles and mestizos gained control over their means of production, forming the foundation of today's Colombian peasants. During the 16th to 18th centuries, productive latifundia with full control of production means prevailed as the dominant production model (Bejarano, 1983; Chonchol, 1994).

Regrettably, the 19th century lacks substantial records detailing the evolution of Colombia's peasantry, with limited information beyond key rural events such as the migration of rural dwellers to cities, territorial expansion, and the establishment of new rural settlements (Fajardo, 1981). In the 20th century, the economic crisis of 1929 triggered a significant reverse migration from urban to rural areas,

fostering small-scale self-sufficient agricultural practices and shaping modern rural-urban relationships. Conversely, social violence, initially linked to Two-Party System (1920-1960) and later illegal armed groups (1950-present), prompted rural-urban migration (Suárez Sánchez, 2015). Today, post-peace-process initiatives focus on rural support, infrastructure, education, and extension, aiming to recognize the long-neglected peasantry's vital role in Colombian society (Cruz Rodríguez, 2019).

Economic features of the peasantry

The peasant economy operates distinctively, centered on multidimensional family units, emphasizing pluriactivity and maximizing family labor for resilience. Farmers aim for self-sufficiency by generating internal resources, showcasing innovation. This economy is characterized by diverse production systems and self-consumption of food, addressing economic needs and food sovereignty. Peasants also excel in organizational and associative capabilities, fostering unity and cohesion through various economic processes such as bartering, labor exchange, and community support (Santacoloma-Varón, 2015; Trujillo Ospina, 2021).

Object of the research

Although there are common features that characterize the peasantry in Colombia, diverse territorial dynamics create certain heterogeneity in this population group. In addition, the last two decades have witnessed economic changes (greater needs, greater monetization of activities, greater market pressure, etc.) and social changes that also affect farmers. In this sense, considering the difference by regions and the changes experienced in the last decades, the purpose of this research is to analyze the specific economic characteristics of the peasants of the Roble Hueco village in the municipality of Bojacá and the Rincón Santo village in the municipality of Zipacón, both located in the department of Cundinamarca. The places of study are located 48 km (Roble Hueco) and 45 km (Rincón Santo) from the center of the city of Bogotá, i.e., relatively close to the largest urban center in Colombia. This, of course, has a marked influence on the specific characteristics of the peasantry, which is worth considering.

Materials and methods

Location of the study

The study was carried out in two villages in the department of Cundinamarca, Roble Hueco (municipality of Bojacá) and Rincón Santo (municipality of Zipacón) (Figure 1 and 2). The following criteria were considered for the selection of the municipalities and hamlets: proximity to Bogotá,

influence of Bogotá's urban area, empirical knowledge of the main researcher about the territories under study, significant presence of peasants among the population of the

hamlets, and the existence of an agricultural and livestock productive structure.

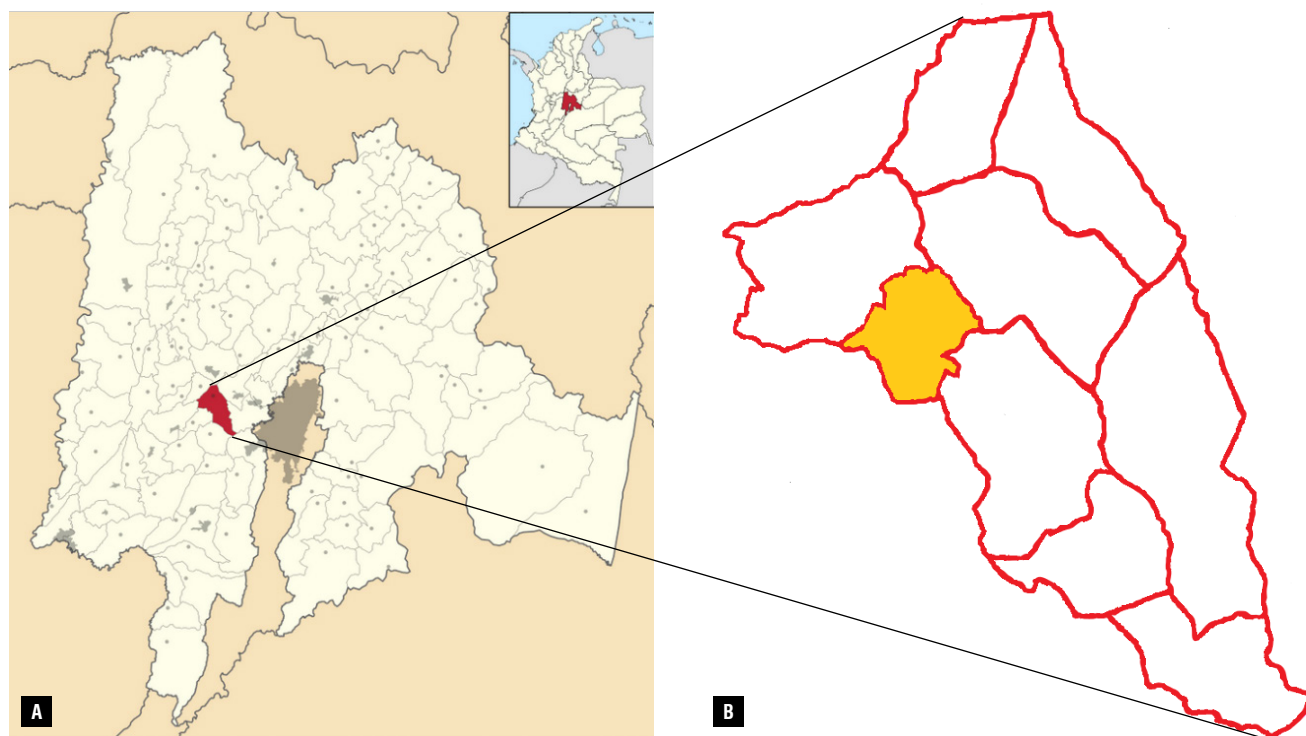


FIGURE 1. Department of Cundinamarca (red) (A), location of Roble Huevo (orange) in the municipality of Bojacá (red) (B). Source: Wikipedia, 2024; own elaboration.

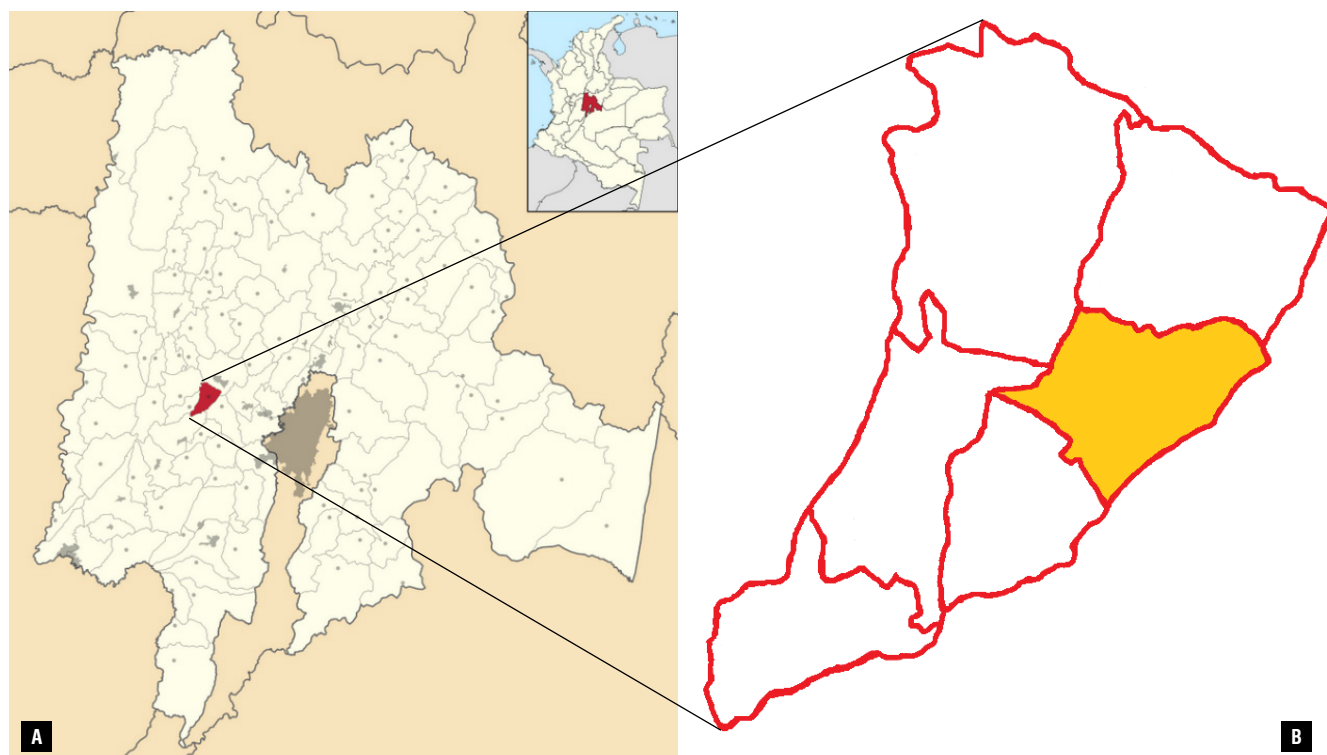


FIGURE 2. Department of Cundinamarca (red) (A) and location of Rincón Santo (orange) in the municipality of Zipacón (red) (B). Source: Wikipedia, 2024; own elaboration, respectively.

The general characteristics of the territories under study are presented in Table 1.

Type of research

This is a non-experimental research of a descriptive-interpretative nature (Benavides, 2014) based on case studies that describe the territorial phenomenon of two peasant groups, based mainly on empirical information provided by farming families and experts (Secretaries of economic development of the mayor's office, Extensionists, Director of the extension office) in the territories under study.

Information required: sources and methods

The information required was divided into three components (Tab. 2). The first component refers to the productive agricultural economy (Schejtman, 1980); the second component refers to the income and expenditures of

the farming family (Aristizábal-Arias & Duque-Orrego, 2008); and the third component is related to the support that farmers receive from state and private organizations (Aristizábal-Arias & Duque-Orrego, 2008).

Most of the required information was collected through a survey and a semi-structured interview (Tab. 3).

The sample taken for the surveys and interviews was of convenience (not random). The criteria considered were: the recommendation of the rural leaders, peasant self-identification and the willingness of the farming families to participate. Additionally, the number of cases is based on the methodology proposed by Eisenhardt (1989), who indicated that the number of cases is terminated at the discretion of the researcher until the theoretical saturation of the sample is achieved (Martínez Carazo, 2007). Since these

TABLE 1. General characteristics of the villages under study.

Variables	Roble Hueco village (Bojacá)	Rincón Santo village (Zipacón)
Location	Southeast of the municipality 4°41'36.48" N, 74°22'25.61" W	Northeast of the municipality 4°45'37.08" N, 74°22'47.57" W
Average altitude	2200 m a.s.l.	2450 m a.s.l.
Weather	Moderate cold with high relative humidity	Cold
Predominant agricultural production systems	Fruit crops, ornamental crops and transient crops. Poultry systems and livestock systems	Transient crops and fruit crops, livestock systems
Population	173 people*	332 people*
Distance from the municipality to the center of Bogotá	48 km	45 km

* Information collected through the municipal Land Use Planning Scheme and economic development secretaries of the municipalities.

TABLE 2. Information required for the research.

Component	Variables	Measurement	Information source	Collection method/ analysis
Family economy	Economic activities	Type and amount of economic activities	Peasant families	Semi-structured interview
	Family incomes	Sales value of products and services, and other incomes	Peasant families	Semi-structured interview
	Family expenditures	Family expenditure value	Peasant families	Semi-structured interview
	Self-consumption	Sales value of products and services for self-consumption	Peasant families	Semi-structured interview
	Economic balance	Incomes minus expenditures	Indirect	Economic balance analysis
Agricultural and livestock economy	Agricultural and livestock production	Products and amount	Peasant families	Survey and family unit records
	Production costs	Costs of production factors	Peasant families	Survey and family unit records
	Commercialization	Distribution channels	Peasant families	Semi-structured interview
	Sale income of agricultural and livestock production	Sales amount and prices	Peasant families	Semi-structured interview
	Profitability	Profits versus costs	Indirect	Through effectiveness cost formula
Organizational economy	Organizational economy	Presence and operation of peasant organizations	Peasant families	Semi-structured interview
	State support	State aid in the peasant economy	Peasant families	Semi-structured interview

Note: - A period of one year is considered for the calculations.

TABLE 3. Number of surveys and interviews.

Variables	Roble Hueco village (Bojacá)		Rincón Santo village (Zipacón)	
	Amount	Information source	Amount	Information source
Surveys	8	Peasant	8	Peasant
Interviews	8	Peasant	8	Peasant
	1	Secretary of economic development of the Mayor's office	1	Secretary of economic development of the Mayor's office
	1	Extension	1	Extension
			1	Director of extension office

are case studies, the results are approximations, and generalizations to the municipality or department are limited.

The surveys consisted of 3 topics and 8 closed questions, while the interviews consisted of 3 topics, 10 closed questions and 8 open questions. The surveys and interviews were conducted between July 15 and August 15, 2022. The survey and interview were applied to each selected person according to previous considerations. The average duration of a survey was 0.5 h and of an interview 1.5 h.

The information obtained through surveys and interviews was analyzed in an Excel matrix. The interpretation of the results was based on literature and analysis of the researcher on the topic of peasant economy. Costs, revenues and profitability were based on monetary values. The non-monetary costs of the farm family's own labor and the value of their own land were not considered.

Results and discussion

General characteristics of the peasant economic units

The survey determined that the average size of the family units is 2.5 ha for Roble Hueco and 3.07 ha for Rincón Santo. This reinforces the smallholding character of the peasant production. Limited areas forces them to be efficient in the use of the soil through polycultures and diversity of cultivated species, following agroecology principles that remain in the peasant productive tradition (Altieri & Toledo, 2010). In 100% of the interviews, agricultural production emerged as the main economic activity. Systems associated with transitory crops, fruit and ornamental crops (foliage crops) are representative. The latter has become relevant in the study areas and is an alternative of economic viability for the peasantry. However, this shift generates risks in food sovereignty as it replaces traditional crops such as blackberry, potato and peas — sources for the food sovereignty of the region. This productive change responds to market

dynamics, especially the demand for agricultural products related to the flower agroindustry in the region.

The peasant families in the areas under study, contrary to the typical logic of peasant reproduction and fertility, are small families, composed of an average of 2.75 members for Roble Hueco and 2.25 members for Rincón Santo. This situation is explained by the migration of the young rural population in the two territories to seek work outside the family unit (Kessler, 2006).

Peasant family economy

Economic activities

Peasant family economy centers on primary agricultural production, with little to no value addition. Nevertheless, family economic analysis reveals that many households, especially with young members, rely on formal employment in established companies. Intensive agricultural systems are utilized by 87.5% of those surveyed, focusing on small-scale partnerships, primarily in fruit, transitory, and ornamental production. Livestock production systems, on the other hand, are present in only 37.5% of the peasant population due to limited property sizes, which hinder the implementation of intensive poultry and cattle production for self-consumption. Additionally, the absence of product transformation leaves farmers vulnerable to fluctuating market prices, occasionally resulting in monetary losses during sales (Tab. 4).

Family income

From the results presented in table 5, it can be deduced that the monthly family income for Roble Hueco is \$1,357,625 Colombian peso (COP), and for Rincón Santo is \$2,058,333.3 COP. Both are within the range of 1 SMMLV and 2 SMMLV (current legal minimum monthly salary). An analysis of each economic activity shows that “other economic activities” represent the highest percentage of total income. This situation is explained by the salaries or pensions that some peasant inhabitants receive which

TABLE 4. Economic activities of the peasant families of Roble Hueco (Municipality of Bojacá) and Rincón Santo (Municipality of Zipacón).

Villages	Economic activities (n=8)						
	Agricultural production	Livestock production	Transformation of agricultural production	Trade	Wages service	Transport	Others
Roble Hueco	6	5	1	0	2	2	3
	75%	63%	13%	0%	25%	25%	38%
Rincón Santo	8	2	0	0	4	8	4
	100%	25%	0%	0%	50%	100%	50%

sustain the family economy. It can also be observed that activities related to “commerce” such as the transformation of agricultural products do not represent significant income in the peasant economy of the territories under study. Agricultural production is the main economic activity in the family economy; however, due to price and market fluctuations, annual income levels from this activity are lower than wage-related activities (generally contributed by the younger members). It is important to note that the economic development of the urban zone, close to the places studied, has expanded the sources of income of the peasant families, thus, reducing the importance of agriculture as a source of income.

TABLE 5. Average annual income in Colombian peso (\$COP) of farming families in Roble Hueco (municipality of Bojacá) and Rincón Santo (municipality of Zipacón).

Economic activities	Roble Hueco village (Municipality of Bojacá)	Rincón Santo village (Municipality of Zipacón)
Agricultural production	\$3,417,500.00	\$8,850,000.00
Livestock production	\$3,291,500.00	\$250,000.00
Transformation of agricultural production	\$325,000.00	
Trade		
Wages services	\$2,071,428.57	\$2,012,500.00
Transport	\$1,500,000.00	\$1,587,500.00
Others	\$5,945,000.00	\$12,000,000.00
Total	\$16,291,500.00	\$24,700,000.00

Family expenditures

In the family agricultural unit, food expenditures dominate, accounting for 48% of total expenditures in Roble Hueco and 37% in Rincón Santo, posing a food sovereignty risk, especially in Rincón Santo, where peasant food depends on transactions (Tab. 6). This situation relates to the new interactions between the territory and the urban areas, which reduces self-consumption production and grows commercial production. Entertainment/Leisure and Transportation expenses hold significance due to rising transportation costs and the importance of socializing for

peasants. Interestingly, health and education expenses are low, as subsidized services are preferred, with occasional expenses for unforeseen situations. The surplus from higher income doesn't always suffice, as it's often directed towards covering production costs and investment in productive systems. This, as reported by the community, highlights the economic challenges faced by peasant families.

TABLE 6. Average annual expenses in Colombian peso (COP) of the peasant families of Roble Hueco (Municipality of Bojacá) and Rincón Santo (Municipality of Zipacón).

Expenditures	Roble Hueco village (Municipality of Bojacá)	Rincón Santo village (Municipality of Zipacón)
Food	\$4,860,000.00	\$5,400,000.00
Housing	\$754,500.00	\$1,988,571.43
Clothes	\$780,000.00	\$697,500.00
Transport	\$1,110,000.00	\$2,227,500.00
Communications	\$463,500.00	\$847,500.00
Education	\$170,000.00	\$1,080,000.00
Health	\$385,714.29	\$210,000.00
Entertainment and leisure	\$1,425,000.00	\$2,130,000.00
Total	\$9,948,714.29	\$14,581,071.43

Self-consumption of agricultural products

Self-consumption is a vital aspect of peasant life, ensuring food sovereignty in rural communities. In Roble Hueco, self-consumption accounts for 33% of agricultural production and 22% of livestock products, particularly poultry (eggs and chicken meat) and dual-purpose livestock milk. In contrast, in Rincón Santo, self-consumption accounts for 2.2% of agricultural production and 83% of livestock products. This lower self-consumption of agricultural production is due to monoculture systems that fail to diversify food offerings and concerns about toxic chemical inputs. In Rincón Santo, Livestock production primarily serves self-consumption, with minimal surplus sold through short distribution channels, unlike Roble Hueco's more commercial approach (Tab. 7). Some of the studied families have agroecological gardens for self-consumption, especially in

TABLE 7. Annual self-consumption versus annual sale of agricultural products in Colombian Peso (COP) of the rural families of Roble Hueco (Municipality of Bojacá) and Rincón Santo (Municipality of Zipacón).

Economic activities	Roble Hueco (Municipality Bojacá)			Rincón Santo (Municipality Zipacón)		
	Self-consumption*	Sale	Total	Self-consumption	Sale	Total
Agriculture production	\$1,153,000	\$3,417,500	\$4,570,500	\$200,000	\$8,850,000	\$9,050,000
Livestock production	\$750,000	\$3,291,500	\$4,041,500	\$1,220,000	\$250,000	\$1,470,000
Transformation of agricultural and livestock production		\$325,000				
Total	\$1,903,000	\$6,709,000	\$8,612,000	\$1,420,000	\$9,100,000	\$10,520,000

* Estimated values based on local market prices.

Roble Hueco. The agroecological production system has not been taken to a commercial scale, because the local market does not pay a premium for these products.

Economic balance (monetary)

The economic analysis of family farming units is based on average monetary costs and income, generating positive results (Tab. 8). However, two crucial aspects must be considered. First, the fluctuation of market prices for agricultural inputs and food affects the value of the balance sheet, as these prices are sensitive to factors such as inflation, supply and demand of agricultural products, and local constraints such as transportation in rural areas. Second, farmers underestimate the value of family labor and internal production resources, making a standardized analysis difficult. Available balances after expenses do not allow for significant savings, limiting the capacity to undertake new projects with external financial support, such as bank loans. Additionally, the balance result indicates the savings capacity of the peasant in this territory. The values presented in Table 8 reflect a low savings capacity that hinders the scalability of productive projects and an increase in crop area for the two study cases. This situation is prevalent in other rural territories, where production costs are high, especially in areas far from urban capitals, where inputs are more expensive due to transportation costs.

TABLE 8. Average annual (monetary) economic balance in Colombian Peso (COP) of the peasant families of Roble Hueco (Municipality of Bojacá) and Rincón Santo (Municipality of Zipacón).

Expenditures	Roble Hueco (Municipality Bojacá)	Rincón Santo (Municipality Zipacón)
Household incomes	\$16,291,500.00	\$24,700,000.00
Household expenses	\$9,948,714.29	\$14,581,071.43
Average monetary costs of agricultural and livestock production	\$3,675,000	\$7,103,000
Balance	\$2,667,785.71	\$3,015,928.57

Agricultural productive economy

Agricultural and livestock production and processed products

Table 9 shows the amount of production represented in terms of sales levels in each of the villages under study according to their production system. The values obtained in Table 9 correspond to the commercial production (without considering the values of self-consumption) under the price perceived by the farmers at the time of the interview. Fruit and transitory crops have a higher percentage in the production measured from the family income, while the highest participation in the production of both villages is bovine production.

Production costs

Table 10 shows the production costs grouped by type of items. Labor, Fertilizers, and Pesticides have the highest percentage weight in the cost structure due to the lack of labor supply in the territories under study caused by youth migration and the gradual depopulation of the villages. Regarding fertilizers and fungicides, it was identified that the territories implement technical assistance systems of a commercial nature, which causes the peasantry to depend on technological packages of high monetary value, translating into purchases of highly effective yet costly chemical fertilizers and phytoprotection inputs. Items related to the cost of land and services connected to the productive activity do not have a weight in the production cost structure, since the land used by the peasantry is their own or family-owned, incurring no monetary expense in the implementation of productive systems. Regarding services such as technical assistance and vaccination, the community chooses to use the state institutional offer linked to local mayor's offices.

Marketing

Marketing and availability for consumption are crucial in food production. The two studied villages presented two types of distribution channels, which vary depending on

TABLE 9. Sum annual agricultural production in Colombian Peso (COP) of the peasant families of Roble Hueco (Municipality of Bojacá) and Rincón Santo (Municipality of Zipacón).

Products	Economic peasant unities								
	1	2	3	4	5	6	7	8	Total
Roble Hueco (Bojacá)									
Blackberry	\$4,800,000	\$1,440,000					\$10,000,000		\$16,240,000
Passion fruit	\$4,000,000								\$4,000,000
Foliage									
Pea	\$1,500,000						\$4,700,000		\$6,200,000
Livestock production			\$2,764,000	\$1,800,000	\$400,000	\$7,100,000		\$7,000,000	\$19,064,000
Poultry production	\$200,000		\$300,000	\$168,000					\$668,000
Pig production							\$6,000,000		\$6,000,000
Honey production	\$600,000								\$600,000
Transformation		\$2,600,000							\$2,600,000
Total	\$11,100,000	\$4,040,000	\$3,064,000	\$1,968,000	\$400,000	\$7,100,000	\$20,700,000	\$7,000,000	\$55,372,000
Rincón Santo (Zipacón)									
Blackberry							\$7,000,000		\$7,000,000
Tomato		\$2,000,000	\$2,000,000						\$4,000,000
Tree tomato							\$1,000,000		\$1,000,000
Aromatics							\$500,000		\$500,000
“Brillantina” (<i>Pittosporum Shiny</i>)							\$1,500,000	\$7,000,000	\$8,500,000
Yellow potato	\$800,000	\$5,000,000	\$3,000,000	\$12,000,000					\$20,800,000
Carrot					\$4,400,000				\$4,400,000
Coriander				\$5,000,000	\$3,600,000				\$8,600,000
Pumpkin				\$5,000,000	\$8,000,000	\$7,000,000			\$20,000,000
Livestock production			\$1,000,000			\$500,000	\$500,000		\$2,000,000
Total	\$800,000	\$7,000,000	\$6,000,000	\$22,000,000	\$16,000,000	\$7,500,000	\$3,500,000	\$7,000,000	\$69,800,000

the product marketed and the organization of institutional events that facilitate the exhibition of harvested food.

Long distribution channels

These distribution channels are the most common in the villages and represent the easiest form of marketing for products such as foliage, dairy products, and fruits. This type of marketing relies on intermediaries who sell directly to consumers or to collection and distribution centers such as “Fruvers” or marketplaces. The selection of this type of distribution channel represents an advantage in sales, as farmers negotiate 100% of their harvest; however, this channel does not offer the best prices, directly affecting the profitability of the production system.

Short distribution channels

These distribution channels are linked to events such as farmer markets or fairs organized by the municipal

governments; some of those surveyed also choose to set up sales stands in the urban centers, taking advantage of the influx of tourists to sell directly to consumers. In Roble Hueco, another option is internal marketing to external agents such as visitors or inhabitants of the village, which eliminates logistics and transportation costs. This type of channel offers better purchase prices, but typically results in lower sales volumes since it is limited to specific periods, such as weekends.

Profitability

A general analysis of the profitability of the peasant production systems in the territories under study showed that they had positive profitability indexes, with an average profitability of 88% for Roble Hueco and 23% for Rincón Santo. It is worth noting that the costs used in the analysis are monetary costs, which indicates that, in the Roble Hueco area, profitability is due to the use of family and community

TABLE 10. Sum of annual agricultural production costs in Colombian Peso (COP) of the peasant families of Roble Hueco (Municipality of Bojacá) and Rincón Santo (Municipality of Zipacón).

Type	Economic peasant unities								Total
	1	2	3	4	5	6	7	8	
Roble Hueco (Bojacá)									
Land									
Labor	\$2,500,000	\$250,000					\$3,500,000		\$6,250,000
Seeds	\$400,000						\$700,000	\$200,000	\$1,300,000
Fertilizers	\$1,500,000	\$600,000					\$3,000,000		\$5,100,000
Pesticides	\$800,000						\$1,500,000	\$100,000	\$2,400,000
Tools and equipment	\$200,000	\$200,000	\$100,000	\$200,000			\$1,000,000		\$1,700,000
Infrastructure maintenance	\$700,000		\$300,000	\$400,000		\$1,000,000		\$1,000,000	\$3,400,000
Food and supplies	\$400,000	\$1,000,000	\$700,000	\$850,000		\$1,700,000		\$2,400,000	\$7,050,000
Maintenance of fields and corrals		\$500,000		\$100,000	\$100,000	\$500,000		\$500,000	\$1,700,000
Services						\$100,000	\$150,000	\$-	\$250,000
Others								\$250,000	\$250,000
Total	\$6,500,000	\$2,550,000	\$1,100,000	\$1,550,000	\$100,000	\$3,300,000	\$9,850,000	\$4,450,000	\$29,940,000
Roble Hueco (Zipacón)									
Land		\$800,000	\$800,000	\$2,000,000	\$1,400,000	\$650,000			\$5,650,000
Labor	\$100,000	\$1,000,000	\$500,000	\$3,000,000	\$2,700,000	\$1,200,000	\$700,000	\$2,000,000	\$11,200,000
Seeds	\$20,000	\$70,000	\$70,000	\$1,000,000	\$800,000	\$100,000	\$1,000,000	\$600,000	\$3,660,000
Fertilizers	\$130,000	\$800,000	\$800,000	\$6,000,000	\$6,000,000	\$2,500,000	\$2,500,000	\$1,500,000	\$20,230,000
Pesticides	\$200,000	\$1,500,000		\$1,200,000	\$2,000,000	\$1,150,000	\$1,000,000	\$800,000	\$7,850,000
Tools and equipment	\$100,000	\$200,000	\$300,000	\$1,500,000	\$900,000	\$600,000	\$750,000	\$250,000	\$4,600,000
Infrastructure maintenance							\$700,000		\$700,000
Food and supplies			\$750,000	\$300,000			\$300,000		\$1,350,000
Maintenance of fields and corrals			\$220,000				\$200,000		\$420,000
Services						\$250,000	\$120,000		\$370,000
Others						\$800,000			\$800,000
Total	\$550,000	\$4,370,000	\$3,440,000	\$15,000,000	\$13,800,000	\$7,250,000	\$7,270,000	\$5,150,000	\$56,830,000

labor that is not paid transactionally, savings in inputs using resources internal to the production unit and the small scale of the production systems. Rincón Santo, unlike the municipality of Bojacá, is transitioning towards commercial agriculture with larger areas, monocultures and distribution channels focused on monetary gain, which is why the production costs are proportionally higher (Tab. 11). In the case of farm number 7 in the municipality of Zipacón, there is a negative profitability indicator, showing that production costs exceed profits by 52%. A specific analysis showed that the strategy of the owner of the farm is to invest in livestock infrastructure and to diversify production in such a way as to ensure food for his family, by investing in agricultural and livestock activities with capital from ancillary labor and transportation activities.

Organizational economics

Economic organization

Organization at the social and economic level is one of the keys to the resilience of peasants in the territories, since through collective groups, peasants can gain market power, access programs and projects and achieve greater visibility not only to government agencies but also to private financing. However, despite the importance of this activity, 100% of those interviewed stated that they are not part of any association or cooperative, commenting that efforts have been made by some local actors without success due to the lack of cohesion and cooperation within the community.

TABLE 11. Annual profits and costs for agricultural production in Colombian Peso (COP) of the peasant families of Roble Hueco (Municipality of Bojacá) and Rincón Santo (Municipality of Zipacón).

Type	Economic peasant units								
	1	2	3	4	5	6	7	8	Total
Roble Hueco (Bojacá)									
Costs	\$6,500,000	\$2,550,000	\$1,100,000	\$1,550,000	\$100,000	\$3,300,000	\$9,850,000	\$4,450,000	\$29,400,000
Incomes	\$11,100,000	\$4,040,000	\$3,064,000	\$1,968,000	\$400,000	\$7,100,000	\$20,700,000	\$7,000,000	\$55,372,000
Profits	\$4,600,000	\$1,490,000	\$1,964,000	\$418,000	\$300,000	\$3,800,000	\$10,850,000	\$2,550,000	\$25,972,000
Profitability	71%	58%	179%	27%	300%	115%	110%	57%	88%
Roble Hueco (Zipacón)									
Costs	\$550,000	\$4,370,000	\$3,440,000	\$15,000,000	\$13,800,000	\$7,250,000	\$7,270,000	\$5,150,000	\$56,830,000
Incomes	\$800,000	\$7,000,000	\$6,000,000	\$22,000,000	\$16,000,000	\$7,500,000	\$3,500,000	\$7,000,000	\$69,800,000
Profits	\$250,000	\$2,630,000	\$2,560,000	\$7,000,000	\$2,200,000	\$250,000	-\$3,770,000	\$1,850,000	\$12,970,000
Profitability	45%	60%	74%	47%	16%	3%	-52%	36%	23%

State support

State support for the communities under study is limited to assistance programs for the delivery of inputs to support productive systems. In Roble Hueco, subsidies are related to the advanced age of the population or pension support as well as some productive initiatives such as the delivery of animals or beehives for beekeeping. In Rincón Santo, the presence of the state has been limited, with only the delivery of plant material to support fruit production systems, ignoring the real needs of the rural population, especially the peasantry.

Conclusions

The study shows that both villages have similarities (land and labor) and differences (income and expenses). The changes in the past and present profile of the farmer include pluriactivity, diversification of income sources, reduction of self-consumption, and monetization of production. These points are detailed below.

The farming families of Roble Hueco (Bojacá) and Rincón Santo (Zipacón) have small production units (2.5-3.0 ha) and limited human resources (2.25-2.75 people per farm) for agricultural and Agroindustrial activities. These activities are a source of income for farming families in both villages; however, they are no longer the main source of income. Proximity to the city has allowed families to diversify their economic activities. The self-consumption generated by agricultural production and processed agricultural products has little monetary value. The peasant units in Rincón Santo generate more income, but also incur more expenses; they have higher profitability in their economic activities than those in Roble Hueco. In both villages, a

monetary surplus is generated per peasant unit. The State supports them with some subsidized materials and inputs, more so in Roble Hueco than in Rincón Santo. Finally, both villages show a low level of community organization, which does not favor their economic development.

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 the new information, systematized the information, and collaborated in the writing; JCBF structured the article, collaborated in the writing, and translated the document. Both authors reviewed the final version of the manuscript.

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Structural, physicochemical, and pasting properties of native cassava (*Manihot esculenta*) and yam (*Dioscorea alata*) starch blends

Propiedades estructurales, fisicoquímicas y de empastamiento de mezclas de almidones nativos de yuca (*Manihot esculenta*) y ñame (*Dioscorea alata*)

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ABSTRACT

Starch blends are a technological alternative aimed at the development of starchy matrices that exhibit improvements in some physicochemical properties from interactions between their individual components. Native cassava and yam starches were mixed in different proportions and the effect of the blend on the structural, physicochemical, and pasting properties was evaluated. The viscosity behavior as a function of temperature revealed a significant non-additive effect on the pasting parameters of all the blends with respect to the individual native starches. Similarly, non-additive variations were evident in the crystallinity index of some mix ratios (NSB-2: 40.11%). Likewise, the difference in the amylose content of each native starch (20.88-25.66%) possibly exerted an effect on the resulting semicrystalline characteristics of the blends and the gelatinization behavior. Hence, the botanical origin and the proportion of starch blends play an important role in the behavior of the resulting physicochemical properties and *in vitro* digestibility. Polymeric blends were obtained with a low tendency to retrogradation and lower crystallinity index values compared to their native counterparts and a regulated water absorption capacity, all potentially desirable characteristics in the food industry.

Key words: tuber starch, crystallinity index, non-additive effect, digestibility.

RESUMEN

Las mezclas de almidones son una alternativa tecnológica dirigida al desarrollo de matrices amiláceas que presenten mejoras en algunas propiedades fisicoquímicas a partir de interacciones entre sus componentes individuales. Por lo tanto, se mezclaron almidones nativos de yuca y ñame en diferentes proporciones y se evaluó el efecto de la mezcla sobre las propiedades estructurales, fisicoquímicas y de empastamiento. El comportamiento de la viscosidad en función de la temperatura reveló un efecto no aditivo significativo sobre los parámetros de pasta de todas las mezclas con respecto a los almidones nativos individuales. De manera similar, las variaciones no aditivas fueron evidentes en el índice de cristalinidad de algunas proporciones de mezcla (NSB-2: 40.11%). Asimismo, la diferencia en el contenido de amilosa de cada almidón nativo (20.88-25.66%) posiblemente ejerció un efecto sobre las características semicristalinas resultantes de las mezclas y el comportamiento de gelatinización. Por lo tanto, el origen botánico y la proporción de mezcla de almidón juegan un papel importante en el comportamiento de las propiedades fisicoquímicas resultantes y la digestibilidad *in vitro*. Se obtuvieron mezclas poliméricas con una baja tendencia a la retrogradación y valores de índice de cristalinidad más bajos en comparación con sus homólogos nativos y una capacidad de absorción de agua regulada, todas ellas características potencialmente deseables en la industria alimentaria.

Palabras clave: almidón de tubérculos, índice de cristalinidad, efecto no aditivo, digestibilidad.

Introduction

Starches from different botanical sources exhibit unique physicochemical properties because of their specific granular size, amylose content, and chain length distribution of their molecular constituents (Waterschoot *et al.*, 2015). The specific characteristics related to the structure and granular composition govern the macroscopic behavior of each

starchy material. In Colombia, starch-rich raw materials such as cassava and yam are cultivated; and their starchy constituents show differences in size and granular shape, chemical composition, and viscosity behavior under an excess of water (Karam *et al.*, 2006). For instance, cassava starch displays an amylose content of approximately 19%; and in yam starches it is around 28% (Karam *et al.*, 2006). Thus, the paste clarity and strength characteristics of native

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cassava starch gels, coupled with the remarkable hot viscosity stability of yam starch pastes, make these polysaccharide materials highly suitable ingredients that are attractive in the formulation of different products related to the food industry (Novelo-Cen & Betancur-Ancona, 2005; Karam *et al.*, 2006).

At the molecular level, starches are reserve macromolecules synthesized in the amyloplasts of different plants as granular particles with variable shapes and sizes, depending on their botanical origin (Ai & Jane, 2015). At the structural level, they have two main polysaccharide constituents: amylose and amylopectin. The first is essentially linear, made up of glucose units linked by α -(1,4) bonds. The second is present in a greater proportion in the starch granule and has abundant branches anchored to the polymer chains of glucose by α -(1,6) bonds. The latter exerts a significant effect on the semi-crystalline properties, swelling power in the granules, insolubility in water and viscosity (Pérez & Bertoft, 2010; Ee *et al.*, 2020). In their native state, starches are used as stabilizing, binding, or thickening materials (Majzoobi & Farahnaky, 2021). However, their industrial application is limited (Maniglia *et al.*, 2021), since they exhibit significant sensitivity to environments with low pH, high temperatures or high shear forces (Dupuis & Liu, 2019).

To develop new functionalities and inhibit some undesirable properties of native starches, modifications of their structural characteristics are made (Dolas *et al.*, 2020) using physical, chemical, or enzymatic methods (Zia-ud-Din *et al.*, 2017). In recent years, the growing consumer demand for clean-label products has generated an increase in research aimed at improving the functionalities of native starches through low-cost methods that use simple and powerful industrial technologies. Zhu *et al.* (2020) suggest a blend of native starches as an alternative process that makes possible the synthesis of polymeric matrices with desirable properties for the food industry (Waterschoot *et al.*, 2015; Hornung *et al.*, 2017) that guarantees the conservation of the category of the resulting starchy material as “food ingredient” instead of “food additive” (Park & Kim, 2021), thus allowing the development of chemical-free starch-based products. Obanni and Bemiller (1997) observe the formation of a continuous phase (native granules) and a discontinuous phase (swollen granules, fragments of granules or retrogradation products) in blends made up of granules of different botanical origin, showing a greater effect of interaction between starches compared to the responses observed in individual starches. Thus, the selection of starches that exhibit significant differences in

their intrinsic properties could cause unpredictable behavior for the resulting blends compared to the properties of their starchy counterparts.

The starch blends derived from various botanical sources are regarded as a mechanism capable of imparting novel functionalities to the industry through the interactions among the individual properties of each starchy component (Oliveira *et al.*, 2018; Wang *et al.*, 2020). In starch blending, additive effects are noticed when the properties of the blend can be predicted from the individual starches. Nevertheless, non-additive effects are associated with interaction phenomena among starches; consequently, predictions do not align consistently with the actual characteristics of the blend (Park & Kim, 2021). Waterschoot *et al.* (2015) establish that significant differences in properties such as gelatinization temperature, granular size, and amylose content of each component in the starch blend could lead to specific interactions, depending on the blend proportions evaluated by Waterschoot *et al.* (2016). Novelo-Cen and Betancur-Ancona (2005) report that the interactions among starch molecules and granular structures in starch blends result in behaviors that resemble the properties developed through chemical modifications. Hence, it is crucial to comprehend the potential types of interactions among starches in blends to enhance their functionalities for industrial applications (Zhu *et al.*, 2020).

Different blend proportions were formulated for native cassava and yam starches in this research. In this case, native cassava and yam starches exhibit significant differences in chemical composition, granular and molecular structure, variations in terms of thermal stability, tendency to retrogradation, as well as limited commercial and industrial exploitation of yam starch (Karam *et al.*, 2006; Salcedo Mendoza *et al.*, 2016). The present study aims to assess the effect of different proportions of cassava and yam starches on the physicochemical, pasting, structural and *in vitro* digestibility properties of the blends, to regulate the tendency towards retrogradation and achieve improvements in stability of the viscosity of the resulting starchy materials. All were evaluated from the analysis of the interactions present in each property. The foregoing is intended to stimulate the investigation of processes that develop polymeric starch matrices that exhibit desirable properties for the food industry. These processes include starchy sources with limited technological use in Colombia, through a mechanism of functional improvement that avoids the use of chemical agents, ultimately facilitating the formulation of clean-label products.

Materials and methods

Materials

The native cassava starch (*Manihot esculenta* cv. M-TAI) was supplied by the company Almidones de Sucre S.A.S. (Induyuca®, Sincelejo, Colombia). The tubers of Creole yam (*Dioscorea alata*) were purchased from the local market in the city of Sincelejo, Sucre, Colombia. Commercial enzymes such as bacterial α -amylase (Lyquozyme Supra-2.2X®) and fungal amyloglucosidase (Dextrozyme®) were purchased from Novozymes (Bagsvaerd, Denmark). Likewise, pancreatic α -amylase with biocatalytic activity ≥ 5 U/mg (Sigma-Aldrich, Type VI-B, USA) was used.

Yam starch extraction

Yam starch was extracted following the methodology described by Salcedo Mendoza *et al.* (2016) with some modifications. At first, the yams were washed, dehusked and cut into cubes and processed in an Oster® domestic blender for 1 min. The resulting slurry was processed using a pilot air disperser bubbling equipment (pump 0.25 hp, air compressor 100 pounds) that promotes the separation between starch and bagasse due to density differences. This facilitates the natural decanting of starch. Later, filtrations were carried out with the intention of separating residual solid impurities, as well as successive washings to finally dry the starches at 35°C for 24 h. The samples were macerated and sieved (≤ 74 μ m), and stored in hermetically sealed bags.

Starch blends

All mix ratios between cassava and Creole yam starches were prepared following the methodology described by Hornung *et al.* (2017) with some modifications. Thus, each proportion of starch blend was developed in a suspension of 30% w/v in distilled water, magnetically stirred (250 rpm) for 15 min to ensure homogeneity (Tab. 1). Afterward, each starchy mixture was centrifuged at 3500 rpm for 7 min, discarding the aqueous supernatant and drying the starches for 24 h at 35°C. Finally, the samples were ground, sieved (200 mesh, which is equivalent to 74 μ m of mesh opening) and stored at room temperature in airtight plastic bags for further analysis.

Apparent amylose content

The apparent amylose content for all samples was determined by the conventional iodine staining spectrophotometric method described by Khoontong and Noomhorm (2015), with minor modifications. One hundred mg of dry basis sample were dissolved in 1 ml of absolute ethanol plus 9 ml of 1M NaOH, heated to 100°C for 30 min. A sample of

the diluted solution was used for estimating the apparent amylose content, mixing it with 200 μ l of 1M acetic acid, 400 μ l of lugol solution (2.0% KI and 0.2% I₂), and distilled water. A UV-VIS spectrophotometer Pharo 300 (Spectroquant®, Darmstadt, Germany) was employed to measure the coloration of the samples at 620 nm. The amylose content was determined using a calibration curve with pure potato amylose as standard (Sigma Aldrich®, USA).

TABLE 1. Percentage of starch used in the binary blends.

Samples	Starch (%)	
	Cassava	Yam
NCS	100	0
NYS	0	100
NSB-1	30	70
NSB-2	50	50
NSB-3	60	40
NSB-4	45	55
NSB-5	70	30

NCS: native cassava starch; NYS: native yam starch; NSB-1: blend of cassava 30%-yam 70%; NSB-2: blend of cassava 50%-yam 50%; NSB-3: blend 60%-yam 40%; NSB-4: blend of cassava 45%-yam 55%; NSB-5: blend cassava 70%-yam 30%.

Morphology and birefringence

An aliquot of 10 μ l (0.1% w/v starch in deionized water) was poured onto a slide to examine the birefringent characteristics of the granules using a binocular microscope (DM1000 LED, Leica, Japan). Micrographs were obtained from polarized light fields at 40X magnification using a digital camera (Leica, ICC50W, Japan).

Diffraction patterns and crystallinity index

X-ray diffraction patterns of starch samples were acquired using a diffractometer (X'Pert Pro-MPD, Panalytical, Italy), employing CuK radiation ($\lambda=1.55$ pm) and a secondary beam graphite monochromator at 30 kV and 30 mA (Colussi *et al.*, 2020). Spectra were obtained over a 2θ Bragg angle range of 4-30°, at an inspection speed of 2° min⁻¹ with a measurement interval of 0.02° for each sample. The crystallinity index was obtained from the ratio of the areas corresponding to the crystalline regions and the total area, obtained by numerical integration methods, using the MATLAB software (MathWorks, R2019a, USA).

Pasting properties

Viscosity profiles were determined following the method proposed by Fonseca-Florido *et al.* (2017) with slight modifications, using a rheometer (Anton Paar, MCR 302, Austria), a cell for analysis of starch suspensions (C-ETD160/ST) and a starch cell geometry (Anton Paar, ST24-2D/2V /2V-30, Austria). Thus, 2.0 g (dry basis) of

each sample were suspended in 25 ml of distilled water. The suspensions were subjected to controlled heating and cooling cycles with a stirring speed of 250 rpm. Initially, each sample was subjected to a temperature sweep at 50°C for 1.0 min, then heated to 95°C in 7.5 min, held at 95°C for 5.0 min, cooled to 50°C in 7.5 min, and finally kept at 50°C for 2.0 min. The rates of ascent and descent were 7.5°C min⁻¹ for each stage. The following parameters were obtained: pasting temperature, peak viscosity, breakdown viscosity, and setback viscosity. Viscosities were recorded in centipoise (cP).

Water absorption capacity (WAC)

WAC values were estimated following the methodologies developed by Yadav *et al.* (2016) with some modifications. Briefly, a starch sample of 1.0 g on a dry basis was suspended in 10 ml of distilled water at room temperature, gently shaken to homogenize, and centrifuged at 3500 rpm for 15 min. The supernatant liquid was discarded, and the precipitated starch was weighed to estimate the percentage of water absorption capacity (Eq. 1).

$$\text{WAC (\%)} = \frac{W - W_0}{W_0} \times 100 \quad (1)$$

where, W_0 (g) is the weight of dry starch, and W (g) is the weight of the sediment after centrifugation.

In vitro digestibility

In vitro starch digestibility was determined as described by Englyst *et al.* (1992) with modifications. Initially, enzyme solutions were prepared by mixing 9 g of pancreatic α -amylase in 60 ml of citrate buffer (Solution I) and a solution of amyloglucosidase with biocatalytic activity of 140 AGU ml⁻¹ (Solution II). Then, 200 mg (dry basis) of starch was dispersed in 25 ml of citrate buffer (pH 5.2) and subjected to gelatinization for 20 min at 90°C. Afterward, 1 ml of Solution III (composed of 54 ml of Solution I and 6 ml of Solution II) was added to the gelatinized suspension and kept at 37°C with stirring at 250 rpm for 120 min. Aliquots were taken at 20 and 120 min to determine the content of glucose released, using the DNS method (3,5-dinitrosalicylic acid). The fractions of rapidly digestible starch (RDS), slowly digestible (SDS), and resistant starch (RS) in the blends were calculated according to Englyst *et al.* (1992).

Thermal properties

Samples of starch (3.0 mg, dry basis) were mixed with 11 μ l of distilled water and evaluated by differential scanning calorimetry (DSC) (Reyes-Atrizco *et al.*, 2019) with slight modifications. In this way, the aluminum capsules

were sealed and stored for 24 h prior to analysis to balance the system. Then, the samples were subjected to thermal treatments with heating from 20 to 120°C at a rate of 10°C min⁻¹ followed by cooling to 20°C with a ramp of 25°C min⁻¹ in a nitrogen atmosphere. The thermal parameters onset temperature, peak temperature, final temperature, and enthalpy of gelatinization (ΔH) were determined from the analysis of the thermogram using the TA Universal Analysis software (TA 2000, TA Instruments Inc., USA).

Statistical analysis

All determinations were expressed as the mean of three replicates \pm standard deviation. Means and significance of differences between samples were established using analysis of variance (ANOVA) and Tukey's test ($P < 0.05$), using the statistical software Statgraphics Centurion (Statgraphics Inc., version XVI, USA).

Results and discussion

Amylose content

The amylose content of native starches and their blends is presented in Table 2. At first, native yam starch (NYS) had a higher amylose content compared to native cassava starch (NCS); these findings corresponding to those reported by Monroy *et al.* (2018) and Duan *et al.* (2020) for NCS and NYS. This could be related to the nature of the crystalline polymorphism of NYS and NCS, which were B-type and A-type. Hornung *et al.* (2017) report that starches with B-type diffraction pattern generally have a high retrogradation, so the greater tendency towards retrogradation shown by NYS pastes on cooling may be associated with its higher amylose content compared to NCS (Fig. 3). The amylose content exerts an important effect on the semi-crystalline characteristics, the gelatinization temperatures, and the paste behavior of starches (Hornung *et al.*, 2017).

The evaluated starch blends presented a possible additive behavior in the amylose content, so that the values obtained were in a range delimited by the amylose content of the individual native starches, guaranteeing a proportional relationship for the amylose content of starch blends as a function of the individual values of each starchy counterpart (Tab. 2). It is possible to predict the behavior of the amylose content of the starch blends from the values exhibited by NCS and NYS, while each mixture presented an additive variation in the content of amylose, corresponding to each mixing ratio between NCS and NYS. Likewise, a similar behavior was reported by Ma *et al.* (2020) for amylose leaching from binary blends between potato starch and high amylose rice starch, where amylose leaching for

each mixing ratio of these starches was between the values exhibited by their individual counterparts, indicating an additive effect. Related to the above, Cruz-Benitez *et al.* (2019) state that the amylose content of each starch affects the physicochemical properties such as water absorption. Consequently, the possible proportional interaction observed in the binary blends of NCS and NYS in the amylose content could contribute to the display of non-additive behaviors in other physicochemical properties, and the absorption capacity of water in the blends.

TABLE 2. Water absorption capacity, amylose content, and crystallinity index.

Samples	WAC (%)	AC (%)	CI (%)
NCS	65.72 ± 1.11 ^a	20.88 ± 0.07 ^a	48.05 ± 0.70 ^a
NYS	93.09 ± 1.14 ^b	25.66 ± 0.08 ^b	45.07 ± 0.42 ^b
NSB-1	94.19 ± 2.89 ^c	24.30 ± 0.09 ^c	43.77 ± 0.53 ^b
NSB-2	78.84 ± 0.55 ^d	23.34 ± 0.04 ^d	40.11 ± 0.16 ^c
NSB-3	81.57 ± 1.12 ^e	22.88 ± 0.06 ^e	44.87 ± 0.46 ^b
NSB-4	78.73 ± 0.65 ^d	23.73 ± 0.11 ^f	44.67 ± 0.66 ^b
NSB-5	77.56 ± 0.92 ^d	22.03 ± 0.04 ^g	45.03 ± 0.43 ^b

WAC: water absorption capacity; AC: amylose content; CI: crystallinity index; NCS: native cassava starch; NYS: native yam starch; NSB-1: blend of cassava 30%-yam 70%; NSB-2: blend of cassava 50%-yam 50%; NSB-3: blend 60%-yam 40%; NSB-4: blend of cassava 45%-yam 55%; NSB-5: blend cassava 70%-yam 30%. Means followed by same letters within a column do not differ statistically according to Tukey's test ($P < 0.05$).

Morphology and birefringence

Polarized light microphotographs of native starches and their blends are illustrated in Figure 1. At first, Figure 1-NYS presents the morphology of the NYS granules, which exhibit an ellipsoidal structure with a “Maltese cross” orientation directed towards the periphery of the granule (He & Wei, 2017), indicating that the position of the “hilum” is decentralized, a typical characteristic for starch granules from rhizomes and yams (Chakraborty *et al.*, 2020).

In contrast, the NCS granules presented a spherical shape, with the “Maltese cross” located in the granular center (Fig. 1, NCS), suggesting the presence of concentrically ordered semi-crystalline structures within the granules (Lin *et al.*, 2020). On the other hand, the micrographs of all blends between NCS and NYS exhibited a granular polydispersity dependent on each mixing ratio evaluated, where the conservation of the birefringent characteristics of each individual starch was appreciable, as well as the typical morphology of the granules in its native state after the mixing process (Fig. 1, NSB-1).

Hornung *et al.* (2017) find that the morphological structure of yam starch granules from different varieties remains unchanged after the mixing process. Wu *et al.* (2016)

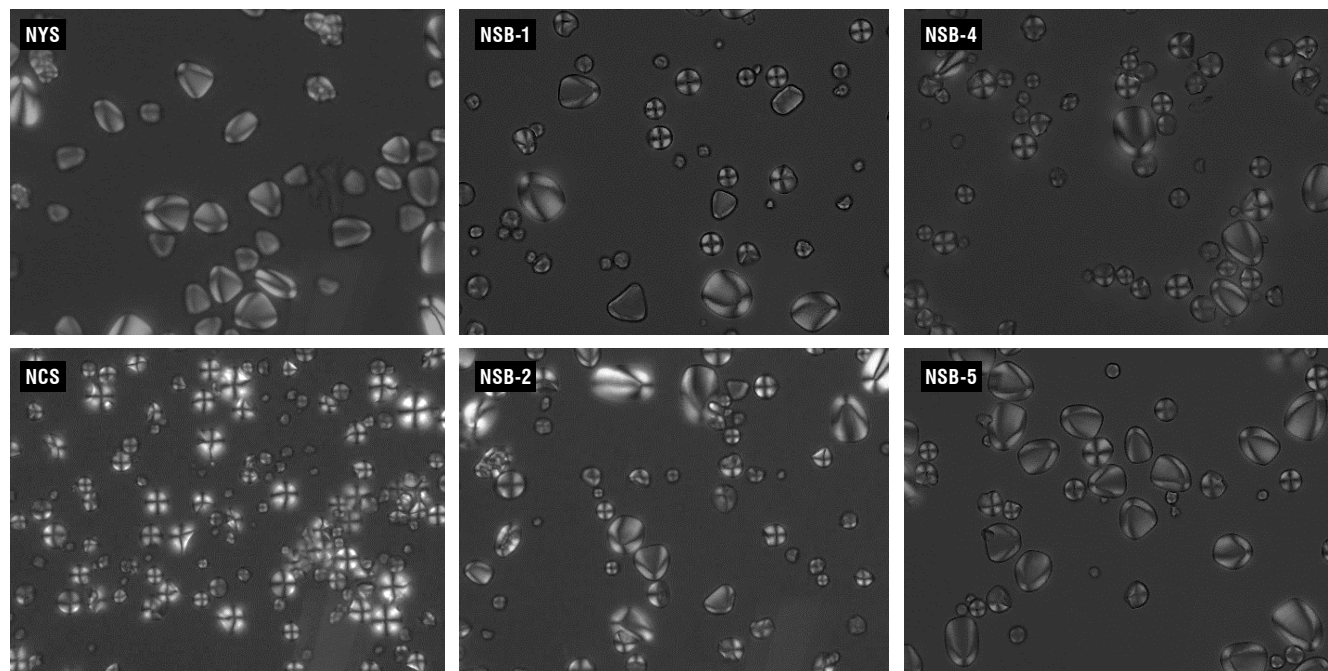


FIGURE 1. Microphotographs under polarized light of native starches and their blends. NCS: native cassava starch; NYS: native yam starch; NCS-1: blend of cassava 30%-yam 70%; NCS-2: blend of cassava 50%-yam 50%; NCS-4: blend of cassava 45%-yam 55%; NCS-5: blend cassava 70%-yam 30%. Magnification 40X.

highlight that the size and shape of the granules have a determining impact on the non-additive behavior resulting in the pasting properties of the blends between native sweet potato and mung bean starches, showing that significant differences in the morphological characteristics of the starches could trigger unpredictable behaviors in some physicochemical properties of the resulting blends. Hence, the differences in size and granular shape manifested by NCS and NYS could be associated with the non-additive behaviors observed in the pasting profiles (Fig. 3), crystallinity index, and *in vitro* digestibility of the starch blends evaluated in this study.

X-ray diffraction (XRD) and crystallinity index (CI)

The semicrystalline order of native starches and their blends can be studied from the diffractograms shown in Figure 2. Firstly, NCS showed crystalline peaks characteristic of an A-type starch, exhibiting high magnitude intensities at the 2θ Bragg angles: 15° , 17° , 18° , and 23° , similar to Sangian *et al.* (2018). In contrast, NYS showed crystallinity peaks at angles 5.7° , 15.1° , 17.1° , and 24.1° , associated with a B-type diffraction pattern, similar to Oliveira *et al.* (2021).

The behavior of the X-ray diffraction patterns of the starch blends suggested the occurrence of variations in the semi-crystalline characteristics compared to their native starchy counterparts (Tab. 2; Fig. 2). All blends between NCS and NYS presented crystalline peaks at Bragg angles 15° , 17° , 18° , and 23° , characteristic of an A-type diffraction pattern similar to that exhibited by NCS; the NSB-1, NSB-3, and NSB-4 blends presented unresolved crystalline peaks

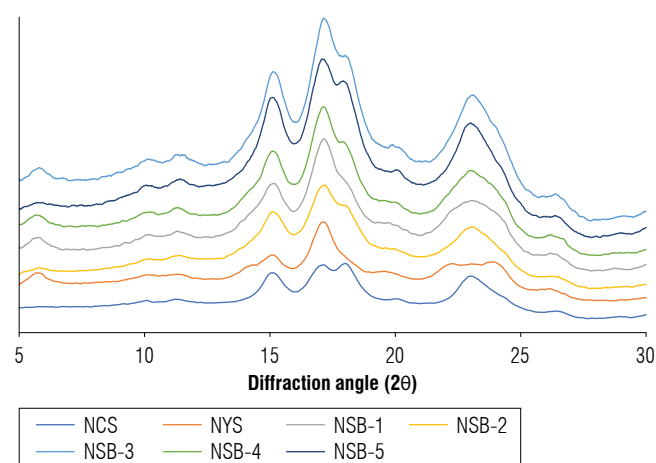


FIGURE 2. X-ray diffraction patterns of native starches and their blends. NCS: native cassava starch; NYS: native yam starch; NSB-1: blend of cassava 30%-yam 70%; NSB-2: blend of cassava 50%-yam 50%; NSB-3: blend 60%-yam 40%; NSB-4: blend of cassava 45%-yam 55%; NSB-5: blend cassava 70%-yam 30%.

at angles 5° , 10° , 12° , and 20° , so that, in these cases, the existence of a C-type pattern can be considered. Oliveira *et al.* (2018) similarly observe an A-type pattern for a binary blend between native potato and sweet potato starches, where the sweet potato A-type diffraction pattern predominates rather than the B-type exhibited by individual potato starch.

However, the differences observed in the intensities of the crystalline peaks present in the diffraction patterns of each blend caused variations in CI compared to the native starches (Tab. 2). At first, NCS and NYS exhibited a crystallinity index of 48.24% and 44.56%, estimates corresponding to Tester *et al.* (2004) and Figueroa-Flórez *et al.* (2019). In this context, the behavior of the crystallinity index presents a correlation with the amylose content of NCS and NYS, where NYS showed a lower CI due to its higher content of amylose compared to NCS. However, the evaluated starch blends showed a non-additive behavior in the CI compared to their native counterparts. Thus, except that NSB-2, blends did not present differences ($P>0.05$) in CI compared to NYS despite being evaluated in different proportions of their starchy constituents, suggested the occurrence of a possible effect of interaction between the starches of each blend. However, NSB-2 showed an unpredictable behavior in CI due to a reduction ($P<0.05$) compared to the rest of the samples evaluated. Similar to the above, Gomes *et al.* (2018) demonstrate the occurrence of a non-additive behavior in the crystallinity index of binary blends between native pea and rice starches, Oliveira *et al.* (2018) in sweet potato-potato blends, and Hornung *et al.* (2017) for a binary blend between starches from yams *Dioscorea piperifolia* and *Dioscorea trifida*. These last authors establish that the developed mixture process alters the semi-crystalline characteristics of the starches that make up the starchy system. Likewise, the previous behaviors in the crystallinity index could be correlated with the non-additive effects observed in the enthalpy of gelatinization of the samples (Tab. 4), since the thermal requirements to trigger the gelatinization of the starch blends would be associated with the semi-crystalline behavior exhibited by each blend.

Water absorption capacity (WAC)

The water absorption capacity of native starches and their blends can be seen in Table 2. At first, NCS and NYS showed differences in WAC with values of 65.72 and 93.09%. Waterschoot *et al.* (2015) report that starches with B-type crystallinity pattern have a greater space between the double helices of the amylopectin chains compared to those starches with A-type diffraction patterns. The space allows a greater capacity for water absorption.

The WAC was within the ranges reported by Ikegwu *et al.* (2009) and Ayetigbo *et al.* (2018) for native cassava starch and by Donaldben *et al.* (2020) for native yam starch. The blends of starches exhibited a non-additive interaction behavior between the starches associated with the in WAC, because the starchy blends exhibit a non-proportional trend in said parameter compared to the individual values of NCS and NYS for each mix ratio. In other words, the WAC of the starch blends cannot be predicted from the mix ratio and the individual values manifested by NCS and NYS. These facts revealed complex phenomena of interaction between the granular populations of each evaluated proportion, possibly associated with the characteristics of the granular surface and the amylose content of each starch present in the blend (Hagenimana & Ding, 2005). The starch blends are a mechanism that allows the development of polymeric matrices with novel and different physicochemical characteristics from the individual starches involved.

Pasting properties

The pasting behavior of native starches and their blends is presented in Figure 3. At first, NCS presented the highest peak viscosity value (2110 cP), indicating a marked tendency for the suspension to reach higher viscosity in the heating section compared to the rest of the starchy samples evaluated, while NYS reached a peak viscosity value (1658 cP) lower than NCS ($P<0.05$). In contrast, high-temperature viscosity was more stable in NYS compared to NCS, exhibiting a lower breakdown viscosity value ($P<0.05$), corresponding to 20 cP and 1348 cP. However, the tendency for retrogradation, related to the setback viscosity, was more evident in NYS (2043 cP) than in NCS (795 cP). Thereby, Hornung *et al.* (2017) report that starches with a B-type diffraction pattern retrograde much more than those with an A-type, as in the case of NYS. Similar pasting profiles have been reported for NCS (Novelo-Cen & Betancur-Ancona, 2005; Lin *et al.*, 2013) and NYS (Huang *et al.*, 2006; Salcedo Mendoza *et al.*, 2016). The behavior of the starch blends viscosities exhibited a non-additive effect on all the pasting parameters compared to their individual counterparts (Fig. 3). All the blend ratios evaluated presented a double peak viscosity, possibly associated with the disparity in granular size and the differentiated water absorption of the two amylaceous species in the blend. In this context, Karam *et al.* (2006) refer to blends of starches in which the swelling of yam starch granules could be restricted due to the presence of other starchy constituents. Waterschoot *et al.* (2015) review that small granules in binary starch blends would fill the voids between the large granules, producing a packed system with reduced swelling power that would be positively correlated with the behavior on peak viscosity

parameter in starch blends (Wu *et al.*, 2016). Similarly, NCS and NYS present significant differences in granular size, where NYS presents larger granules than NCS, so that it can also be inferred that there was a possible interaction phenomenon between NCS and NYS swelling granules in the heating section for all starch blends, leading to a generalized reduction in the peak viscosity parameter of the evaluated ratios. This was presumably associated with the possible formation of a packed system between small and large granules. However, Obanni and Bemiller (1997) speculate that the double viscosity peak observed in the viscosity profiles of starch blends was a consequence of the significant differences between the breakdown viscosities of the individual starch constituents, a hypothesis that could also be supported by the results obtained in this work. Likewise, similar double peak viscosity behaviors have been reported for binary blends of potato-maize (Ai & Jane, 2015), cassava-sweet potato (Li *et al.*, 2019), and potato-waxy maize starches (Waterschoot *et al.*, 2014). Moreover, the non-additive behavior of the blends between NCS and NYS was more evident in the setback and breakdown viscosity parameters. For example, NSB-2 presented values for breakdown and setback viscosities of 429.9 cP and 655.9 cP, respectively, instead of 684 cP and 1419 cP, where the latter will be the expected values for a 50:50 binary blend for the case that there is an additive behavior of their individual counterparts. The foregoing suggests that it is not possible to establish predictions in the pasting parameters for the blends of NCS and NYS because there are interaction phenomena in the behavior of the viscosities of the starches when they are blended. Thus, non-additive reductions were evident in the pasting parameters of the evaluated starch blends, depending on the proportion of the individual counterparts. Thus, similar results were reported by Zhu *et al.* (2020), where a binary blend of potato:quinoa (0.67:0.33) presented no additive behavior in the viscosities of the suspension at 95°C and 50°C, showing higher values than those exhibited by the individual potato and quinoa starches.

Interaction phenomenon was observed in the behavior of setback and breakdown viscosities of the starch blends. The blends evaluated exhibited similarity to NCS in the viscosity profile on cooling so that the values of setback viscosity of the blends showed a marked proximity to NCS. This suggested the occurrence of synergistic effects in said parameter ($P<0.05$), and in practical terms they refer to the fact that the blends of starches showed a response with a low tendency to retrogradation compared to NYS. Likewise, the breakdown viscosities of the starch blends showed greater proximity in said parameter to NYS. Thus,

the starch blends presented greater stability of the hot viscosity compared to NCS ($P<0.05$).

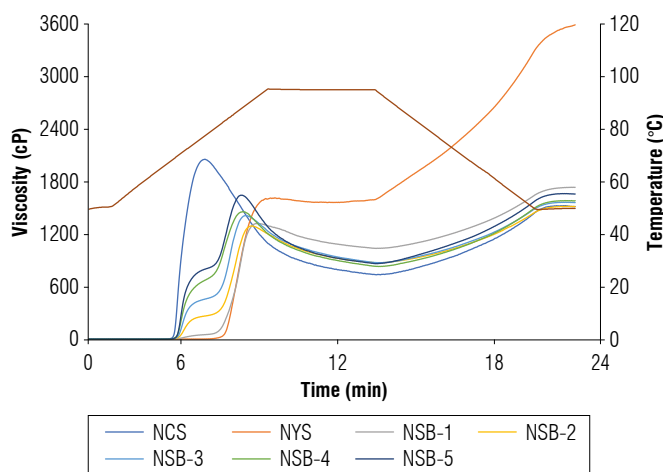


FIGURE 3. Viscosity profiles of native starches and their blends. NCS: native cassava starch; NYS: native yam starch; NSB-1: blend of cassava 30%-yam 70%; NSB-2: blend of cassava 50%-yam 50%; NSB-3: blend 60%-yam 40%; NSB-4: blend of cassava 45%-yam 55%; NSB-5: blend cassava 70%-yam 30%.

The blends exhibited greater viscosity stability against thermal stress. This was possibly due to the presence of NYS, as well as a reduced tendency to retrograde compared to NYS, perhaps associated with the proportion of NCS in each case. There was non-additive behavior in the pasting parameters of the starch blends that is not likely to be predicted from the viscosities of the individual counterparts. Likewise, Zhu *et al.* (2020) considered that the non-additive behavior present in the pasting parameters of the potato, sweet potato and quinoa starch blends could be attributed to the differences in the chemical, structural and granular composition of the starchy components, which applies to the blends of NCS and NYS, where the individual starches exhibited significant differences in morphology, and amylose content among themselves. In

contrast, Zhang *et al.* (2011) report that no variations were observed in breakdown and setback viscosities of blends between potato and corn starches. This could be the result of interactions between granular components leached during the gelatinization of the blend.

Thermal properties

The gelatinization parameters of NCS, NYS and their blends are shown in Table 3. At first, the transition temperatures in the NCS gelatinization were lower than those observed in NYS. This could be related to the crystalline polymorphism of NYS that was B-type, different from NCS, which was A-type. Consequently, the presence of abundant amylopectin chains with a high degree of polymerization in B-type starches could cause a lower thermal energy requirement to trigger gelatinization in NYS compared to NCS (Karam *et al.*, 2006). The NYS gelatinization enthalpy was lower than that observed in NCS (Tab. 4). Similar results were reported for the transition temperatures of native cassava and yam starches (Chen *et al.*, 2011; Duan *et al.*, 2020). Likewise, the gelatinization enthalpies for NCS and NYS were 9.39 and 5.77 J g⁻¹. These results are consistent with Jyothi *et al.* (2005) and Duan *et al.* (2020).

All the starch blends exhibit the presence of two peak temperature values (Tab. 4), probably due to the independent gelatinization of the starches in each blend. Waterschoot *et al.* (2015) report that the DSC tests for binary blends of starches with intermediate moisture contents (35-65%), show two endotherms in the DSC profiles. In the present study, the first peak is related to the onset gelatinization temperature of NCS and the second corresponds to NYS.

The onset temperatures T_0 for each blend showed values like NCS, whereas the final temperatures T_f were close to NYS. Therefore, the evident independence in the gelatinization of each starch in the mixture suggests additive

TABLE 3. Gelatinization parameters of native starches and their blends.

Samples	T_0 (°C)	T_{p1} (°C)	T_{p2} (°C)	T_f (°C)	ΔH (J g ⁻¹)
NCS	67.88 ± 0.03 ^a	74.18 ± 0.05 ^a	-	82.84 ± 0.49 ^a	9.39 ± 0.06 ^a
NYS	79.45 ± 0.01 ^b	83.37 ± 0.02 ^b	-	89.48 ± 0.30 ^b	5.77 ± 0.05 ^b
NSB-1	67.60 ± 0.06 ^b	72.18 ± 0.01 ^c	82.56 ± 0.01 ^a	86.65 ± 0.56 ^c	5.73 ± 0.02 ^b
NSB-2	67.76 ± 0.01 ^b	72.13 ± 0.01 ^c	83.38 ± 0.01 ^b	88.20 ± 0.48 ^b	6.82 ± 0.06 ^c
NSB-3	67.45 ± 0.01 ^c	72.32 ± 0.01 ^d	83.25 ± 0.43 ^b	88.29 ± 0.41 ^b	7.59 ± 0.38 ^d
NSB-4	67.40 ± 0.32 ^c	72.51 ± 0.01 ^e	83.37 ± 0.13 ^b	88.07 ± 0.04 ^{bc}	6.81 ± 0.02 ^c
NSB-5	67.45 ± 0.03 ^c	72.24 ± 0.01 ^f	82.56 ± 0.24 ^{ab}	88.89 ± 1.00 ^b	7.31 ± 0.02 ^b

T_0 : onset temperature; T_p : peak temperature; T_f : final temperature; ΔH : enthalpy of gelatinization; NCS: native cassava starch; NYS: native yam starch; NSB-1: blend of cassava 30%-yam 70%; NSB-2: blend of cassava 50%-yam 50%; NSB-3: blend 60%-yam 40%; NSB-4: blend of cassava 45%-yam 55%; NSB-5: blend cassava 70%-yam 30%. Means followed by same letters within a column do not differ statistically according to Tukey's test ($P<0.05$), average ± standard deviation.

behavior in the transition temperatures. However, a non-additive effect was observed in gelatinization enthalpies of the blends between NCS and NYS, so that the thermal energy required to trigger the gelatinization of the mixtures could not be predicted from the individual values of each counterpart because of certain phenomena of interaction with each other. Obanni and Bemiller (1997) report non-additive behavior in the gelatinization enthalpy of binary blends of cassava-wheat and potato-rice starches, where the differences in granular size in each case could cause the stochastic behavior for ΔH . The non-additive variations observed in the crystallinity index of starch blends may be related to the unpredictable behavior observed in ΔH , while the thermal requirements to trigger gelatinization respond to the semi-crystalline characteristics of starchy materials. Furthermore, the concentration of starch present in the DSC analyzes of the present study (33%) could also play an important effect on the resulting non-additive behavior of ΔH .

***In vitro* digestibility**

The fractions of rapidly digestible starch (RDS), slowly digestible (SDS), and resistant starch (RS) in gelatinized samples of NCS, NYS and their blends are represented in Table 3. At first, NCS and NYS showed differences between them ($P<0.05$) in terms of the proportion of RDS and RS, where NYS presented a higher content of RDS compared to NCS. It is relevant to show that the behavior of the starches *in vitro* digestibility indicates significant variations when the samples are gelatinized, demonstrating an increase compared to the native samples without gelatinizing. Similar results have been reported for RDS in native cassava (Jyothi *et al.*, 2005) and yam starches (Zhou & Kang, 2018).

RDS, SDS, and RS for the starch blends showed stochastic differences compared to their individual components. The foregoing is related to non-additive behavior of the

blends in some parameters related to RDS, SDS, and RS. For example, NSB-2 and NSB-3 presented RDS values significantly higher than the individual values of NCS and NYS, suggesting the interactions between the starchy constituents of the blend that cause unpredictable behaviors in the digestion of starch fractions in the first 20 min of the process. Similarly, the SDS fractions of NSB-3 and NSB-4 showed non-linear reductions relative to the individual counterparts. Ma *et al.* (2020) also report non-additive variations on *in vitro* digestibility parameters for blends of rice starches with different amylose content, where stochastic increases and reductions in RDS, SDS, and RS of some mix ratios were appreciable. Thus, the variations in the amylose content of NCS and NYS, together with the differences in granular size and crystalline pattern, could have influenced the resulting non-additive behavior of the mix ratios of starches evaluated in the present study.

Conclusions

There were changes in the physicochemical and structural properties of the cassava and yam starch blends compared to their native counterparts, as well as a general conservation of the granular morphological characteristics after the mixing process. The significant differences in the intrinsic properties of each starch exhibited a prevalent additive-type effect on amylose content and gelatinization temperatures, in contrast to the effects observed on the pasting parameters and crystallinity index of all the mix ratios evaluated that were non-additive. Specifically, the significant differences in amylose content and granular morphology of each individual starch would be responsible for the various resulting effects exhibited by mixing ratios. Thereby, the process of mixing native starches could be considered as an alternative mechanism in the polymeric matrix design with improved physicochemical properties from interaction

TABLE 4. *In vitro* digestibility in native starches and their blends.

Samples	RDS (%)	SDS (%)	RS (%)
NCS	77.91 \pm 0.63 ^a	10.52 \pm 1.13 ^a	11.56 \pm 0.74 ^a
NYS	84.02 \pm 1.22 ^b	11.25 \pm 1.63 ^{ab}	4.72 \pm 0.40 ^b
NSB-1	86.17 \pm 1.03 ^b	11.50 \pm 0.21 ^{ab}	2.31 \pm 0.86 ^b
NSB-2	88.48 \pm 0.39 ^c	7.45 \pm 1.50 ^{ab}	4.06 \pm 1.71 ^b
NSB-3	89.23 \pm 0.91 ^c	6.29 \pm 0.30 ^b	4.47 \pm 1.06 ^b
NSB-4	85.40 \pm 0.67 ^b	9.87 \pm 1.62 ^a	4.72 \pm 0.94 ^b
NSB-5	86.19 \pm 0.42 ^b	9.56 \pm 0.30 ^a	4.24 \pm 0.19 ^b

RDS: rapidly digestible starch; SDS: slowly digestible starch; RS: resistant starch. NCS: native cassava starch; NYS: native yam starch; NSB-1: blend of cassava 30%-yam 70%; NSB-2: blend of cassava 50%-yam 50%; NSB-3: blend 60%-yam 40%; NSB-4: blend of cassava 45%-yam 55%; NSB-5: blend cassava 70%-yam 30%. Means followed by same letters within a column do not differ statistically according to Tukey's test ($P<0.05$).

phenomena between individual starches where the resulting blends showed in the present case a low tendency for retrogradation, lower crystallinity index values compared to their native counterparts, and a regulated water absorption capacity, all potentially desirable characteristics in the food industry. However, the proportionate choice of each starch in the mixture is considered a determining factor of the resulting behaviors in the physicochemical and structural properties, therefore, the selection criteria of a particular mix ratio must be evaluated depending on the application or further processes of modification.

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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

EDAD, JAFF, ECC designed the experiments; EDAD and JAFF carried out the experiments and data collection in laboratory experiments. EDAD, ERS, MACR and ECC contributed to the data analysis. EDAD, JAFF, JGSM, and ERS wrote the article. All authors reviewed the final version of the manuscript.

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Value chain approach for research on *Moringa oleifera* as a nutritional supplement in the human diet

Enfoque de cadena de valor de la investigación de *Moringa oleifera* como suplemento nutricional en la dieta humana

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ABSTRACT

Research in Cuba suggests a value chain approach for the production of *Moringa oleifera* that might identify the strengths and weaknesses that influence food production and the advantage of applying a value chain model to integrate the productive links. As part of these strengths, we established an evaluation of *Moringa oleifera* quality using analytical methods to diagnose the value chain of the industrial process as a nutritional supplement for human use, increase production and product quality, and improve manufacturing and production. Potentialities were found that diversified the products in different areas, demonstrated by the high content of metabolites that positively influence human health using products supplemented with moringa. An approach that applied the value chain defined the steps in moringa industrial processing that guaranteed and expanded the moringa product portfolio that impacted effectiveness, safety, and quality for the consumer.

Key words: urban agriculture, sustainable development, nutraceuticals.

RESUMEN

La investigación en Cuba sugiere un enfoque de cadena de valor para la producción de *Moringa oleifera* que podría identificar fortalezas y debilidades que influyen en la producción de alimentos y la ventaja de aplicar un modelo de cadena de valor para integrar los eslabones productivos. Como parte de estas fortalezas se estableció la valorización de la calidad de *Moringa oleifera* a través de métodos analíticos para el diagnóstico de la cadena de valor del proceso industrial como suplemento nutricional para uso humano e incrementar la producción, la calidad de los productos y mejorar su manufactura y procesos productivos. Se encontraron potencialidades que diversificaron los productos en diferentes ámbitos, evidenciadas por el alto contenido de metabolitos que influyen de forma positiva en la salud humana con productos suplementados con moringa. La aplicación de cadena de valor expuso los pasos del procesamiento industrial de la moringa que garantizan y amplían la cartera de productos de moringa con impacto en el consumidor en términos de efectividad, seguridad y calidad.

Palabras clave: agricultura urbana, desarrollo sostenible, nutracéuticos.

Introduction

Moringa oleifera is a plant native to India that is rich in nutrients. The plant can be grown for its green biomass for human or animal consumption (Foidl *et al.*, 2001). The only difference between its use in a human or animal diet lies in measures applied for the microbiological control of the cultural practices, industrial processing, and the recurrent process of obtaining the seeds. *Moringa oleifera* crops differ in their genetic features (Gutiérrez *et al.*, 2015), edaphoclimatic conditions, cultural practices, spacing, and harvest periods. This analysis focused on using Moringa to produce green biomass for human use and applying a value chain approach to produce dry leaves as nutraceuticals for human consumption (Almora-Hernández *et al.*, 2021).

The value chain provides a general application model that allows the representation of activities of any organization based on the concepts of cost, value, and profit. It consists of productive processes that provide a scheme for diagnosing the enterprise's position concerning its competitors and possible actions leading to a sustainable competitive advantage (Tumbaco-Laje *et al.*, 2022).

Nowadays, Cuba promotes a value chain approach that increases yields in food production, food quality, and nontoxic nature, including all the chain links that meet consumer requirements (Murgueitio Escobar, 2005). This efficiently contributes to the sustainability of the national economy and establishes food security as a national priority, as declared in 2007 (Castro, 2007).

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Various studies in Cuba demonstrate the potential use of different parts of the *Moringa* tree for food applications. Leaves can be used as a dietary supplement to prepare soups (Liu, 2018) as well as infusions for medicinal purposes (Bancessi *et al.*, 2019) and crackers (Almora-Hernández *et al.*, 2021).

The development of functional foods and their associated science mark guidelines in industrial research. Scientific research on *Moringa* is important to discover specific foods that principally clarify benefits leading to a reduction of degenerative chronic diseases, such as diabetes, hypertension, and obesity, as well as the need to find functional foods that improve the living quality of the population (Aguirre, 2019).

Applying a model for the value chain is beneficial since it considers actors, critical points, access to supporting services, and the search to increase competitiveness and equity. It identifies factors leading to success and value creation (Murgueitio Escobar, 2005).

This research defined the value of *Moringa oleifera* tree parts through strategic actions based on the diagnosis of the value chain of industrial processing of this plant as a nutritional supplement for human use. This increased the product's production, quality, and non-toxicity and improved the manufacturing process, productive areas, and goods.

Materials and methods

Plant material

The plant material used was the leaves of the Criolla, Supergenius, Plain, and Nicaragua ecotypes of *Moringa oleifera* Lam. 1783, Moringaceae (Bocarando-Guzmán *et al.*, 2020). The ecotypes Supergenius, Plain, and Nicaragua were planted from seeds. Supergenius and Plain were donated by India, and the Nicaragua ecotype was donated by Nicaragua. The Criolla seeds came from the national germplasm collection. All ecotypes are preserved by the National Germplasm Collection from the Basic Productive Unit (BPU) "El Pitirre," Los Palacios, Pinar del Rio provinces, belonging to the Research Center on Protein Plants and Bionatural Products (CIPB, Cuba). The leaves of the four ecotypes were harvested in areas of the BPU "Futuro Lechero" in Havana, also part of the CIPB, which is devoted to crops for nutraceutical purposes. The area established for these crops is located at 23°04'20" N, 82°29'20" E. The

climate in this area is classified as tropical, with an average temperature of 33°C and a rainy season from May to June.

Cultural practices used with these crops are similar, and leaf drying used the following procedure. After 45 d of growth, the leaves from the upper 1/3 part of the plant from the ground were collected (without the apical tips). The *M. oleifera* plants used were obtained from a study that quantified secondary metabolites (Lago Abascal *et al.*, 2020). The age of the plant was noted for this study since previous studies show that metabolites vary according to the age of the leaves. This demonstrates that the state of development of the plants affects the biosynthesis and accumulation of chemical compounds, and the young mature leaves synthesize the most significant amount. The phenols and flavonoids have higher contents in the leaves of the medial parts of the plants (Cabrera-Carrión *et al.*, 2017). The harvest of the ecotypes Criolla, Supergenius, and Plain took place in June 2019, and that of the ecotype Nicaragua took place in October 2020.

Leaf collection occurred manually using latex gloves (according to Good Agricultural Practices - GAP). The leaves were placed vertically on aseptic plastic boxes supported on canvas in the field (Sánchez *et al.*, 2014). These were transported in a closed vehicle to the processing site, where they were manually peeled wearing latex gloves (Good Manufacturing Practices - GMP). The plant fragments were immediately subjected to continuous washing and mechanical centrifuging. Drying occurred on stainless steel trays, using solar ovens CONA at a controlled temperature of 45°C.

The GAP and GMP correlated with the set of agricultural strategies of *Moringa* growers and the guarantee of having microbiological control over the vegetal fragments and repetition of the procedures.

Strengths, opportunities, weaknesses, and threats to the industrial processing of *Moringa* were identified following a value chain approach, as shown in Figure 1. This value chain approach consisted of several stages, from agriculture to final product marketing. The first stage of crop establishment began with seed propagation and sowing that was carried out on selected soils, free of chemical contaminants, under conditions of temperature varying from 25°C to 35°C, with a pH of 5.9, and well-drained. After first pruning at 30-50 cm from the ground after 6 months, the crop began the second productive stage: every 45-60 d, the new leaves were collected and pruned again.

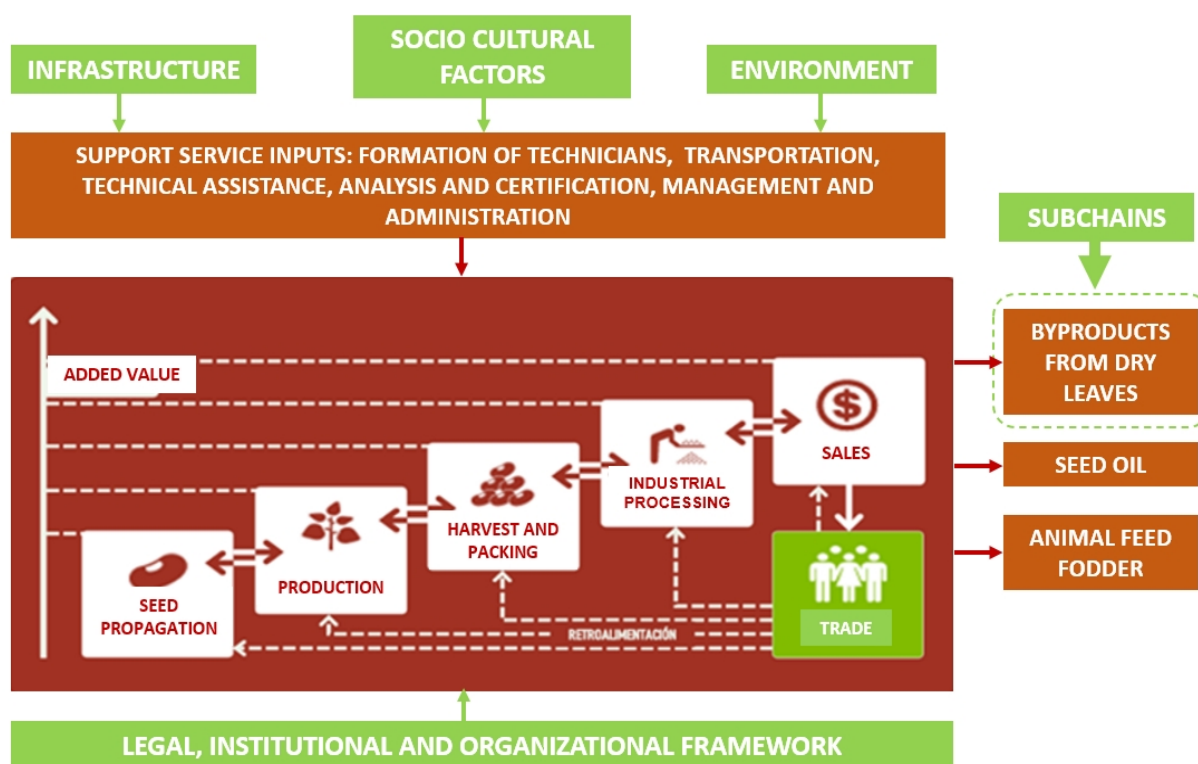


FIGURE 1. Value chain of *Moringa oleifera* industrial processing.

The third stage of the industrial process began with the reception and weighing of the raw material (harvested leaves). The selected material (leaves and rachis) was washed and dried. The final products derived from the raw material were dried leaves, dried leaf powder, dried rachis, and dried rachis powder. For subsequent marketing, these products acquired a sanitary registry from the Institute of Hygiene, Epidemiology and Microbiology (Inhem) of Cuba.

Throughout the stages, the strengths, opportunities, weaknesses, and threats of the *Moringa* industrial process were identified with a value chain approach based on an analysis of Figure 1. This study did not discuss the sub-chains for producing seed oil and animal feed fodder (Fig. 1) since each depends on differences during the first stage.

Quantification of metabolites in the ecotypes of *Moringa oleifera*

The quantification of metabolites in leaves was carried out according to Lago Abascal *et al.* (2020). The content of total polyphenols was determined by forming tungsten and molybdenum salts using pyrogallol (Merck) as a standard. The reaction was carried out at room temperature for 30 min in darkness, and absorbance was measured at 760 nm with a spectrophotometer (Shimadzu UV160-A). Results were expressed as milligram-equivalents of pyrogallol per

gram of dry extract. Quercetin was used as a standard to determine flavonoids, and the samples were treated with ethanol, aluminum chloride, and potassium acetate. The reaction was carried out at 30°C for 30 min, and absorbance was measured at 415 nm with a spectrophotometer (Shimadzu UV160-A). The result was expressed as milligram-equivalents of quercetin per gram of dry extract. All assays were performed in triplicate.

Statistical analysis

All tests were triplicated. For the statistical analysis, means and standard deviations were used as descriptive measures of the central trend and data dispersal. Before processing, data normality and variance homogeneity were evaluated through Kolmogorov-Smirnov and the Bartlett test. A comparison among groups was made through an analysis of variance of simple classification (ANOVA). Means were compared using ANOVA. Statistical analyses were accomplished using the statistical package SPSS 10.0.1.

Results and discussion

The DAFO matrix analysis of the process (–D– weaknesses, –A– threats, –F– strengths, and –O– opportunities) is shown in Table 1.

TABLE 1. Evaluation of the industrial process of *Moringa oleifera* using a value chain approach.

Aspects	Strengths	Weaknesses
Internal	<ul style="list-style-type: none"> • The plant is present in Cuba • Over 10 years of experience with the crop • Propagation is either by seeds or cuttings • Drought resistant • Adaptability of the crop to a wide range of soils • There is experience for the crop, harvest, and processing of the plant • There is governmental support to expand the crop • High nutritional value • Preventive and curative properties • All the parts of the plant can be used • Plant parts have different uses • The national sanitary registration is available for the products derived from the plant • There is a national association of <i>Moringa</i> growers • The domestic market has prices for the products • Imports and exports throughout the year can be controlled 	<ul style="list-style-type: none"> • There are few growers in the country • Access to funding to consolidate production is difficult • Plant production is not stable throughout the year • Little incentives by growers to adopt this new crop • It is an “extractive” plant that demands fertilization of soil
Aspects	Opportunities	Threats
External	<ul style="list-style-type: none"> • It generates natural products that are accepted by the population • It generates scientific research • It creates permanent employment • There are interests in the food and cosmetic industries • Diversification of the crop is possible • There is quantitative data on <i>Moringa</i>’s annual exports and imports • There are established prices for the final products • Low-level technology is required for agricultural and industrial processing 	<ul style="list-style-type: none"> • Climate change affects plots, either by flooding or extreme drought. The former one is the most severe problem affecting the crop (manageable) • Hurricanes can destroy crop plots

Products generated from the green biomass

As a result of research on various *Moringa* parts and the diverse use of the plant, it is possible to diversify products in other branches. In the Cuban pharmaceutical industry, *Moringa* is part of the basic basket of natural medicines available in powder from dry vegetal fragments for direct consumption as a protein source, vitamins and minerals, and its formulation in 500 mg gel capsules (Lago Abascal, Thu Huong *et al.*, 2021) and dry plant fragments for tea (Lago Abascal, Almora Hernández, González García, Hernández Rivera *et al.*, 2021). Integral rice crackers are also manufactured and supplemented with fresh apical buds (Almora Hernández *et al.*, 2020). All natural products made from plant leaves are sanitarily registered by the Institute of Hygiene, Epidemiology, and Microbiology (Inhem), the National Regulatory Organ of the Ministry of Public Health (Minsap) for foods for human consumption.

In the food industry, dry vegetal fragments powder, rachis powder, and other products derived from seeds are used to fortify meat sausages, snacks, and preserves; in cosmetics, the *Moringa* oil is used for shampoos and soaps.

Identification of the biological effects of the different parts of the plant, specifically of the leaves that are present in the dry plant fragments after processing and that have been underlined in animal models after consumption (Lago Abascal, Thu Huong *et al.*, 2021), confirms the value chain

approach of research carried out to develop uses for this plant. Antimicrobial effects (Monteagudo Borges *et al.*, 2022) and antioxidant effects are known (Lago Abascal, Almora Hernández, González García, Hernández Rivera *et al.*, 2021) as well as hypolipemiant (Lago Abascal, Thu Huong *et al.*, 2021) and anti-inflammatory (Lago Abascal, Menéndez Soto del Valle *et al.*, 2021) effects. These effects are attributed to flavonoids and polyphenols present in the dry plant fragments after the manufacturing process, based on results where the different evaluated ecotypes showed a similar composition (Tab. 2) without expressing significant differences among levels. These results are the basis for performing clinical tests made in humans (Lago Abascal *et al.*, 2020).

Studies of *Moringa* plant fragments demonstrate many beneficial compounds for human health that favor the enrichment of foods with this plant (Lago Abascal *et al.*, 2020). Powder from dry *Moringa* plant fragments is used by the food industry. It can be stored for some months at room temperature without losing nutritional value (Srinivasamurthy *et al.*, 2017). *Moringa* sprouts, after harvesting, can be used for human nutrition as integral rice crackers; these are increasingly accepted (Almora-Hernández *et al.*, 2021) and as fresh vegetables.

The value chain approach of the research on the nutraceutical uses of *Moringa* consists of generating products

that are beneficial for health from the properties present in the plant. The objective is to obtain new products from the circular economy (Cosme, 2022). The production of dry plant fragments involves using only two-thirds of the plant leaves. The apical third of the plant can also extract metabolites with biological effects. The stems can be used to organically fertilize the crop. There are ongoing projects with Project MOR-*e* and the University of Zaragoza, Spain, and Cubaenergia (Cuba) to design a technology for the production of fuel pellets that can be used to heat water on a boiler that, in turn, will supply heat to warm the air used in plant fragments drying. This is an added value for this product that integrates into the circular economy. Rachis from the leaf peeling process are also processed through drying and are used as expanders and strengtheners for other products like coffee or tea with positive acceptance (Lago Abascal *et al.*, 2020; Almora-Hernández *et al.*, 2021; Lago Abascal, Thu Huong, *et al.*, 2021).

Quantification of metabolites of the ecotypes of *Moringa oleifera* grown in Cuba

All the ecotypes generally showed an elevated secondary metabolite content in their leaves (Tab. 2). The highest flavonoid content corresponded to the Supergenius ecotype, and polyphenols corresponded to the Nicaragua ecotype. However, among all these levels, no significant differences were found, confirming the possibility of using any of the four ecotypes in the production procedure of dried plant fragments to be used as nutraceuticals for human consumption.

The contents of polyphenols and flavonoids in this study were superior to that of Cabrera-Carrión *et al.* (2017) where the polyphenol contents were 13.00 mg-eq g⁻¹ and flavonoid contents were 22.88 mg-eq g⁻¹. They are also superior to those Campo-Fernández *et al.* (2020) describe, who recorded polyphenol values of 21.27 mg-eq gallic acid g⁻¹ DW and flavonoids of 37.60 mg-eq quercetin g⁻¹ DW.

The similarities found in the metabolite contents between these ecotypes coincided with the phytochemical evaluations previously made for three of these ecotypes. Such

evaluations confirmed that *Moringa* effectively accumulates phenolic compounds (Lago Abascal, Almora Hernández, González García, Campa Huergo *et al.*, 2021). These ecotypes showed antimicrobial properties against pathogens of biological interest (Lago Abascal *et al.*, 2020).

The high metabolite content in *Moringa* leaves of this study might be due to factors relating to the plant's age and harvest practices (Yusof, 2015). Our research showed that the four ecotypes had similar results under field conditions for the composition of compounds used for human consumption.

Food products fortified with polyphenols have high impacts for human health; they prevent neurodegenerative diseases (Farzaei *et al.*, 2018) and help to control high blood pressure (Oudot *et al.*, 2019). They also influence the oxidized form of the low-density cholesterol responsible for increased lipid levels in arteries (Frankel & Meyer, 2000).

This plant can regulate the content of secondary metabolites in its photosynthetic organs, given possible climate changes that might occur with increased temperatures and ultraviolet radiation (Ballaré *et al.*, 2011). All the above confirms the value of *Moringa oleifera* as a food enrichment and/or its direct consumption as a nutritional supplement.

Conclusions

Applying the value chain approach breaks down the steps of both agricultural and industrial processing of *Moringa oleifera*. It guarantees a portfolio of products for this plant that impacts the consumer for effectiveness, security, and quality.

Recommendations

Undoubtedly, the use of the value chain is one of those organizational approaches that help identify and develop all the links in manufacturing products originating from *Moringa oleifera*.

TABLE 2. Quantification of secondary metabolites in leaves of ecotypes of *Moringa oleifera*.

Ecotype	Flavonoid content (mg-eq quercetin g ⁻¹ DW)	Polyphenol content (mg-eq pirogallol g ⁻¹ DW)	Harvest date	Reference
Criolla	80.91±1.64	71.23±0.38	June 2019	Lago Abascal <i>et al.</i> (2020)
Supergenius	97.67±0.97	94.54±1.67		
Plain	110.12±0.75	93.77±0.65		
Nicaragua	82.55±2.43	98.50±4.76	October 2020	Lago-Abascal <i>et al.</i> (2024)

P > 0.05, there is no statistical difference among ecotypes and metabolites at the 95% probability level.

Conflict of interest statement

The authors declare no conflicts of interests regarding the publication of this article.

Author's contributions

VLA and ERJ: conceptualization; VLA and EAH: data curation; VLA and EAH: formal analysis; VLA and EAH: research; VLA and EAH: methodology; VLA and EAH: software; ERJ and CC: supervision; VLA: visualization; VLA: writing – original draft; ERJ: writing – review & editing. All authors reviewed the final version of the manuscript.

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Research article length should not exceed 5,200 words, whereas scientific notes should have no more than 4,000 words. As review articles contain a large amount of detailed information, their length may be greater than research articles but should not exceed 8,000 words, or 10,000 words including the list of references. For review articles, the list of references (Literature cited section) should include at least 50 references. Tables and figures, that is to say, diagrams, drawings, schematic and flow diagrams, pictures, and maps should be consecutively numbered (Table 1 ...Table n; Figure 1... Figure n, etc.).

Texts and tables should be prepared using the MS Word® processor. Manuscripts including tables as embedded images will not be published. All text should be double-spaced including table headers, figure captions and cited literature. All pages must be numbered consecutively. Line numbering on each page is mandatory. Tables and diagrams of frequency (bar and circular diagrams) should be included in the mentioned Word file as well as in their original MS-Excel® or other graphic formats but maintaining a high resolution. Other figures, including photographs and drawings should be submitted in digital JPG (or JPEG) compression format, with a minimum resolution of 300 dpi.

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The journal's official language is English. Regarding measurement units, the metric system (SI) should be consistently used through the manuscript, unless the need is seen to apply any specific units that are of frequent use by the scientific community. Multiplication followed by negative superscript (e.g., kg ha⁻¹) can only be used with SI units. The slash (/) is a mathematical operation symbol that indicates "divided by". Anyway, in sciences it is used as a substitute for the word "per", and it is used to indicate rates. Use the slash to connect SI to non-SI units (e.g., 10°C/h or 10 L/pot).

Decimal fractions should be separated by a point (.), not a comma (,).

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With regards to the tenses, the most commonly used ones are the past, for the introduction, procedures and results; and the present, for the discussion.

Title and authors

The title in English, as well as its corresponding Spanish translation, shall not exceed 15 words. The scientific names of plants and animals shall be italicized and lowercased, except for the first letter of the genus (and of the species author), which must be uppercased.

The authors (including first and second names) shall be listed in order of their contribution to the research and preparation of the manuscript, in completely justified text format (filling the whole line, or, if necessary, the next

one below) under the translated version of the title. At the bottom of the article's first page, only the name and city location of the employer or supporting institution(s), and the e-mail address of the corresponding author should be included.

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The Abstract should be written in English with Spanish translation for the "Resumen". Both texts should contain brief (no longer than 200 words in a single paragraph) and accurate descriptions of the paper's premise, justification, methods, results and significance. Both language versions shall be mandatorily provided with a list of (maximum six) key words that have not appeared in the title or abstract, and included in the Agrovoc thesaurus by Agris (FAO).

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The introduction must include the delimitation and current status of the problem, the theoretical or conceptual basis of the research, the literature review on the topic, and the objectives and justification of the research. Common names must be accompanied by the corresponding scientific ones, plus the abbreviation of the species author surname when mentioned for the first time.

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Besides a clear, precise and sequential description of the materials used for the research (plant or animal materials, plus agricultural or laboratory tools), this section illustrates the procedures and protocols followed, and the experimental design chosen for the statistical analysis of the data.

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Averages should be accompanied by their corresponding Standard Error (SE) values. The discussion shall be

complete and exhaustive, emphasizing the highlights and comparing them to the literature data.

This section should briefly and concisely summarize the most important findings of the research.

Conclusion (optional)

A short conclusion section is useful for a long or complex discussion. It should provide readers with a brief summary of the main achievements from the results of the study. It can also contain final remarks and a brief description of future complementary studies that should be addressed.

Acknowledgments

When considered necessary, the authors may acknowledge the researchers or entities that contributed - conceptually, financially or practically - to the research: specialists, commercial organizations, governmental or private entities, and associations of professionals or technicians.

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