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Fax: 316 5176

E-mail: agrocol_fabog@unal.edu.co

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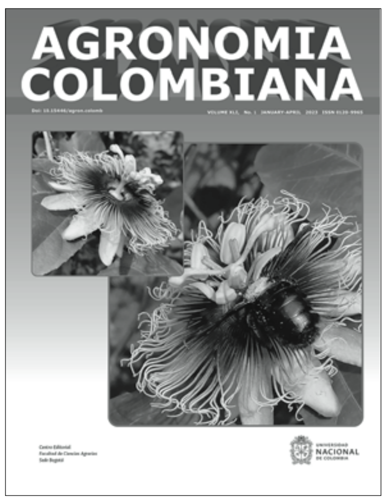

Our cover:

Responses of landraces and commercial cultivars of yellow passion fruit to the prevalence of *Fusarium oxysporum*

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Low water availability has a greater influence on the development of coffee seedlings than an increase in temperature

La baja disponibilidad hídrica tiene mayor influencia sobre el desarrollo de plántulas de café que el aumento de temperatura

Fabiola Rubí León-Rojas[1], Deyna Valderrama-Palacios[1], Ricardo Borjas-Ventura[1*], Leonel Alvarado-Huaman[1], Alberto Julca-Otiniano[1], Lourdes Tapia y Figueroa[1], Viviana Castro-Cepero[1], Sara Malpica Ninahuanca[2], and Alan Cardoza-Sánchez[3]

ABSTRACT

Coffee is an important product in the world, essential for thousands of producing families. However, climate change has generated variations in temperature and precipitation that negatively impact the maturation of crops. To quantify the combined effect of drought stress and elevated temperatures, plants of *Coffea arabica* cv. Ouro Verde IAC H5010-5 were evaluated under the climatic conditions of La Molina, Peru, with four treatments (WT: 100% available water + air temperature (22.7°C); -WT: < 50% available water + air temperature (22.7°C); W+T: 100% available water + elevated air temperature (22.7 + 2.5°C), and –W+T: < 50% available water + elevated air temperature (22.7 + 2.5°C)). In general, morphological indices were decreased by -WT and –W+T ($P≤0.05$). Plants subjected to -W+T, significantly affected quality indices like root:shoot ratio, robustness, and Dickson ($P≤0.05$). The -WT plants had a nitrogen content of 2.46%, the highest nitrogen content compared to other treatments. Isolated and combined stress had negative differential effects on plant development, and water scarcity (as an individual factor) was the repercussion, in most cases, that was more prominent than the effect of high temperature.

Key words: drought, growth, photosynthesis, nitrogen content, *Coffea arabica* L.

RESUMEN

El café es un producto importante en el mundo, esencial para miles de familias productoras. No obstante, el cambio climático ha generado variaciones en los patrones de temperatura y precipitación que impactan negativamente el desarrollo del cultivo. Por lo tanto, con el fin de cuantificar el efecto combinado del estrés por sequía y de altas temperaturas, se evaluaron plantas de *Coffea arabica* cv. Ouro Verde IAC H5010-5, bajo condiciones de La Molina, Perú con cuatro tratamientos (WT: 100% de agua disponible + temperatura ambiental (22.7°C); -WT: < 50% agua disponible + temperatura ambiental (22.7°C); W+T: 100% agua disponible + temperatura ambiental elevada (22.7 + 2.5°C), y –W+T: < 50% agua disponible + temperatura ambiental elevada (22.7 + 2.5°C)). Los resultados mostraron que las variables de crecimiento fueron menores bajo -WT y -W+T ($P≤0.05$). Asimismo, -WT afectó significativamente índices de calidad como relación parte aérea:raíces, robustez y Dickson ($P≤0.05$). Las plantas – WT tuvieron un contenido de nitrógeno superior a otros tratamientos. Tanto el efecto aislado como combinado de ambos estreses tuvo una repercusión negativa sobre el desarrollo de la planta, siendo la deficiencia de agua (como efecto aislado), en la mayoría de los casos, más determinante que el efecto de la alta temperatura.

Palabras clave: sequía, crecimiento, fotosíntesis, contenido de nitrógeno, *Coffea arabica* L.

Introduction

Coffee is one of the most internationally traded food products and the second most consumed beverage in the world behind water. Its contribution to the socioeconomic development of tropical countries is a key factor with about 70% of the world production of coffee beans produced by small farmers (Fridell *et al*., 2008). In Peru, the coffee crop occupies almost 350,000 ha and generates employment for 2 million growers (Junta Nacional del Café, 2020). But one of the most important threats to coffee production is climate change which will increase temperatures by an average of 4.8°C by the year 2100 (DaMatta *et al*., 2018). It is anticipated that rising temperatures will be coupled with variations in precipitation patterns, intensifying

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[1] Departamento de Fitotecnia, Universidad Nacional Agraria La Molina, La Molina, Lima (Perú).
[2] Departamento de Suelos, Universidad Nacional Agraria La Molina, La Molina, Lima (Perú).
[3] Cooperativa Bosques del AltoMayo-COOPBAM, Rioja, San Martín (Perú).
[*] Corresponding author: rborjas@lamolina.edu.pe

drought conditions in many agricultural areas. Also, the occurrence of extreme weather events and fluctuations in the distribution of rainfall in both time and space are contributing factors to alterations in soil moisture levels, leading to an increase in saline soils (Corwin, 2021). According to Corso *et al.* (2020), drought causes a decrease or cessation of cell expansion, leading to a delay in plant growth. In plants, this produces wilting, chlorosis (yellowing of leaves), and changes in the number of leaves and leaf area. Additionally, elevated temperatures may increase the respiratory rate, disrupt the synthesis of chlorophyll, decrease net photosynthesis, and stimulate the synthesis of reactive oxygen species (Sharkey & Schrader, 2006; Wang *et al.*, 2018; Mittler *et al.*, 2022).

Likewise, during previous decades research focused on limiting the detrimental effects of coffee leaf rust, caused by *Hemileia vastatrix* L., a critical pathogen in coffee. For that reason, breeders have produced plants with resistance to this disease (Zambolim, 2016). However, low attendance has been addressed to produce plants with tolerance or resistance to the harsh conditions caused by climate change that may become increasingly problematic in the future. Despite the effects of heat and drought on plants that have been widely studied for important crops (Borjas *et al.*, 2020; Handayani & Watanabe, 2020; Alsamir *et al.*, 2021), information on coffee is still restricted, particularly to new cultivars in Perú, such as Ouro Verde IAC H 5010-5. This cultivar is susceptible to coffee leaf rust but has high vigor, productivity, and organoleptic quality (Fazuoli *et al.*, 2000).

It is important to study the combined effect of stresses to quantify and document the physio-morphological response of the seedlings of coffee cv. Ouro Verde IAC H 5010-5 to low availability of water and elevated temperature, since many scientific reports have proved that the responses of plants to single and combined stress are different. Both elevated temperatures and drought have a negative impact on coffee production. Subsequently, these factors force small farmers to migrate to other suitable areas for the cultivation of this crop (Hussain *et al.*, 2021) and alter the morphology, physiology, biochemistry, and yield of tropical crops (Borjas *et al.*, 2019). The severity of impact of elevated temperature and drought on crops depends on weather conditions, crop management, levels of stress, and genetic material (Ullah *et al.*, 2019; Reshma *et al.*, 2021; Yang *et al.*, 2021). Consequently, it is convenient and necessary to conduct future studies on different cultivars to increase our knowledge of their responses to climate change.

Materials and methods

Plant material used was the coffee cultivar Ouro Verde IAC H 5010-5, obtained from the Peruvian Coffee Gene bank located in San Ramón (province of Chanchamayo, department of Junín). This assessment was carried out at the Universidad Nacional Agraria La Molina (Lima, Peru) (12°04'55" S; 76°56'53" O) from December 2021 to May 2022.

Once harvested, mature fruits were pulped manually to avoid damage to the seeds. After that, fruits were fermented for 24 h to facilitate mucilage removal. The bean parchment was discarded. To stimulate germination, the seeds were soaked in water for 24 h. They were then sowed in seed trays under a controlled environment that maintained the optimal temperature for growth. Once the seedlings reached the appropriate stage after 50 d, they were transferred to 1 L polyethylene bags, 25.4 cm high and 12.7 cm wide. The substrate used was composed of *Sphagnum* peat moss and 15% perlite.

Adequate seedling nutrition was guaranteed by supplying 100 ml of solution prepared by combining 2 ml of micronutrients and 5 ml of macronutrients per liter of "Solución nutritiva hidropónica La Molina®" (Fundación para el Desarrollo Agrario - FDA) nutrient solution. The fertilization started 78 d after sowing and was done once a week for six weeks.

Two levels of water availability and temperature were studied in this work. For this purpose, plants were grown for three months with a water input in weight (g) corresponding to the amount of water present at 0.3 bar in the substrate, representing 100% available water. After that period the plants were subjected to different treatments with weekly evaluations for the following seven weeks. Water availability had two levels: well-watered (100% available water) (W) and drought stress (< 50% available water) (-W), and the temperatures examined were air temperature: 22.7°C (T) and elevated temperature (~2.5°C above air temperature) (+T). The combination of the levels resulted in four treatments with 14 replicates, one plant per experimental unit (Tab. 1).

TABLE 1. Treatments tested in coffee cv. Ouro Verde IACH 5010-5.

Treatments	Description
WT	100% available water + air temperature (22.7°C)
-WT	< 50% available water + air temperature (22.7°C)
W+T	100% available water + elevated air temperature (22.7 + 2.5°C)
-W+T	< 50% available water + elevated air temperature (22.7 + 2.5°C)

To determine the volume of water needed to reach 50% of available water, we followed Wang *et al.* (2017). First, it was necessary to calculate the percentage of available water in weight (%) at 15 and 0.3 bars, representing the two moisture levels that determine the available water, as well as the bulk density of the substrate. With these data, we calculated the approximate dry weight of the substrate in the bags, to which a calculated amount of water was added to maintain a state of water stress below 50% of available water. To determine the reference weight for this stress treatment, the average total weight of the bag with substrate and water was considered under stress conditions. Subsequently, the bags subjected to this treatment were weighed weekly to ensure that they did not exceed the predetermined stress state weight.

To increase the temperature, a 1 m-tall tunnel was designed using PLASTERMIC DH2A (SOTRAFA S.A.), a transparent agricultural plastic film that allows light to enter. The coffee seedlings grew into this tunnel. Furthermore, the temperature into and out of the tunnel was recorded using a datalogger Elitech RC-4HC (Elitech UK Ltd, UK).

Growth

After seven weeks of treatment, the number of leaves was recorded. Height (cm) was measured using tape from the stem base to the apical bud, and for stem diameter (mm) measuring, a vernier caliper was used at the stem base. Root length (cm), and leaf area (cm^2) for each seedling were quantified, employing ImageJ software. Dry biomass was measured. To obtain the dry weight of the shoot and roots, the coffee seedlings were placed in an oven at 70°C for 2 d.

Growth indices

With the information from the evaluation of growth, some indices such as the sturdiness index (SI) (Enquist & Niklas, 2002), Dickson index (DI) (Dickson, 1960), and dry shoot: root dry biomass ratio (Sd/Rd), were calculated.

The following equations were used:

$$SI = \frac{\text{Length}}{\text{diameter of shoot}} \quad (1)$$

$$DI = TDB / [(SI) + (Sd/Rd)] \quad (2)$$

where:
TDB = is total dry biomass (g);
SI = sturdiness index (cm/mm);
Sd = shoot dry biomass;
Rd = root dry biomass (g).

Content of total nitrogen

At the Soil Laboratory "Sven Villagarcía Hermosa" – Universidad Nacional Agraria La Molina, we used the micro Kjeldahl method (Bazan, 1996). The total leaves were collected for this analysis. Foliar material was dried on a stove at 70°C for 24 h and later ground for an easier digestion process. From the processed leaf tissue, 0.1 g per sample was taken that was digested with K_2SO_4, $CuSO_4$, and H_2SO_4. The resulting product was subjected to distillation in the presence of 50% NaOH and collected in 2% H_3BO_3. This distillate was titrated with H_2SO_4 at 0.02 N. This acid expenditure (V) was entered into the equation to obtain the percentage of foliar nitrogen.

$$\%N: \frac{(V \times 0.02 \times 0.014 \times 100)}{0.1} \quad (3)$$

Photosynthesis and water use

The photosynthetic performance of coffee under high temperatures and drought was examined with the CIRAS-3 DC CO_2/H_2O gas analyzer that was used to evaluate the content of CO_2 in the intercellular space of the leaves (Ci, µmol mol^{-1}), net CO_2 assimilation rate (A, µmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s, mmol H_2O m^{-2} s^{-1}), transpiration rate (E, mmol H_2O m^{-2} s^{-1}), vapor pressure deficit (VPD, KPa), and water use efficiency (WUE, mmol CO_2 mol^{-1} H_2O). The measurements were made in mature leaves (from the middle part of the plants) at 71 d (first sampling), 78 d (second sampling), 85 d (third sampling), and 93 d (fourth sampling) after transplanting. All assessments were taken in the morning between 9-11 a.m.

Climate data

Temperature variation was recorded every 15 min throughout the day during the experiment. Air temperature (T) varied from 13.7°C to 38.5°C and had an average of 22.7°C. The treatments with elevated temperature (+T) varied from 14.7°C to 42.3°C with a mean temperature of 25.2°C (Fig. 1).

Statistical analysis

A completely randomized design was used. Every treatment had 14 replicates. ANOVA was applied to the data. A comparison of means was done using the Scott Knot test (95%).

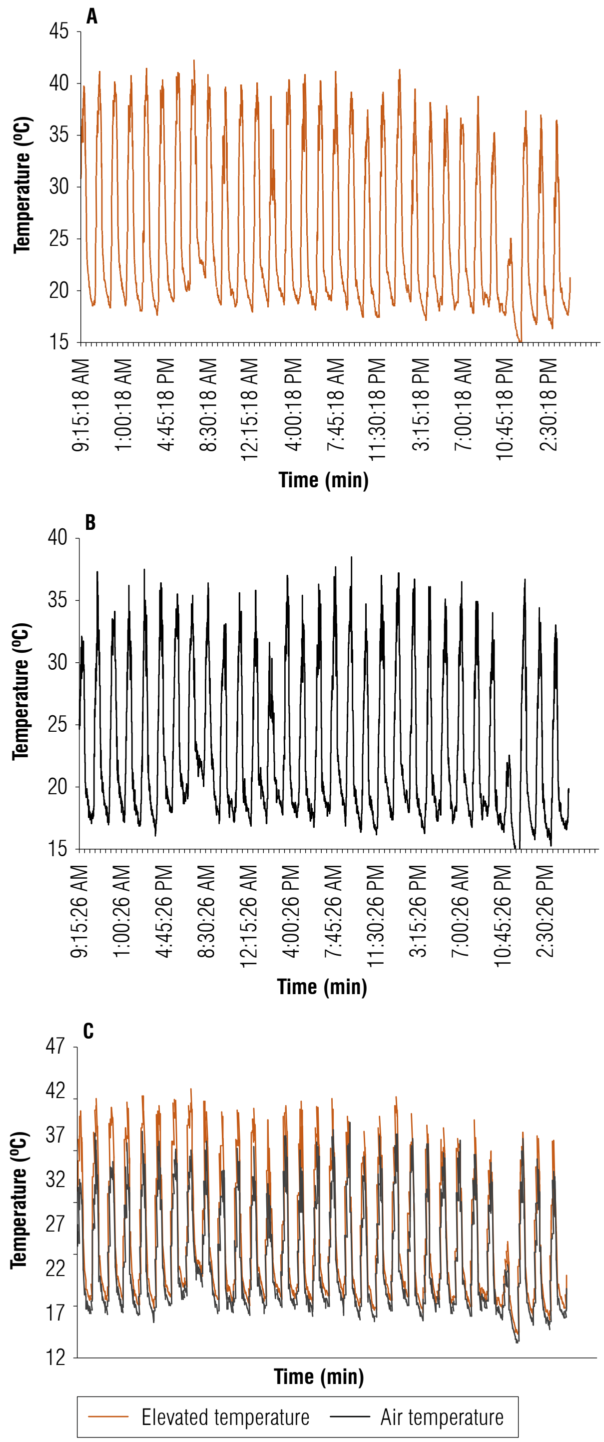

FIGURE 1. Variation of the temperature in treatments studied in the experiment. A) elevated temperature; B) air temperature; C) comparison between air temperature and elevated air temperature.

Results and discussion

To deepen the knowledge of the impact of isolated and combined drought and heat, we studied the effects of two levels of both water (100% available water [W] and < 50% of available water [-W]) and temperature (air temperature [T] and elevated temperature ~2.5°C above air temperature [+T]) on the growth, the content of nitrogen, and photosynthesis of coffee seedlings cv. IAC H 5010-5.

During the experiment growth was disrupted by the [-W T] and [-W +T] treatments, both decreasing the height and diameter of the shoot, root length, and the number of leaves per plant (NL) ($P \leq 0.05$) (Figs. 2-3). Additionally, the effect of water scarcity was more prominent than the increased temperature for plant height, root length, and NL.

Drought is considered the most adverse environmental factor that modifies plant growth (Seleiman *et al.*, 2021) by altering its physiology, biochemistry, and chemical composition and the allocation of photosynthetic products (Wang *et al.*, 2018; Hussain *et al.*, 2019; Borjas *et al.*, 2020; Schönbeck *et al.*, 2021). Growth reduction is the first visual indicator that plants are under drought stress, as informed in previous experiments with corn (Van Nguyen *et al.*, 2022), soybean (Imran *et al.*, 2021), and coffee cvs. IAPAR 11260, IPR 100 and IPR 103 (Carvalho *et al.*, 2017). However, there are few studies on the cultivar IAC H 5010-5.

The plants subjected to the treatments [-W T] and [-W +T] had a lower leaf area and shoot dry biomass than the other ones ($P \leq 0.05$) (Figs. 4-5). While both [W +T] and [-W +T] plants caused a reduction in the root dry weight. For this latter variable, temperature acted individually, suggesting that the effects of high temperature on plant development are water-dependent (Fahad *et al.*, 2017). Indeed, plants well-watered and under high temperatures can allocate more photosynthetic products toward the aerial part than the roots (Shinohara & Leskovar, 2014; Reddy *et al.*, 2017) as observed in this study, where plants under [W +T] showed high shoot dry weight but low shoot dry biomass ($P \leq 0.05$). This result can be related to the fact that the same treatment increased the shoot dry: root dry biomass ratio ($P \leq 0.05$) (Fig. 6).

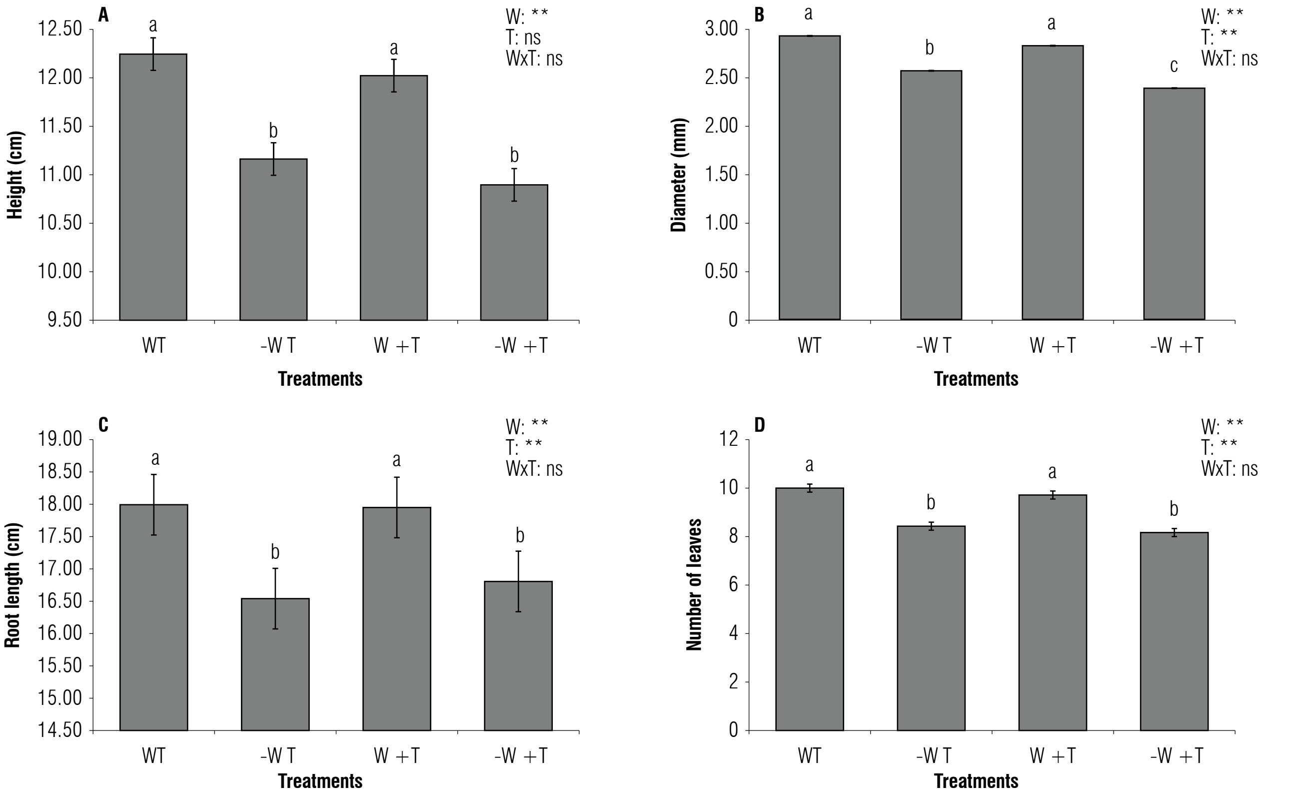

FIGURE 2. Growth of coffee seedlings cv. IAC H 5010-5 subjected to levels of water and temperature. A) Plant height; B) stem diameter; C) root length; D) number of leaves. W: 100% available water; –W: < 50% of available water; T: air temperature (22.7°C); +T: elevated air temperature (22.7 + 2.5°C). Different letters indicate statistical differences, and error bars represent the standard error values (Scott & Knott test at 95%).

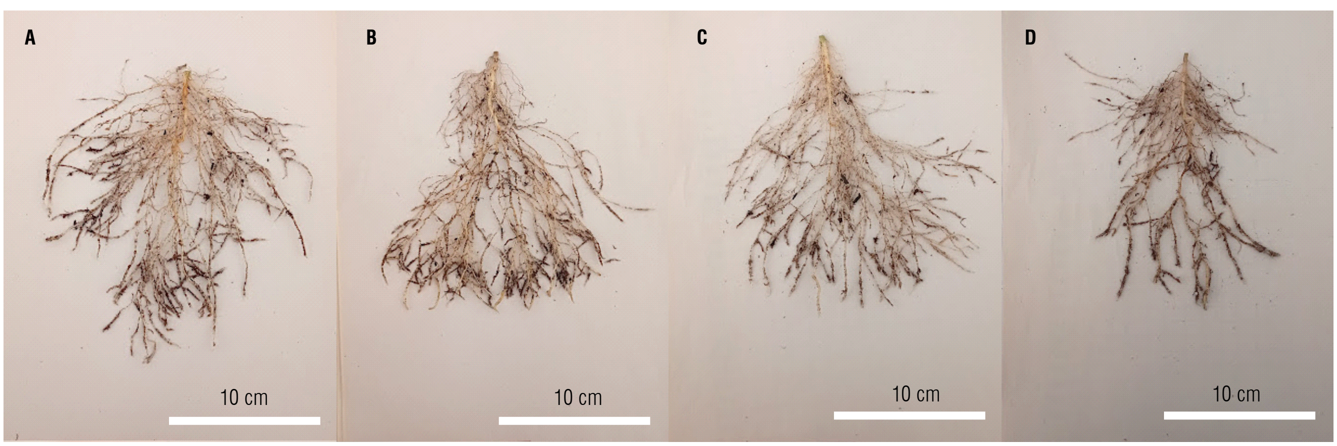

FIGURE 3. Coffee cv. Ouro Verde IAC H 5010-5 roots subjected to treatments: A) WT; B) -WT; C) W+T; D) -W+T, where W: 100% available water; –W: < 50% of available water; T: air temperature (22.7°C); +T: elevated air temperature (22.7 + 2.5°C).

Conversely, when the high temperature is accompanied by water scarcity, both can be extremely harmful since they can disrupt the adequate physiology of plants leading them to decrease biomass production as recorded in this assessment where shoot and root dry biomass reduced in plants subjected to [-W +T] ($P \leq 0.05$) (Fig. 5) (Raja *et al.*, 2020). Our results agree with Shinohara and Leskovar (2014), and Zhou *et al.* (2017) for *Cynara cardunculus* L. var.

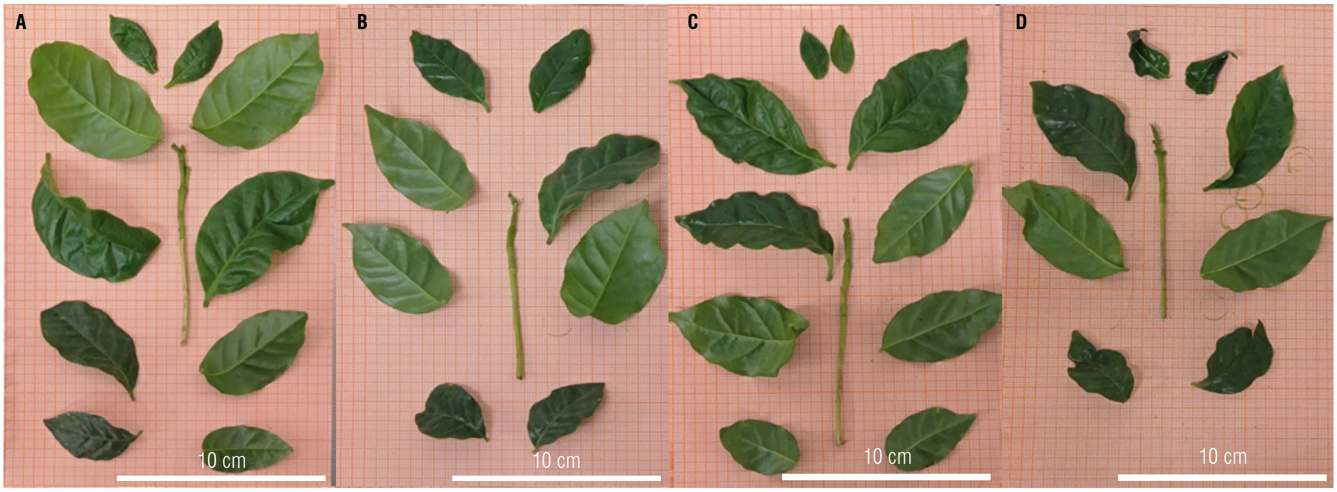

FIGURE 4. Coffee cv. Ouro Verde IAC H 5010-5 shoot subjected to treatments: A) WT; B) -WT; C) W+T; D) -W+T, where W: 100% available water; –W: < 50% of available water; T: air temperature (22.7°C); +T: elevated air temperature (22.7 + 2.5°C).

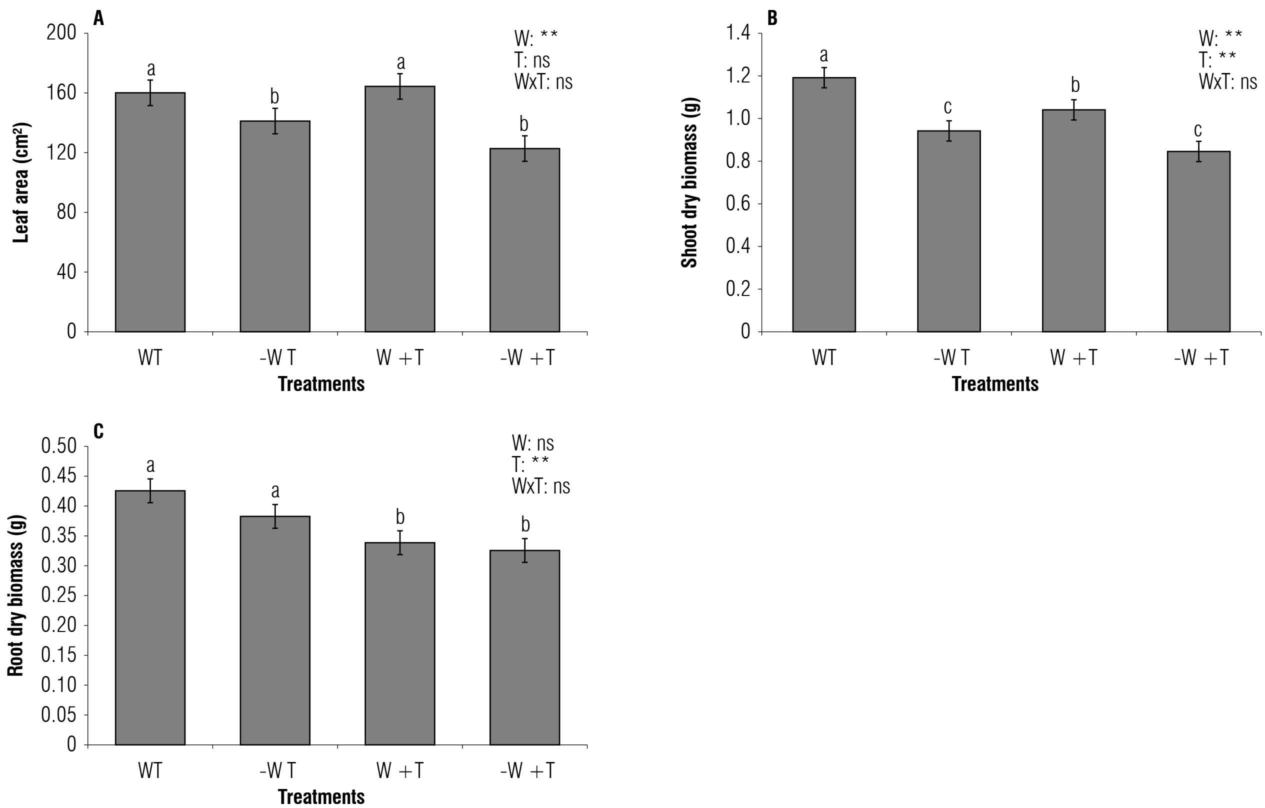

FIGURE 5. Shoot and root growth parameters of coffee seedlings cv. IAC H 5010-5. A) Leaf area; B) dry biomass of the shoot; C) dry biomass of the roots. W: 100% available water; –W: < 50% of available water; T: air temperature (22.7°C); +T: elevated air temperature (22.7 + 2.5°C). Different letters indicate statistical differences, and error bars represent the standard error values according to the Scott & Knott test (95%).

scolymus (L.) Fiori and *Solanum lycopersicum*. The changes in morphological traits caused by [-W +T] increased the sturdiness index (SI). High values of SI suggest that coffee seedlings are less able to resist abiotic stress conditions. Likewise, [-W +T] decreased the Dickson quality index that confirmed those plants under both stresses have not adapted to tolerate harsh conditions in the field (Lin *et al*., 2019) (P≤0.05) (Fig. 6).

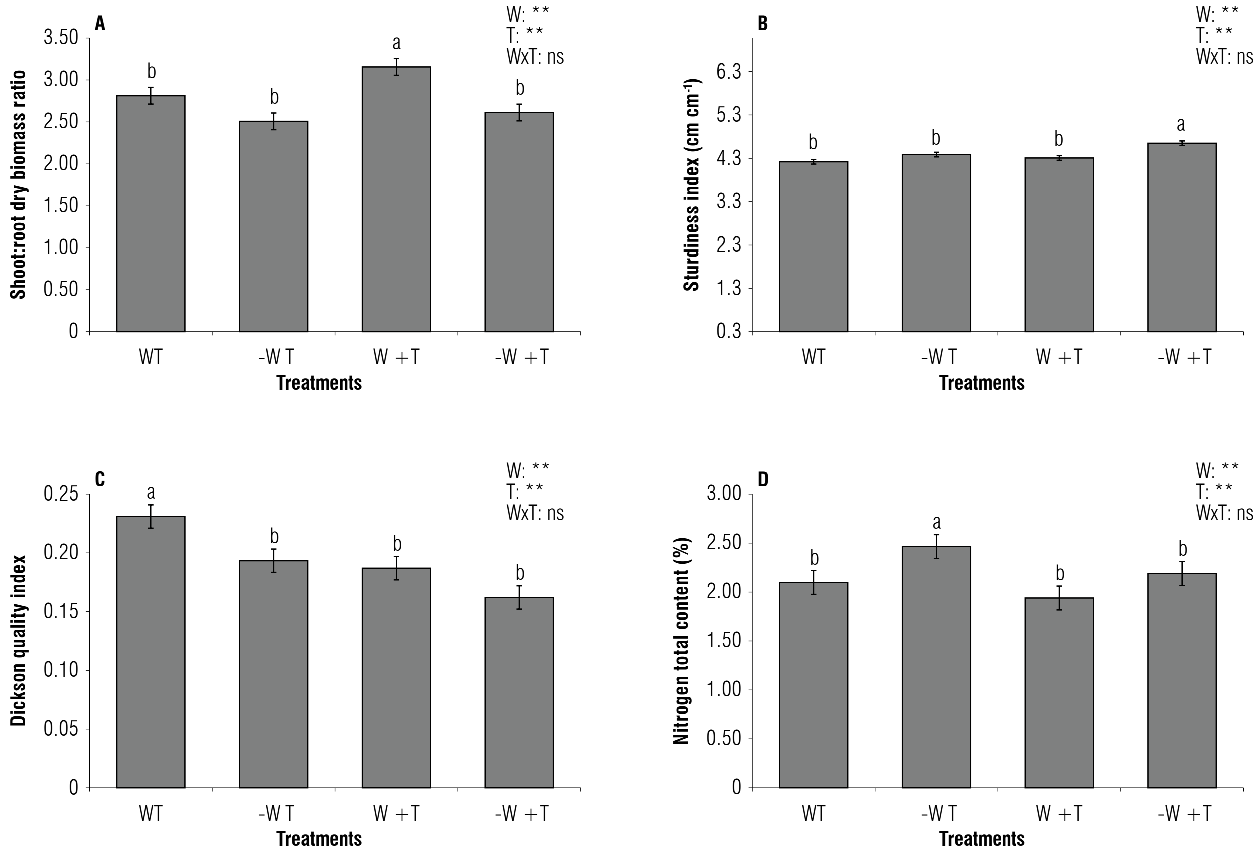

FIGURE 6. Variables measured in coffee seedlings cv IAC H 5010-5. A) Shoot dry: root dry biomass ratio; B) sturdiness index; C) Dickson quality index; D) total nitrogen content. W: 100% available water; –W: < 50% of available water; T: air temperature (22.7°C); +T: elevated air temperature (22.7 + 2.5°C). Different letters indicate statistical differences and error bars represent the standard error values according to the Scott & Knott test (95%).

On the other hand, nitrogen (N) is an essential nutrient related to plant development and productivity (Hessini *et al*., 2019; Tadesse *et al*., 2019; Xiong *et al*., 2021). The availability of N in the soil is subject to climatic conditions, particularly, the levels of water in the soil. This means that low levels of water in the soil can limit N uptake by plants (Liu *et al*., 2013; He & Dijkstra, 2014; Borjas *et al*., 2019). Nevertheless, an unexpected result was noticed since the highest content of N (%) was recorded in the plants exposed to [-W T] with 2.46 compared to the 1.9 for [W +T] treatment (Fig. 6).

The quantity of N in plants under the drought treatments can be explained by the activation of the genes responsible for the uptake and assimilation of nitrogen (*AMT1;3, AMT1;1b, NRT1;2* and *NRT2;5, NR, GS2*, and *GSI;2*), that enhance the absorption of N as reported in maize where drought stress increased the level of N in mature leaf and roots (Wang *et al*., 2017). Also, a higher percentage of total nitrogen is most likely a result of the accumulation of nitrate in the leaves that is brought about by the impact of stress that had a greater effect on assimilation as opposed to the transport and/or absorption of nitrogen (Martínez *et al*., 2020).

Photosynthesis is defined as the use of sunlight to synthesize carbohydrates to meet the needs of plants. The evaluation of photosynthesis in this research included the quantification of net CO_2 assimilation rate (*A*), the CO_2 content in the intercellular space of the leaves (Ci), stomatal conductance (g_s), transpiration rate (*E*), vapor pressure deficit (VPD), and the use of water use efficiency (WUE) (Fig. 7).

Drought can have a detrimental effect on cell turgidity, decreasing the photosynthetic area, while stomatal closure leads to decreasing gas exchange limiting the functioning of the photosynthetic apparatus (Semedo *et al*., 2018; Helm *et al*., 2020). Similar effects were reported by Rodrigues *et al*. (2018) in coffee subjected to high temperatures.

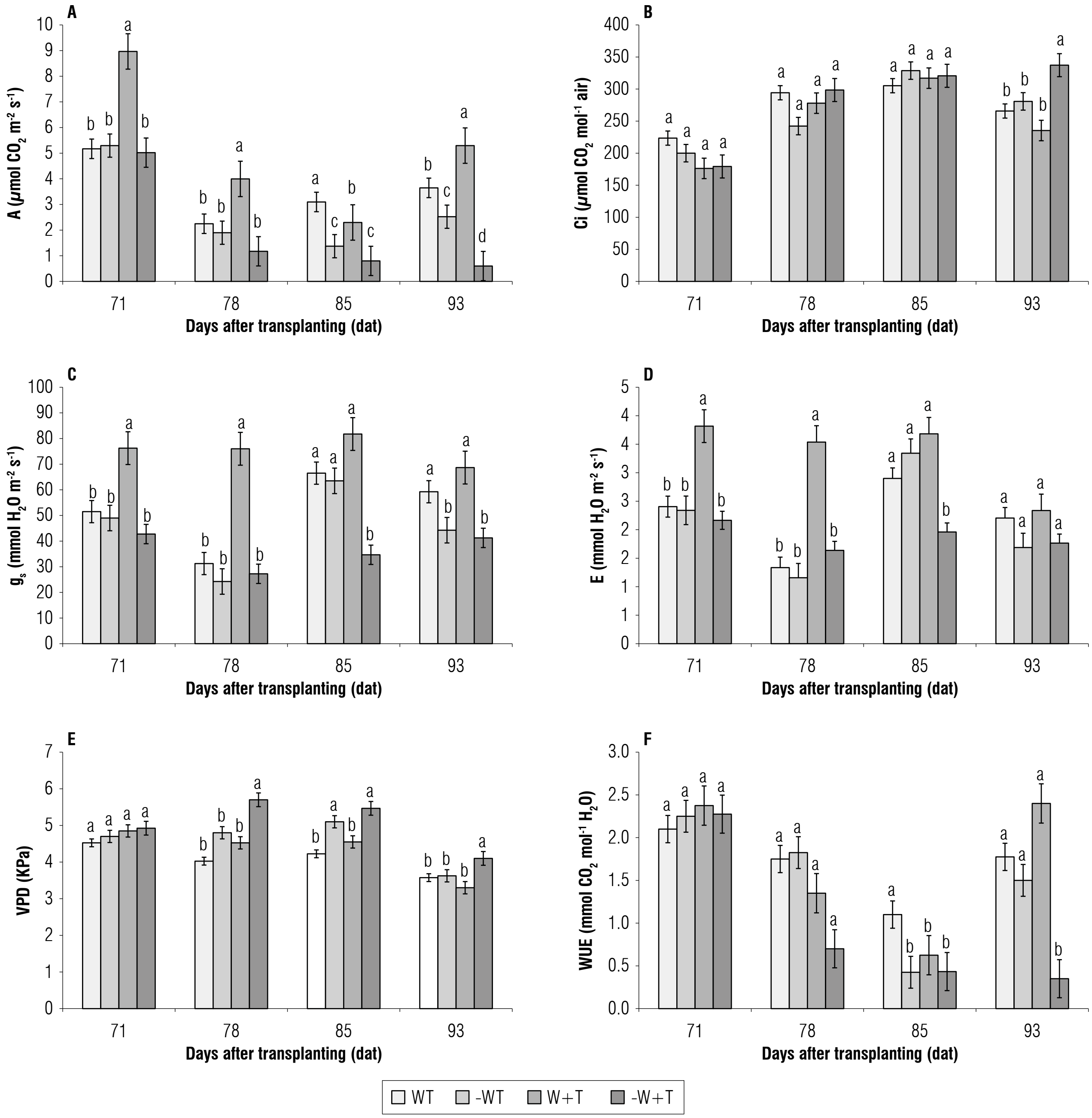

FIGURE 7. The variable measured in coffee seedlings cv. IAC H 5010-5. A) *A*: net CO_2 assimilation rate; B) Ci: content of CO_2 in the intercellular space of the leaves; C) g_s: stomatal conductance; D) *E*: transpiration rate; E) VPD: vapor pressure deficit; F) WUE: water use efficiency. W: 100% available water; –W: < 50% of available water; T: air temperature (22.7°C); +T: elevated air temperature (22.7 + 2.5°C). Different letters indicate statistical differences and error bars represent the standard error values according to Scott & Knott test (95%).

Our research demonstrated the modulator effect of the water levels in soil on plant responses to high temperatures (Ayub *et al*., 2021). In fact, [W +T] increased *A* and g_s suggesting that with high water availability C3 plants, particularly coffee, can have their growth enhanced. In contrast, the combination of high temperatures and water scarcity [-W +T] decreased *A*, g_s, *E*, and WUE ($P \leq 0.05$) at 85 and 93 d after transplanting (third and fourth sampling dates). This response was also reported by Hussain *et al*. (2019) and Raja *et al*. (2020) in maize (C4) and tomato (C3). The fall of carbon net assimilation, caused by the reduction in the carboxylation activity of RuBisCo (Souza *et al*., 2020; Almeida *et al*., 2021; León *et al*., 2022), increased the quantity of carbon intracellular and the VDP ($P \leq 0.05$) (Fig. 7).

Conclusion

Assessing the morphology, nitrogen content, and physiology of coffee seedlings cv. IAC H 5010-5 under increased temperature and water scarcity demonstrated that the isolated and combined occurrence of these stresses produced differentiated responses that were more prominent than the effects of combined stresses. In general, the impact of the less than 50% available water was more predominant than increased temperatures in reducing plant growth and physiology. Likewise, the effect of temperature was water-dependent. Furthermore, coffee seedlings under drought showed high nitrogen content suggesting that this cultivar possesses physiological strategies to face the low availability of water in the soil.

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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

FLR, SMN, and RBV designed the experiments, FLR carried out the field and laboratory experiments, FLR and DVP contributed to the data analysis, FLR and RBV wrote the manuscript, LAH, AJO, VCC, and LTF supervised and validated the data, and ACS acquired funding for the research. All authors reviewed the final version of the manuscript.

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Responses of landraces and commercial cultivars of yellow passion fruit to the prevalence of *Fusarium oxysporum*

Respuestas de maracuyá nativo y sus cultivares comerciales frente a la incidencia de *Fusarium oxysporum*

Juan Pablo Bernal Moreno[1]* and Nohra Rodríguez[2]

ABSTRACT

Yellow passion fruit (*Passiflora edulis* f. *flavicarpa*) is a fruit of high economic potential in Colombia, but the prevalence of some diseases often makes growers stop cultivating it. Also, varieties tolerance against some pathogens that have high prevalence in Colombia have not been released. The objective of this study was to contribute to the characterization of 63 populations of yellow passion fruit, including 46 cultivars and 17 landraces. The plants were evaluated using morphoagronomic descriptors (leaves, flowers, and fruits) as well as ecophysiological parameters (stomatal conductance, chlorophyll content, and degree of chlorosis) evaluated against the 21-02129 strain of *Fusarium oxysporum* isolated from purple passion fruit (*gulupa*, in Spanish). Results showed low levels of germination (55% landraces and 50% cultivars). The landrace populations showed greater morphological variability and greater tolerance to the pathogen expressed by the chlorophyll concentration from intact leaf samples on 28 d post inoculation (dpi) (landraces: 458±108 μmol m^{-2} and cultivars: 411±125 μmol m^{-2}) and stomatal conductance (landraces: 90.8±14.9 mmol m^{-2} s^{-1} and cultivars: 87.1±34.1 mmol $m^{-2}s^{-1}$). The study revealed a good potential for tolerance to this pathogen in landraces, so it is necessary to carry out research aimed at preserving this diversity *in situ* and *ex situ* as well as a continuous analysis of these populations.

Key words: stomatal conductance, chlorophyll content, chlorosis degree, Fusarium wilt, *Passiflora edulis* f. *flavicarpa*.

RESUMEN

El maracuyá (*Passiflora edulis* f. *flavicarpa)* es una fruta de alto potencial económico en Colombia, pero la prevalencia de algunas enfermedades hace que muchas veces los agricultores desistan de cultivarla. Asimismo, no se han liberado variedades tolerantes a algunos patógenos que tienen alta prevalencia en Colombia. El objetivo de este estudio fue contribuir a la caracterización de 63 poblaciones de maracuyá, incluyendo 46 cultivares y 17 nativas. Las plantas se evaluaron utilizando descriptores morfoagronómicos (hojas, flores y frutos) al igual que parámetros ecofisiológicos (conductancia estomática, contenido de clorofila y grado de clorosis) evaluados frente a la cepa 21-02129 de *Fusarium oxysporum* aislada de maracuyá morado (gulupa). Los resultados mostraron bajos niveles de germinación (55% nativas y 50% cultivares). Las poblaciones nativas presentaron mayor variabilidad morfológica y mayor tolerancia al patógeno expresada por la concentración de clorofila de muestras de hojas intactas a los 28 d post inoculación (nativas 458±108 μmol m^{-2} y cultivares 411±125 μmol m^{-2}) y la conductancia estomática (nativas: 90.8±14.9 mmol m^{-2} s^{-1} y cultivares: 87.1±34.1 mmol m^{-2} s^{-1}). El estudio reveló un buen potencial de tolerancia a este patógeno en poblaciones nativas, por lo que es necesario realizar estudios encaminados a preservar esta diversidad *in situ* y *ex situ*, así como un análisis continuo de estas poblaciones.

Palabras clave: conductancia estomática, contenido de clorofila, grado de clorosis, fusariosis, *Passiflora edulis* f. *flavicarpa*.

Introduction

Passiflora edulis f. *flavicarpa* Deneger is commonly known as yellow passion fruit (López *et al*., 2006) in reference to the passion of Christ. The fruit has its origins in an ancestral form known in Colombia as *gulupa* (*P. edulis* f. *edulis* Sims), commonly known in English as round passion fruit or purple passion fruit and distributed in the Brazilian Amazon region (Lima & Da Cuhna, 2004). The center of passion fruit diversity is the Andean region (Ocampo *et al.* 2013). The fruit's aroma, acidity, and content of passiflorine have made it one of the most promising fruit crops in Colombia (MADR, 2006; MinTIC, 2019; Procolombia, 2021). Among all national passionflower production, passion fruit cultivation corresponds to 35% of the most cultivated passionflower plants in Colombia (MADR, 2021).

In Colombia in 2019 the passion fruit crop was mainly cultivated in the departments of Meta (35,000 t), Antioquia (32,000 t), and Huila (17,000 t) with a total annual

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[1] GEBIMOL-Grupo de Investigación en Genética y Biología Molecular, Universidad Pedagógica y Tecnológica de Colombia, Tunja, Boyacá (Colombia).
[2] BPM-Grupo de Investigación en Biología de Plantas y Microorganismos, Universidad del Valle, Cali (Colombia).
* Corresponding author: juan.bernal04@uptc.edu.co

production of 137,000 t (Treid, 2022). In Boyacá, the municipalities of Miraflores, Tenza and Covarachía were also engaged in production of this fruit that in 2021 reached 1,345 ha, with a production of 9,439 t and a yield of 14 t ha^{-1} for various passion fruit crops (MinTIC, 2020; MADR, 2021). During 2019, only 252 t of the fruits were exported from the 137,000 t produced nationally (MADR, 2020). Exports were mainly destined for Spain, France, and the Netherlands (MADR, 2020). Although production is high, exports are not very significant due to different adverse impacts observed on the crops, including *Fusarium* sp. (MADR, 2021).

Fusarium is a saprophytic filamentous phytopathogenic fungus that produces toxins such as fumonisins, trichothecenes, and zearalenone (Agrios, 1988). Species like *F. oxysporum* have been used to control illicit crops. That is why their dispersion is expanding throughout many parts of the world and particularly in Colombia (Jelsma, 2000; Garcés *et al*., 2001; Orozco & Garcés, 2007). The presence of this fungus in soils affects crops and causes a disease called Fusarium wilt (Ortiz & Hoyos, 2012). This results in losses that reach up to 100% of passionflower crops in some municipalities of the departments Cundinamarca and Antioquia (Torres *et al*., 1999). Worldwide losses from this pathogen in other crops, such as beans (*Phaseolus vulgaris*) and bananas (*Musa acuminata*) are seen in Indonesia (121 million USD), Taiwan (253 million USD), and Malaysia (14 million USD) (Fontagro, 2020).

So far, an evaluation of pathogen resistance has only been reported for *Passiflora maliformis;* and, therefore, it has been used as rootstock for passion fruit and *gulupa* crops (Forero *et al*., 2016); but it is important to search for materials resistant to *F. oxysporum* within the intra-specific variety of passion fruit for genetic improvements (Silva *et al*., 2013).

The presence of *F. oxysporum* in passion fruit crops not only reduces the quality of the fruits but can also lead to the total loss of the crops (Torres *et al*., 1999). However, some landraces may show a tolerant response to the pathogen compared to cultivars that are genetically homogeneous. For this reason, the current study aims to contribute to the characterization of passion fruit populations by evaluating the responses of some landraces and cultivated varieties against this pathogen as well as its relationship to morphological and genetic diversity.

Materials and methods

Collection of *Passiflora edulis* f. *flavicarpa* and its morphological evaluation

We collected 63 populations of *P. edulis* f. *flavicarpa* in five departments of Colombia, and we classified them as cultivars or landraces (Tab. S1). The sampling of the cultivars consisted of collecting one to five individuals per site that were registered as a single accession. The landraces were every plant that was found on the roadsides and in reserve areas and orchards and that were not part of crops. For landraces and cultivars, mature fruits were evaluated for volume (cm^3), fresh weight (g), fruit shape, pulp, and seed color and by degrees Brix (°), using a 0-90% Brix Atc (± 0.2%) refractometer (Biomed Instruments). For the seeds, the following characters were evaluated: percentage germination (number of germinated seeds / number of seeds planted) * 100% (ISTA, 1976) at room temperature and germination rate at 3 months, number of seeds per fruit, and number of seed pits by cm^2 (Rodríguez Melgarejo *et al*., 2020). For leaves, the following characteristics were determined: leaf area (leaa), leaf invagination angle (leaang), leaf petiole length (pelon). For flowers, the following characteristics were determined: sepal area (separ), floral peduncle diameter (pedd), sepal length (proleng), petal area (petar), operculum diameter (opd), androgynophore length (andleng), filament length (filleng), anther length (antleng), style length (styleng), ovary longitudinal diameter (ovalond), ovary transverse diameter (ovatrad), transverse stigma length (trastileng), and longitudinal stigma length (lonstileng) (Crochemore *et al*., 2003; Ángel-Coca *et al*., 2011; Castro *et al*., 2012). Based on the diversity found, 18 accessions were selected for evaluation against *F. oxysporum*.

Activation of *F. oxysporum* to produce the inoculum

The *F. oxysporum* was isolated from *P. edulis* plants and confirmed by the OCATI plant pathologist (Sr. Ricardo Mora, pers. comm.). The *F. oxysporum* was cultivated in a potato dextrose agar (PDA) culture medium at 25°C for one week. For strain activation, a sample of the material was cultured on Clavel agar (CLA) to facilitate the observation of macroconidia at a temperature of 28°C for 48 h.

Evaluation of the response of *Passiflora edulis* f. *flavicarpa* to *F. oxysporum*

Seeds of the selected accessions of three replicates were germinated according to the protocol proposed by Cardona *et al.* (2005). The seeds were first disinfected. Sterile organically fertilized soil in black bags of 1 kg was used for adequate development of the plants. The emerging seedlings were kept under greenhouse conditions with temperature between 20±1°C (measured with a thermo-hygrometer Traceable Brand, Fisher Scientific, Pittsburgh, PA, USA). Relative air humidity 77.5±2% and luminosity between 650 µmol m^2 s^{-1} were measured with a luxmeter Apogee MQ-100 (Apogee Instrument, Logan, UT, USA); these were favorable conditions for the establishment of the *P. edulis* and the pathogen (Ramos *et al.*, 2017).

Inoculations were made on 5-month-old plants with stem diameters of 10-12 mm using the average of 10 leaves. Tests were carried out by performing a stem incision on completely healthy plants. A deep cut was made at the base of the stem with a sterile blade and the inoculum was added per 5 ml per plant with a concentration of $3x10^6$ macroconidia/ml (Ángel-García *et al.,* 2018).

Mature leaves of the middle third stratum were evaluated for stomatal conductance measured by porometer (Decagon Devices, Pullman, WA, USA) (±10%), and chlorophyll content was measured with the Apogee chlorophyllometer MQ-100 (Apogee Instrument, Logan, UT, USA) (± 1%). The pre-inoculation symptoms at 4, 12, 20, and 28 d after inoculation (dpi) were monitored. Physiological values were taken at predawn (04:00 am) and at midday (12:00 pm) (Pérez & Melgarejo, 2014). Three plants for each accession were used as a negative control and were inoculated similarly with distilled water. The response was evaluated morphologically by identifying the degree of chlorosis in the leaves and by measuring the area with the ImageJ as follows: 0% without symptoms; 10% to 40% of the leaf area with yellowing; 60% to 80% of the leaf area with mild leaf and stem with necrosis; and 100% necrosis.

To confirm the presence of the pathogen and its association with the symptoms, a re-isolation was performed from the leaves and roots with a destructive method in PDA culture medium (Hernández *et al.,* 2019) followed by a subsequent evaluation of color and shape of the colony and conidia to comply with Koch's postulates (Fig. S1).

Statistical analysis

Inoculations were carried out under a completely randomized design using three seedlings that were 5 months old for each accession as well as the control group. Phenotypic variables were evaluated using descriptive statistics, normality tests (with logarithmic transformation for variables without normal distribution), and analysis of variance to determine variations between the landraces and cultivars. To determine variables that explained the diversity among the varieties to a greater extent, the variance inflation factor (VIF) was determined; and a principal component analysis was performed using the RWizard package (Guisande *et al*., 2014). The results of the qualitative and quantitative descriptors were analyzed simultaneously using the Ward-MLM method to group the landraces and similar cultivars (Franco *et al*., 1998; Paiva *et al*., 2014). This procedure was carried out in the SAS v. 9.4 program (SAS Institute Inc, Cary, NC).

Results

Morphological and physiological descriptors of landraces and cultivars

A total of 46 cultivars and 17 local varieties were collected, and each was labeled as an accession. The samples were collected in the departments of Boyacá, Casanare, Cundinamarca, Meta, and Quindío at altitudes between 400 and 1,900 m a.s.l. Averages of the morphophysiological evaluations are presented in Table 1, indicating the mean, standard deviation, normality, and the variance inflation factors (VIF). The representative values are depicted in red. Considering the VIF, only 15% of the variables showed variability between cultivars and landraces (Tab. 2). Among the morphological descriptors, the following stand out for the cultivars (first value shown) and landraces (second value): °Brix (13.06±3.6 and 11.85±3.1), the percentage of germination (49.85±25.6 and 55±24.3%), the number of seeds per fruit (168.19±8.4 and 150.85±4.3), the leaf area (142.23±9.6 and 84.08±6.5 cm^2), the length of the flower filament (1.0±0.1 and 0.88±0.1 cm) and of the anther (1.30±0.2 and 1.28±0.1 cm), and the length of the style (1.42±0.2 and 1.34±0.3 cm). Similarly, the physiological parameters that show variation between these groups (cultivars and landraces) are the evaluations carried out at midday for stomatal conductance (4.74±7.9 and 69.09±4.8 mmol m^{-2} s^{-1}) and chlorophyll content (481.07±30.4 and 497.31±25.8 µmol m^{-2}).

According to the normality test (Shapiro test with a significance level of 95%), the descriptors of leaf area, anther length, and transverse stigma length did not have a normal distribution, according to the *P*-value of 0.05. For this reason, these variables were transformed for subsequent analyses.

TABLE 1. Quantitative descriptors evaluated in landraces and cultivars of *P. edulis* f. *flavicarpa*.

Descriptor	Abbreviation	Average±σ		Shapiro-Wilk (P-value)	Variance inflation factor (VIF)
		Commercial cultivar	Landrace		
° Brix	Brix	13.06±3.6	11.85±3.1	0.38	4.28
Germination (%)	ger	49.85±25.6	55±24.3	0.09	4.96
Number of seeds per fruit	seefru	168.19±8.4	150.85±4.3	0.94	4.22
Number of seed pits /cm^2	fov	335.36±3.5	348.16±1.5	0.08	1.29
Fruit weight (g)	fruwei	143.14±7.6	141.29±5.4	0.08	1.82
Leaf angle (°)	leaang	99.53±14.5	62.32±43.7	0.10	2.31
Leaf area (cm^2)	leaa	142.23±9.6	84.08±6.5	$2.03x10^{-4}$***	4.12
Leaf petiole length (cm)	lengpe	4.10±1.2	2.46±1.9	0.22	1.79
Fruit diameter (cm)	fd	8.84±1	9.04±1.3	0.12	1.21
Sepal area (cm^2)	separ	4.37±1.1	3.79±0.4	0.42	1.56
Peduncle diameter (cm)	pedd	0.32±0.04	0.33±0.06	0.32	2.80
Sepal length (cm)	proleng	0.49±0.1	0.75±0.1	0.09	3.96
Petal area (cm^2)	petar	3.03±0.9	2.46±0.9	0.85	3.45
Operculum diameter (cm)	opd	1.01±0.07	1.00±0.2	0.15	3.95
Androgynophore length (cm)	andleng	0.94±0.1	1.00±0.2	0.44	2.96
Filament length (cm)	filleng	1.0±0.1	0.88±0.1	0.65	5.07
Anther length (cm)	antleng	1.30±0.2	1.28±0.1	0.03*	4.10
Style length (cm)	lengsty	1.42±0.2	1.34±0.3	0.21	5.54
Longitudinal diameter of the ovary (cm)	ovalond	0.94±0.2	0.90±0.2	0.28	2.47
Ovary transverse diameter (cm)	ovatrad	0.64±0.1	0.60±0.1	0.39	2.74
Transverse stigma length (cm)	trastileng	0.28±0.1	0.34±0.2	$2.04x10^{-4}$***	3.21
Longitudinal stigma length (cm)	lonstileng	0.58±0.2	0.56±0.2	0.13	3.96
Stomatal conductance (predawn) (mmol m^{-2} s^{-1})	scp	20.90±6.4	24.31±8.4	0.22	1.25
Stomatal conductance (midday) (mmol m^{-2} s^{-1})	scm	64.74±7.9	69.09±4.8	0.06	8.29
Chlorophyll (predawn) (μmol m^{-2})	Chlp	459.25±22.6	480.9±24.6	0.05	2.54
Chlorophyll (midday) (μmol m^{-2})	Chlm	481.07±30.4	497.31±25.8	0.20	4.28

The values correspond to the average n=3. σ – standard deviation. Values in red indicate significant differences according to the VIF test.

Some qualitative descriptors also showed variations between the landraces and cultivars; thus, the landraces produced seeds that were rounder than they were oval (Fig. 1A). The proportion of these was similar between the cultivars as well the color of the mature fruits (Fig. 1B). The most frequent color of the landraces was a yellowish-orange with a percentage greater than 40%, and none displayed an orange color. Conversely, the cultivars only had 10% fruits showing a yellowish orange color and there were no fruits with a red-orange color.

The color “orange” for the pulp of the landraces was dominant compared to the other colors (Fig. 1C). Orange-yellow was not represented for this group. In sharp contrast, the cultivars had the highest prevalence of orange-yellow color, while red-orange was not found. The color of the seeds (Fig. 1D) was very similar for cultivars and landraces: They exhibited light-brown and brown-black seeds.

In the analysis of principal components performed for the flower descriptors (Fig. 2), the principal component CP1 explains 42.12% of the variation in the data where the landraces (UPTC 1, 7, 19 and 20) are located with differential values for two of the cultivars (UPTC 3 and 10). Similarly, the vectors of style length (styleng), stigma length (lonstileng), and operculum diameter (opd) were more relevant for CP2, explaining 14.58% of the variation in the data. Here, the vectors of the variables regarding the

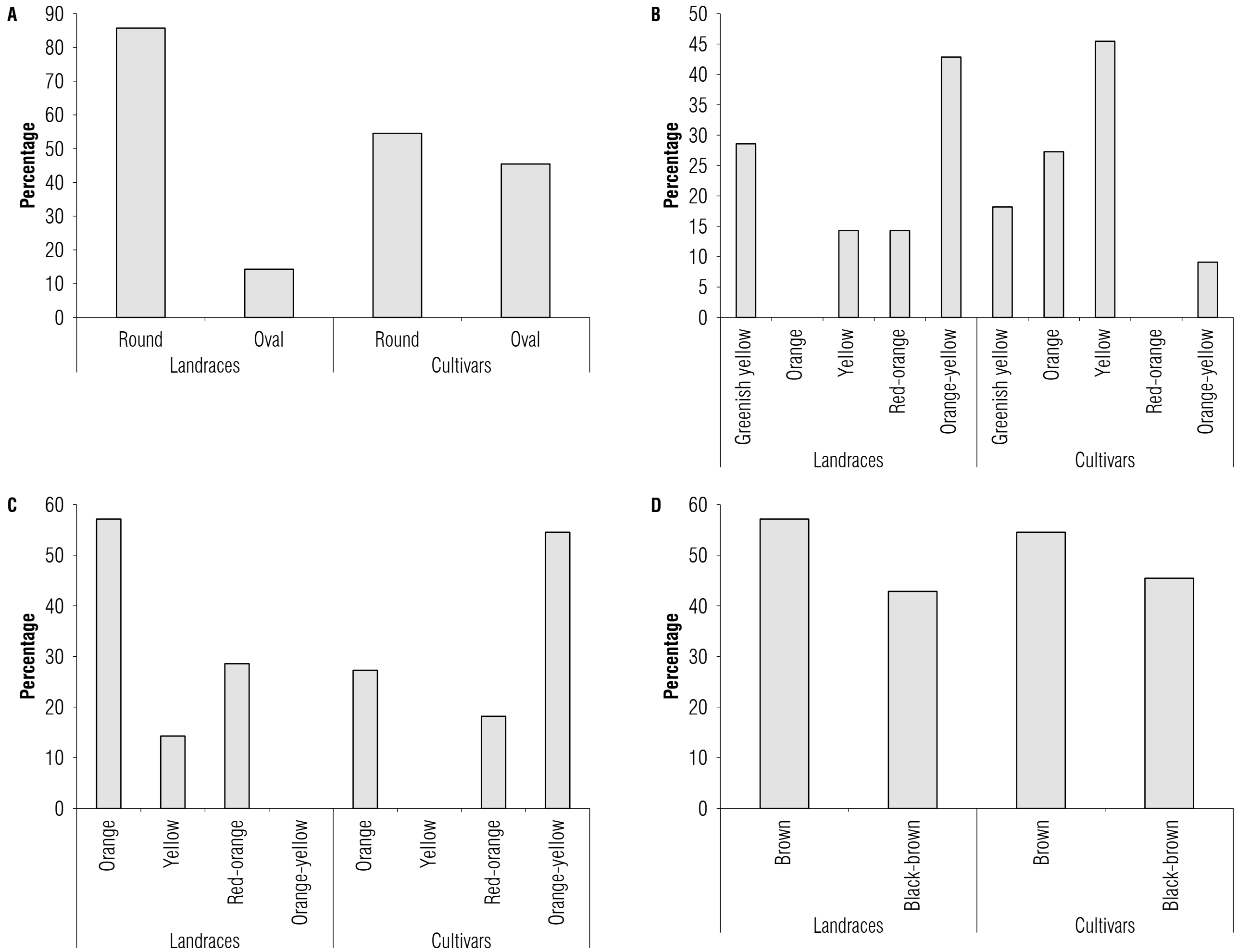

FIGURE 1. Qualitative fruit descriptors (%)for landraces and cultivars of *Passiflora edulis* f. *flavicarpa*. A) fruit shape, B) ripe fruit color, C) pulp color, and D) seed color. An average of 46 cultivars and 17 landraces are presented. n=3.

length of the sepal length (proleng) and filament length (filleng) were more relevant.

Response of landraces and commercial cultivars to *Fusarium oxysporum*

According to the results of morphological variation, 18 accessions including 12 cultivars and 6 landraces were selected (landraces: UPTC 1, 7, 8, 9, 9, 19 and cultivars: UPTC 2, 3, 4, 5, 6, 10, 11, 12, 14, 15, 16, 20) and were inoculated with *F. oxysporum*. Prior to inoculation with *F. oxysporum* the plants had the chlorophyll content with average values of 574±130 µmol m^{-2} for the landraces and 596±156 µmol m^{-2} for the cultivars. Evaluations were carried out every 8 d for one month, and they indicated that the concentration of chlorophyll was reduced for 28 dpi to 481±125 µmol m^{-2} in the landraces and 458±108 µmol m^{-2} in the cultivars. Similar values for the landraces were seen in the control group, where the chlorophyll concentration fluctuated between 478.5±51.9 and 570.1±128.1 µmol m^{-2} (Fig. 3).

The plant water status associated with the potential photosynthetic rate evaluated through stomatal conductance showed that the average values were higher in the landraces on each of the sampling days compared to the cultivars (Fig. 4). These values indicated that the midday stomatal conductance before inoculation totaled an average of 37.2 mmol $m^{-2} s^{-1}$ in the landraces, with a daily range that fluctuated between 15 and 180 mmol $m^{-2} s^{-1}$, and an average of 100.4±28 mmol $m^{-2} s^{-1}$ in the cultivars, with a daily range that fluctuated between 25 and 230 mmol $m^{-2} s^{-1}$. These averages held even without many variations during the 20 dpi, while on day 28 dpi, the average conductance in the landraces was reduced to 98.1±35 mmol $m^{-2} s^{-1}$, while in the cultivars the average increased to 126±29 mmol $m^{-2} s^{-1}$. The cultivar UPTC 14 was the one that had an atypical value of 251±29 mmol $m^{-2} s^{-1}$. Comparatively, the control group showed a much lower conductance value than both groups, exhibiting values that ranged between 19.7±6.7 and 48.2±15.1 mmol $m^{-2} s^{-1}$.

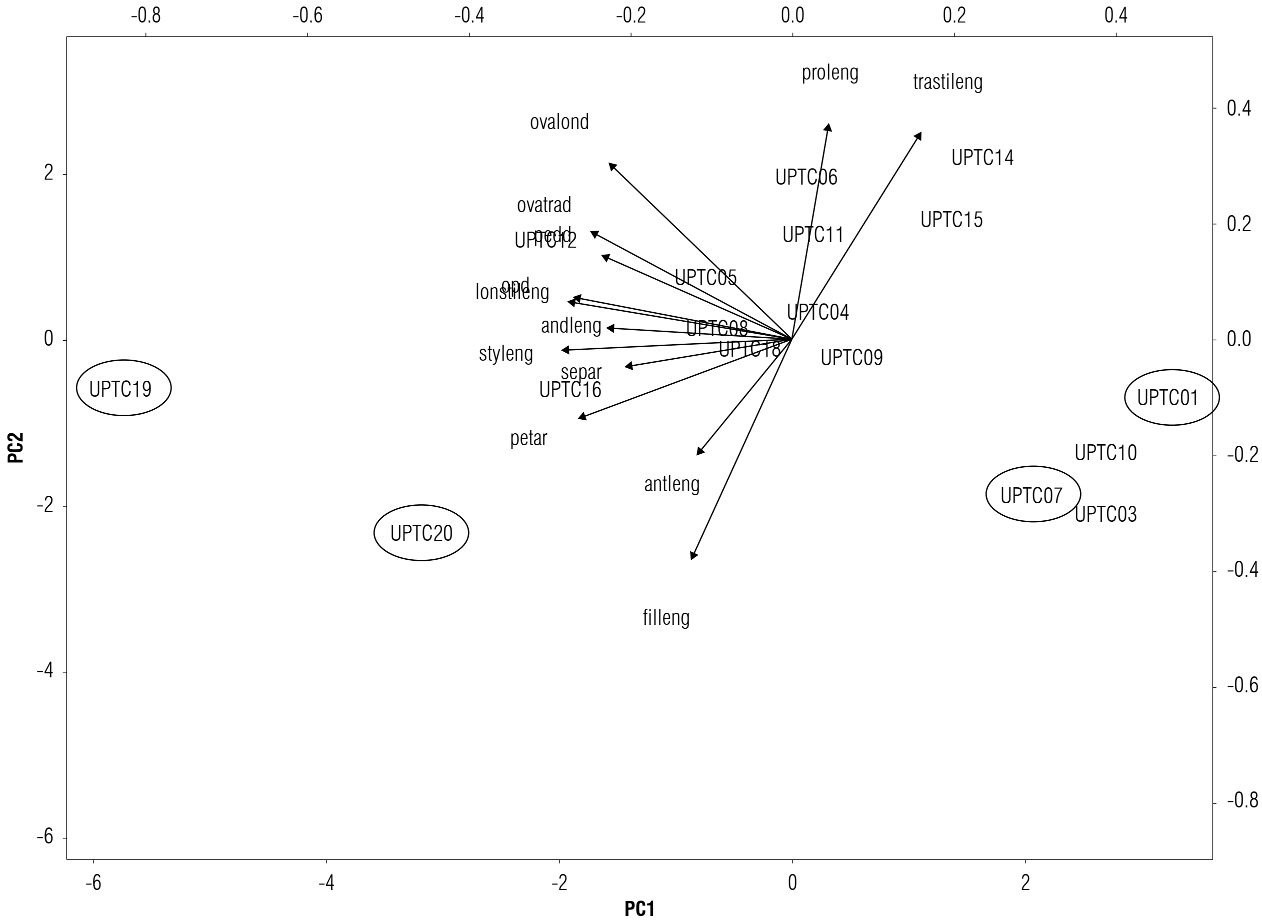

FIGURE 2. Principal component analyses (PCA) of the morphological characteristics of the flowers: sepal area (separ), floral peduncle diameter (pedd), sepal length (proleng), petal area (petar), operculum diameter (opd), androgynophore length (andleng), flower filament length (filleng), anther length (antleng), style length (styleng), ovary longitudinal diameter (ovalond), ovary transverse diameter (ovatrad), transverse stigma length (trastileng), and longitudinal stigma length (lonstileng). An average of 46 cultivars and 17 landraces of *Passiflora edulis* f. *flavicarpa* is shown. n=3. The accessions were named as UPTC.

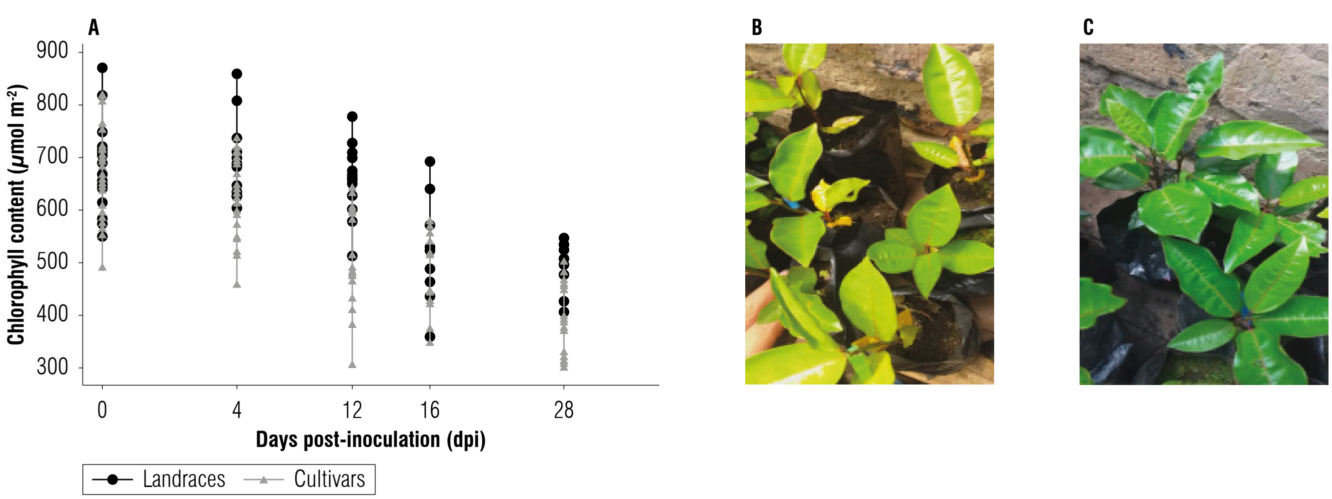

FIGURE 3. Relative chlorophyll contents (μmol m^{-2}) in leaves of *Passiflora edulis* f. *flavicarpa*. A) evaluation from day 0 (prior to inoculation) up to 28 dpi with *F. oxysporum*of landraces (black circle) and cultivars (grey triangle). An average of 12 cultivars and 6 landraces is shown, each collection equal to n=3. Degree of chlorosis of cultivars (B) is compared to that of landraces (C) at 28 dpi.

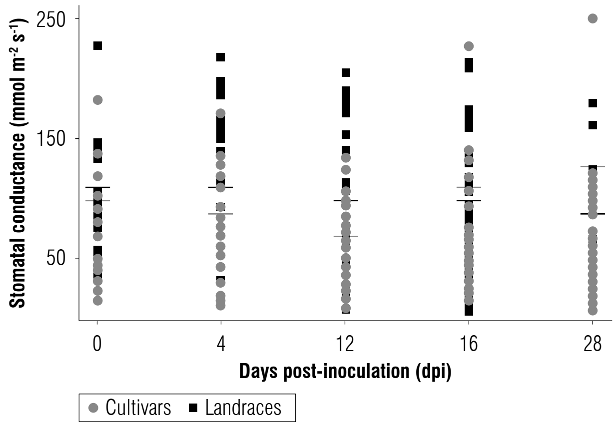

FIGURE 4. Stomatal conductance (g_s) measured in landraces (black box) and cultivars (grey circle) of *Passiflora edulis* f. *flavicarpa* (mmol m^{-2} s^{-1}) from day 0 (before inoculation) to 28 dpi with *F. oxysporum*. The black line shows the average of the landraces, while the gray line shows the average of the cultivars. An average of 12 cultivars and 6 landraces is presented, each collection represents n=3.

As a result of the above, we show that the ecophysiological parameters of chlorophyll content and stomatal conductance had variations between the landraces and cultivars in the predawn and midday samplings. Variations were also seen in relation to the symptomatologic scale after inoculation with *F. oxysporum* (Fig. 5). Chlorophyll values remained similar during predawn and midday, although landraces always had a higher chlorophyll content than cultivars. The average predawn chlorophyll content for landraces and cultivars was 481±85 and 458.4±63.0 µmol m^{-2} and at midday it was 497.3±125.0 and 481±143 µmol m^{-2}. Likewise, the degree of chlorosis between landraces and cultivars had a percentage of 0.43±0.20% and 17.3±3.2% and reached values of 48% in the cultivars. Stomatal conductance significantly varied (P<0.05) between predawn and midday, with small variations between landraces and cultivars with the average values 26.0±2.9 and 21.7±5.7 mmol m^{-2} s^{-1} at predawn and 90.8±14.9 and 87.1±34.1 mmol m^{-2} s^{-1} at midday. Additionally, Pearson's correlation analysis showed a direct relationship between chlorosis and chlorophyll content at midday (r=0.81, P-value=0.01) and predawn (r=0.76, P-value=0.02) in landraces, and midday (r=0.76, P-value=0.02) and predawn (r=0.66, P-value=0.01) for the cultivars. In the same way, chlorosis was directly related with stomatal conductance at midday (r= 0.76, P-value=0.02) and predawn (r=0.69, P-value=0.01) for landraces and at midday (r= 0.73, P-value=0.01) and predawn (r=0.67, P-value=0.01) for cultivars.

The landraces and cultivar accessions were separated according to morphological descriptors (qualitative and quantitative) and their response to *F. oxysporum* with physiological parameters. Two groups or clusters were defined according to Ward-MLM procedure (Tab. 3). The distances found indicated that there was greater diversity between the varieties of Cluster 2 (1.41) than between the varieties of Cluster 1 (0.32), while the variation between the

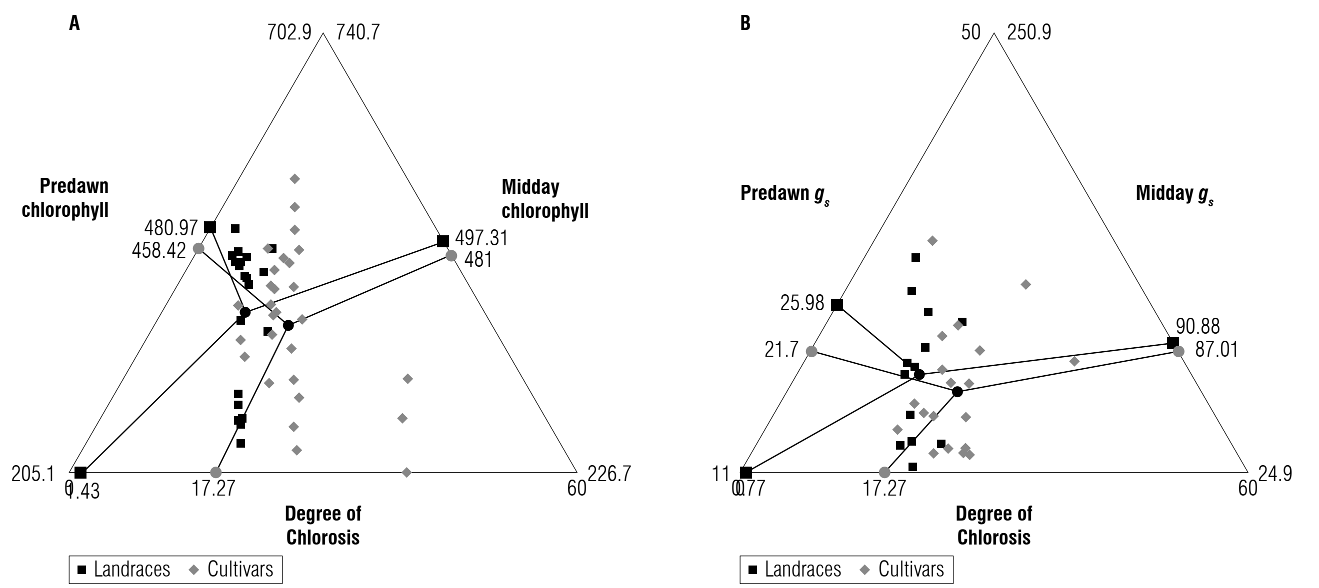

FIGURE 5. Correlation between ecophysiological parameters and the degree of chlorosis of *Passiflora edulis* f. *flavicarpa* at 28 dpi with *F. oxysporum*. A) Triplot showing chlorophyll content at predawn=4:00 h (left) and midday =12:00 h (right) in relation to the degree of chlorosis (bottom), B) triplot showing stomatal conductance (g_s) at predawn=4:00 h (left) and midday=12:00 h (right) in relationship to the degree of chlorosis (bottom). The landraces are shown with a black square and the cultivars are shown with a gray circle. An average of 12 cultivars and 6 landraces is shown, each accession comprises n=3.

two clusters was 4.26. This discrimination was confirmed with the Euclidean distance between the clusters that was 6.3 (not tabulated).

TABLE 3. Distance between the clusters formed by Ward-MLM and the intracluster distance on the diagonal in red. An average of 11 cultivars and 7 landraces of *Passiflora edulis* f. *flavicarpa* is shown, each collection consists of n=3.

Cluster	1	2
1	0.32	4.26
2		1.41

Cluster 1 was made up of the cultivars UPTC 2, 3, 10, and x14 (Fig. 6). All of the cultivars came from municipalities in Boyacá. While Cluster 2 contained all the landraces in one subgroup UPTC 19, 20, 8, 7, 9, 9b, 1, the other cultivars contained cultivars in another subgroup, UPTC 4, 5, 15, 16, 6, 12, and 11.

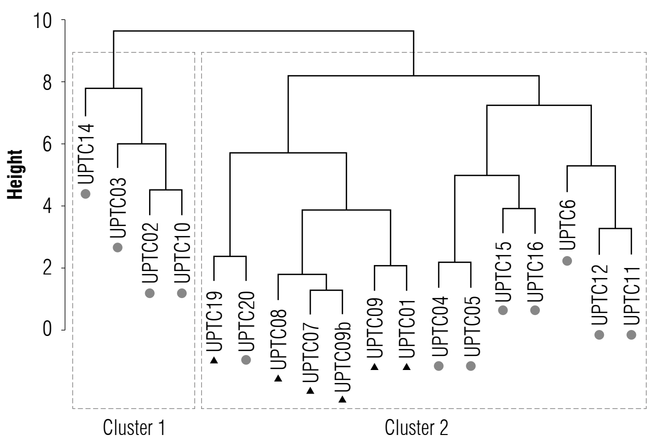

FIGURE 6. Grouping dendrogram of varieties of *P. edulis* f. *flavicarpa* evaluated, n=3.

Discussion

The morpho-agronomic descriptors did not show high variations between landraces or cultivars, except for 23% of the descriptors (6 variables). Some characteristics of cultivars such as Brix degrees, number of seeds, and leaf area are highlighted that show higher averages due to selection processes of passionfruit populations (Jaramillo *et al.*, 2009; Ocampo *et al.*, 2013; Rodríguez Ambachew *et al.*, 2020). Within the descriptors that did not show variation were the size and weight of the fruits. Under favorable conditions, the weight of these fruits range between 150 and 200 g (Dorado *et al.*, 2013). The weights reported in our research were lower, with landraces weighing 141.3±5.4 g and cultivars weighing 143.1±7.6 g. This may be because most of the landraces that were part of our study were located at higher altitudes in the department of Boyacá. They are new crops, meaning that good practices are still being established which include protocols for fertilizer application and other practices (Colombia Gobernación de Boyacá, 2017; MinTIC, 2020). In the landraces, low values are expected since mineral nutrients must be provided by mineralization of the soil´s organic matter (Julca-Otiniano *et al.*, 2006).

Other descriptors that distinguished these groups are related to the flower, such as the length of the anthers and the diameter of the stigma. Passionfruit is an allogamous plant that has structures in the flower to generally attract bumblebees that assist in pollination (Arias-Suárez *et al.*, 2014). Thus, in the principal component analysis carried out with the floral morphological data (Fig. 2), it was possible to associate the characteristics that passionflowers use to facilitate fruit production. These include the dimensions of the stigma and the androgynophore that are both relevant (Bonilla *et al.*, 2015) and whose vectors are located on the PC1. This explains most of the data. The diameter of the operculum and style length were also added (Rodríguez Ambachew *et al.*, 2020; Ocampo *et al.*, 2021). For PC2, the anther performs a different movement from the stigma, promoting attraction of pollinators (Bonilla *et al.,* 2015). Also, the length of the filament and the length of the unifacial process of the sepal are important for pollination (Rodríguez Ambachew *et al.*, 2020). For the ACP (Fig. 2), it was not possible to show a clear association of the landrace variations with respect to the cultivars; even so, the landraces UPTC 1, 7, 19, and 20 are located with high values on the PC1, indicating the importance of exploring these populations.

The characteristics associated with color, size, shape, and scent of the flowers make them efficient in pollination (Ramírez, 2006; Siqueira *et al.*, 2009). Therefore, no matter how small the variations in these descriptors (according to our measurement systems and equipment) it is likely that pollinators have a different appreciation for these mechanisms. One expects that there is a wider range of pollinators in landraces than in cultivars (Ricketts *et al.*, 2008). This is partly due to the use of agrochemicals that have drastically impacted pollinating insects to the point that, for many passionflower crops, manual pollination is necessary, increasing the costs of the crop due to the increase in labor (Bogdanski, 2008; Calle *et al.*, 2010).

An evaluation of the responses of passion fruit accessions to *F. oxysporum* was carried out according to the symptomatologic scale (Tab. 1). The landraces and cultivars behaved differently (Figs. 3B-C and 6A) since the landraces showed on average less than 1% chlorosis, indicating the absence of symptoms, while the cultivars showed 17% chlorosis with moderate yellowing. In general, these values were low and were like those obtained for another passionflower, where 2.5-month-old *Passiflora maliformis* plants at 28 dpi showed leaf decay and mild generalized chlorosis (Forero *et al*., 2016). This chlorosis can be caused by *F. oxysporum* advancing through the roots and plugging the vascular bundles that serve as transport of essential mineral nutrients for the plants. This influences on the content of many molecules, including chlorophyll (Fischer & Rezende, 2008). The content of chlorophyll was evaluated because it is a molecule that allows the determination of the physiological status of the plant. Chlorosis was not caused by a deficiency of mineral nutrients (such as magnesium) in the soil since a fertilized soil was used for this study. Chlorophyll is responsible for light absorption and through photosystems it allows photosynthesis (Lodish *et al*., 2016). An evaluation of this pigment identifies the level of stress that plants reach because of pathogens (Aguilar *et al*., 2012; Pérez & Melgarejo, 2014; Carmona *et al*., 2020). Our results showed a reduction in chlorophyll content from day 12 and up to 28 dpi (Fig. 3). This is associated with a reduction in the photosynthetic rate so that its efficiency is impacted (Rodríguez & Cayón, 2008). The plant uses its protein structure to activate its defenses against pathogens, restricting other metabolic processes. The level of decrease in chlorophyll content was on average 300 μmol m^{-2} for landraces and cultivars (Fig. 3A) and was observed in relation to the degree of severity (Fig. 3B-C) that was measured by the external characteristics (Tab. 1). Chlorosis was evident for the cultivars, reaching a range of moderate chlorosis that differed from the landraces since these did not present external change. As no reference values were found for the chlorophyll content in this species when measured with a chlorophyll meter, we estimated that this reduction places the leaves in a range of moderate chlorosis. Additionally, similar studies where the degree of chlorophyll (extraction and quantification by spectrometry) also measured the severity of the disease caused by *Fusarium* revealed a mild generalized chlorosis at 28 dpi, after which the level of chlorosis increased (Ortiz & Hoyos, 2012; Forero *et al*., 2016).

Stomatal conductance is a parameter that indicates the condition associated with water deficit due to stomatal closure that can be caused by pathogens such as *F. oxysporum* (Fischer *et al*., 2009; Nankishore & Farrell, 2016; Carmona *et al*., 2020). This affects the normal transport of water and minerals through the xylem and causes variations in gas exchange that is consistent with the results observed for the chlorophyll content (Bishop & Cooper, 1983; Carmona *et al*., 2020; Aguilar *et al*., 2012). This is because stomatal closure also reduces CO_2 entry and, therefore, carbon fixation, suggesting fewer active photosystems and reduced chlorophyll content (Aguilar *et al*., 2012). However, stomatal conductance values did not show significant variations between the landraces or cultivars with respect to this parameter (P=0.26) (Fig. 4). Research demonstrated that the decrease in the rate of net chlorophyll assimilation was associated with low values of stomatal conductance, limiting the gas exchange of the *gulupa* (*Passiflora edulis* f. *edulis*) plants once they were affected by *F. oxysporum*, with average initial values of 70 mmol m^{-2} s^{-1} before inoculation (Aguilar *et al.*, 2012). At 28 dpi, the values were less than 10 mmol m^{-2} s^{-1} that contrasts with the results obtained in our study showing an average of around 100 mmol m^{-2} s^{-1}, in general (Fig. 4).

As evaluated through measurements at predawn and midday for landraces and cultivars (Fig. 5B), stomatal conductance values varied throughout the daily cycle. Values close to 20 mmol m^{-2} s^{-1} were reported for the cultivars at predawn, and at midday they increased to a range of 50 to 90 mmol m^{-2} s^{-1}. The landraces had higher values since they reached an average maximum of 40 mmol m^{-2} s^{-1} at predawn and a range of 78 to 110 mmol m^{-2} s^{-1} at midday. Studies carried out on *gulupa* indicate that midday stomatal conductance have higher values compared to the morning values, since this phenomenon is normal in C3-type plants (Sánchez *et al*., 2013; Pérez & Melgarejo, 2014).

Based on the simultaneous analysis of all the morphoagronomic and ecophysiological parameters, two clusters were organized. The first cluster was made up of cultivars (UPTC 02, 03, 10, and 14) from two municipalities in the department of Boyacá, where these crops were recently being introduced. The second cluster brought together the other cultivars (UPTC 04, 05, 06, 11, 12, 15, and 16) and all the landraces. This group was then separated into two subgroups, one comprising a landraces collection (UPTC19) originating from the department of Quindío together with a cultivar (UPTC20) from the department of Cundinamarca. The other subgroup was made up only of the landraces (UPTC 01, 07, 08, and 09) from Boyacá and Meta.

In addition, from the morphological variability it was evident there was a wide physiological response to the pathogen. These results confirmed the importance of continuing with the evaluation of landraces and wild populations that could provide greater genetic diversity to strengthen passion fruit cultivation against diseases such as those caused by phytopathogenic fungi such as *F. oxysporum* (Cerqueira-Silva *et al.*, 2016). This is of greater importance in allogamous plants that need an increase in their variability to guarantee the sustainability of their cultivation and commercialization and to continue organizing seed banks in the areas where the species is naturally distributed and is currently affected by livestock farming (Hernández & García, 2006; Ocampo, 2013).

Conclusions

The morphological and ecophysiological characterization of yellow passion fruit populations against the effects of phytopathogenic organisms such as *F. oxysporum* allows an adequate analysis of the potential of landraces and cultivars. It is necessary to find a more efficient selection process, supported by the fact that some of the landraces have potential tolerance against this pathogen that is superior to that of the cultivars. This has led to an adaptation of the different populations and their associated biotic conditions. Therefore, it is necessary to expand evaluation studies of the cultivars and landraces to continue advancing research programs aimed at preserving this diversity *in situ* and *ex situ* in pro of food security.

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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

JPM and NRC conceived, conceptualized, collected, and analyzed data, wrote, reviewed, and edited the manuscript. All authors reviewed the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

TABLE S1. Landraces and cultivars populations of *Passiflora edulis* f. *flavicarpa* collected in five departments of Colombia.

Accession	Department	Township	Coordinates	Altitude (m a.s.l.)	Relative air humidity (%)	Origen
UPTC001	Meta	Restrepo	04°12'41.0" N, 73°29'47.6" W	434	52	Landrace
UPTC002	Boyacá	Miraflores	05°13'02.6" N, 73°09'57.2" W	1482	60	Cultivar
UPTC003	Boyacá	Miraflores	05°13'14.3" N, 73°10'12.4" W	1450	60	Cultivar
UPTC004	Boyacá	Miraflores	05°13'04.0" N, 73°09'28.6" W	1444	60	Cultivar
UPTC005	Boyacá	Miraflores	05°13'00.2" N, 73°09'30.8" W	1462	60	Cultivar
UPTC006	Boyacá	Miraflores	05°12'53.5" N, 73°09'42.5" W	1512	60	Cultivar
UPTC007	Boyacá	San Luis de Gaceno	04°51'33" N, 73°15'09" W	459	50	Landrace
UPTC008	Boyacá	San Luis de Gaceno	04°50'21" N, 73°36'28" W	510	50	Landrace
UPTC009	Boyacá	San Luis de Gaceno	04°49'10" N, 73°08'51" W	463	50	Landrace
UPTC010	Boyacá	San Luis de Gaceno	04°53'12" N, 73°09'53" W	456	50	Cultivar
UPTC011	Boyacá	San Luis de Gaceno	04°54'25" N, 73°15'46" W	471	50	Cultivar
UPTC012	Boyacá	San Luis de Gaceno	04°50'38" N, 73°14'37" W	458	50	Cultivar

Continued

Accession	Department	Township	Coordinates	Altitude (m a.s.l.)	Relative air humidity (%)	Origen
UPTC013	Boyacá	San Luis de Gaceno	04°48'41" N, 73°13'21" W	461	50	Cultivar
UPTC014	Boyacá	San Luis de Gaceno	04°47'50" N, 73°12'19" W	439	50	Cultivar
UPTC015	Boyacá	San Luis de Gaceno	04°50'67" N, 73°11'03" W	412	50	Cultivar
UPTC016	Boyacá	San Luis de Gaceno	04°51'71" N, 73°10'41" W	473	50	Cultivar
UPTC017	Casanare	Villa Nueva	04°36'31" N, 72°55'44" W	420	40	Landrace
UPTC018	Casanare	Villa Nueva	04°36'31" N, 72°55'44" W	420	40	Landrace
UPTC019	Quindío	Tebaida	04°27'08" N, 75°47'12" W	1200	80	Landrace
OCA001	Cundinamarca	Mesitas del Colegio	04°55'06" N, 74°48'28" W	1322	75	Cultivar
OCA002	Cundinamarca	Mesitas del Colegio	04°53'89" N, 74°41'08" W	990	80	Cultivar
OCA003	Cundinamarca	Mesitas del Colegio	04°53'89" N, 74°41'08" W	990	80	Cultivar
OCA004	Cundinamarca	Mesitas del Colegio	04°53'89" N, 74°41'08" W	990	80	Cultivar
OCA005	Cundinamarca	Cogua	05°03'43" N, 73°58'46" W	1900	53	Cultivar
OCA006	Cundinamarca	Mesitas del Colegio	04°53'89" N, 74°41'08" W	990	80	Cultivar
OCA007	Cundinamarca	Cogua	05°03'43" N, 73°58'46" W	1900	53	Cultivar
OCA008	Cundinamarca	Cogua	05°03'43"7 N, 3°58'46" W	1900	53	Cultivar
OCA009	Cundinamarca	Cogua	05°03'43" N, 73°58'46" W	1900	53	Cultivar
OCA010	Cundinamarca	Mesitas del Colegio	04°53'89" N, 74°41'08" W	990	80	Cultivar
OCA011	Cundinamarca	Mesitas del Colegio	04°53'89" N, 74°41'08" W	990	80	Cultivar
OCA012	Cundinamarca	Mesitas del Colegio	04°53'89" N,74°41'08" W	990	80	Cultivar
OCA013	Cundinamarca	Mesitas del Colegio	04°53'89" N, 74°41'08" W	990	80	Cultivar
OCA014	Cundinamarca	Mesitas del Colegio	04°53'89" N, 74°41'08" W	990	80	Cultivar
OCA015	Cundinamarca	Mesitas del Colegio	04°53'89" N,74°41'08" W	990	80	Cultivar
OCA016	Cundinamarca	Mesitas del Colegio	04°53'89" N, 74°41'08" W	990	80	Cultivar
OCA017	Cundinamarca	Mesitas del Colegio	04°53'89" N,74°41'08" W	990	80	Cultivar
OCA018	Cundinamarca	Mesitas del Colegio	04°53'89" N, 74°41'08" W	990	80	Cultivar
OCA019	Cundinamarca	Mesitas del Colegio	04°53'89" N,74°41'08" W	990	80	Cultivar
OCA020	Cundinamarca	Mesitas del Colegio	04°53'89" N, 74°41'08" W	990	80	Landrace
OCA021	Cundinamarca	Mesitas del Colegio	04°53'89" N, 74°41'08" W	990	80	Cultivar
OCA022	Boyacá	Miraflores	05°13'06" N, 73°09'57.2" W	1482	60	Cultivar
OCA023	Boyacá	Miraflores	05°13'02.6" N, 73°09'57.2" W	1482	60	Cultivar
UPTC043	Casanare	Yopal	05° 22' 12'' N, 72° 25' 15'' W	312	80	Landrace
UPTC044	Casanare	Yopal	05° 22' 09'' N, 72° 25' 18' W	315	81	Landrace
UPTC045	Casanare	Yopal	05° 21' 12'' N, 72° 25' 23'' W	310	80	Landrace
UPTC046	Casanare	Yopal	05° 21' 13'' N, 72° 25' 28'' W	312	80	Landrace
UPTC047	Casanare	Yopal	05° 21' 33'' N, 72° 25' 21'' W	315	77	Landrace
UPTC048	Casanare	Yopal	05° 22' 21'' N, 72° 24' 14'' W	340	83	Cultivar
UPTC049	Casanare	Yopal	05° 22' 16'' N, 72° 24' 09'' W	342	80	Cultivar
UPTC050	Casanare	Yopal	05° 22' 36'' N, 72° 24' 10'' W	343	83	Cultivar
UPTC051	Casanare	Yopal	05° 22' 42'' N, 72° 24' 11'' W	341	77	Cultivar
UPTC052	Casanare	Yopal	05° 22' 45'' N, 72° 24' 29'' W	351	77	Cultivar
UPTC053	Casanare	Yopal	05° 22' 14'' N, 72° 24' 46'' W	350	77	Cultivar
UPTC054	Casanare	Yopal	05° 22' 07'' N, 72° 24' 33'' W	356	77	Cultivar

Continued

Accession	Department	Township	Coordinates	Altitude (m a.s.l.)	Relative air humidity (%)	Origen
UPTC055	Casanare	Yopal	05° 22’ 11’’ N, 72° 24’54’’ W	360	77	Cultivar
UPTC056	Casanare	Yopal	05° 22’ 06’’ N, 72° 24’ 3’’ W	357	77	Cultivar
UPTC057	Casanare	Yopal	05° 22’ 28’’ N, 72° 24’ 07’’ W	351	77	Cultivar
UPTC058	Casanare	Yopal	05° 22’ 58” N, 72° 24’ 51’’ W	352	77	Cultivar
UPTC059	Meta	Granada	03°32’50” N, 73°42’31” W	400	45	Landrace
UPTC060	Meta	Granada	03°32’50” N, 73°42’31” W	400	45	Landrace
UPTC061	Meta	Granada	03°32’50” N, 73°42’31” W	400	45	Landrace
UPTC062	Meta	Restrepo	04°12’41.0” N, 73°29’47.6” W	434	52	Landrace
UPTC063	Meta	Restrepo	04°12’41.0” N, 73°29’47.6” W	434	52	Landrace

FIGURE S1. Confirmation of Koch’s postulates from root explants of yellow passion fruit in medium PDA according to the protocol proposed by Hernández *et al*. (2019).

Field management of *Phelipanche ramosa* on tomatoes by plowing depth or resistance strategies

Manejo en campo de *Phelipanche ramosa* en tomates por profundidad de arado o estrategias de resistencia

Grazia Disciglio[1*], Annalisa Tarantino[1], Francesco Lops[1], and Laura Frabboni[1]

ABSTRACT

Two separate independent experiments were carried out on agronomic approaches for controlling infestation by *Phelipanche ramosa*: the evaluation of two soil plowing depths (30 and 50 cm) and determining the resistance of two tomato cultivars (SV5197 and SV8840) to this parasitic plant. The experiments were performed in 2014 and 2018 seasons in naturally heavily infested fields in the province of Foggia (Apulia Region, south-eastern Italy). Based on our results, the 50 cm moldboard plowing depth reduced parasite infestation by 41.2% compared to that of 30 cm depth, commonly adopted by local farmers. Higher attachments to the host plants were observed in SV5197 than in SV8840, which could be correlated with the different level of resistance of the host plant. In both experiments, the highest *Phelipanche* infestation reduced the tomato yield but did not affect the fruit quality.

Key words: branched broomrape, tillage depth, plant resistance, achlorophyllous plant.

RESUMEN

Se llevaron a cabo dos experimentos independientes separados sobre enfoques agronómicos para controlar la infestación por *Phelipanche ramosa:* la evaluación de dos profundidades de arado del suelo (30 y 50 cm) y la determinación de la resistencia de dos cultivares de tomate (SV5197 y SV8840) a esta planta parásita. Los experimentos se realizaron en las temporadas 2014 y 2018 en campos naturales altamente infestados en la provincia de Foggia (región de Apulia, sureste de Italia). Según nuestros resultados, la profundidad de arado con vertedera de 50 cm redujo la infestación de parásitos en un 41.2% en comparación con la profundidad de 30 cm, comúnmente adoptada por los agricultores locales. Se observaron mayores adherencias a las plantas hospedantes en SV5197 que en SV8840, lo que podría estar correlacionado con el diferente nivel de resistencia de la planta hospedante. En ambos experimentos, la mayor infestación de *Phelipanche* redujo el rendimiento del tomate, pero no afectó la calidad del fruto.

Palabras clave: jopo ramudo común, profundidad de labranza, resistencia de plantas, planta aclorófila.

Introduction

Tomato (*Solanum lycopersicum* L.) is the vegetable crop with the highest demand and greatest economic value in the world. Tomato trade and production have particular importance in tropical, subtropical, and temperate regions of the world, for both fresh and processing markets (Gaytosso-Barragán *et al*., 2019). Italy plays an important role in tomato cultivation worldwide, with 13% of the world's production and 53% of Europe's production (ISMEA, 2022). In Italy, as in other areas of the world, the tomato crop is undergoing increased attack by a holoparasitic plant belonging to the Orobanchaceae family, the *Phelipanche ramosa* (L.) Pomel (formerly classified as *Orobanche ramosa* L.), and commonly known as a branched broomrape. The economic damage that it causes to infested crops is worrisome.

Phelipanche seed germination occurs after a preconditioning period (moist and suitable temperatures for several days) and in response to metabolites exuded by the host plant roots (Sato *et al.,* 2003). Following germination, this holoparasitic plant remains concealed underground for its life cycle, thus inaccessible to conventional agricultural control methods, such as tillage and contact herbicides. Following germination, the seedlings attach to the host roots by the production haustoria that penetrate the host tissues until they reach the vascular system for uptake of water, nutrients, assimilates, growing at the expense of the host plant's resources (Joel *et al*., 2007). Once connected to a host plant, broomrape grows rapidly, forming a tubercle (a storage organ for nutrients and water extracted from the host) underground.

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[1] Department of Agricultural Sciences, Food, Natural Resources and Engineering, University of Foggia, Foggia (Italy).
[*] Corresponding author: grazia.disciglio@unifg.it

Multiple shoots (up to 20) develop from the tubercle and emerge above the soil surface, then reach from 15 to 30 cm in height. After several weeks of underground development, the holoparasitic plant emerges above the soil surface, having already done most of the damage to the host, and develops flowering stems, which produce seeds within a short period of time. Most of the seeds in the soil will not germinate immediately, instead forming a seed bank for the next cropping seasons. They can remain viable in the soil for more than 10 years; thus, if host crops are frequently cultivated, the seed bank in the soil increases tremendously, leading to the failure of cultivating host crops (Fernández-Aparicio *et al.,* 2008, 2016). The main difficulties in the control of *Phelipanche* seed bank arise from their immense number, diminutive size, extreme longevity, and ease of dispersal. These cause rapid increase in the parasite soil seed banks, even when the original infestation area is very limited. The amount of seed in the soil can be reduced only when the introduction of new seeds is lower than those produced that do not germinate or die naturally or by the action of pathogens or predators (Grundy *et al.,* 2003; Van Mourik *et al.,* 2003; Rubiales *et al.,*2009; Van Mourik*et al*., 2011). For this reason, understanding the fate of seeds in the seed bank can be an important component of overall weed control. When weed seeds enter the seed bank, several factors influence their duration. Seeds can sense the surrounding environment in the seed bank and use these stimuli to become dormant or initiate germination (Hossain & Begum, 2016).

The control of *P. ramosa* in the field is important not only to reduce direct damage to the host crop but also to limit the production of seeds, which will replenish the local seed bank and at the same time increase the risk of the seed dispersal to uninfested areas.

To control this parasite, there are several methods (preventive, physical, chemical, and biological) that help to avoid germination, infection, or effective reproduction of the weed (Rubiales *et al*., 2009; Habimana *et al*., 2014). For the reduction of the seed bank in the soil, various environmentally friendly approaches are possible (Disciglio *et al*., 2016, 2018; Lops *et al*., 2021), including those related to soil management and the resistant varieties.

Soil management practices can directly influence the seed environment in the soil and can, thus, be used to manage the seed longevity and germination behavior. The soil management must aim at reducing the seed bank, while minimizing the production of new seeds and their dispersal to new sites. In this regard, it is widely recognized that the primary tillage influences distribution of weed seed in the soil tillage layer (Skuodienė *et al*., 2013). Inversion plowing results in burial of a large proportion of seed in the tillage layer, placing them at a depth from which they cannot germinate, although they do remain viable in the deep soil for a long period of time (Roberts & Feast, 1972; Mohler & Galford, 1997; Benvenuti *et al.,* 2001). Deep plowing has been suggested to bring seeds of parasitic weeds to a soil depth with less oxygen availability and, therefore, a reduction in their germination capacity (Van Delft *et al*., 2000; Eizenberg *et al*., 2007). Furthermore, some research findings suggest that, in newly infested fields, soil inversion or the adoption of no-till seeding may reduce the replenishment of the seed reserves in soil (Hamad *et al*., 2014).

Mohammed-Ahmed (1995) found that the largest number of *P. ramosa* attachment on roots of tomato were from the seeds buried at 3-4 cm compared to shallower –or deeper– buried seeds. Also, the experiments carried out on tomato in pots (Eizenberg *et al.,* 2007) showed that deep-plowing delayed parasite emergence and prevented flower initiation and seed set.

The use of resistant varieties is another sustainable method to control *Phelipanche* (Rubiales *et al*., 2003; Abbes *et al*., 2007; Disciglio *et al*., 2016; Bai *et al*., 2020). In addition, it is a useful component of an integrated approach (Haussmann *et al*., 2000), because it is easy to combine with other measures such as soil fertility amendments, land preparation or soil tillage. Tomato crops are usually susceptible to the destructive *Phelipanche* weed, but some cultivars are potentially resistant. The selection of resistant cultivars is among the most promising approaches to deal with this problem (Bai *et al*., 2020; Albanova *et al*., 2023). In the host, response and resistance to parasitic plants are of molecular nature, which are still not completely understood (Mutuku *et al*., 2021). The mechanisms of resistance to parasitic plants vary depending on the host species and cultivars (Mutuku *et al*., 2021; Jhu & Sinha, 2022; Albanova *et al*., 2023). Some mechanisms underlying the resistance have been described Pérez-de-Luque *et al*., 2008; 2009; Pérez-de-Luque & Rubiales, 2009). Some host varieties exude stimulants of the seed germination at low levels (Labrousse *et al*., 2001) or excrete chemical inhibitors of germination (Serghini *et al*., 2001), while others exhibit strengthening and lignification of root cell walls (Jhu *et al*., 2022) that could reduce the holoparasitic infection. Other varieties are able to tolerate the infection with lower production losses due to lags between the development phases of the parasite compared to the productive phases of the host (Grenz & Sauerborn, 2005; Fernández-Aparicio *et al*., 2012) or due to the biochemical changes (low levels of amino acids in the phloem) that induce low performance of the parasite when it attacks the roots. This limits the absorption strength of the parasite and yield losses of host due to its parasitism (Abbes *et al*., 2009).

One underestimated aspect is the cross-resistance/tolerance to multiple stresses as well as the interaction between them (Jhu *et al.,* 2022). In the case of tolerant varieties, their cultivation can continue to increase the seed bank of the parasite.

The number and dry weight of parasitic plants per host plant and the number of emerged shoots per surface unit can be used as indicators to monitor the *Phelipanche* infection (Rubiales *et al*., 2006). Some authors (Cubero, 1991; Rubiales *et al*., 2003; Abbes *et al*., 2011) indicate that the best indicator is the number of parasite shoots per host plant.

This study assessed two crop production practices in naturally infested fields: 1) the effect of a 50 cm soil plowing depth compared to the 30 cm depth generally used by farmers to control seed germination of *P. ramosa*, and 2) the resistance level of two commercially available tomato varieties to *P. ramosa* infection.

Materials and methods

Site description and experimental setup

The study was conducted on tomato crops in the Foggia Province (Apulia Region, Southern Italy) on heavily naturally infested soils (Typic Chromoxerert, fine, thermic, according to the Soil Taxonomy-USDA-NRCS,1999; silty-clay Vertisol of alluvial origin (1.20 m depth).

The tomato seedlings (with 4-5 leaves and a height of 100-150 mm) were obtained from a nursery and manually transplanted to the field in double rows at 0.4 m apart which were spaced at 2 m intervals, with the plants positioned 0.3 m apart within each single row. This provided a theoretical plant density of 3.3 plants/m^2. Drip irrigation with the lines between each pair of plant rows was used. The water volume for each irrigation was 100-300 m^3 ha^{-1} depending on crop growth stage, with a watering interval of 3-4 days. Weed and pest control and the other agricultural management employed were those commonly adopted by local farmers (the weed control is carried out mechanically). For fertilization at pre-transplanting, 35 kg ha^{-1} N and 70 kg ha^{-1} P_2O_5 were applied. Throughout the tomato crop cycles, 75 kg ha^{-1} N and 100 kg ha^{-1} P_2O_5 were added with fertirrigation. Regarding pest and disease management, tomato pinworm was controlled by chlorantraniliprole 18.5% SC (Coragen®SC) at a rate of 0.3 ml L^{-1} (during the flowering and fruit setting stages). Fruit borers and *Helicoverpa* spp. were controlled by emamectin benzoate 0.5 g L^{-1} SC + dichlorvos 76% EC at 1 ml L^{-1}. Early blight was controlled by spraying metalaxyl 8% + mancozeb 63%WP at 2 g L^{-1} of water, and late blight of tomato was controlled by spraying cymoxanil 8% + mancozeb 64% WP fungicide (Curzate M8) at 2 g L^{-1} (applied in the growth phase of the plants).

P. ramosa infestation was monitored as: (I) number of emerged branched holoparasitic shoots (hereafter referred to as "shoots"); (II) attachment of tubercles on tomato roots. The shoots that emerged above the soil were counted at various days after transplanting (DAT). At tomato-fruit harvest, the numbers and dry weight of mature holoparasitic shoots and tubercles were recorded as reported by Qasem and Kasraw (1995).

The tomato fruits were harvested at full-maturity stage in August (a transplanted crop has a cycle of 100-120 d). At the tomato harvest, the marketable yield and the major qualitative parameters were evaluated as described by Disciglio *et al.* (2018). In particular, the following major qualitative and quantitative yield parameters were determined on 10 fruits for each plot: mean fresh weight (g), dry-matter content (% fruit fresh matter); soluble solids content (°Brix), pH, and titratable acidity (g citric acid per 100 ml fresh fruit juice) (AOAC, 1990) The color parameters were measured using a spectrophotometer (CM-700d; Minolta Camera Co. Ltd., Osaka, Japan) as the CIELAB coordinates (i.e., L*, a*, b*) on four randomly selected areas of the fruit surface. Only the a*/b* ratio has been reported, which represents an index that describes the color differences of tomato fruit (Francis & Clydesdale, 1975; Favati *et al*., 2009).

The monthly climatic parameters were recorded daily at the nearest meteorological stations of the Consorzio per la Bonifica della Capitanata, located at Foggia (Tavernola countryside). The site of the research was in a typical semiarid zone, characterized by a Mediterranean climate, which is classified as an accentuated thermomediterranean climate (UNESCO/FAO, 1963), with temperatures that may fall below 0°C in the winter and exceed 40°C in the summer. Rainfall is unevenly distributed throughout the year and is mostly concentrated in the winter months, with a long-term annual average of 559 mm (Ventrella *et al*., 2012).

In Table 1, the main climatic data (May-September) for the two experimental seasons (2014 and 2018) are reported. Differences occurred in air temperature and rainfall between the two years of tomato cultivation. The maximum and minimum temperatures were relatively higher in 2018 than in 2014, whereas this last season was slightly drier than that of 2018 (Tab. 1).

TABLE 1. Monthly maximum and minimum temperature (Tmax and Tmin) and total precipitation (P) during the 2014 and 2018 seasons.

Month	2014			2018		
	Tmax	Tmin	P	Tmax	Tmin	P
	(°C)	(°C)	(mm)	(°C)	(°C)	(mm)
May	22.7	10.2	43.6	26.1	13.4	58.3
June	29.0	14.8	56.8	30.0	12.1	88.2
July	29.3	17.1	6.8	33.3	19.6	16.8
August	32.1	17.4	15.0	32.7	20.0	39.1
September	24.4	13.6	65.8	29.1	17.1	80.0
Mean (°C)	27.5	14.6		30.2	16.4	
Total (mm)			188.0			282.4

Effect of the plowing depth

To assess the effect of two plowing depths on the infestation of *P. ramosa* on tomato for processing crop (cv. DRES F1 produced by Clause Vegetable Seeds Company), an experiment was performed in 2014 in the countryside of Foggia (41°65' N, 12°48' E, altitude 54 m a.s.l.) at two different fields (field A and B, about 2 km apart) of different private farms ("Ortuso" and "Pazienza" properties). The soils were silty-clay Vertisol of alluvial origin (1.20 m depth) (Typic Chromoxerert, fine, thermic, according to the USDA Soil Taxonomy (USDA-NRCS, 1999), whose physical-chemical characteristics were reported in Lops *et al.* (2021). In particular, the soil texture in field A was clayey-loamy, while that in field B the soil was of medium texture.

The effect of 50 cm plowing depth was compared to the 30 cm depth (control) normally used by the farmers. The two plowings were carried out in spring, just before the tomato transplant; the soil was then refined with a disc harrow. The tool used to reverse the soil at both depths was the moldboard plow.

In both fields, the tomato plants were transplanted on May 5, 2014, to the soil cultivated in the previous year with wheat. Each experiment was laid in a randomized block design, with three replications, using the plowing depth plot of 100 m^2 (10 × 10 m), which included five tomato twin rows (330 plants).

P. ramosa infestation was measured by counting the emerging shoots during the tomato growing cycles, at 56, 78 and 92 DAT on three sampling areas of 4 m^2 for each plot. At tomato harvest (on August 5, 2014), the numbers and dry weight of mature holoparasitic shoots and tubercles and fruit yield from 12 tomato plants (~4 m^2 of sampling area where the parasite infestation was detected) were recorded.

P. ramosa flowering stems

Only in field A, at tomato harvest, a sample of 100 *P. ramosa* holoparasitic shoots was chosen at random in the field, the number of stem spears per branched plant was counted, and the frequency distribution of classes of the 2-amplitude interval was determined.

Effect of tomato resistance

The resistance of two commercially tomato cultivars (SV5197 and SV8840), produced by Bayer Vegetables Italia Company, to the *P. ramosa* infection was evaluated in 2018, in the countryside of Rignano (Foggia province, 41°40' N, 15°35' E, altitude 50 m a.s.l.).

The two varieties belonging to the pear-shaped fruit and round-shaped fruit typology, respectively, are widely grown in the agricultural areas where the experiment was carried out.

The transplant of the tomato cultivars was carried out on May 10, 2018. The experiment was arranged according to a completely randomized design, with three replicates, using the tomato cultivars treatments as plots of 60 m^2 (6 × 10 m); each plot included three tomato twin rows (200 plants). The *P. ramosa* infestation was measured by counting the emerging shoots during the tomato growth cycle at 45, 76, and 96 (harvest) DAT on three sampling areas of 4 m^2 (along the central row) for each plot. Moreover, at tomato harvest on August 14, 2018, the final number of parasites per host plant was counted. Dry weight of holoparasitic shoots and tubercles was also recorded. The dry weight was determined by drying samples in a ventilated stove at 70°C for about 12 h.

Statistical analysis

All data were submitted to ANOVA by using the GLM Procedure of SAS software, version 6 (SAS Institute, 1999). When the main effects were significant, differences between means were compared with the Tukey's test at $P \leq 0.05$. Standard deviations (SD) were calculated using Excel software of the Office 2007® suite. Percentage values of fruit dry matter were transformed to arcsine prior to analysis of variance.

Results and discussion

Plowing depth

P. ramosa infection and density

Figure 1 shows the average number of emerged shoots per m^2 at 56, 78, and 92 DAT of 30 cm (control) and 50 cm plowing depth treatments in both fields.

At 56 DAT, the number of emerged shoots was low in both fields with no significant difference between plowing depth treatments. At 78 and 92 DAT, the infestation increased, albeit with some variations between the two fields, particularly at 78 DAT, with a significantly higher number of emerged shoots at 30 cm plowing depth than at 50 cm . From 78 up to 92 DAT, the shoot density slightly increased for the 30 cm plowing depth in both fields, while, for the 50 cm plowing depth in field B, the infestation continued to increase. Significant higher emerged shoots were measured at the tomato harvest (92 DAT) in 30 cm plowing in both fields A and B (34.8 and 44.7 m^2, respectively) compared to the 50 cm plowing (18.2 and 27.8 m^{-2}, respectively).

In Table 2, the average number of *P. ramosa* emerged shoots at different days after transplant of the two fields are reported.

In both field trials, in accordance with what has been described for the emerging shoots, the mean values of total attachments, dry weights of sprouts and tubercles were significantly higher in the 30 cm plowing than in the 50 cm plowing (Tab. 3). Some differences were noted between the two fields. In field A, the number of total attachments, dry weight of shoots, and dry weight of tubercles for deeper plowing (50 cm) respect to shallower plowing (30 cm) were

TABLE 3. Mean of total attachments per m^2, dry weight of shoots, and dry weight of tubercles (g) per tomato plant for 30 and 50 cm plowing depths in each field, for each plowing depth and for each field .

Field	Plowing depth (cm)	Total attachments	Shoot (DW) (g)	Tubercles (g)
Field A	30	9.7±2.4 a	56.9±12.9 a	106.1±11.8 a
	50	5.1±1.5 b	29.9±6.7 b	56.1± 8.2 b
Field B	30	12.8±2.8 a	73.0±16.4 a	140.7± 15.6 a
	50	7.9±1.6 b	46.2±10.4 b	87.4± 9.7 b
Average plowing depth	30	11.2±2.6 a	64.9±14.6 a	123.4±9.0 b
	50	6.5±1.5 b	38.0±8.4 b	71.7±8.9 b
Field averaged	Field A	7.4±1.9 a	43.4±9.8 a	81.1±10.0 b
	Field B	10.3±2.2 a	59.6±13.4 a	114.0±12.6 a

Data are means±SD, n=30. Different letters in each column of each field and plowing treatment indicate significant differences at $P\leq 0.05$ according to the Tukey's test.

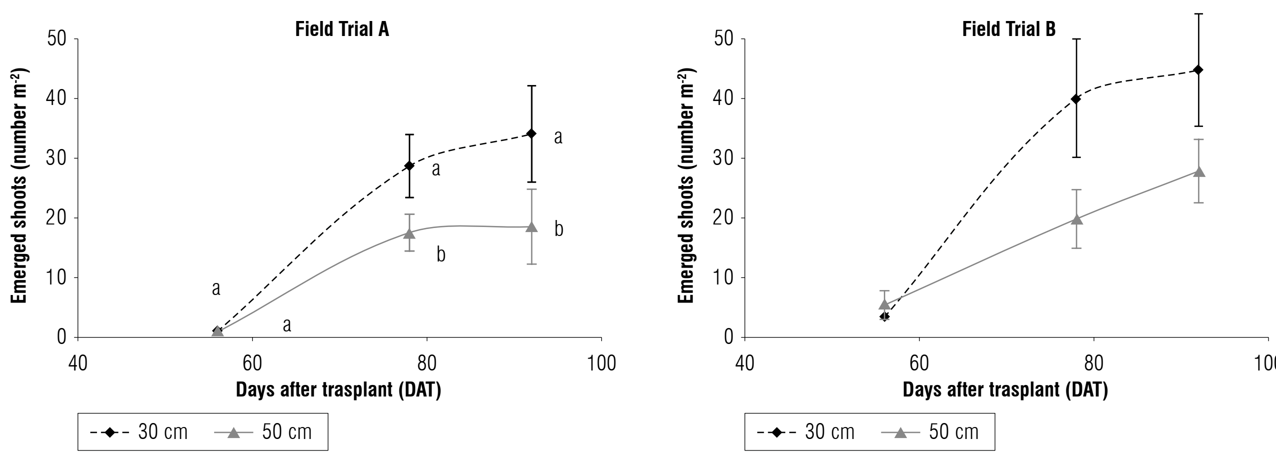

FIGURE 1. Shoot density (plants/m^2) of *P. ramos*a for 30 and 50 cm plowing depth at different days after transplant. Values are means±SD, n=3. Different lowercase letters indicate significant differences between plowing depth treatments in each day at $P\leq 0.05$ according to the Tukey's test.

TABLE 2. Average shoot density (plants/m^2) of *P. ramosa* for each 30 and 50 cm for each plowing depth and for each field at different days after transplant (DAT).

Fields	Plowing depth (cm)	56 DAT	78 DAT	92 DAT (harvest)
Average plowing depth	30	2.1±0.6 a	3.0±1.2 a	39.3±8.7 a
	50	3.0±1.2 a	18.6±4.0 b	23.1±5.8 b
Field averages	Field A	0.7±0.2 b	23.0±4.0 a	26.2±7.0 b
	Field B	4.4±2.0 a	29.9±6.9 a	36.2±7.6 a

Data are means±SD, n=9. Different letters on cumulative values of each DAT and in each column of each field and treatment indicate significant differences at $P\leq 0.05$ according to the Tukey's test.

lower (-52.6%, -52.5%, and -52.9%, respectively) than those in field B (-61.7%, -63.3%, and -62.1%, respectively). This may be due to differences in the level of *Phelipanche* infestation and/or of soil characteristics between the two fields.

As an overall average between fields of each plowing depth (Tab. 3), the number of total attachments, dry weight of shoots, and dry weight of tubercles were lower in the 50 cm plowing than the 30 cm plowing by 58.0%, 58.5%, and 58.1%, respectively. Moreover, as an overall average between plowing depths of each field, the same parameters in field B showed an increase compared to field A of 39.2%, 37.2%, and 40.6%, respectively. Although these *P. ramosa* reductions are to be considered positive, they are lower than those reported on tobacco by Habimana *et al.* (2014) (80-90%), using the same 50 cm deep plowing. Our results are in accordance with those of Eizenberg *et al.* (2007) in which deep inversion plowing (to more than 30 cm) would bury broomrape seeds to a depth below the soil layer where attachment to tomato root can occur.

P. ramosa flowering stems

Multiple shoots develop from the tubercle and emerge above the soil surface, then grow into stems from 15 to 30 cm in height. Figure 2 shows the frequency class distribution in the range of 2 amplitudes of emerging flowering stems per *P. ramosa* branched plant. The number of flowering stems ranged from 1 to 20, but most ranged from 1 and 10. The highest percentage (32.3%) was shown by 6 stems, followed in order by 4 (20.3%), 8 (16.9%), 2 (14.9%) and 10 stems (6.2%).

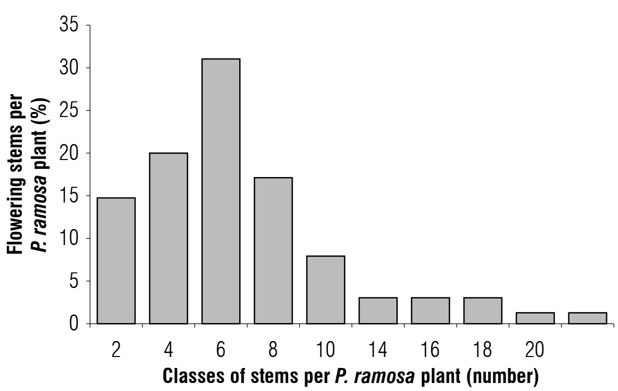

FIGURE 2. The frequency distribution for classes of 2-amplitude interval of flowering stems per *P. ramosa* emerged shoots.

Tomato fruit yield and qualitative traits

Results of the tomato productive traits for the two compared plowing depths are reported in Table 4. In both fields A and B, the 50 cm plowing depth provided significantly higher marketable yield (66.9 and 93.1 t ha^{-1}, respectively) than the 30 cm plowing depth (55.5 and 74.3 t ha^{-1}, respectively). No significant differences were found for the qualitative characteristics of the fruits between plowing depths and fields.

In Figure 3, the negative correlations between marketable yield and *P. ramosa* emerged shoots and of both fields A (R^2 = 0.83) and B (R^2 = 0.94) are shown.

In general, the tomato marketable yield and *P. ramosa* infestation were related; the smaller numbers of emerged shoots resulted in increased marketable yield. This result agrees with previous research performed on tomato (Conversa *et al.*, 2017; Disciglio *et al.*, 2018). The regression lines show

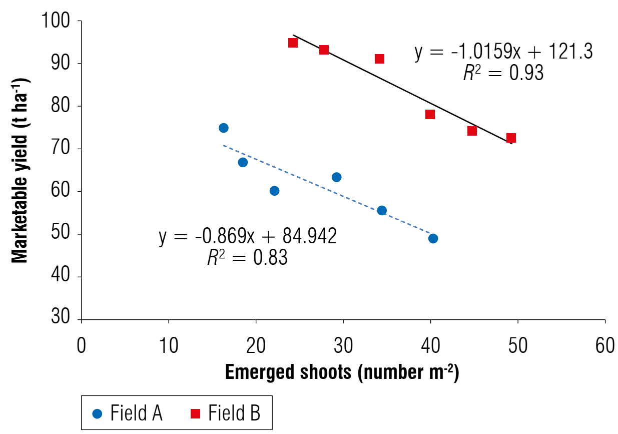

FIGURE 3. Linear regression between tomato marketable yield and number of emerged shoots of *P. ramosa* detected at tomato harvest in each field (data used in the analysis refer to the single plot of each field).

TABLE 4. Mean values of quanti-qualitative traits of processing tomato fruit for 30 and 50 cm plowing depth treatments in both fields A and B.

Field	Plowing depth (cm)	Marketable yield (t ha^{-1})	Mean fruit weight (g)	Fruit dry matter (%)	Soluble solids (°Brix)	pH	Titratable acidity (g citric acid/100 ml of juice)	Fruit color (a/b)
Field A	30	55.5±5.8 b	58.3±5.2	6.5±0.3	5.6±0.2	4.2±0.3	0.38±0.01	1.1±0.8
	50	66.9±5.8 a	56.7±4.8	6.5± 0.3	6.5± 5.3	4.1±0.3	0.33 ±0.01	1.2±0.8
Field B	30	74.3±5.8 b	62.0±5.3	5.6±0.2	4.3±0.2	4.6±0.3	0.36±0.01	1.1±0.03
	50	93.1±5.8 a	63.3±5.3	5.4±0.2	4.3±0.2	4.5±0.3	0.40±0.01	1.1±0.03

Data are means±SD, n=3. Different letters in each column of each field and plowing indicate significant differences at P≤0.05 according to the Tukey's test.

almost similar slopes in both fields A and B (-0.87 and -1.01, respectively), thus, highlighting a similar depressive effect due to the variation of the infestation.

Tomato resistance to *P. ramosa*

P. ramosa infection and density

In this experiment, the level of resistance of two tomato cultivars to *P. ramosa* was different. According to Table 5, *P. ramosa* infestation was detected at 45, 76, and 96 DAT. On all sampling dates, significant higher emerged shoots in the cv. SV 5197 than cv. SV8840 were observed. At 96 DAT, a 65.1% reduction of emerging shoots in cv. SV8840 compared to cv. SV 5197 was measured.

TABLE 5. Mean values of *P. ramosa* emerged branched shoots (plants/m^2) in each tomato variety at different days after transplant (DAT).

Cultivar	45 DAT	76 DAT	96 DAT (harvest)
SV5197	1.8±0.8 a	14.6±9.7 a	21.2±5.7 a
SV8840	0.1±0.1 b	1.9±0.8 b	7.4±2.0 b

Data are means±SD, *n*=9. Different letters on cumulative values of each DAT and in each column indicate significant differences at $P \leq 0.05$ according to the Tukey's test.

Table 6 reports the number of *P. ramosa* total attachment on tomato roots and the dry weight of emerged and underground branched shoots per host plant, detected at the end of the experiment. Differences in responses of tomato cultivars to *P. ramosa* infection were observed for these measured parameters. The results, in accordance with what has been described for the emerging shoots, showed that, for the three measured parameters, higher values were obtained for the SV5197 cultivar than for SV8840 cultivar, which could be correlated with the different level of resistance of the host plant. Differences in susceptibility to infection were also seen among tomato varieties based on their difference in the number of emerged shoots of *Phelipanche* and *Phelipanche* plant dry weight (shoots and tubercles) (Tokasi *et al.,* 2014). The SV8840 cultivar could exude stimulants at a lower level and, therefore, could be suitable for reducing *Phelipanche* infection. These results agree with previous studies by Kasrawi and Abu-Irmaileh (1989), Qasem and Kasrawi (1995), and Mariam and Suwanketnikom (2004).

TABLE 6. Total attachments, dry weights of emerged shoots and tubercles of *Phelipanche ramosa* per tomato host plant of the two varieties.

Cultivar	Total attachments (number/plant)	Shoot dry weight (g)	Tubercle dry weight (g)
SV5197	6.4±1.8 a	37.5±4.3 a	70.3±8.2 a
SV8840	2.2±1.0 b	12.9±2.5 b	24.2±4.6 b

Data are means±SD, *n*=30. Different letters in each column indicate significant differences at $P \leq 0.05$ according to the Tukey's test.

Tomato productivity

Results of the tomato productive traits for the two compared tomato cultivars are reported in Table 7. The SV8840 cultivar, which showed the lowest *P. ramosa* infection, had significantly higher yield than the SV5197 cultivar. This could be due to the low infestation, as seen previously, although other factors dependent on the two genotypes used in the experiment may have been involved. In this last respect, higher mean weight and width, and lower length were shown from the SV8840 round-shaped fruit compared to the SV5197 pear-shaped ones. Finally, fruit characteristics were not significantly different between the tested cultivars.

TABLE 7. Quanti-qualitative traits of the fruits of SV5197 and SV8840 tomato cultivars.

Parameters evaluated	Cultivar	
	SV5197	SV8840
Yield (t ha^{-1})	71.3±3.4 b	78.9±2.9 a
Mean fruit weight (g)	57.9±6.1 b	72.6±5.9 a
Fruit length (cm)	6.6±0.4 a	5.51±0.3 b
Fruit width (cm)	3.9 ±0.2 b	4.8±0.3 a
Dry matter (%)	6.1±1.0 a	6.2±0.8 a
Soluble solids (°Brix)	5.3±1.0 a	5.5±0.7 a
pH	4.4 ±0.1 a	4.3±0.1 a
Titratable solids (g citric acid/100 ml juice)	0.30±0.1 a	0.22±0.1 a
Color index (a/b)	2.05±0.1 a	2.00±0.1 a

Data are means±SD, *n*=3. Values in the same row followed by different letters are significantly different at $P \leq 0.05$ according to the Tukey's test.

Conclusions

This study addressed the agronomic strategies of depth of plowing of the soil and use of resistant varieties to reduce the infestation by *P. ramosa* in processing tomato cultivation. Inversion plowing or deep burial of weed seed using a moldboard plow is a very effective method of decreasing seedling emergence. This is consistent with the results of many ecological studies which showed that weed seedling emergence is inversely related to the depth of seed burial and that maximum emergence is from shallow depths of around 1 cm for most species. Small-seeded weeds can only emerge from shallow depths while large-seeded ones can germinate from greater depths if conditions are suitable, but rarely do they emerge from depths of 15 cm or more. In our environments, plowing is usually carried out in autumn or spring. In the latter period, it is performed just before sowing, with the main objective of avoiding hardening of the clods, caused by the gradual appearance of the sun and from the sun heat.

Based on our data obtained from two experiments in natural infested fields on clay-loamy and medium-texture soils, for the same tomato cultivar (DRES F1), the 50 cm plowing depth overall reduced infestation by about 41% compared to the plowing depth of 30 cm, commonly adopted by farmers in Italy. However, this reduction was slightly lower (- 52.7%) in clay-loamy soil than in medium texture one (- 62.4%). Multiple shoots develop from the tubercle and emerge above the soil surface. Most of them (70%) ranged from 4 to 8.

Moreover, in another experiment, different infection levels by *P. ramosa* were detected between two commercial tomato varieties (SV5197 and SV8840) . The SV8840 results in a tolerant variety capable of enduring infection.

In both experiments, the largest number of *Peliphanche* emerged shoots provided a significantly reduced market yield, while no significant influence was found on the qualitative characteristics of the fruits.

Therefore, the results obtained in this study, carried out under infested field conditions, show that agronomic strategies, such as soil plowing depth and resistant tomato cultivars are suitable to reduce *P. ramosa* infestation. For the continuing reduction of parasite seed bank in the soil, the utilization of both the aforementioned methods could be even more efficient if they are used jointly.

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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

GD and LF conducted the research; GD, AT and FL analyzed the data; GD, FL, AT and LF wrote the manuscript; GD supervised the research. All authors approved the final version of the manuscript.

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Spatial variability of dendrometric parameters in a native tree *Mabea fistulifera* Mart. and its relationship with soil physical properties

Variabilidad espacial de parámetros dendrométricos del árbol nativo *Mabea fistulifera* Mart. y su relación con las propiedades físicas del suelo

Alessandra Ferreira Cortes[1], Ana Patrícia Lima Sampaio[1], Gloria Milena Rojas Plazas[2*], Carlos Matheus Santos da Costa [3], Rafael Montanari[3], and Job Teixeira de Oliveira[1]

ABSTRACT

Mabea fistulifera Mart. (common name: canudo-de-pito) belongs to the Euphorbiaceae family and is a native tree species in Brazil showing a high potential to recover degraded lands. This study aimed to evaluate the distribution and spatial correlation between the dendrometric parameters of the *M. fistulifera* plants and the physical attributes of the soil through geostatistics. The study was carried out at the Paulista State University (UNESP), in the city of Selvíria, MS, Brazil, in a typical dystrophic Red Oxisol with a clayey texture. The following properties were analyzed: for soil, penetration resistance, gravimetric moisture, particle density, and, for plants, circumference and diameter at breast height (measured at 130 cm above the ground), tree height, and total volume of the tree. An experiment grid was introduced with 35 sample points spaced 13 m x 13 m. Two soil samples were taken at each point, at 0.00 - 0.10 m, and 0.10 - 0.20 m depth. Descriptive data analysis and spatial dependence analysis were carried out through semivariogram adjustments and kriging maps. The dendrometric properties of the species *M. fistulifera* and the soil gravimetric moisture content showed spatial dependence. The spherical semivariogram model best explained the spatial structure of circumference at breast height, diameter at breast height, tree volume, and soil gravimetric moisture. There was an emphasis on the correlation between the total volume of the tree as a function of the diameter at breast height, showing a moderate spatial dependence. Furthermore, the tree diameter at breast height proved to be a good indicator for determining the total height of the *M. fistulifera* tree.

Key words: precision agriculture, geostatistics, kriging maps, canudo-de-pito.

RESUMEN

Mabea fistulifera Mart. (nombre común: canudo-de-pito) pertenece a la familia Euphorbiaceae y es una especie arbórea nativa de Brasil que presenta un alto potencial para ser utilizada en la recuperación de áreas degradadas. Este estudio tuvo como objetivo evaluar la distribución y correlación espacial entre parámetros dendrométricos de plantas de *M. fistulifera* y los atributos físicos del suelo a través de geoestadística. El estudio fue realizado en la Universidade Estadual Paulista (UNESP) en la ciudad de Selvíria, MS, Brasil; se utilizó un Oxisol rojo distrófico típico de textura arcillosa. Los atributos analizados del suelo fueron: resistencia a la penetración, humedad gravimétrica, densidad de partículas, mientras que en plantas se analizaron circunferencia y diámetro a la altura del pecho (medido a 130 cm del suelo), altura del árbol y volumen total del árbol. Se introdujo una malla experimental con 35 puntos de muestreo espaciados en 13 m x 13 m. Se tomaron dos muestras de suelo en cada punto, una de 0.00 - 0.10 m de profundidad y otra de 0.10 - 0.20 m de profundidad. El análisis de datos descriptivos y el análisis de dependencia espacial se realizaron mediante ajustes de semivariograma y mapas de kriging. Las propiedades dendrométricas de la especie *M. fistulifera* y la humedad gravimétrica mostraron dependencia espacial. El modelo de semivariograma esférico explicó mejor la estructura espacial de la circunferencia y diámetro a la altura del pecho, el volumen total del árbol y la humedad gravimétrica del suelo. Se hizo énfasis en la correlación entre el volumen total del árbol en función del diámetro a la altura del pecho, mostrando una dependencia espacial moderada. Además, el diámetro del árbol a la altura del pecho resultó ser un buen indicador para determinar la altura total de la planta de *M. fistulifera*.

Palabras clave: agricultura de precisión, geoestadística, mapas kriging, canudo-de-pito.

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[1] Federal University of Mato Grosso do Sul-UFMS, Campus Chapadão do Sul, Mato Grosso do Sul (Brazil).
[2] Federal University of Viçosa-UFV, Campus Viçosa, Minas Gerais (Brazil).
[3] Paulista State University, Campus de Ilha Solteira, São Paulo (Brazil).
[*] Corresponding author: gloria.plazas@ufv.br

Introduction

Mabea fistulifera Mart. belongs to the Euphorbiaceae family and is a native tree species popularly known in Brazil as "canudo-de-pito". Its trees reach height from 6 to 15 m with trunks up to 30 cm in diameter. It is found mainly in riverside areas in Brazil (Reflora, 2019). The wood of this species is used for small interior projects. The plant is not demanding in terms of soil fertility, showing a high potential to be used to recover degraded lands. It has the characteristics of improving the physical and chemical conditions of the soil and the microclimatic conditions of the area (Giácomo *et al.,* 2019).

According to Barroso *et al.* (2021), *M. fistulifera* can be used in phytoremediation programs for soils contaminated with hormonal herbicides. Their results showed that it can be used to compose riparian forests, preventing the entry of herbicides into the water. In a complementary manner, this tree species proved to be tolerant to the presence of hormonal herbicides and can be used for the recovery of natural areas in phytoremediation programs.

In agroforestry systems, planting *M. fistulifera* increases the soil organic matter content due to frequent pruning carried out on the plants (Farias & Souza, 2009). It is a plant with the ability to adapt, develops in open environments (forest edges), and accommodates itself in the forest shade by adapting its canopy architecture to small spaces (Carvalho *et al*., 2018). Also, its great economic potential for pollen production stands out, with flowering peaking from April to May, which coincides with the beginning of the dry season in the region and, consequently, a time of greater food scarcity for insects. During this period, many insect species use pollen as a food source (Daud & Feres, 2004).

Sustainable agricultural production and conservation of natural resources are parameters to be considered in the implementation of new agricultural practices. The current trend of an increasingly demanding market is to produce safer food, with reduced labor, and less environmental contamination, factors that contributed to the increase in the use of precision agriculture (Bassoi *et al*., 2019). Precision agriculture techniques are based on spatial variability and allow the application of fertilizers, correctives, seeds, and pesticides in specific areas, enabling rational use of inputs by identifying the location and correct dose of application, generating economic and environmental benefits (Ezenne *et al*., 2019).

Physical attributes of the soil are good indicators of the soil quality and assist management practices (Oliveira *et al*., 2021). The use of geostatistics characterizes the spatial variability of soil attributes that can determine management practices, such as fertilization and liming, and verify the vertical and horizontal heterogeneity of the soil (Gelain, 2021). Therefore, geostatistics, using semivariograms and kriging maps, can establish which soil attribute is associated with the development and production of plants.

Oliveira, Oliveira, Valente *et al.* (2020), studying the spatial correlation between agricultural productivity and mechanical resistance to penetration, found that crop development is negatively influenced by soil compaction. Therefore, regions where there is an increase in soil density, with a reduction in its porosity, show lesser development of the trees.

The study of forest species that are adapted to anthropized areas, such as *M. fistulifera*, is paramount for regeneration and protection projects in degraded lands (Gomes Júnior & Lopes, 2017). Information on the spatial variability of soil and plant properties in a *M. fistulifera* plantation is still limited. Thus, this study aimed to evaluate the distribution and spatial correlation of the *M. fistulifera* dendrometric properties and the physical attributes of the soil using geostatistics tools.

Materials and methods

The study was carried out in an experimental area at the Teaching, Research, and Extension Farm of the Faculty of Engineering of Ilha Solteira – Paulista State University (UNESP), in the city of Selvíria, MS, Brazil. The trees were planted in 2011, with spacing of 3 m x 3 m between plants, for recovery purposes in a degraded area.

The soil of the experimental area is classified as typical dystrophic Red Oxisol of clayey texture (Santos *et al*., 2018). The city is located at 20°20' S and 51°23' W, with an altitude of 335 m a.s.l. According to Köppen's precepts, mentioned by Alvares *et al.* (2014), the climate of the region is megathermic moisture tropical (Aw), with a rainy summer and a dry winter, with the rainiest months from December to March and the driest from June to September. The average annual temperature is 22.7°C with annual rainfall ranging from 1,200 mm to 1,500 mm (Martins & Montanari, 2021).

Initially, on April 12, 2019, a georeferencing was carried out with demarcation of 35 points distributed every 13 m divided into 5 rows of 7 points each (Fig. 1).

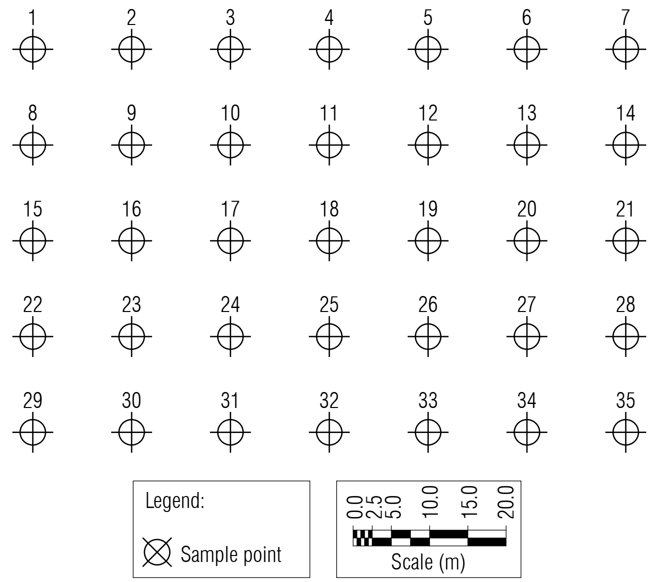

FIGURE 1. Detail of the sampling grid performed in *Mabea fistulifera* plantation at the Teaching, Research, and Extension Farm of the Faculty of Engineering of Ilha Solteira FEPE - UNESP, Brazil.

Then, a manual auger was used to remove 2 soil subsamples at each sampling site, in the 0.00-0.10 m, and 0.10-0.20 m layer. Subsequently, the 2 subsamples were mixed to obtain a representative sample of each sampling point. They were packed in plastic bags and sent to the laboratory.

The collected soil samples were crushed and passed through a 2.0 mm sieve. Then the samples were placed to dry on a paper for 1 d for later analysis.

The analyzed attributes in the soil layer 0.00-0.20 m were: penetration resistance (PR) (MPa), gravimetric moisture content (GM) (kg kg^{-1}), and particle density (PD) (kg dm^{-3}).

For the determination of gravimetric moisture, a soil sample was collected, simultaneously the PR was determined, using a cup auger. Gravimetric moisture content was calculated according to Equation 1 (Teixeira *et al*., 2017):

$$GM = \frac{WW - DW}{DW} \quad (1)$$

where: GM=gravimetric moisture content (kg kg-^{1}), WW=wet weight of soil (g), DW=oven-dry weight of soil (g) at 105ºC.

Soil particle density (PD) was obtained through the volumetric flask method (Teixeira *et al.,* 2017). The volume of alcohol needed to complete the capacity of a 50 ml volumetric flask containing oven-dried soil was determined. Then the PD was calculated using Equation 2 (Teixeira *et al*., 2017):

$$PD = \frac{MSD}{50 - V} \quad (2)$$

where: PD=particle density (g cm^{-3}), MSD=mass of the soil dried at 105ºC (g), and V=volume of alcohol used (m^3).

The soil penetration resistance (PR) was evaluated by the impact penetrometer and calculated according to Equation 3 (Rosa Filho *et al*., 2009):

$$PR = (5.581 + 6.891)\left(\frac{N}{P-A}10\right)0.981 \quad (3)$$

where PR = penetration resistance of the soil (MPa), N = number of impacts given by the penetrometer hammer to obtain the readings, A and P are the readings before and after the impacts (cm), respectively. The Penetration Resistance 1 (PR1) was obtained in the 0.00-0.10 m layer, the Penetration Resistance 2 (PR2) was obtained in the 0.10-0.20 m layer, and the Average Soil Penetration Resistance (APR) was obtained in the 0.00-0.20 m layer.

The dendrometric variables of the plant included: trunk circumference at breast height (CBH), measured at 1.30 m above the ground with the aid of a tape measure; trunk diameter at breast height (DBH), calculated from CBH (cm) using the formula: CBH/π. Plant height (PH) (m) was measured with a Haglof® electronic clinometer at 10 m away from the tree, and tree crown volume (TV) was calculated from the stem + crown dimensions using Equation 4 (Martins & Montanari, 2021):

$$TV = \frac{\pi(DBH)^2}{4} * (PH) * 0.45 \quad (4)$$

Statistical analysis

Pearson correlations were used to study the dendrometric properties and physical attributes of the soil. A network of correlations was obtained and tabled. The positive correlations were highlighted in green, while the negative ones were highlighted in red. Line thicknesses express the magnitude of the correlation between the attributes; thicker lines represent high magnitude, thinner lines indicate low magnitude.

For all attributes analyzed, a descriptive analysis of parameters was carried out, including mean, minimum, maximum, standard deviation, coefficient of variation (CV), kurtosis, asymmetry, test probability, and frequency distribution (FD). Pimentel-Gomes and Garcia (2002) follow a classification based on CV values: low CV < 10%;

medium (10% < CV < 20%); high (20% < CV < 30%) and very high (CV > 30%). To test the hypothesis of normality of the attributes, the Shapiro-Wilk (1965) test was used at a 5% error probability. In it, the W statistic tests the null hypothesis that considers the sample coming from a population with sampling distribution (Dalchiavon, 2012). All analyses were carried out using the RBio software (Bhering, 2017).

Spatial dependence was analyzed through semivariogram adjustments (Vieira *et al.*, 2002), based on the stationarity assumption of the intrinsic hypothesis, which is estimated by Equation 5:

$$\bar{y}(h) = \frac{1}{2N(h)} \sum_{i=1}^{N(h)} . [Z(xi) - Z(xi + h)]^2 \quad (5)$$

where: N(h)= the number of observed experimental pairs $Z(x_i)$ and $Z(x_i + h)$ separated by a distance h. The semivariogram is represented by the graph ŷ (h) *versus h* whose adjustments were made by mathematical theoretic models with the aid of the GS+ 7.0 software that characterized the parameters of the semivariogram and the spatial dependency of the attributes.

The adjustments of the models were carried out by selecting the sum of the square of the residue (SSR), coefficient of determination (r^2), and spatial dependence evaluator (SDE).

The following parameters were used to adjust a mathematical model to the data: nugget effect (C_0), contribution (C_1), sill ($C_0 + C_1$), and range (A_0). The spatial dependence evaluator (SDE) was calculated using Equation 6:

$$SDE\ (\%) = \left(\frac{C_0}{C_0 + C1}\right) * 100 \quad (6)$$

According to Cambardella *et al.* (1994), the SDE values have the following classification: strong spatial dependence (SDE(%) ≤ 25%), moderate spatial dependence (25% < SDE(%) ≤ 75%), and weak spatial dependence (SDE(%) > 75%).

For the adjustment of the semivariograms, cross-validation was carried out to evaluate alternative models of simple and crossed semivariograms, using kriging and cokriging maps (Oliveira, Oliveira, Oliveira *et al.*, 2020), which are very informative descriptors in terms of detail in the graphics. In this technique, each point contained within the spatial domain is excluded individually, and its value is estimated as if it did not exist. In this way, it is possible to construct a graph of estimated values versus observed values for all analyzed points.

To obtain the ideal number of neighbors, the data were interpolated, and then the spatial dependence and interdependence between the attributes were evaluated, using kriging and cokriging maps.

Results and discussion

The junctions between the dendrometric properties of the species *M. fistulifera* and the physical attributes of the soil were determined using Pearson's correlation coefficients to construct a network of correlations. Circumference at breast height (CBH) was positively correlated with the diameter at breast height (DBH), and CBH and DBH were positively correlated with the tree crown volume TV and plant height PH (Fig. 2). This result shows the close relationship existing between the dendrometric indices of the plants studied. Due to the plant's arboreal size, it is used at the edges of forests with accentuated anthropic impact as a windbreak, as well as in the recovery of degraded lands, improving the physical and chemical conditions of the soil (Daud & Feres, 2004).

APR expressed a positive correlation with PR1, as well as with PR2. Notably, Oliveira, Oliveira, Valente *et al.* (2020) report similar results and highlight the importance of studying penetration resistance to better understand the development of agricultural crops.

The GM had low and negative correlations with APR, PR1, PR2, and PD. The increased GM eases the penetration of the penetrometer, as reported by Oliveira *et al.* (2021) in studies of soil moisture, porosity, and density in an Oxisol. These regions of lower resistance to penetration are precisely those that have greater ease of root development for agricultural crops.

The statistical parameters of the dendrometric attributes in Table 1 tended to be normal for CBH and DBH; normal for PH; undetermined for TV. The frequency distribution of the physical attributes was normal for GM, PD, PR1, and indeterminate for PR2. APR showed a distribution tending to normal.

The dendrometric properties of this species have a positive effect on the recovery of degraded areas, emphasizing the soil protection against adverse climatic agents, soil conservation, addition of organic matter to the soil, mobilization and recycling of mineral nutrients, and soil biological activity (Giácomo *et al.*, 2019). Although the observed average values of penetration resistance, APR of 4.40 MPa, PR1 of 4.37 MPa, and PR2 of 4.43 MPa, were very high, they were not enough to limit the timber yield of the forest studied, as also seen by Barbosa *et al.* (2012). This verification is important because it evidences the capacity of the *M. fistulifera* to develop in compacted soils, deepening the roots even in situations of reduced soil porosity. Once again, it clarifies the species ability to recover degraded areas, contributing to the environment and improving the soil quality around planting sites.

The CV values for the attributes TV, PR2, PR1, APR, and GM were very high (74.01%, 49.48%, 47.57%, 37.84%, and 33.61%, respectively). The coefficient of variation for CBH and DBH was 26.85%. PH (18.72%) showed medium CV, whereas PD had a low CV value (3.82%), as shown in Table 1.

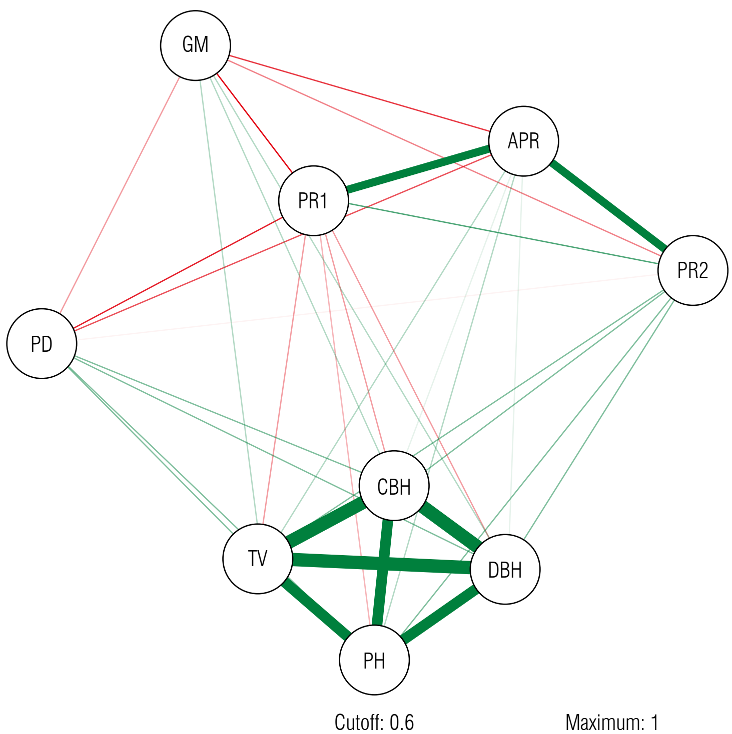

FIGURE 2. Pearson correlations networks of variables studied: CBH – tree circumference at breast height (cm); DBH – tree diameter at breast height (cm); PH - plant height (m); TV - tree crown volume (m^3); GM – soil gravimetric moisture content (kg kg^{-1}); PD – soil particle density (g cm^{-3}); APR - average soil penetration resistance (MPa); PR1 – soil penetration resistance 1 (MPa); PR2 – soil penetration resistance 2 (MPa).

TABLE 1. Descriptive statistics for the dendrometric properties of the *Mabea fistulifera* and physical attributes of the soil.

Variable[a]	Value		Mean	Standard	Coefficient			Test probability	
	Minimum	Maximum		deviation	Variation (%)	Kurtosis	Asymmetry	Pr>F[b]	FD[c]
CBH	12.300	35.60	20.59	5.53	26.85	-0.17	0.73	0.046	TN
DBH	3.915	11.33	6.55	1.76	26.85	-0.17	0.73	0.046	TN
PH	4.700	11.20	8.05	1.51	18.72	-0.69	0.18	0.575	NO
TV	0.003	0.05	0.01	0.01	74.01	1.24	1.37	8.69E-05	UN
GM	0.001	0.08	0.05	0.02	33.67	0.48	-0.30	0.65	NO
PD	2.133	2.82	2.53	0.10	3.82	7.40	-1.25	4.39E-06	UN
APR	1.900	9.69	4.40	1.66	37.84	1.171	1.046	0.0256	TN
PR1	1.224	10.69	4.37	2.08	47.57	1.229	1.115	9.71E-03	UN
PR2	1.900	9.88	4.43	2.19	49.48	0.322	1.126	2.83E-04	UN

[a] CBH – tree circumference at breast height (cm); DBH – tree diameter at breast height (cm); PH - plant height (m); TV - tree crown volume (m^3); GM – soil gravimetric moisture content (kg kg^{-1}); PD – soil particle density (g cm^{-3}); APR - average soil penetration resistance (MPa); PR1 – soil penetration resistance 1 (MPa); RP2 – soil penetration resistance 2 (MPa).

[b] Shapiro-Wilk test.

[c] FD = frequency distribution: NO - normal, TN - tending to normal, UN - undetermined.

TABLE 2. Estimated parameters for the simple and crossed semivariogram of the dendrometric properties of the *Mabea fistulifera* and physical attributes of the soil.

Attribute	Model	Range			r^2	SSR	SDE		Cross-validation		
		Nugget (C_0)	Sill (C_0+C)	(A_0) (m)			(%)	Class	a	b	R
Simple semivariogram											
CBH	sph	14.30	33.30	52.4	0.946	4.860	57.1	Moderate	1.34	-6.72	0.570
DBH	sph	1.43	3.37	52.2	0.947	0.049	57.5	Moderate	1.33	-2.11	0.571
PH	gauss	1.09	2.53	34.3	0.680	0.357	57.0	Moderate	0.91	0.78	0.457
TV	sph	6.20E-05	1.48E-04	74.5	0.998	5.42E-12	58.4	Moderate	1.22	0	0.480
GM	sph	1.50E-04	3.06E-04	74.8	0.925	6.51E-10	51.0	Moderate	0.79	0.001	0.356
PD	pne	9.31E-03	9.31E-03	-	-	-	-	-	-	-	
APR	pne	2.61	2.61	-	-	-	-	-	-	-	
PR1	pne	4.22	4.22	-	-	-	-	-	-	-	
PR2	pne	4.41	4.41	-	-	-	-	-	-	-	
Crossed semivariogram											
TH-f(DBH)	sph	1.42	2.80	73.00	0.909	0.493	49.3	Moderate	0.84	1.33	0.546
TV-f(DBH)	sph	9.52E-02	2.16E-02	68.8	0.990	4.19E-07	56.0	Moderate	0.90	0	0.571
TV-f(PH)	sph	8.14E-02	1.69E-02	79.0	0.929	2.26E-06	51.7	Moderate	0.90	0	0.571

CBH – tree circumference at breast height (cm); DBH – tree diameter at breast height (cm); PH - plant height (m); TV - tree crown volume (m^3); GM – soil gravimetric moisture content (kg kg^{-1}); PD - soil particle density (g cm^{-3}); APR - average soil penetration resistance (MPa); PR1 – soil penetration resistance 1 (MPa); PR2 – soil penetration resistance 2 (MPa); gauss - gaussian; sph - spherical; pne - pure nugget effect; SSR - sum of squared residue; SDE - spatial dependence evaluator.

The kurtosis and asymmetry values measure the average dimension of the deviation of the values of a set of data in relation to a certain Measure of Central Tendency as well as how these data are allocated around this Measure (Oliveira, Oliveira, Valente *et al*., 2020). Thus, we observed in Table 1 that GM and PD were negative asymmetrical and the attributes CBH, DBH, and PH have a more pointed histogram.

The parameters of the simple and crossed semivariograms for the phenological indices of the *M. fistulifera* plant and the physical attributes of the soil are shown in Table 2 and Figure 3. The parameters evaluated for the models adjusted to the semivariograms showed high values of r^2, which indicates high reliability in the settings. The spherical model was the fittest for the dendrometric properties CBH, DBH, TV, and the soil attribute GM, as in Costa *et al*. (2020) and Dantas *et al*. (2020). Notably, Costa *et al*. (2020) also reported the spherical model as the fittest for the dendrometric variables of diameter at breast height and volume in the study of the spatial variability of *Swietenia macrophylla* in an agroforestry system in the Brazilian Amazon.

Dantas *et al*. (2020) studied volumetric prediction through kriging to reduce sampling effort in forest inventories and concluded that the spherical model is the fittest for studying tree crown volume. Greco *et al*. (2005) mentioned that the spherical model is more identified in soil science studies. PH fitted best to the Gaussian model, in agreement with the results obtained by Barbosa *et al*. (2012) and Costa *et al*. (2020), who also obtained the Gaussian model as the fittest for the study of height in forest tree species. The soil attributes PD, APR, PR1, and PR2 obtained a pure nugget effect.

According to the classification by Cambardella *et al*. (1994), SDE values of <25%, 25%-75%, and >75% have a weak, moderate, and strong spatial dependence, respectively. Based on this classification, the values of the variables CBH, DBH, PH, TV, and, GM showed moderate SDE.

The range of the semivariogram corresponds to the maximum distance that correlation or spatial dependence can be found, being the main parameter provided by geostatistics. Furthermore, the spatial behavior of the variable is completely random (Behera *et al*., 2018). The evaluated properties ranged from 0.01 (tree volume, m^3) to 8.05 (plant height, m) average. The ranges found can be used to guide future sampling plans in studies related to plant dendrometric properties and soil physical attributes in a *M. fistulifera* crop.

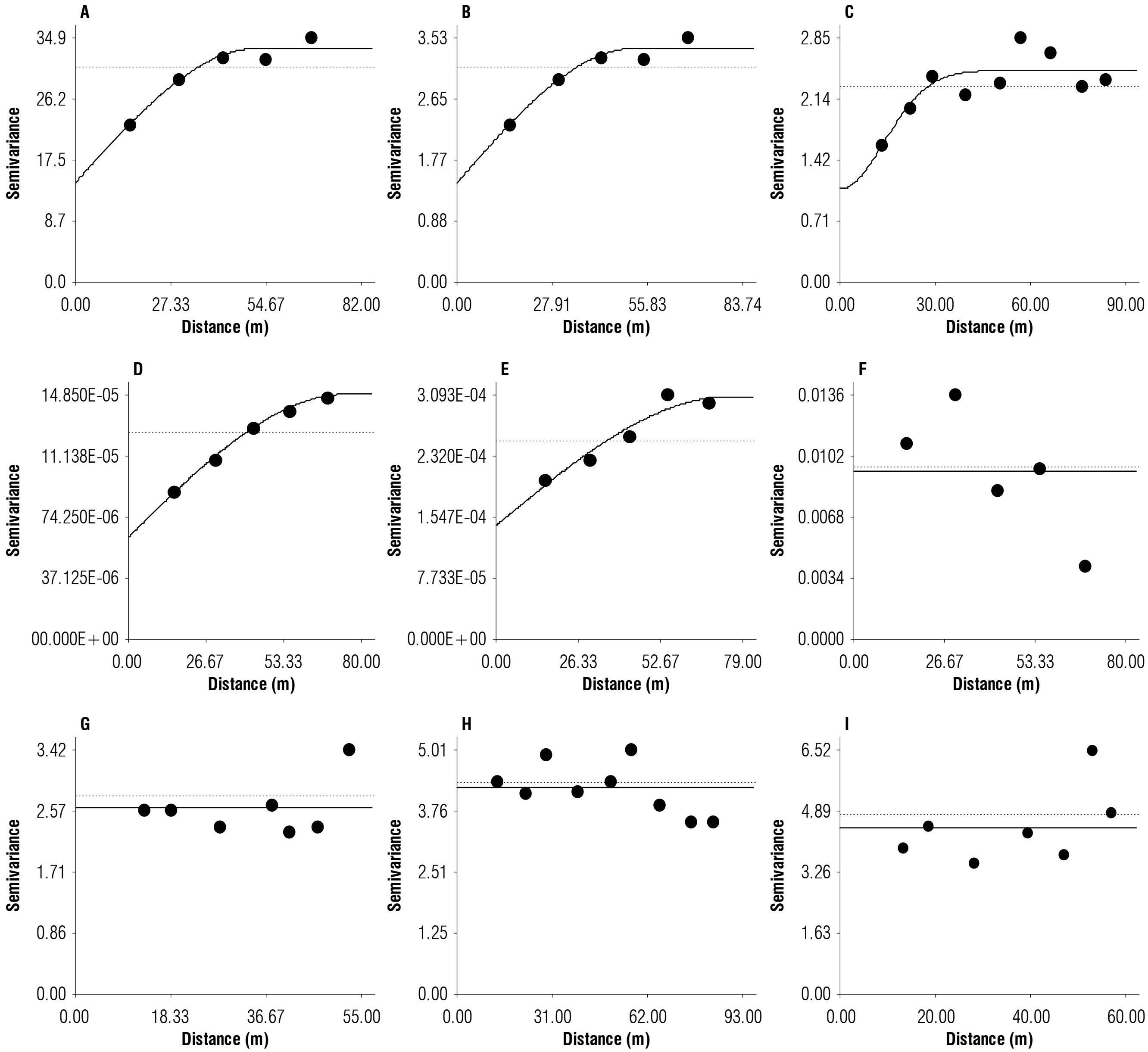

FIGURE 3. Simple semivariograms adjusted for dendrometric properties and soil physical attributes in a *Mabea fistulifera* crop, where: A) tree circumference at breast height (cm); B) tree diameter at breast height (cm); C) plant height (m); D) tree volume (m^3); E) soil gravimetric moisture content (kg kg^{-1}); F) soil particle density (g cm^{-3}); G) average soil penetration resistance (MPa); H) soil penetration resistance 1 (MPa); I) soil penetration resistance 2 (MPa).

Figure 4 shows the pattern for spatial variability of the dendrometric properties of the *M. fistulifera* and the gravimetric soil moisture content estimated through ordinary kriging. The variability maps showed a relationship between DBH, PH, and TV variability.

Regarding the soil physical attribute (GM) evaluated in the present study, no link that could influence the development of the *M. fistulifera* was found in the spatial variability pattern. Rosa Filho *et al.* (2011) emphasized that it was not possible to establish a cause-effect relationship between the dendrometric properties of the forest trees under study and the physical attributes of the soil (gravimetric moisture content and penetration resistance).

In the crossed semivariogram (Fig. 5), among the dendrometric properties of the *M. fistulifera,* PH-f(DBH), TA-f(DBH), and TV-f(PH), moderate spatial dependence

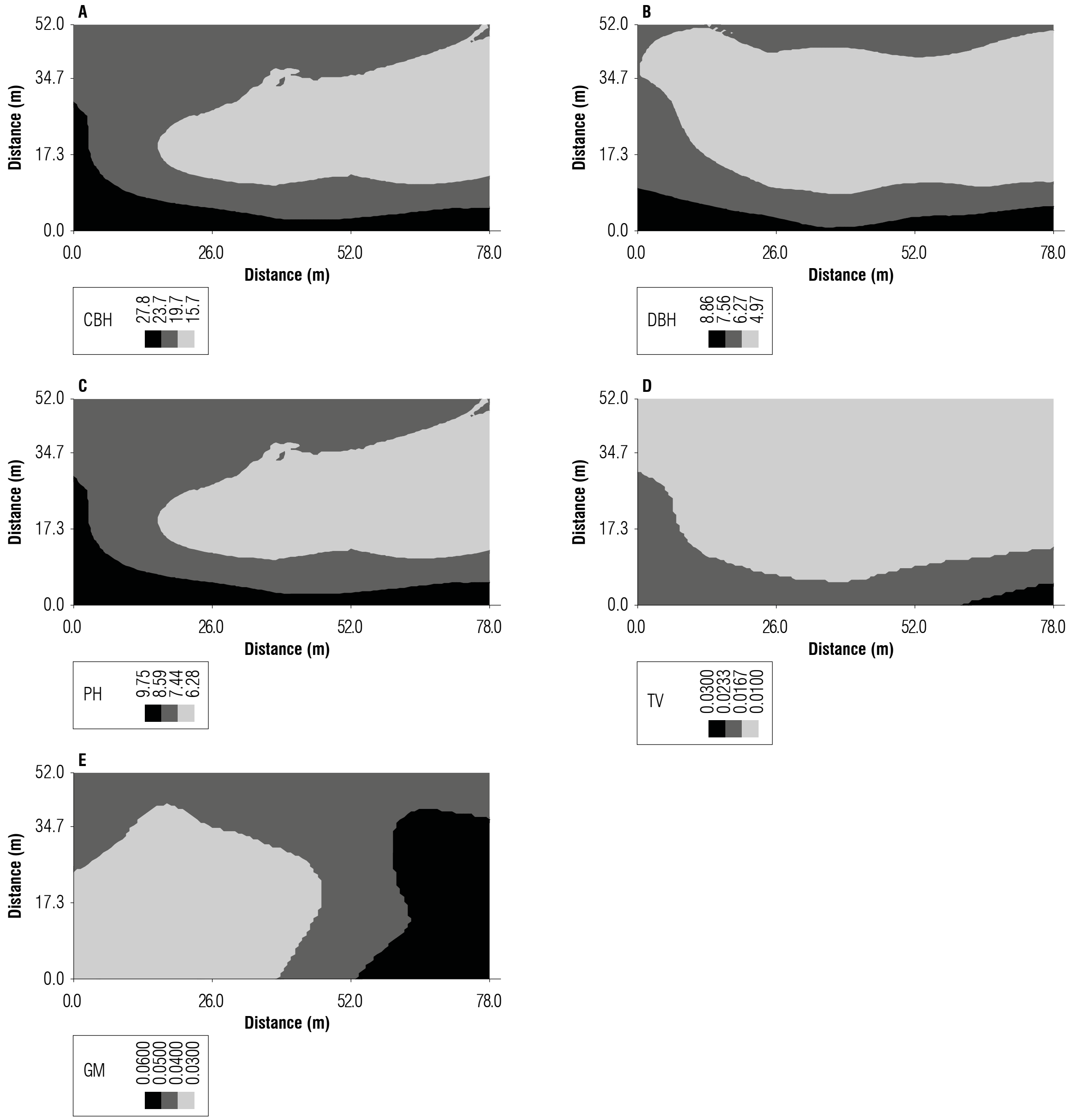

FIGURE 4. Simple kriging maps of dendrometric properties and soil physical attributes in a *Mabea fistulifera* crop, where: A) tree circumference at breast height (cm); B) tree diameter at breast height (cm); C) plant total height (m); D) tree volume (m^3); E) soil gravimetric moisture content (kg kg^{-1}).

was obtained (49.3, 56.0, and 51.7, respectively) and the fittest model was the spherical one for the three cases. The cokriging map (Fig. 5) shows a similar spatial structure between the volume and the one observed in simple kriging (Fig. 4). Based on the r^2 in Table 2 (0.990 and 0.929 for TV- f(DBH) and TV-f(PH), respectively) and SDE, it can be inferred that DBH is the best estimator for the TV of the *M. fistulifera*.

DBH was also shown to be a useful explanatory variable for estimating the PH of the *M. fistulifera*, with an r^2=-0.909. This is important, as determining DBH in the field may be easier than determining PH.

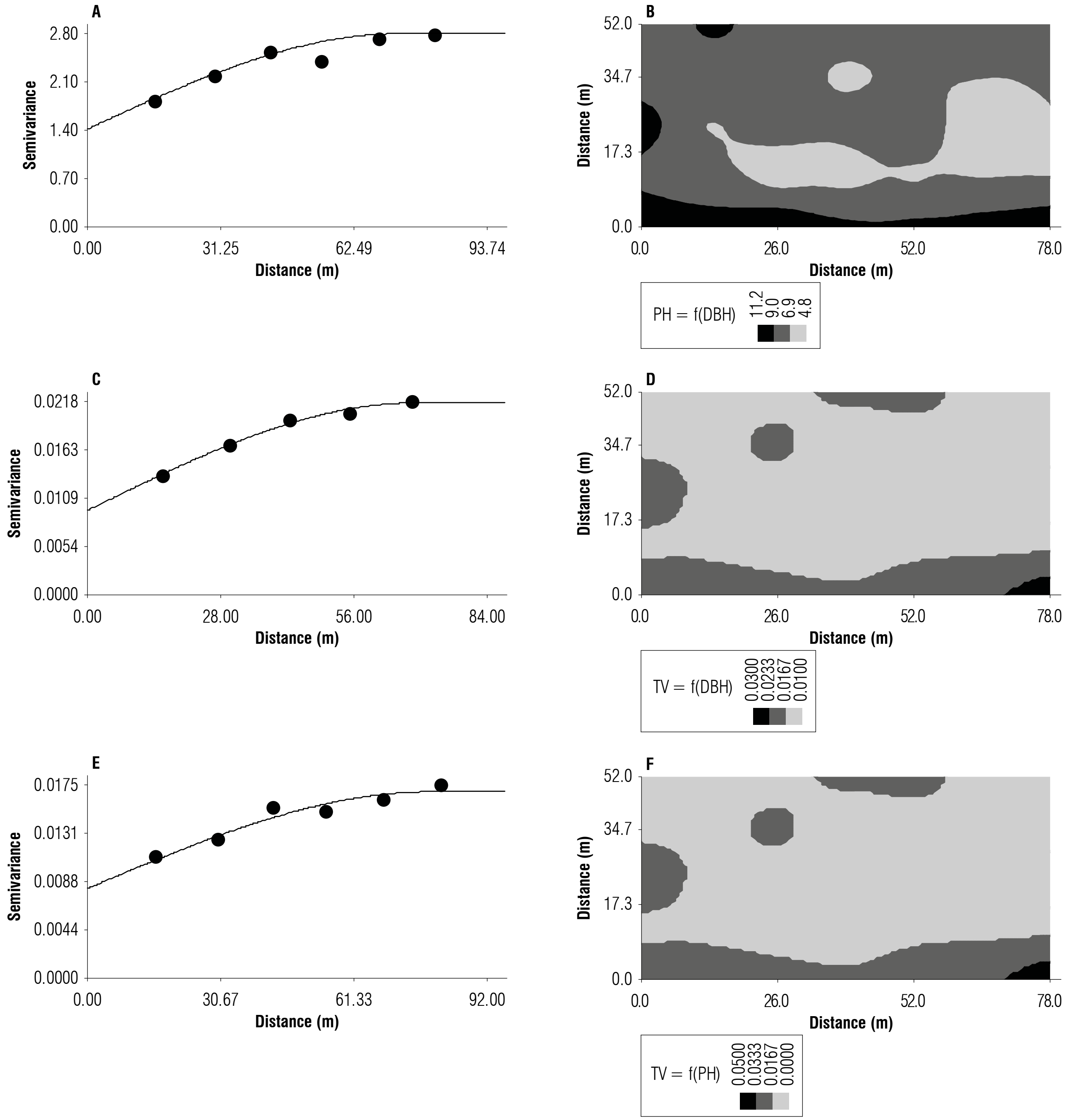

FIGURE 5. Crossed semivariogram and kriging map for: (A) tree height as a function of diameter at breast height; (B) tree crown volume as a function of diameter at breast height; and (C) tree crown volume as a function of height.

Conclusions

The dendrometric properties CBH, DBH, PH, TV, and the soil physical attribute GM showed spatial dependence. The spherical semivariogram model best explained the spatial structure of CBH, DBH, PH, TV, and GM.

There was a correlation of dendrometric properties between TV and DBH, showing moderate spatial dependence. Additionally, DBH proved to be an effective indicator for determining the PH of the *Mabea fistulifera* plant.

Conflict of interest statement

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

Author's contributions

CMSC and RM designed and conducted the experiments. AFC, APLS, GMRP, and JTO performed the data analysis and wrote the manuscript. JTO edited the manuscript. All authors reviewed the final version of the manuscript.

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Spatial correlation of soybean yield with the chemical attributes of an Oxisol

Correlación espacial del rendimiento de soya con los atributos químicos de un Oxisol

Luiz Claudio Rodrigues[1], Tulio Russino Castro[1], Cassiano Garcia Roque[1], Fernando França da Cunha[2], Fabio Lima Abrantes[1], Gabriela Vitória Souza[1], and Job Teixeira de Oliveira[1*]

ABSTRACT

Soybean is currently the most cultivated oilseed in the world. The objective of this study was to analyze the chemical attributes of Oxisol correlated with soybean productivity. The attributes evaluated were grain productivity, soil properties of organic matter contents, phosphorous, pH, potassium, calcium, magnesium, hydrogen + aluminum, aluminum, sum of bases, cation exchange capacity (CEC), base saturation, liming requirement; penetration resistance at 0.0-0.10 m, 0.10-0.20 m, 0.20-0.30 m, and 0.30-0.40 m. Soil chemical attributes were submitted to a descriptive classic analysis with the help of the RBio software. In the semivariogram analysis, the attributes calcium, magnesium, aluminum, sum of bases, base saturation, liming requirement, and penetration resistance at 0.30-0.40 m showed a pure nugget effect, meaning no spatial dependence. In conclusion, CEC was the attribute that best described the soybean yield data in dystrophic Red Latosols. Attributes of grain productivity, soil organic matter, phosphorus, pH, potassium, hydrogen + aluminum, CEC, penetration resistance at 0.00 to 0.30 m showed spatial dependence.

Key words: soil properties, semivariogram, kriging, cokriging.

RESUMEN

La soya es actualmente la oleaginosa más cultivada en el mundo. El objetivo de este estudio fue analizar los atributos químicos de Oxisol correlacionados con la productividad de soya. Los atributos evaluados fueron productividad de grano y las propiedades edáficas contenido de materia orgánica, fósforo, pH, potasio, calcio, magnesio, hidrógeno + aluminio, aluminio, suma de bases, capacidad de intercambio catiónico (CIC), saturación de bases, requerimiento de encalado; resistencias a la penetración 0.0-0.40 m, 0.10-0.20 m, 0.20-0.30 m y 0.30-0.40 m. Los atributos químicos fueron sometidos a un análisis descriptivo clásico, con la ayuda del programa RBio. En el análisis del semivariograma, los atributos calcio, magnesio, aluminio, suma de bases, saturación de bases, requerimiento de encalado y resistencia a la penetración 0.30-0.40 m mostraron un efecto pepita puro, es decir, sin dependencia espacial. En conclusión, la CIC es la propiedad que mejor describió los datos de rendimiento de soya en el Latosol Rojo distrófico. La productividad del grano, contenido de materia orgánica, fósforo, pH, potasio, hidrógeno + aluminio, CIC, y la resistencia a la penetración de 0.00 a 0.30 m mostraron dependencia espacial.

Palabras clave: propiedades de suelo, semivariograma, kriging, cokriging.

Introduction

Among the current wide variety of oilseeds in the world, soybean (*Glycine max* [L.] Merrill) is the main cultivated crop. Due to its high levels of adaptability in tropical and subtropical climates, combined with high productive potential, soybean has become the most produced grain in the world (Piemontez *et al.,* 2021). Added to this is the important use of it as a natural grain, industrialized food, and biofuel.

In recent years, because of high demand in the productive sector, soybean production has increased exponentially worldwide. Notably in Brazil, production follows the global advance. According to estimates of the *Coompanhia Nacional de Abastecimento* - Conab (2022), the planted area of the crop in the 2021 and 2022 would reach 40.58 million ha, an increase of 35% compared to the 2020 and 2021 harvests.

Agricultural areas in the Cerrado region are ideal for the development of Brazilian agriculture and production, mainly due to the favorable tropical climate and soils, especially Oxisols with good characteristics that facilitate mechanization and management practices (Vilela *et al*., 2020; Ratke *et al*., 2023).

Among the great need to expand soil cultures, mineral resources in the soil must be maintained so that high

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[1] Universidade Federal de Mato Grosso do Sul, Chapadão do Sul (Brazil).
[2] Universidade Federal de Viçosa, Viçosa (Brazil).
[*] Corresponding author: job.oliveira@ufms.br

productivity is always achieved. Knowing and understanding all the spatial variability of soil attributes is essential because it allows for more coherent decision-making regarding the management that will be adopted. It is important to point out that the chemical, physical, and biological conditions of soil are extremely important for obtaining satisfactory agricultural production.

Roots can homogeneously exploit the soil for root development under ideal conditions when the soil volume is relatively greater than in soils with compaction problems (Valadão *et al*., 2015). Some compaction states cause changes in soil structure, resulting in increased soil density and soil resistance to soil penetration (Cortez *et al*., 2018; Oliveira *et al.,* 2020).

In geostatistics, studies used in precision agriculture from simple and crossed interpolation of data (kriging and cokriging) refers to the physical attributes of the soil that allow an estimate of the spatial variability of a given variable by means of another variable that allows ease of determination (Montanari *et al*., 2015).

This study aimed to correlate the soil resistance to penetration and chemical properties of a dystrophic Red Latosol, performing mapping, and evaluating the correlation between soybean yield and soil attributes.

Materials and methods

This research was carried out in the municipality of Chapadão do Céu-GO, on the premises of the Fazenda Independência and its arable area during the 2017/2018 harvest period. The experimental site is located in the southern mesoregion of Goiás at the following geographic coordinates: 18º24'17"S, 52º44'57"W at 825 m a.s.l. The region has a megathermic humid tropical climate (Aw), according to the Köppen classification with two well-defined seasons: dry winter and humid summer with rainfall concentrated from September to March and annual averages between 1,400 and 2,200 mm and air temperatures ranging from 17ºC to 30ºC. The soil was classified as typical dystrophic Red Latosol with a very clayey to moderate epidystrophic texture (Santos *et al.,* 2018).

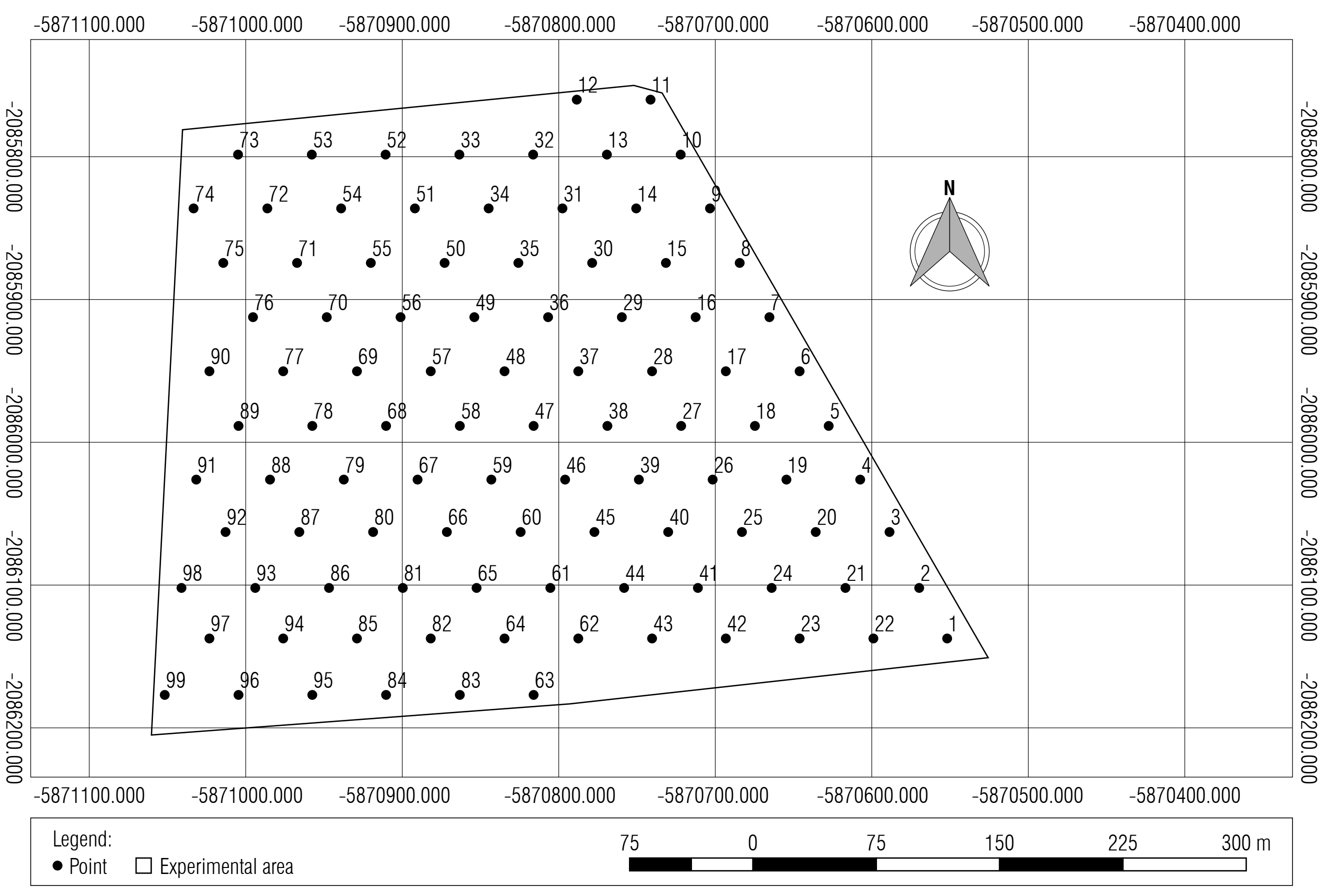

FIGURE 1. Representation of the sampling mesh of the planting bed.

Soil liming was carried out in July 2015, and the sequence of crops adopted was soybean and subsequently millet until the start date of the experiment. The soybean cultivar used was Desafio with pre-planting treatment. Sowing took place on November 15, 2017 under millet straw in a no-tillage system. The plant density was 310 thousand plants/ha.

Soybean fertilization was carried out as recommended with fertilizer applied at sowing using 78 kg ha^{-1} of P_2O_5 and 16 kg ha^{-1} of N (monoammonium phosphate—MAP). At 30 d after sowing (V4 stage—four fully expanded leaves, *i.e.*, fourth trifoliolate), and 80 kg ha^{-1} of K_2O (KCl) was applied in topdressing.

Soybean seeds used in this experiment were pretreated with pyraclostrobin + methyl thiophanate + fipronil (at a dose of 2 ml kg^{-1}) and then inoculated with efficient *Bradyrhizobium* spp. strains at a dose of 3.0 ml kg^{-1} as recommended by the supplier.

The total area of the experiment equaled 22 ha, and we collected soil samples from points in a regular grid of 40.0 × 40.0 m, totaling 99 points (Fig. 1). The deformed soil samples were collected on March 20, 2018 using a mug auger (0.0-0.2 m) with 4 simple sub-samples collected to obtain a composite sample.

Subsequently, the soil samples were sent for determination of chemical properties (Teixeira *et al*., 2017). The chemical attributes were as follow: pH_{CaCl2}, phosphorus (P) (mg dm^{-3}), potassium (K) (mmol$_c$ dm^{-3}), calcium (Ca) (mmol$_c$ dm^{-3}), magnesium (Mg) (mmol$_c$ dm^{-3}), aluminum (Al) (mmol$_c$ dm^{-3}) and sum of bases (SB) (mmol$_c$ dm^{-3}), acid potential (H-Al) (mmol$_c$ dm^{-3}), cation exchange capacity (CEC) (mmol$_c$ dm^{-3}), base saturation (V) (%), organic matter contents (OM) (g dm^{-3}) and the amount of liming required (LR) to increase the soil base saturation to 70%. Grain productivity (GP) was obtained from the harvest carried out in the total area of the experiment by a John Deere® harvester model S and totaled 670 kg ha^{-1} on March 5, 2018.

Mechanical resistance to penetration of soil was determined in kPa with a digital Falker® penetrolog at the soil depths of 0.0-0.10 m (PR1), 0.10-0.20 m (PR2), 0.20-0.30 m (PR3), and 0.30-0.40 m (PR4).

Statistical analysis

Soil chemical properties were submitted to a descriptive classical analysis using Rbio software (Bhering, 2017). Mean, minimum, and maximum values, standard deviation, coefficient of variation, kurtosis, asymmetry, and frequency distribution were calculated. To test the hypothesis of normality, the Shapiro & Wilk (1965) test at 5% probability was used. To assess the degree of relationship between the variables involved in the modeling process, Pearson's correlation analysis was performed, aiming to perform simple linear regressions for the combinations, two by two, among all the attributes studied. Combinations with the highest linear correlation were selected for regression modeling.

For the classification of the coefficient of variation (CV), the following variability classes and magnitudes were adopted: low (CV≤10%), medium (10%<CV≤20%), high (20%<CV≤30%), and very high (CV>30%) (Pimentel-Gomes & Garcia, 2002).

An analysis of spatial dependence of the soil chemical properties was performed by calculating the semivariogram, based on the stationarity assumptions of the intrinsic hypothesis using the Gamma Design Software GS$^+$.

For each attribute, the nugget effect (C_0), the reach (A_0), and the threshold (C_0 + C) were related. A dependency evaluator analysis (DEA) was performed following Cambardella *et al.* (1994) and according to Equation 1:

$$DEA = [C/(C + C0)] \times 100 \quad (1)$$

The proposed interpretation for the DEA was: 0≤DEA<0.25 = weak; 0.25≤DEA<0.75 moderate; 0.75≤DEA<1 = strong.

Results and discussion

Figure 2 shows the network of correlations between the attributes studied, where V% has significant correlations of the same intensity with Ca, Mg, SB, the same as Al and H-Al. In this case, differences are the types of correlation with each character. Related to the Ca, Mg, and SB, the correlation was positive and strong; and with Al and H-Al, the correlation was negative and strong. Cations such as Ca and Mg are adsorbed on the surface of the soil colloids, and the percentage of these cations that occupy the CEC is called base saturation (V%) (Gorski *et al*., 2023). When V is high, that is, greater than or equal to 50%, the soil is eutrophic, rich in nutrients, especially in Ca and Mg.

The data from the descriptive analysis of the attributes under study are shown in Table 1. From these analyses, we see a considerable variation. However, simply knowing this change is not enough to identify high-value locations for any attribute, nor low-value locations. Geostatistics must be used to determine if there is spatial variability and how

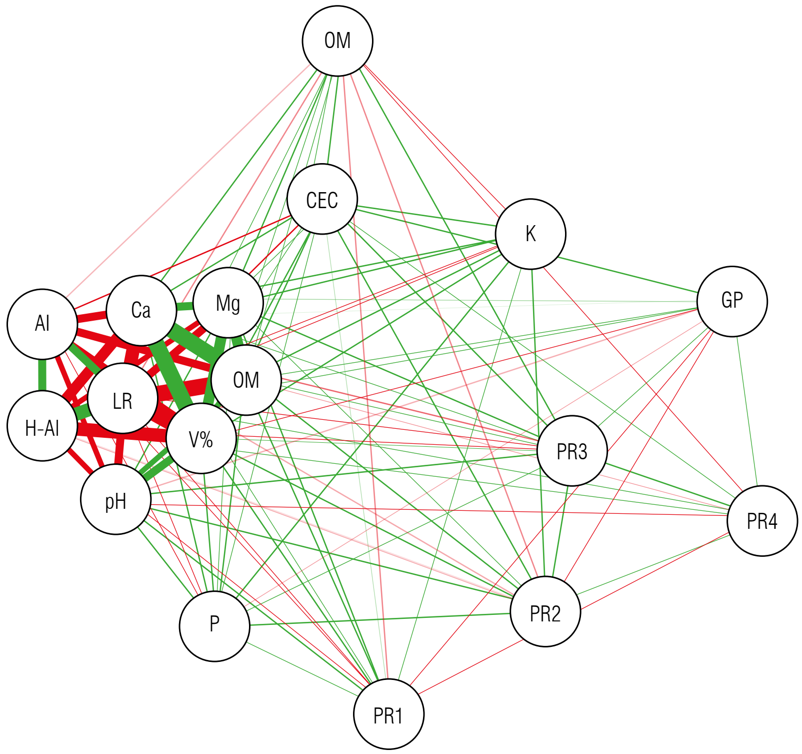

FIGURE 2. Correlation network of the attributes studied: grain productivity (GP) in kg ha^{-1}; soil organic matter (OM) in g dm^{-3}; phosphorus (P) cmol$_c$ dm^{-3}; hydrogen potential (pH); potassium (K) in cmol$_c$ dm^{-3}; calcium (Ca) in cmol$_c$ dm^{-3}; magnesium (Mg) in cmol$_c$ dm^{-3}; hydrogen + aluminum (H-Al) in cmol$_c$ dm^{-3}; aluminum (Al) in cmol$_c$ dm^{-3}; sum of bases (SB); cation exchange capacity (CEC); base saturation (V%); liming requirement (LR) in t ha^{-1}; penetration resistance 0.0-0.10 m (PR1) in kPa; penetration resistance 0.10-0.20 m (PR2) in kPa; penetration resistance 0.20-0.30 m (PR3) in kPa; penetration resistance 0.30-0.40 m (PR4) in kPa.

this variability occurs in the study area, and subsequently maps must be created to manage the necessary field interventions more accurately.

Table 1 presents the descriptive analysis of the productive components studied. The variability of a property can be classified according to the magnitude of its coefficient of

TABLE 1. Descriptive statistics for soil properties and soybean productivity.

Property	Average	Min	Max	Standard deviation	Variation (%)	Kurtosis	Asymmetry	Pr<w
GP	9433.69	3915.2	12875.7	1872.91	19.85	0.24	0.48	1.3775
OM	31.16	26.5	35	2.08	6.70	-0.86	-0.22	0.3676
P	44.59	20	105	16.46	36.92	1.17	1.03	1.9999
pH	4.85	4.5	6.6	0.26	5.42	18.01	3.19	0.3900
K	1.51	0.7	2.3	0.28	18.93	0.04	-0.01	0.9615
Ca	30.54	17	46	6.64	21.75	-0.78	0.22	1.6291
Mg	9.67	5	15th	1.94	20.116	0.01	0.32	1.8151
H-Al	39.48	22	52	6.88	17.43	-0.32	-0.11	0.6363
Al	1.51	0	3	0.77	51.15	-0.39	-0.49	0.0478
SB	41.94	24.5	62.8	8.69	20.72	-0.72	0.29	1.7682
CEC	81.22	72.2	96	4.63	5.70	-0.19	0.20	1.5958
BS	51.29	32	72	09/19	17.92	-0.58	0.20	1.5865
LR	1.67	0.01	3.23	0.80	47.77	-0.56	-0.18	0.4593
PR1	0.47	0	1.6	0.33	71.90	0.56	1.11	1.9999
PR2	3.31	1.6	5.8	1.00	30.37	-0.71	0.51	1.9602
PR3	3.17	1.5	4.6	0.68	21.51	-0.13	-0.32	0.1944
PR4	3.07	1.4	4.4	0.54	17.63	0.52	-0.50	0.0417

Grain productivity (GP) in kg ha^{-1}; soil organic matter (OM) in g dm^{-3}; phosphorus (P) cmol$_c$ dm^{-3}, hydrogen potential (pH); potassium (K) in cmol$_c$ dm^{-3}; calcium (Ca) in cmol$_c$ dm^{-3}; magnesium (Mg) in cmol$_c$ dm^{-3}; hydrogen + aluminum (H-Al) in cmol$_c$ dm^{-3}; aluminum (Al) in cmol$_c$ dm^{-3}; sum of bases (SB); cation exchange capacity (CEC); base saturation (BS); liming requirement (LR) in t ha^{-1}; penetration resistance 0.0-0.10 m (PR1) in kPa; penetration resistance 0.10-0.20 m (PR2) in kPa; penetration resistance 0.20-0.30 m (PR3) in kPa; penetration resistance 0.30-0.40 m (PR4) in kPa.

variation (CV) (Pimentel-Gomes & Garcia, 2002). The OM, pH and CEC were determined to be low. The properties GP, K, H-Al, V%, and RP4 were classified as average. The CV, Ca, Mg, SB, and PR4 were determined as high. Finally, P, Al, NC, PR1, and PR2 were classified as very high. High values for coefficients of variation referring to phosphorus are completely normal due to its availability in the soil but the values vary noticeably. Lautharte *et al.* (2021) explain that in the case of phosphorus, the sorption of this nutrient is common in soils of this type, as it has low mobility in the soil and high retention capacity for clay minerals and oxides, which is the general case for Latosols that are relatively rich in Fe and Al oxides.

The average soybean yield was 9433.7 kg ha^{-1} and within the values found in soybean with high technology. Similar results in the same soil type were observed by Machado *et al.* (2018) who found average soybean yields of 4786.5 kg ha^{-1}.The average productivity among producing states in Brazil is 3517.5 kg ha^{-1} (Conab, 2022).

The range values related to the semivariograms found by the variables ranged from 66.0 m (potassium) to 337.0 m (pH). The spatial variability observed in the crops can positively or negatively influence the crops used in rotation, so that the homogenization of the stand is essential for increasing the productive potential (Della Flora *et al.,* 2021).

In the cross semivariogram presented in Table 3, between soybean productivity and soil attributes, we found a positive correlation. For grain yield as a function of soil cation exchange capacity (GP=f(CEC)) there was a strong spatial dependence of 0.998% on the adjusted Gaussian model. This result shows a direct relationship between productivity and CEC.

The coefficients of spatial determination (r^2) were all higher than 0.940 for the semivariogram adjustments of the cross validations. Thus, there was a significant linear correlation between GP and soil attributes. Therefore, analyzing Table 3, as an example, high CEC rates might serve as a good indicator when destined for productivity projections in distrophic Red Latosol.

After adjusting the semivariograms (Fig. 3A-J) for each attribute, values were estimated using ordinary kriging. In this way, it was possible to build maps of spatial distribution for all variables in this study (Fig. 4A-J) that allowed visualizing the spatial variability in the area.

TABLE 2. Parameters estimated for the simple semivariogram of the productive components of the soybean crop.

Property	Model	Nugget effect	Level	Range (m)	r^2	RSS	SDE (%)	Class
GP	Exp	221698.0	468970.0	280.0	0.80	5.36E09	0.52	Moderate
OM	Exp	2.7	4.7	270.0	0.59	0.81	0.42	Moderate
P	Exp	112.0	282.0	90.0	0.35	0.60	0.58	Strong
pH	Exp	3.0E-2	8.0E-2	337.0	0.62	1.04E-03	0.56	Moderate
K	Eph	1.0E-4	8.0E-2	66.0	0.58	4.98E-04	0.99	Strong
Ca	Pne	x	x	x	x	x	x	X
Mg	Pne	x	x	x	x	x	x	X
H-Al	Shp	34.0	51.0	300.0	0.44	343.00	0.33	Weak
Al	Pne	x	x	x	x	x	X	X
SB	Pne	x	x	x	x	x	x	X
CEC	Exp	11.0	22.0	174.0	0.51	21.20	0.50	Moderate
BS	Pne	x	x	x	X	x	x	X
LR	Pne	x	X	x	x	x	x	X
PR1	Exp	7.0E-2	0.1	260.0	0.66	6.90E-04	0.46	Moderate
PR2	Gau	0.2	1.0	68.0	0.47	1.19E-01	0.85	Strong
PR3	Sph	0.1	0.5	76.0	0.84	3.66E-03	0.78	Strong
PR4	Pne	x	x	x	x	x	x	X

Grain productivity (GP) in kg ha^{-1}; soil organic matter (OM) in g dm^{-3}; phosphorus (P) $cmol_c\,dm^{-3}$; hydrogen potential (pH); potassium (K) in $cmol_c\,dm^{-3}$; calcium (Ca) in $cmol_c\,dm^{-3}$; magnesium (Mg) in $cmol_c\,dm^{-3}$; hydrogen + aluminum (H-Al) in $cmol_c\,dm^{-3}$; aluminum (Al) in $cmol_c\,dm^{-3}$; sum base (SB); cation exchange capacity (CEC); base saturation (BS); liming requirement (LR) in t ha^{-1}; penetration resistance 0.0-0.10 m (PR1) in kPa; penetration resistance 0.10-0.20 m (PR2) in kPa; penetration resistance 0.20-0.30 m (PR3) in kPa; penetration resistance 0.30-0.40 m (PR4) in kPa; residual sum of square (RSS); spatial dependence evaluator (SDE); exponential (Exp); gausian (Gau); spherical (Sph); pure nugget effect (Pne).

TABLE 3. Parameters estimated for the cross semivariogram of the productive components of the soybean crop.

Property	Model	Nugget	Level	Range (m)	R^2	RSS	SDE%	Class
GP = f(CEC)	Gau	1.00	623.00	285.00	0.940	33994	0.998	Strong

Residual sum of square (RSS); spatial dependence evaluator (SDE).

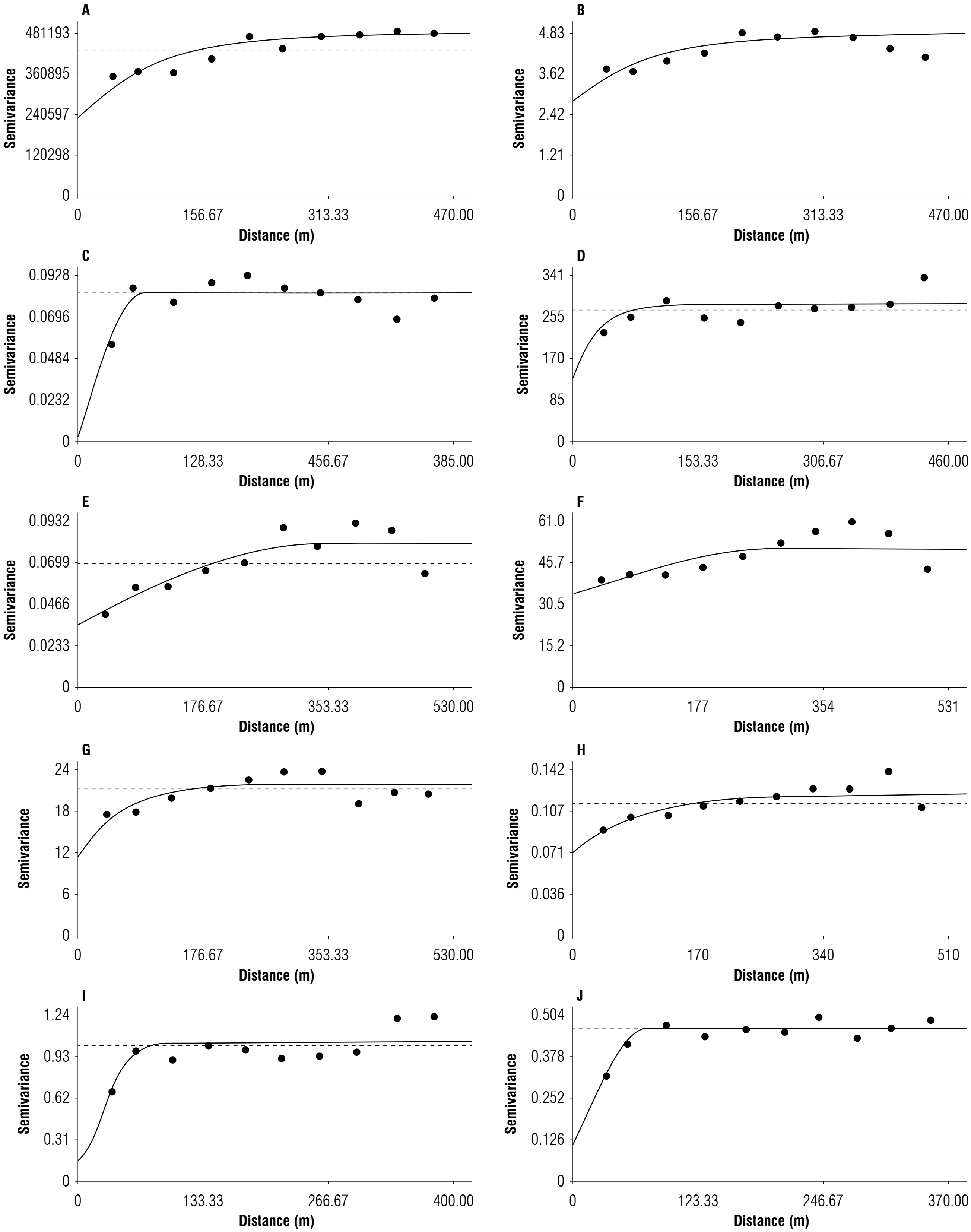

FIGURE 3. Semivariograms ajustaded: (A) grain productivity (GP) in kg ha^{-1}; (B) soil organic matter (OM) in g dm^{-3}; (C) phosphorus (P) cmol$_c$ dm^{-3}; (D) hydrogen potential (pH); (E) potassium (K) in cmol$_c$ dm^{-3}; (F) hydrogen+aluminum (H-Al) in cmol$_c$ dm^{-3}; (G) cation exchange capacity (CEC); (H) penetration resistance 0.0-0.10 m (PR1) in kPa; (I) penetration resistance 0.10-0.20 m (PR2) in kPa; (J) penetration resistance 0.20-0.30 m (PR3) in kPa.

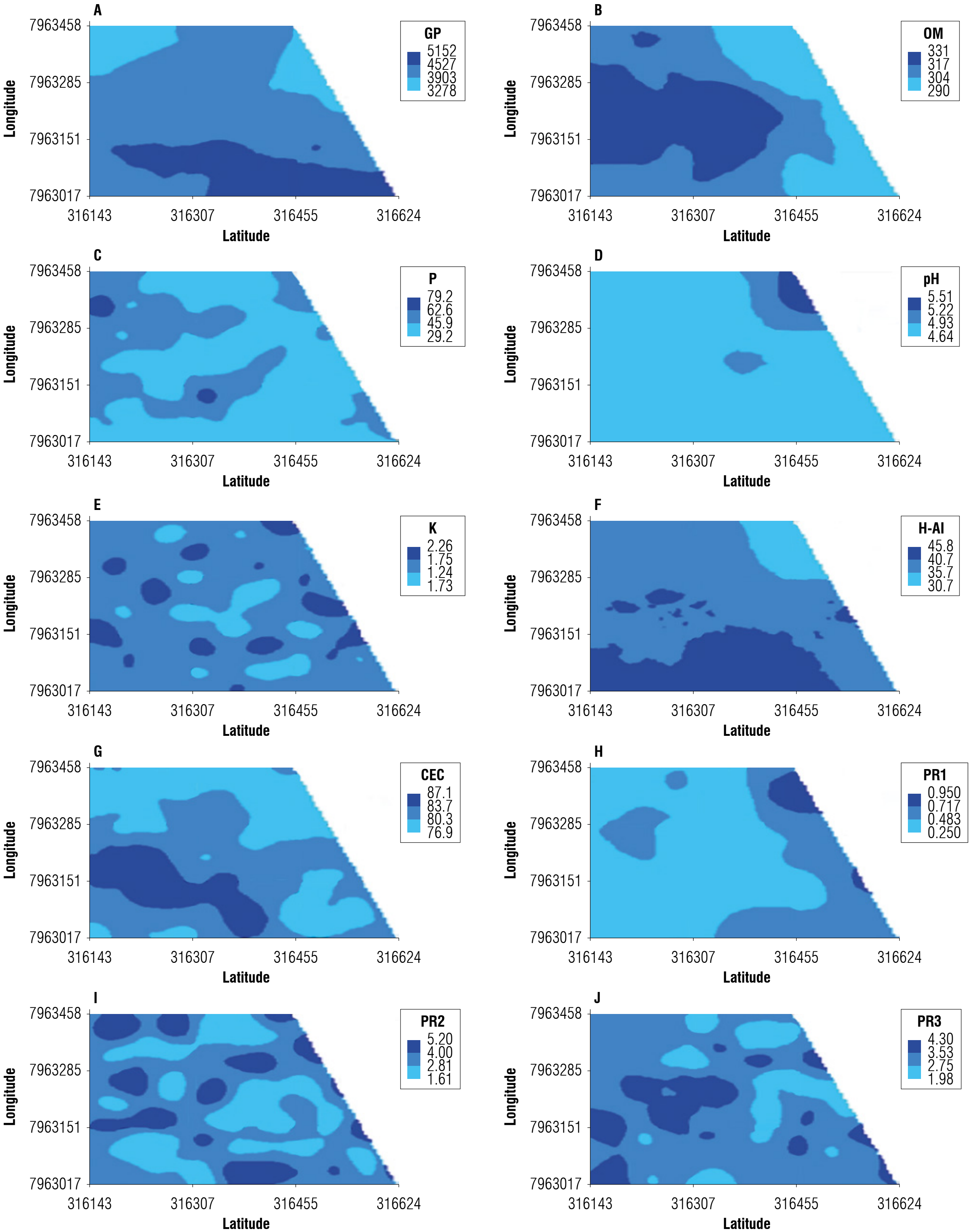

FIGURE 4. Kriging maps of parameters: (A) grain productivity (GP) in kg ha^{-1}; (B) soil organic matter (OM) in g dm^{-3}; (C) phosphorus (P) cmol$_c$ dm^{-3}; (D) hydrogen potential (pH); (E) potassium (K) in cmol$_c$ dm^{-3}; (F) hydrogen + aluminum (H-Al) in cmol$_c$ dm^{-3}; (G) cation exchange capacity (CEC); (H) penetration resistance 0.0-0.10 m (PR1) in kPa; (I) penetration resistance 0.10-0.20 m (PR2) in kPa; (J) penetration resistance 0.20-0.30 m (PR3) in kPa.

Analyzing the spatial variability map of productivity, the southern regions of the experimental area had the highest rates of crop productivity. The observation of a productivity map (Fig. 4A), together with the observation of other types of maps, such as the physical attributes of the soils, can help to find the reasons for the occurrence of production variability. With this identification, the possibility of correction for failures arises, leaving the possibility of minimizing problems in the crop. Oliveira *et al.* (2018) explain that, in this way, the grower can take advantage of historical information of the area from previous mappings to make the necessary decisions for crop management, identifying regions where there is a greater or lesser need for intervention, whether in the soil or on the plants.

Observing correlated maps of GP (Fig. 3A), K (Fig. 3E) and CEC (Fig. 3G), we can analyze a higher incidence in the graph from the same region and towards the south. More incisively, there was a greater aggregate of GP correlated with the CTC map. Gazola *et al*. (2017) explain that, in this sense, areas with high CEC have a greater capacity to retain cations (K, Ca, and Mg) and, consequently, a greater supply of these cations are available for the plants, in view of lower leaching losses. Consequently, we can expect higher productivity.

Interpreting the map of organic matter (Fig. 3B), we see a higher incidence in the central and western regions of the cultivated area. So, it is possible to correlate areas with higher rates of organic matter available in the soil with high productivity figures. The increase in soil organic matter content is the most appropriate recommendation for maintaining soil structure and increasing water retention in the soil, favoring adequate plant development and providing better results (Oliveira *et al.,* 2022).

Analyzing the Figure 4H, an incidence of PR1 (0.10-0.20 m) in the northeast region is classified as strong; and, in the entire eastern sector it is classified as moderate. This resistance in the surface layers of the soil hinders and restricts the results of the initial development of the plant. An important strategy to achieve soil decompaction consists of the adoption of cover crops with a robust root system as an important "biodecompaction" soil agent of soil since with the disturbance in the surface layers of the soil at planting in the direct planting system, combined with compaction because of the use of agricultural implements, the subsurface layers tend to become denser, reducing the pore space causing compaction (Oliveira *et al.,* 2021). The cultivation of cover crops in the off-season of grain production is a practice much appreciated for Oxisols in the Cerrado region (Ratke *et al.,* 2023).

Even with kriging providing information regarding spatial distribution and management zones, the pure nugget effect is common in soil analysis. Due to this, the attributes Ca, Mg, Al, BS, V% showed this result, that is, an absence of spatial dependence. The nugget effect is an important parameter of the semivariogram and indicates unexplained variability, considering the sampling distance used (Vieira, 2000). The greater the difference of the nugget effect in relation to the semivariogram level, the greater the continuity of the phenomenon and the smaller the variance of the estimate or the greater the confidence that can be had in the estimate.

Conclusions

Soybean productivity, soil organic matter content, phosphorus, pH, potassium, potential acidity, cation exchange capacity and soil penetration resistance from 0.00 m to 0.30 m showed spatial dependence.

The cation exchange capacity was the attribute that best described soybean productivity data in the studied dystrophic Red Latosol. Geographic points where the verified locations show higher potential acidity values have low productivity rates and consequently high CEC values correspond to places with higher productivity.

Conflict of interest statement

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

Author's contributions

LCR, CGR, TRC, and JTO conducted the experiments. LCR, CGR, FFC, and GVS performed the data analysis and wrote the manuscript. LCR, CGR, FFC, and GVS edited the manuscript. All authors reviewed the final version of the manuscript.

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Technical, economic, social, and environmental implications of the organic panela production in Nocaima, Colombia: The ASOPROPANOC case

Implicaciones técnicas, económicas, sociales y ambientales de la producción orgánica de panela en Nocaima, Colombia: Caso ASOPROPANOC

Ruth Mary Ibarra Guevara[1], Juan Carlos Barrientos Fuentes[2]*, and Wilmer Andrés Gómez Guerrero[3]

ABSTRACT

Strategies to overcome the sugarcane crisis in recent years are based on technological improvements in production, product diversification, exports, associativity and organic production. The purpose of this research was to analyze the technical, economic, social, and environmental implications of organic production of panela using ASOPROPANOC as a case study. The comparative analysis between the certified organic production system and the traditional system was based on documentary review, surveys with producers of the association, interviews to experts of the municipality, and observation in farms and sugarcane mills. The results show that the organic panela production system improves yields by 11% due to new varieties introduced and a more orderly production culture. Producing organic panela requires increases in investment by 46% but compensates with higher income and profits (16%); it also generates more jobs, improves worker-producer safety, their health and organization, and offers a better product to the consumer. The organic production system reduces between 50% and 150% the use of fuel materials per kg of panela produced as well as greenhouse gas emissions (between 1 and 91%) and water use (72%). Producing organic panela is an attractive option for growers.

Key words: productivity, costs and benefits, health and safety, gas emissions.

RESUMEN

Las estrategias de superación de la crisis panelera de los últimos años se fundamentan en mejoras tecnológicas de producción, diversificación de productos, exportación, asociatividad y producción orgánica. El propósito de esta investigación fue analizar las implicaciones técnicas, económicas, sociales y ambientales de la producción orgánica de panela tomando como estudio de caso a ASOPROPANOC. El análisis comparativo entre el sistema de producción orgánica certificada y el tradicional se basó en revisión documental, encuestas a productores de la asociación, entrevistas a expertos del municipio, y observación en fincas y trapiches paneleros. Los resultados muestran que el sistema orgánico de producción de panela mejora en 11% los rendimientos debido a nuevas variedades introducidas y una cultura productiva más ordenada. Producir panela orgánica incrementa la inversión en un 46%, pero compensa con mayores ingresos y ganancias (16%); también genera más empleos, mejora la seguridad, salud y organización del trabajador-productor, y ofrece un mejor producto al consumidor. El sistema de producción orgánica reduce entre 50% y 150% el uso de materiales combustibles por kilogramo de panela producida, así como reduce la emisión de gases de efecto invernadero (entre 1 y 91%) y el uso de agua (72%). Producir panela orgánica es una opción atractiva para los paneleros.

Palabras clave: productividad, costos y ganancias, salud y seguridad, emisión de gases.

Introduction

The agricultural sector is one of the strategic areas of the Colombian economy despite its decreasing share in the Gross Domestic Product (GDP) in recent decades. It contributes to the national economy with job generation, with 15.7% of 22,104,000 jobs at the national level. Its contribution to GDP was 6.29% in 2018 (DANE, 2020, February 14; DANE, 2020, March 31) and its contribution to exports was 18.6% in 2019 (Contexto Ganadero, 2020; DANE, 2020, August 20); likewise, it plays a role in reduction of poverty and gender equity, among others. One of the branches of this sector is the sugarcane production of "panela", a traditional consumer product in Colombia. For 2019, the national agricultural production area of sugarcane was 5,345,794 ha, of which 239,873 ha or 4.49% of the total (EVA-UPRA, 2019) was used for panela production. This crop had a five-year fluctuation of agricultural area with a

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[1] Agricultural and Forestry Chains, Ministry of Agriculture and Rural Development (Colombia).
[2] Department of Rural and Agrifood Development, Faculty of Agricultural Sciences, Universidad Nacional de Colombia, Bogotá (Colombia).
[3] PUR Project.
* Corresponding author: jcbarrientosf@unal.edu.co

slight downward trend. The panela sugarcane is cultivated in 28 departments distributed in five production nuclei: The Occidental nucleus, whose main producer is Antioquia; the Western-Central nucleus, whose main producers are Caldas, Quindío, Risaralda, Cauca, and Valle del Cauca; the Southern nucleus, whose main producers are Nariño, Huila, and Tolima; the North-eastern nucleus, whose main producers are Boyacá and Santander; and the Central nucleus, whose main producer is Cundinamarca. Panela sugarcane crops are distributed in 511 municipalities, where the raw material is processed in approximately 18,000 sugar mills (registered), with 117 of these being the axis of the economy (MADR, 2019). The panela production in 2019 was 1,364,523 t, with an average yield of 6.41 t ha^{-1} (EVA-UPRA, 2019). More than 350,000 families participated in the production of panela and 287,000 direct jobs were generated (MADR, 2019). In 2018, only 7,654 t of panela (<1%) were exported, which indicates that almost all panela production is destined to domestic consumption (Agronet, 2020). In terms of panela consumption, Colombia ranks first in the world. For 2017, in Colombia, the estimated annual apparent consumption per capita was calculated between 19 to 22 kg of panela (Celis, 2017).

The Cundinamarca department is the major national producer of panela with 39,324 ha of sugarcane planted and 37,353 ha harvested, with a yield of 5.45 t ha^{-1}, and 203,631 t of panela produced (EVA-UPRA, 2019). In Cundinamarca, the region with the highest production of panela is Gualivá. There, the most important municipality is Nocaima, which has 1,777 ha planted and harvested sugarcane, with a production of 10,662 t and an average yield of 6 t ha^{-1}, which is somewhat less than the national yield average (EVA-UPRA, 2019).

At the national level, the panela sector faces limitations in the technical and economic aspects with negative consequences in the social sphere, which have been accentuating in recent years. The main cause is the fall in prices because of a saturation of the national market, which is almost the only demand field for this product. According to López (2019), the price of panela in June 2016 was approximately 2,800 Colombian pesos (COP) per kg, while in June 2019 it was 1,662 COP per kg, which makes a price reduction of approximately 40% in three years. According to Celis (2017), the annual demand per capita fell from 32 kg in 2002 to 19 kg in 2017 (approximately 40%), while the supply barely increased by 7% in 12 years, from 1,272,855 t in 2007 to 1,364,523 t in 2019. This situation is disadvantageous for producers because it generates a low profit margin. The low economic benefits are also due to the relatively low productivity of the crop, high unit production costs, low degree of differentiation, and reduced bargaining power at the producer level.

The difficulties mentioned above were approached, among others, by scientific studies. A reference sample of 17 studies during the last ten years, mainly undergraduate thesis, were found through Google and Google Scholar. These studies show a tendency to search for practical improvements of the disadvantaged situation in the panela industry via three main strategies: technological improvement of production, organic production, and exportation. One more strategy can be added to these, which is a creation of the producer association oriented towards marketing (Galeano & Marín, 2010; Román & Prieto, 2013; Melo Hernández, 2014; García, 2015; Guerrero & Escobar, 2015; Gutiérrez & Triviño, 2015; Cárdenas *et al.*, 2016; Franco, 2016; Arcos, 2017; Arévalo, 2017; Castañeda *et al.*, 2017; Galindo, 2017; Agudelo Retavisca, 2019; Betancourt, 2019; Bolívar, 2019; Díaz, 2019; Ruíz *et al.*, 2019; Castaño & Ramírez, 2022).

For several years, both state and private funding institutions encouraged organization of agricultural producers in associations. Within the framework of these actions, the Nocaima Panela Producers Association – ASOPROPANOC was created more than 17 years ago. Its members produce organic and conventional (traditional) panela according to the market demands. The commercialization carried out by this association is aimed at the national market through neighborhood stores, chain stores, final consumers of the same municipality and some health food stores, and at international markets through marketing agents such as Doña Panela® and Comerpanela®. The latter exports panela to Canada and the USA. According to Agronet (2020), the countries involved in the most of the panela exports (partially organic panela) from Colombia in 2018 were the USA with 2,379 t and Spain with 2,248 t.

The production and export of organic panela is one of the alternatives proposed to overcome the crisis in the panela industry. According to FEDEPANELA (2019), until 2019 there were 217 panela producers certified in organic production. Most members of ASOPROPANOC are certified in organic production by the BCS OKÖ GARANTIE certifying agency. The production of organic panela, guaranteed through third-party certification, has advantages and disadvantages for producers. The advantages are referred mainly to better prices for producers, access to new markets, better product quality for consumers, and reduction of negative effects on the environment, among others. The disadvantages, among others, are the monetary and temporal costs of conversion to the organic system, technological conversion of production, and standardization and improvement of product quality. Many traditional and conventional panela producers could become organic panela producers, but they have doubts about the benefits of switching to the certified organic production system. This study aims to provide empirical information that would help the producers to decide whether to produce certified organic panela by answering the following question: What are the technical, economic, social, and environmental implications of the organic panela certification?

The general objective of this study was to determine the technical, economic, social, and environmental implications of certified organic panela production as compared to its traditional (standard) production. The specific objectives deal with each technical, economic, social, and environmental implication. This research aimed to generate empirical information that supports and guides the transition from a traditional or conventional panela production system to a certified organic system.

Materials and methods

This study had a non-experimental design and operated mainly with descriptive data with explanatory additions. Thirty-two panela producers associated with ASOPROPANOC were studied; five were traditional producers and the remaining 27 were certified organic panela producers or were in the process of obtaining certification in organic production. This organization is located in the Nocaima municipality, Cundinamarca department of Colombia. This municipality is located to the northwest and 75 km from Bogotá D.C.; it has a total area of 69 km^2 distributed in 21 townships. Its average altitude is 1,200 m a.s.l. and average air temperature is 24°C in much of the territory (Alcaldía de Nocaima, 2012).

The research was carried out in four phases. The first phase was based on a documentary study to define the dimensions (technical, economic, social, and environmental) and determine the analysis variables. The following variables were considered: 9 in the technical component, 14 in the economic component, 4 in the social component, and 3 in the environmental component. In this phase, the formats to collect field information were prepared. In the second phase, the field work was carried out, between July and September 2018, consisting of a census of the 32 ASOPROPANOC producers through a questionnaire with 20 closed questions and 10 open questions. This census also included a questionnaire with 10 closed questions applied to 8 experts on the research topic of panela production. Additionally, during the census, observations were made about the farms and panela production, and tables of production costs were filled out. The third phase was the systematization and analysis of the information collected in the field through descriptive statistics. The fourth phase was the presentation and interpretation of results. In almost all the results, a comparative analysis was made between the certified organic production system and the traditional system (Ibarra, 2019).

Results and discussion

Technical implications

The technical aspects of certified organic production involve changes that must be applied both in the production of sugarcane for panela production and the transformation of sugarcane into panela. These changes are technological changes, improvements in the producer skills and technical knowledge, and improvements in the awareness of panela producers on panela production. According to Jordan (2015), the certification of organic products must be done according to established governmental standards, which must ensure the conservation of natural resources, avoiding residuality in the products obtained.

According to the analyses by the producers and technical assistants of FEDEPANELA, the labor involved in the sugarcane crop renovation and the technological package implemented in the organic system explain the higher productive margins achieved with said model, since the fertilization directly affects crop productivity (Osorio, 2007). The results referring to the two systems are presented below.

Sugarcane crop management for panela production

The difference between the production of the traditional crop system compared to that of the certified organic system is evident (Tab. 1). The most relevant difference consists in annual productivity, which is almost 0.5 t ha^{-1} (>10%). The most determining factors in improving productivity in certified organic systems relate to technological change, including introduction of improved varieties of higher yield, crop renewal, higher frequency of cutting, lower crop age, and organic fertilization. This change also implies a prior process of changing the production system from traditional to certified organic, which can take up to three years. Technological change also involves an increase in labor (days) and in cost of production (investment) per ha. These two aspects are detailed in the section corresponding to the

TABLE 1. Technical aspects of the sugarcane production for panela under two systems: traditional and certified organic in the ASOPROPANOC (Nocaima, Cundinamarca).

Variables		Production systems (average)	
Complex variable	**Simple variable**	**Traditional (5 growers)**	**Certified organic (27 growers)**
General characteristics of the crop	Average farm area (ha)	5	12.8
	Average area planted with sugarcane crop (ha)	3.9	8.2
	Number of sugarcanes lots	1.6	3.7
	Division of lots	Not carried out	With live fences with native species
	Crop yield (t ha^{-1} $year^{-1}$)	47.6	52.5
	Panela yield (t ha^{-1})	4.76	5.25
Plant material	Varieties cultivated	POJ 2878 RD75-11 MZC 74-275	POJ 2878 RD75-11 MZC 74-275 Cenicaña 937510 Cenicaña 937711
	Crop age (months)	182	52
	Crop renovation	Not carried out	Done in batches with the new varieties of sugar cane for the production of panela
	Renovation cutting time (months)	72	55
Crop management	Fertilization	Not carried out	With organic products approved by the certification agency
	Control of weeds	Mechanized and chemical	Manual
	Control of pests and diseases	Biological and chemical	Biological
Harvest	Type	Thinning cutting	Thinning cutting
	Harvest periods	Permanent as a function of the income flow	Permanent as a function of the income flow

economic aspects. Additionally, assistance and technical support by the certification entity must be included in the certified organic system. Technical support and certification promote establishment of a new production culture in growers that implies increase in personal discipline and greater environmental and social awareness.

Panela production

Compared to the traditional system, the production of certified organic panela suggests technological improvements and a change in the production culture as well as increasing environmental and social awareness of the producers. The technological improvement of the organic production begins with the change of to either an improved traditional stove or a “Cimpa”, which has greater efficiency and performance (Rodríguez & Gottret, 1998; García & Cortés, 2011; Ramírez & Arenas, 2016). Logically, this costs more, which implies additional investment. According to Arévalo (2017), a determining factor in the panela making process is the technology innovation, especially, with the use of evaporators that improve the quality and safety of the panela product.

Another technological improvement is standardization of transformation and packaging process, which includes, among others, management of temperatures and dosages and use of stainless-steel trays and utensils. The foregoing activities ensure compliance with Resolution 779 of 2006 of the Ministry of Social Protection (Colombia) and other regulations for organic panela production, which include, among others, compliance with standards in infrastructure, facilities, equipment, materials and supplies, and personnel handling the product. The personnel linked to the production process must comply with regulations of protection, hygiene, and health elements as well as training requirements for their participation in a panela production unit.

An important factor in panela production is the temperature reached in the oven at the time of boiling of sugar honey (“punteo” in Spanish), where traditional ovens can reach temperatures of 116 to 122°C. This temperature can increase between 1 and 3°C when the altitude of the site is below 1000 m a.s.l.; Nocaima is located at 1200 m a.s.l.

TABLE 2. Technical variables of panela production under traditional and certified organic systems at ASOPROPANOC (Nocaima, Cundinamarca).

Variables		Production systems (average)	
Complex variable	**Simple variable**	**Traditional (5 growers)**	**Certified organic (27 growers)**
Previous activities	Stacking	3-4 d before grinding	1 d before grinding
Equipment	Number of sugarcane mills	5	15
Frequency of grinding	Monthly	0	1
	Biweekly	5	14
Type of stove	Traditional	5 (100%)	1 (7%)
	Traditional improved	0	8 (53%)
	Technified "Cimpa"	0	6 (40%)
Sanitary requirements for panela production	Complying Resolution 779 of 2006 of Ministry of Social Protection	0 (0%)	15 (100%)
Use of fuels	Principal	Bagasse	Bagasse
	Auxiliaries	Firewood, tires	Firewood
Clarification	Clarifiers	Lime and tree species	Lime
	Dosage	The operator experience	Measured as a function of juice volume
Evaporation and concentration	Cooking temperature (123-125°C)	The operator experience	Measure
	Application of Coconut oil	The operator experience	Applied depending on temperature (125°C)
Boiling	Cleaning of honey	West Indian elm, balsa tree	Stainless steel mesh
	Temperature	97°C	97°C
Shake	Trough and utensils	Wood	Stainless steel
Molding	Use of stainless-steel elements	Wooden table using canvas, where the skull is not disinfected until the end of the process	The molding table is made of stainless steel and the skulls are disinfected at each point
Package	Packaging	Without individual cover	Covered with plastic, where the product is packed and shrink-wrapped and packed in wavy cardboard boxes
	Presentation	Standard	According to customer
	Package (20 kg)	Carton box (20 kg)	Plastic basket or cardboard box (20 kg)- Polypropylene bag

Economic implications

Production and costs

In sugar cane production, switching from the traditional production to the certified organic production implies an increase of 88% in the production costs per ha during the establishment phase (Tab. 3). In the following years, the cost of production decreased and then increased to just over 3 million COP (Tab. 5). For all factors of production, differences were found between the two systems, especially in labor expenses, establishment and maintenance of live fences, nutrition and health management. As for inputs, the differences consisted in the acquisition of improved seeds, mineral elements for fertilization, and health management. In services, the differences consisted in transportation, technical assistance, soil analysis, and certification. Regarding this last factor, the annual certification cost per farm is relatively affordable for the producers, given that the certification is done for a grower association and not for a farm. According to Ordoñez-Díaz and Rueda-Quiñonez (2017), small producers of panela must reduce costs within the production process to become competitive at the globalized market.

In addition, the traditional "trapiche" system employs people residing on the farm (family), while some manual operation tasks are done by contracted workers. The system of improved sugarcane mills receives cane to grind and hires workers for various activities (Ramírez & Arenas, 2016). According to Rodríguez and Gottret (1998), technology at this level represented about 29% of the total benefit generated in its productive activity and 13.1% reduction in the production cost. These data provide a quantitative reference.

TABLE 3. Production costs (Colombian peso) of sugar cane for panela in the establishment phase in two production systems: traditional and certified organic.

Production factors	Production systems (average)	
	Traditional (5 growers)	Certified organic (27 growers)
Labor	2,010,675 (67.2%)	3,340,000 (59.5%)
Drainage construction	171,675	80,000
Fallowing	44,000	120,000
Collection of plant material	35,000	35,000
Establishment and maintenance of live fences	0	105,000
Tracing and augering	600,000	880,000
Seed preparation	80,000	80,000
Seed repotting	80,000	80,000
Manual control of weeds (weeding)	600,000	880,000
Parasitoid release	40,000	40,000
Fertilizer application	200,000	160,000
Amendments application	80,000	720,000
Reseeding	80,000	160,000
Inputs	641,500 (21.5%)	1,530,585 (27.3%)
Organic fertilizer	121,500	250,000
Lime	180,000	208,000
Herbicides	90,000	0
Lydella fly (120 pulp)	0	39,960
Trichoderma (200 inch)	0	52,000
Cane seed	250,000	480,000
Live fence seedlings	0	500,625
Materials and equipment	70,000 (2.3%)	67,593 (1.2%)
Tools in general	70,000	67,593
Services	268,000 (9.0%)	676,200 (12.0%)
Seed transport	106,000	212,200
Internal seed transport	88,000	105,000
Inputs transport	74,000	96,000
Technical assistance	0	120,000
Soil analysis	0	120,000
Certification	0	23,000
TOTAL	2,990,175	5,614,378

Note: For the calculation of costs, 2020 prices were used.

As for the labor requirement in the crop establishment stage, given the increased activities compared to the traditional system, the certified organic production has a higher wage expense, approximately 39.5%. During the crop maintenance practices, the certified organic system requires 31% more labor than the traditional system. In the transformation phase for panela production, a correlative difference to yield appears, that is, although the transformation process becomes standardized, the organic system produces more panela per surface unit per year (Tab. 4). Logically, this generates higher costs but could be also considered as an opportunity for creating new jobs.

TABLE 4. Labor expenditure (wages per ha) in the production of 1 ha of sugarcane for panela (establishment - year 1 and maintenance - year 3): traditional system and certified organic system.

Labor (wages per ha)	Production systems (average)	
	Traditional (5 growers)	Certified organic (27 growers)
Establishment phase	26	35
Crop maintenance phase	84	125
Processing	54	54
Total	164	214

Although the production costs of the certified organic system are higher than those of the traditional system, these are compensated by its performance and price of the product. The yield of both the sugarcane crop and panela is approximately 10% higher, while the price is higher by about 15%. With these two elements, the average profit margin of the certified organic system is approximately COP 1,000,000 ha^{-1} per year compared to the traditional one, which generates approximately COP 400,000 ha^{-1} per year. The difference in the average annual profit margin per ha is 150%. The benefit-cost ratio for the traditional system is approximately 1.1, while it is approximately 1.3 for the certified organic system.

Commercialization

The traditional and certified organic panela systems have different product and marketing channels (Tab. 2). While the traditional system obtains a panela of a single type of presentation and of variable quality, the certified organic system produces a panela in two presentations, molds in 20 kg boxes and granules in polypropylene bags of 30 to 40 kg, with uniform quality guaranteed through certification and seal. Traditional panela is sold through two marketing channels: a producer takes it directly to the local market, where it has little bargaining power and generates additional transaction costs, or a producer takes it to an association of growers for sale. The certified organic panela is sold almost entirely by the grower associations, which already accumulates greater bargaining power and reaches

TABLE 5. Production costs of sugarcane and panela and profit margin (COP per ha): traditional (T) and certified organic (CO) production systems.

Year	Production system	Cost of sugarcane production (COP/ha)	Cost of panela production (COP/ha)	Crop yield (t ha^{-1})	Panela yield (t ha^{-1})	Panela price (COP/kg)	Cost of panela (COP/ha)	Income per panela (COP/ha)	Benefit-cost ratio (COP/ha)
1	T	2990175	0	0	0	2350	2990175	0	0.00
	CO	5614378	0	0	0	2711	5614378	0	0.00
2	T	2808100	383300	23.8	2380	2350	3191400	5593000	1.75
	CO	4314761	1165500	26.25	2625	2711	5480261	7116375	1.30
3	T	4048100	6254886	47.6	4760	2350	10302986	11186000	1.09
	CO	5934761	5284449	52.5	5250	2711	11219210	14232750	1.27
4	T	4048100	6254886	47.6	4760	2350	10302986	11186000	1.09
	CO	5814761	5284449	52.5	5250	2711	11099210	14232750	1.28
5	T	4048100	6254886	47.6	4760	2350	10302986	11186000	1.09
	CO	5934761	5284449	52.5	5250	2711	11219210	14232750	1.27

Note: COP - Colombian peso. For the calculation of costs, 2020 prices were used.

national and international markets. The current difficulty of associations at the international markets is their small and constant volume of supply. Additionally, different qualities and markets also have different prices (Tab. 5). Certified organic panela costs on average 15% more than traditional panela. This is an interesting incentive for the producers, in addition to access to a less competitive market such as an international market. On the other hand, the market for organic products in Colombia is relatively small and slow growing. Due to the higher prices of organic products, an average Colombian consumer prefers to buy the conventional or traditional products. In the USA and European markets, the main destination for Colombian panela, prices for organic products are currently declining.

Social implications

Employment generation

As shown in Table 4, the certified organic sugarcane production for panela (214 d ha^{-1} year^{-1}) requires an average of 31% more labor than the traditional system (164 d ha^{-1} year^{-1}). While this implies an increase in the average wage requirement of just over 50 wages ha^{-1} year^{-1}, it could mean the opportunity to generate new jobs in the area. However, this situation can produce two contrary effects. If there are farm workers searching for a job in the area, this system change could be advantageous, but if there is a labor shortage in the area, it can have a negative effect on production. Another possible effect could be the fluctuations in the wage price. According to Ordoñez and Rueda (2017), the phenomenon of rural-urban migration generates the need to create committees or societies that work together and guarantees the self-sustainability of the crop.

Producer protection

Producer protection refers to a social insurance, that is, health insurance, pension, and labor accident insurance. At the producer level, both the traditional system and the certified organic system are in similar conditions in terms of social security. Most employment contracts are informal; therefore, they do not include monetary contributions to health, pension, and insurance against labor accidents, although 40% of the producers surveyed were beneficiaries of the Identification System for Potential Beneficiaries of Social Programs (SISBEN in Spanish). However, at the level of certified organic panela producers, there is a tendency to improve this situation by formalizing fixed-term labor contracts with all legal obligations, although this is only in the case of administrators of panela farms (4 of 27 productive units). Additionally, the certified organic production, unlike the traditional system, commonly offers higher protection against physical injuries and occupational accidents, either through improved practices or appropriate protective equipment. Eighty percent of the operators who work in the mill of the certified organic system have a helmet, glasses, and overalls as well disposable masks and hats during the shaking, molding, and packing of panela, while those of the traditional system do not have these protective measures. Apart from protecting the producer, the personal protection measures mentioned above ensure delivering of a safe product. These aspects are also differentiating at the final product level.

Consumer protection

In the food market, consumers consider product quality, price, and safety. In Colombia, the safety of panela is demonstrated through its INVIMA Registry (National Institute for the Surveillance of Medicines and Food, Colombia), which complies with current regulations of Resolution 779 of 2006 for panela productions. This registry allows the producer to sell its product, for example, in supermarkets. Only 1% of the traditional panela producers have INVIMA registry, while 100% producers of certified organic panela have it. Additionally, the latter have the organic certification that guarantees the non-use of chemical synthesis products in production. The INVIMA registry and organic certification give organic panela an advantage in quality over traditional panela; they allow access to more demanding markets with quality control and to specialized markets such as organic ones. The certification assures the consumer that the product complies with following parameters: i) prevents the degradation of natural resources, ii) protects the environment, and iii) is healthy for human consumption (Abarca & Sepúlveda, 2001).

Stimulus for collective action

According to Gómez (2018), collective action is the effort of two or more people to achieve a desirable result for the participating parties. In the case of the ASOPROPANOC organization, by marketing panela collectively, which is one of the main objectives of this organization, participation becomes more active and individual responsibility increases. The planning, organic production, certification, and marketing for export are carried out jointly by 90% of organic producers. These activities have strengthened the organization. Several of these cooperative activities occurred during the transition from the traditional system to the certified organic production. Traditional panela producers do not receive as many benefits as the "organic" ones, so their permanence in the grower association may be discouraged to the point of abandonment or diminishment. In the last 10 years, the association has increased from 10 members to 32 members because of the support provided to the association by FEDEPANELA, AGROSAVIA, and other entities to implement the organic production system.

Environmental implications

Contamination of the environment

The cultivation of sugarcane for panela, like many other agricultural activities, has effects on the environment. The negative effects are mainly related to the use of agro-inputs, especially those of chemical synthesis, due to their greater persistence and toxicity compared to organic inputs (Rodríguez *et al.*, 2017). The use of chemical synthesis products, in the case of the traditional system, occurs mainly during fertilization and control of weeds and pests (Fonseca, 2002). The lack of management protocols for these inputs in the traditional system, creates a possibility of overdosing and contamination of surface and groundwater through inadequate discharges (Bernal, 2019). In the case of certified organic systems, handling of agro-input containers follows a protocol to reduce the environmental impacts (Tab. 6).

TABLE 6. Indicators of environmental contamination in sugarcane cultivation for panela in traditional and certified organic systems.

Indicator	Production systems (average)	
	Traditional (5 growers)	Certified organic (27 growers)
Follow defined fertilization protocol	No	Yes
Follow defined sanitary management protocol	No	Yes
Uses chemical synthesis inputs	Yes	No
Uses sustainable inputs for crop management	No	Yes
Performs sustainable management of containers	No	Yes
Uses combustion sources other than firewood and bagasse	Yes	No
Produces bagasse	Yes	Yes
Generates wastewater for processing	Yes	Yes
Emits greenhouse gases	Yes	Yes
Emits discharges	Yes	Yes

The transformation of sugarcane juice into panela requires energy; in this case, from combustion. In turn, production of panela generates gaseous emissions, discharges, and by-products such as bagasse (Murcia, 2012; Ordoñez-Díaz & Rueda-Quiñonez, 2017). The bagasse produced during transformation serves, together with wood (firewood) and other materials, as fuel for the transformation process (Tab. 2). Additionally, washing of transformation equipment (troughs, "remillones" [utensil to remove foam], molding table, pan, bottoms, and installations) generates discharges in the crop area or areas near the houses in the case of the traditional system. In the certified organic system, these discharges are generally done in a water treatment system, which is a certification requirement to mitigate the environmental impact. Not all production units have a permit to discharge these residues (the exact data are not available). On this problem, ASOPROPANOC developed a project that implements a treatment system for wastewaters from the production process. Additionally, combustion of bagasse, firewood, and other materials, such as tires, in the case of the traditional system, generate greenhouse gas emissions. The most significant environmental impacts are evident during the stage of evaporation and concentration of juice, mainly due to the use of traditional stoves (Ordóñez-Díaz & Rueda-Quiñones, 2017).

Use of combustible materials

The energy to heat the sugarcane juice and evaporate water comes from the combustion of bagasse, firewood, and, in some cases, vehicle tires (García & Cortés, 2011). The traditional system uses tires, while the certified organic does not. The efficiency of the combustion of bagasse and firewood depends on the quality or type of combustion chamber. These chambers could be traditional, improved traditional, or Ward-Cimpa. The traditional chamber generates less heat (Tab. 7) and produces more carbon monoxide (6-10% of CO); while the Ward-Cimpa generates more heat and produces less carbon monoxide (Guerrero & Escobar, 2015). This combustion efficiency also means that, in the certified organic system, less combustion material is required than in the traditional system.

TABLE 7. Consumption of combustion materials (kg ha^{-1} year^{-1}) in production of traditional and certified organic panela.

Indicator	Production systems (average)	
	Traditional (5 growers)	**Certified organic (27 growers)**
Combustion chamber type	Traditional (Plain)	Traditional improved – Cimpa type
Combustion chamber combustion efficiency (burner)	650-850°C	850-1,200°C
Amount of wet bagasse (kg) required to produce 1 kg of panela	6	4
Quantity of firewood (kg) required to produce 1 kg of panela	1.5	0.5
Quantity of tires (kg) required to produce 1 kg of panela	*	0
Yield of panela (kg ha^{-1} year^{-1})	4,760	5,250
Total amount of wet bagasse required (kg ha^{-1} year^{-1})	28,560	21,000
Total amount of firewood required (kg ha^{-1} year^{-1})	7,140	2,625
Number of tires total required (kg ha^{-1} year^{-1})	*	0

* No exact data available.

Greenhouse gas emission

The greenhouse gases generated during panela production (Tab. 8) include carbon monoxide (CO), carbon dioxide (CO_2), nitrogen oxides (NOx), methane (CH_4), and volatile organic compounds other than methane (NMVOC). The following factors differentiate the two systems: combustion technology (burner type), type of fuel material (bagasse, firewood, and tires) and its quantity, which, in turn, depends on the combustion technology. The new stoves generally have less heat availability than traditional stoves, mainly due to reduced fuel consumption (Castillo, 1996; Pandraju *et al.*, 2021). The energy generation technology of the certified organic system, due to its greater efficiency, allows it to emit less greenhouse gases than the traditional system. Additionally, the non-use of tires reduces the amount of production of the greenhouse gases.

TABLE 8. Greenhouse gas emissions (kg ha^{-1} year^{-1}) from the production of traditional and certified organic panela.

Indicator	**Production systems (average)**	
	Traditional (5 growers)	**Certified organic (27 growers)**
Quantity of CO (g) emitted per 1 kg of panela produced	0.023	0.021
Quantity of CO_2 (g) emitted per 1 kg of panela produced	0.712	0.638
Quantity of NOx (g) emitted per 1 kg of panela produced	2.380	2.131
Quantity of CH_4 (g) emitted per 1 kg of panela produced	7.135	0.639
Quantity of NMVOC (g) emitted per 1 kg of panela produced	1.190	1.065
Quantity of MP (g) emitted per 1 kg of panela produced	2.513	2.250
Panela yield (kg ha^{-1} year^{-1})	4,760	5,250
Quantity of CO (g) emitted per 1 ha of sugarcane	109	110
Quantity of CO_2 (g) emitted per 1 ha of sugarcane	3,389	3,350
Quantity of NOx (g) emitted per 1 ha of sugarcane	11,329	11,188
Quantity of CH_4 (g) emitted per 1 ha of sugarcane	3,396	3,355
Quantity of NMVOC (g) emitted per 1 ha of sugarcane	5,664	5,591
Quantity of MP (g) emitted per 1 kg of sugarcane	11,962	11,813
Yield of sugarcane for panela (kg ha^{-1} year^{-1})	47,600	52,500

Note: Based on Ibarra (2019) and García *et al.* (2011). NMVOC - volatile organic compounds other than methane, MP – Particulate matter.

There is a reduction in greenhouse gas production when switching from the traditional system to the certified organic panela production system, especially in the transformation phase. For sugarcane production, the average reduction is from 5,975 g ha^{-1} year^{-1} to 5,901 g ha^{-1} year^{-1}; while for panela production, the reduction is from 2,326 g kg^{-1} to 1,124 g kg^{-1} of panela produced.

Water consumption

The consumption of water during the panela production is oriented to washing of drawers, "remillones" (utensils to remove foam), bottoms, and other elements (Ubaque, 2013). The production technology also makes a difference in water consumption. The certified organic system consumes barely a quarter of what the traditional system consumes (Tab. 9). This implies savings in water costs and, potentially, in the production costs. Additionally, 97% of the productive units studied have a local aqueduct service, which does not measure consumption, which indicates that there is still no restriction on the use of this natural resource. This has a positive effect on production costs, since higher consumption does not necessarily lead to a higher cost of the water consumed.

TABLE 9. Water use (L kg^{-1}) in the production of traditional and certified organic panela.

Indicator	Production systems (average)	
	Traditional (5 growers)	Certified organic (27 growers)
Water use per productive unit (L ha^{-1} $year^{-1}$)	14,462	3,979
Panela yield (kg ha^{-1} $year^{-1}$)	4,760	5,250
Water use per 1 kg of panela produced (L kg^{-1})	3.04	0.76

Argumentative balance of the certified organic panela production system

The arguments for and against organic sugarcane and panela production in the study area of Nocaima are presented below. As can be seen, the arguments in favor outweigh those against.

Conclusions

The change from the traditional panela production system to the certified organic system has favorable technical, economic, social and environmental implications for ASOPROPANOC's sugarcane producers. From the technical point of view, the change in the panela production system increases the productivity of sugarcane and panela due to the introduction of new genetic materials for the crop and the favorable change in production culture and environmental awareness of the producers. Economically, it implies higher production costs (investment), but also increases the net benefits due to improvements in the price for quality. Socially, it increases employment (wages), improves the physical safety of workers and the functionality of their organizations, as well as improving the quality of the product for consumers. And, from the environmental point of view, the use of combustible materials such as bagasse and firewood per kg of panela produced is reduced as well as the emission of greenhouse gases and the use of water.

TABLE 10. Arguments for and against organic production of panela in technical, economic, social and environmental dimensions.

Arguments in favor	Arguments against
Technical	
• A technological improvement is generated in both sugarcane production and panela production • A new production culture based on technical-scientific knowledge is generated • The yield of panela increases per ha and per year • The quality of the product is improved without the use of agrochemicals, being more homogeneous and with certification	• The organic production process requires a wide range of specific technical knowledge • The panela organic production system needs a period of 3 years to complete the transition process towards organic production • More labor activities are required • More labor with the crop is required
Economic	
• Higher price in the market • Access to more stable markets (organic and exportation) • Higher profit margin • Greater possibility of export • Easy access to credit as an association	• Production costs are higher • An investment is required to switch to the organic production system • The product must be sold in organic markets
Social	
• Generates more job offers • Protects more the safety and health of the producers • Offers more protection to the consumers • Stimulates more collective action through associations and alliances with the state	• Requires more labor
Environmental	
• Less contamination of soil and water through agrochemicals and discharges • Less use of fuel material (firewood and bagasse) per ha and year • Lower emission of greenhouse gases • Lower water consumption per ha and year	• The emission of greenhouse gases is not eliminated

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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

RMIG provided the new information and collaborated in the writing, JCBF structured the article and collaborated in the writing, and WAGG wrote the draft of the manuscript. All authors reviewed the final version of the manuscript.

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Effect of physical and thermal pretreatments on enzymatic activity in the production of microporous cassava starch

Efecto de pretratamientos físicos y térmicos sobre la actividad enzimática en la producción de almidón microporoso de yuca

Jorge Antonio Figueroa-Flórez[1*], Eduardo David Arroyo-Dagobeth[1], Edith Cadena-Chamorro[2], Eduardo Rodríguez-Sandoval[2], Jairo Guadalupe Salcedo-Mendoza[3], and Héctor José Ciro-Velásquez[2]

ABSTRACT

Cassava starch is modified to increase porosity and lacerations that are limited when only enzymatic treatments are used. This study proposes to improve enzymatic activity of α-amylase and amyloglucosidase on the polymer chains of cassava starch by implementing physical and thermal pretreatments below the gelatinization temperature and before the hydrolytic process. The pretreatments increased the biocatalytic action of the enzymes, causing significant changes in the morphology of the granules, and superficial lacerations were found in samples of starches pretreated with ultrasound (UTS) or annealing and ultra-rapid freezing (ANN-C). At the structural level, the modified starches revealed substantial changes as the infrared spectra reflected a displacement of the absorption bands in the region from 900 to 1100 cm^{-1}. This is associated with an alteration and reorganization of the amorphous and crystalline zones of the granules and is consistent with a decrease in amylose content (from 19.53% to 17.64%) and an increase in the crystallinity index. The thermal behavior of the starches was also modified by increasing the peak temperature (from 68.22°C to 75.38°C) and reducing the gelatinization enthalpy (from 19.34 to 15.79 J/g). UTS and ANN-C pretreatments significantly improved the mesoporous and hydrophilic properties of the modified cassava starches.

Key words: annealing, crystallinity, gelatinization, hydrolysis, hydro-thermal treatments.

RESUMEN

El almidón de yuca es modificado para aumentar su porosidad y laceraciones, las cuales son limitadas cuando sólo se utilizan tratamientos enzimáticos. Por lo tanto, este estudio propone mejorar la actividad enzimática de la α-amilasa y la amiloglucosidasa sobre las cadenas poliméricas del almidón de yuca utilizando pretratamientos físicos y térmicos por debajo de la temperatura de gelatinización antes del proceso hidrolítico. En este caso, los pretratamientos aumentaron la acción biocatalítica de las enzimas, provocando cambios significativos en la morfología de los gránulos, y se encontraron marcadas laceraciones superficiales en muestras de almidones pretratados con ultrasonido (UTS) o recocido y congelación ultrarrápida (ANN-C). A nivel estructural, los almidones modificados revelaron cambios sustanciales dado que los espectros infrarrojos reflejaron un desplazamiento de las bandas de absorción en la región de 900 a 1100 cm^{-1}. Esto está asociado con una alteración y reorganización de las zonas amorfa y cristalina de los gránulos y es consistente con la disminución del contenido de amilosa (desde 19.53% hasta 17.64%) y el aumento del índice de cristalinidad. El comportamiento térmico de los almidones también se modificó al aumentar la temperatura pico (desde 68.22 hasta 75.38°C) y reducir la entalpía de gelatinización (desde 19.34 hasta 15.79 J/g). Los pretratamientos UTS y ANN-C mejoraron significativamente las propiedades mesoporosas e hidrofílicas de los almidones de yuca procesados.

Palabras clave: recocido, cristalinidad, gelatinización, hidrólisis, tratamientos hidrotérmicos.

Introduction

Starch is a homopolymer composed of units of α-D-glucopyranose that can be linked by α-D-(1,4) and α-D-(1,6) glycosidic bonds to form two different polymeric structures: amylose and amylopectin. Amylose and amylopectin are densely packed in a semi-crystalline state that affects the susceptibility of starch to enzymatic attack (O'Brien & Wang, 2008). Compared to cereal starches, for example, tuberous root granules have a smoother surface free of porosities (Chen *et al.*, 2011). Cassava starch has been widely incorporated in the formulation of food matrices as a stabilizer, texture modifier, fat emulator, or encapsulating material for bioactive compounds (Jayakody &

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[1] Facultad de Ciencias, Posgrado en Biotecnología, Universidad Nacional de Colombia, Medellín (Colombia).
[2] Facultad de Ciencias Agrarias, Departamento de Ingeniería Agrícola y Alimentos, Universidad Nacional de Colombia, Medellín (Colombia).
[3] Facultad de Ingeniería, Departamento de Ingeniería Agroindustrial, Universidad de Sucre, Sincelejo (Colombia).
[*] Corresponding author: joafigueroafl@unal.edu.co

Hoover, 2008). To improve the properties of thermo- or photosensitive substances as carriers of micronutrients or encapsulating materials, their adsorption properties should be enhanced through modification that promotes the development of structures with excellent micro- or mesoporous characteristics.

There are many methods to produce porous starches that involve physical, chemical, and enzymatic treatments, or a combination of them. Enzymatic modification is characterized by polymer degradation, total or partial loss of the native morphology, and molecular disorganization, altering the physicochemical properties of the granules (Rocha *et al.*, 2010; Figueroa-Flórez *et al.*, 2019). These biocatalytic processes offer several advantages: they are efficient, safe, and ecologically sustainable. In addition, the enzymatic modification involves the use of polypeptides in the production of porous starches, whose granules have lacerations, cracks, or pores that run from the surface to the interior (Xie *et al.*, 2019) and are used in the industry for carrying or encapsulating pigments, antioxidants, fatty acids, etc. (Gao *et al.*, 2013; Dura *et al.*, 2014; Xie *et al.*, 2019).

Several studies have reported the use of endo- or exo-amylases from different botanical sources in the production of porous starches (O'Brien & Wang, 2008; Wu *et al.*, 2011; Zhang *et al.*, 2012; Gao *et al.*, 2013; Dura *et al.*, 2014; Wang *et al.*, 2016; Benavent-Gil & Rosell, 2017; Jung *et al.*, 2017; Xie *et al.*, 2019). Although few articles in the literature have examined the biocatalytic action of amylolytic enzymes in the production of porous cassava starches using α-amylase or amyloglucosidase, the articles have concluded that tuberous root starches are less susceptible to enzymatic attacks because they show small superficial lacerations and shallow holes (Rocha *et al.*, 2010; Chen *et al.*, 2011; Benavent-Gil & Rosell, 2017; Figueroa-Flórez *et al.*, 2019).

Other authors have proposed the use of physical treatments in the production of porous corn starches to increase the size and volume of the pores and improve the adsorption capacity of the starches. Zhao *et al.* (2018) explored a freezing-cooling combination and obtained significant changes in the crystallinity index, thermal, and morphological properties, as well as in the water adsorption capacity and solubility of corn granules. Thermal processes such as annealing have been implemented to optimize enzymatic hydrolysis; as a result, significant changes in structural properties and granules with a microporous morphology have been reported (Tukomane *et al.*, 2007; O'Brien & Wang, 2008; Shariffa *et al.*, 2017; Xie *et al.*, 2019).

Previous studies have identified the presence of lacerations or small porosities in enzymatically modified cassava granules (Shariffa *et al.*, 2009; Chen *et al.*, 2011; Dura *et al.*, 2014). However, few authors have evaluated the effect of physical pretreatments on the enzymatic hydrolysis to develop porous cassava starches. We hypothesize that physical-thermal pre-treatments alter the native semi-crystalline packing that favors the enzymatic absorption phenomenon, endo-corrosion processes, and the formation of micro-porous granules. The literature does not include reports on the characteristics of the pores in cassava-modified starches in terms of type, size, and pore volume. The aim of this study was to evaluate the effect of physical and thermal pretreatments on the degree of hydrolysis and the structural and morphological properties of native cassava starches during enzymatic modification.

Materials and methods

Materials

Native cassava starch (*Manihot esculenta* cv. M-Tai) was supplied by Almidones de Sucre S.A (Induyuca®, Sincelejo, Colombia). Amyloglucosidase from *Aspergillus niger* (Dextrozyme® GA, Novozymes, Denmark) and α-amylase from *Bacillus licheniformis* (Liquozyme® Supra 2.2X, Novozymes, Denmark) were used in the experiment. Analytical grade potato amylose (AO512, Sigma Aldrich, Germany) was also used.

Physical and thermal pretreatments

Native cassava starch (NCS) with a moisture content of ~50% (dry weight basis), was subjected to the following pretreatments:

- Annealing (ANN): Heating for 60 min at 60°C under constant agitation at 250 rpm in a thermostatic bath (MaxQ® 4450, Thermo Scientific, USA);
- Homogenization (HMG): Pretreatment using an ultra-turrax disperser (T25 Basic®, IKA, Germany) under stirring at 3000 rpm for 60 min at 60°C;
- Ultrasound (UTS): Process performed in an ultrasonic cleaner (Model CPX3800H, Branson, USA) at a frequency of 40 kHz and 60°C for 60 min;
- Annealing and ultra-rapid freezing (ANN-C): Heating at 60°C with shaking for 240 min with subsequent storage in an ultra-low temperature at -12°C for 8 h (Kaltis®, F390, USA). Pretreated starch was recovered and dried in a forced convection oven (UFB500, Memmert, Germany) at 35°C for 8 h.

Enzymatic hydrolysis of starch

Pretreated-starch suspensions at 20% (w/v) were subjected to enzymatic hydrolysis with the simultaneous application of α-amylase (15 U/g starch) and amyloglucosidase (10 U/g starch) in a sodium citrate/citric acid buffer solution at pH 5.0, 60°C and 250 rpm for 6 h (Figueroa-Flórez *et al.*, 2019). Subsequently, hydrolysates were removed by centrifugation at 5000 rpm for 15 min. Samples were washed with ethanol and distilled water to remove the residual enzyme content. Finally, the starch was recovered and dried in a forced convection oven at 35°C for 8 h.

Native cassava starch (NCS) and non-physically pretreated starch granules (NPS) were referred to as control treatments. The NPS treatment corresponded to samples of enzymatically modified starch without physical and thermal pretreatment.

Hydrolysis degree (HD)

The degree of hydrolysis was evaluated from the reaction of reducing sugars released with 3,5-dinitrosalicylic acid (DNS) by UV-Vis spectrophotometry (UV-2550, Shimadzu, Japan) at a wavelength of 540 nm (Salcedo-Mendoza *et al.*, 2018). We used 500 µl of sample to react with an excess amount of DNS for 8 min at 80°C with subsequent cooling to 10°C to stop the reaction. The degree of hydrolysis was expressed as the ratio between weight at the beginning and at the end of the period of enzymatic action (Figueroa-Flórez *et al.*, 2019). The concentration of sugars was expressed in glucose equivalents (GE).

Starch morphology

Starch granules were fixed in a sample holder with electrically conductive carbon tape covered with a platinum/gold alloy (Chen *et al.*, 2011). Samples were observed on a scanning electron microscope (JEOL, JSU LV-5600, Japan) under conditions set at 15 KV, 30 mA and an amplitude margin 3000X.

Determination of granular size

Granular size was determined by light scattering using a Mastersizer particle analyzer (Model 3000E, Malvern, UK) and expressed as a function of the average diameter D_X (50). Measurements were performed in triplicate at room temperature with a refractive index of 1.52 (Monroy *et al.*, 2018). Pore characteristics were determined using an automatic specific surface area and porosity analyzer (Quantachrome Inc., NOVA 2000e, USA). Pure nitrogen (> 99.99%) was used to determine adsorption-desorption isotherms. The starch samples were dried for 20 h under vacuum at 100°C, degassed at 125°C for 24 h, and immersed in liquid nitrogen (-196°C) in the range of relative pressure P/Po ≈ 5-19% (Gao *et al.*, 2013). The mean pore diameter and the specific surface area (A_S) were calculated with the BET method (Brunauer-Emmett-Teller). The total pore volume (ν) was estimated with the BJH (Barrett-Joyner-Halenda) method (Guo *et al.*, 2020).

Amylose content and infrared spectroscopy (FTIR)

Amylose content was estimated by the colorimetric iodine method using a UV-Vis spectrophotometer (UV-2550, Shimadzu, Japan) at a wavelength of 620 nm (Salcedo-Mendoza *et al.*, 2018). Infrared spectra were obtained in the region of 500 to 4000 cm^{-1} making 32 readings at a resolution of 4 cm^{-1} using a spectrometer (Nicolet IS50 FT-IR, Thermo Scientific, USA). The order degree (OD) was estimated as the absorbance ratio in the 1047/1022 cm^{-1} bands, expressed as a percentage (Ma *et al.*, 2018).

XRD diffraction patterns

Diffraction patterns were obtained with an XRD diffractometer (X'Pert Pro-MPD, Panalytical, Italy) in the range of 4-35°, operating at 1.8 kW with a current of 40 mA (Salcedo-Mendoza *et al.*, 2018). The crystallinity index (CI) was calculated by the ratio of areas of the absorption peaks (crystalline zone) over the total area using Origin Lab v8.0 (OriginLab Corporation, USA).

Water solubility index (WSI), water absorption index (WAI), and swelling power (SP)

WSI, WAI, and SP were determined with slight modifications according to Rocha *et al.* (2010). One g of starch was dispersed in 25 ml of distilled water under agitation at 60°C for 30 min, then centrifugation was performed at 5000 rpm for 15 min. The pellet was removed, and weight was quantified. The SP was estimated as the ratio between the gel weight and the initial weight of dry starch, using the equation reported by Figueroa-Flórez *et al.* (2019). The resulting gel was weighed to estimate WAI. An aliquot of 10 ml was taken from the supernatant, poured into Petri dishes, and evaporated in an oven at 70°C for 16 h. WSI was calculated as the amount of dry solids recovered by evaporating the water absorption test supernatant.

Thermal properties

Gelatinization properties were determined using a differential scanning calorimeter (DSC-Q2000, TA Instruments, USA) and a suspension in an aluminum pan with a 2 mg of starch and 6 mg of water ratio (Xie *et al.*, 2019). Capsules were sealed and stored at 25°C for 24 h to balance the system. Subsequently, they were heated from 20°C to 120°C at a speed of 10°C min^{-1} and cooled to a ramp of 25°C min^{-1}

under a nitrogen atmosphere. Onset temperature (T_O), peak temperature (T_P), and conclusion temperature (T_C) were determined from the DSC curves. The gelatinization enthalpy (ΔH) was estimated based on the endothermic peak area expressed in J/g.

Experimental design

A unifactorial categorical design was implemented where each level is related to a specific pretreatment. Results were analyzed using statistical tools such as analysis of variance (ANOVA) and Tukey test for comparison of means with a level of significance of 5% using the Statgraphics software (Centurion XVI, Statgraphics Technologies Inc., USA).

Results and discussion

Effect of pretreatments on the enzymatic hydrolysis of starch granules

The hydrolysis degree oscillated between 11.99 ± 0.65% and 24.92 ± 0.40% (Tab. 1). Similar results have been reported during hydrolysis with α-amylase and/or amyloglucosidase in starches from various amylaceous sources (Rocha *et al*., 2010; Shariffa *et al*., 2017). This behavior is probably due to the degradation of amylose and amylopectin polymer fractions by the synergistic action of amylolitic enzymes, affecting structural and morphological characteristics of the granules (Chen *et al*., 2011).

A decrease in the degree of hydrolysis was found in the UTS treatment (21.40 ± 0.82%) with respect to the NPS treatment (11.99 ± 0.65%). These results were lower than those reported in the hydrolysis of corn starch through the application of ultrasonic technology (Li *et al*., 2018). However, treatment of UTS can alter swelling and solubility properties, changing the interaction of polymer chains with water molecules and the semicrystalline order of the granules, and forming granules less susceptible to enzymatic attack (Wu *et al*., 2011). This same behavior associated with the redistribution or recrystallization of amylose and amylopectin chains during ANN-C treatment could explain the molecular resistance of the granules to enzymatic degradation and the decrease in the degree of hydrolysis (16.71 ± 0.60%).

The results of the degree of hydrolysis correlate with morphological changes of granules. In Figure 1, NCS granules with oval or spherical morphologies of relatively smooth surfaces without lacerations can be seen, with truncated ends possibly inherent to the extraction process (Salcedo-Mendoza *et al*., 2018). But, after the enzymatic treatment, granules showed erosion on their external surface and generalized cavities with apparent depth (Fig. 1NPS), as well as a rupture or fragmentation, probably associated with the physical and thermal pretreatments established before the biocatalytic process. Similar results have been reported previously, where the same pretreatments facilitated the release of starch components from the amorphous zone affecting the morphological characteristics of the granule on a superficial level (Chen *et al*., 2011; Wu *et al*., 2011; Shariffa *et al*., 2017; Zhao *et al*., 2018; Wang *et al*., 2022).

The microphotographs of the starches pretreated with UTS (Fig. 1UTS) suggest a granular surface and microstructural modifications. Likewise, the starches modified with the ANN treatment showed more generalized superficial granular lacerations with respect to NPS samples (Fig.1NPS), in line with the results reported for hydrolyzed cassava and sweet potato starches pretreated under gelatinization temperature for 72 h (Shariffa *et al*., 2017). With the HMG pretreatment there were deep surface erosions, presumably due to the enzymatic action (Fig. 1HMG) and a generalized loss of granule integrity due to exposure to shear stress. These data are consistent with those reported for mechanically modified cassava starches (He *et al*., 2014). After the ultrasonic pretreatment noticeable changes were observed in granular morphology that presented greater cracks and superficial lacerations at a lower degree of hydrolysis with respect to its NPS counterpart.

TABLE 1. Hydrolysis degree, amylose content, and physicochemical properties in native and modified cassava starches.

Treatment	HD (%)	Amylose (%)	WSI (g/g)	WAI (g/g)	SP (g/g)
NCS	-	19.53 ± 0.32^{a}	2.17 ± 0.08^{a}	3.84 ± 0.16^{a}	3.99 ± 0.10^{a}
NPS	11.99 ± 0.65^{a}	18.76 ± 0.19^{b}	3.50 ± 0.12^{b}	4.36 ± 0.11^{b}	3.20 ± 0.14^{b}
ANN	15.24 ± 0.47^{b}	18.71 ± 0.28^{b}	3.80 ± 0.17^{b}	4.29 ± 0.12^{b}	2.92 ± 0.06^{c}
HMG	24.92 ± 0.40^{c}	18.93 ± 0.14^{b}	4.22 ± 0.07^{c}	4.86 ± 0.05^{c}	2.65 ± 0.18^{d}
UTS	21.40 ± 0.82^{d}	17.97 ± 0.22^{c}	4.61 ± 0.11^{d}	4.85 ± 0.09^{c}	2.28 ± 0.10^{e}
ANN-C	16.71 ± 0.60^{be}	17.64 ± 0.16^{c}	4.84 ± 0.19^{d}	4.41 ± 0.07^{b}	2.55 ± 0.15^{df}

HD - hydrolysis degree; WSI - water solubility index; WAI - water absorption index; SP - swelling power. Native cassava starch (NCS); non-pretreated enzymatic modified starch (NPS); modified starch pretreated by annealing (ANN); modified starch pretreated by homogenization (HMG); modified starch pretreated by ultrasound (UTS), and modified starch pretreated by annealing and ultra-rapid freezing (ANN-C). Different superscripted letters in the same column indicate significant differences for Tukey test (P<0.05).

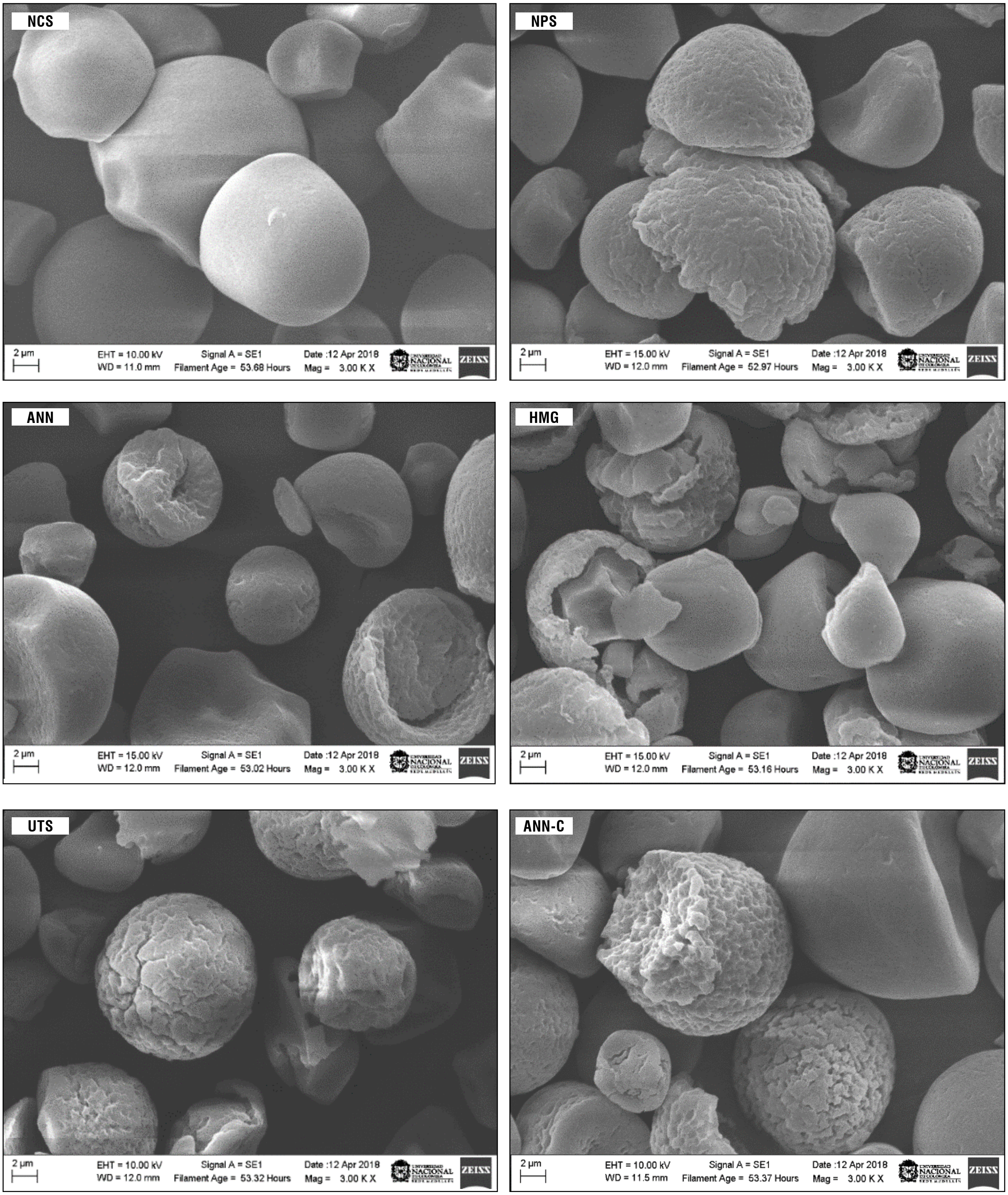

FIGURE 1. Microphotographs of native and modified cassava starches (3000x). Native cassava starch (NCS); non-pretreated enzymatic modified starch (NPS); modified starch pretreated by annealing (ANN); modified starch pretreated by homogenization (HMG); modified starch pretreated by ultrasound (UTS); modified starch pretreated by annealing and ultra-rapid freezing (ANN-C).

Similar observations have been reported regarding corn starches modified using various ultrasound wave frequencies (Li *et al*., 2018). With the ANN-C treatment, an extended and uniform porosity was observed in the granules, accompanied by more pronounced lacerations or cracks in their surfaces (Fig. 1ANN-C). Experiments conducted with sweet potato and waxy rice starches have revealed an analogous behavior after applications of annealing or successive freezing (Tonon *et al*., 2009; Tao *et al*., 2015).

The annealing treatment produces cracks in the granules of sweet potato starch that exhibit a greater proportion of porous granules that preserve the integrity of their morphology (Tonon *et al*., 2009). Tao *et al*. (2015) consider that the presence of some fragmented granules is due to the powerful compression force exerted by the formation of ice crystals during freezing. Therefore, a greater generation of pores and lacerations is a consequence of thermal treatments that increase the surface area and favor the enzymatic action on polyglucan chains (Zhao *et al*., 2018).

The granule size had a bimodal distribution between 5 and 100 μm for NCS, and the mean diameter D_X (50) ranged from 17.05 to 21.65 μm for all samples (Fig. 2, Tab. 2). The diameters D_X (50) decreased after the enzymatic treatment in the following order: HMG<UTS<ANN-C<ANN<NPS. The samples of modified starch pretreated with HMG exhibited the smallest diameters (17.05 ± 0.35 μm), possibly caused by shear force action (Hossen *et al*., 2011). These results are consistent with the morphological changes of the granules analyzed by microscopy (Fig. 1HMG).

TABLE 2. Physical characteristics of starch granule populations in samples.

Treatment	D_X (50) (μm)	ν (cm^3/g) x 10^{-3}	∅ (nm)	As (m^2/g)
NCS	21.65 ± 0.35^{a}	0.14 ± 1E-5^{a}	0.90 ± 0.00^{a}	0.58 ± 0.02^{a}
NPS	20.45 ± 0.48^{b}	0.31 ± 1E-5^{a}	1.12 ± 0.01^{b}	0.88 ± 0.07^{a}
ANN	19.95 ± 0.26^{b}	0.67± 0E-5^{b}	0.97 ± 0.06^{c}	1.94 ± 0.01^{a}
HMG	17.05 ± 0.21^{c}	2.39 ± 3E-5^{c}	1.86 ± 0.03^{b}	1.03 ± 0.12^{a}
UTS	17.65 ± 0.37^{d}	2.05 ± 0E-5^{d}	2.01 ± 0.01^{d}	2.66 ± 0.06^{a}
ANN-C	17.55 ± 0.24^{e}	2.09 ± 1E-5^{d}	1.96 ± 0.05^{e}	3.19 ± 0.09^{a}

D_X (50) - average diameter of microporous granule; ν - total volume of microporous granule; ∅ - average diameter of microporous granule; A_S - specific surface area. Native cassava starch (NCS); non-pretreated enzymatic modified starch (NPS); modified starch pretreated by annealing (ANN); modified starch pretreated by homogenization (HMG); modified starch pretreated by ultrasound (UTS), and modified starch pretreated by annealing and ultra-rapid freezing (ANN-C). Different superscripted letters in the same column indicate significant differences for Tukey test (P<0.05).

UTS and ANN-C exhibited significantly reduced diameter D_X (50) in NCS. Ultrasonic cavitation and molecular reorganization (caused by ice crystals during freezing), probably, triggered structural changes (Wang *et al*., 2022)

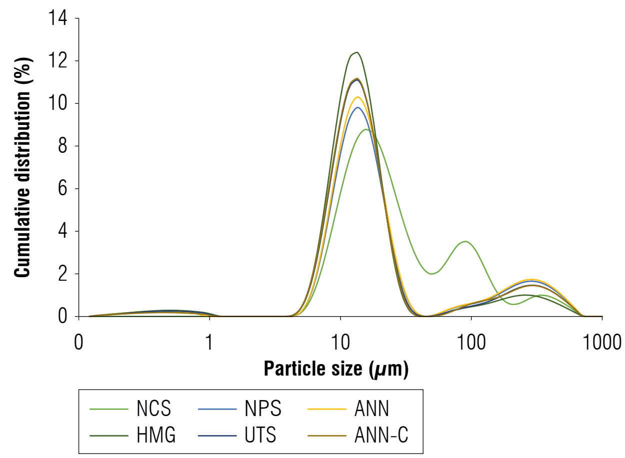

FIGURE 2. Particle size distribution of native and modified cassava starches. Native cassava starch (NCS); non-pretreated enzymatic modified starch (NPS); modified starch pretreated by annealing (ANN); modified starch pretreated by homogenization (HMG); modified starch pretreated by ultrasound (UTS), and modified starch pretreated by annealing and ultra-rapid freezing (ANN-C).

that favored hydrolysis, with morphological changes in granular size, pore size, and volume increase (Tab. 2). The ANN treatment only caused a slight reduction in the diameter D_X (50) with respect to its NPS counterpart. These results confirm that physical and thermal treatments reduce granular size and increase the porosity of the granules based on the depolymerization of the unstable amorphous materials present in the granular surface and the endogenous action of α-amylase hydrolyzing from the surface to the hilum (Tukomane *et al*., 2007; Monroy *et al*., 2018; Zhao *et al*., 2018).

Modification processes caused a significant increase in specific surface area, volume, and pore diameter (P<0.05) (Tab. 2). The N_2 adsorption isotherms of the modified starches were type IV with a hysteresis curve type H3 (results not shown) that is characteristic of mesoporous materials (Rouquerol *et al*., 1994). In this study, pore size was greater than 2 nm. Therefore, according to the classification presented by Xie *et al*. (2019), the material was mesoporous. The mesopores identified and estimated in NCS samples were probably caused by the presence of intrinsic porosities of the granule in its native state or the action of endogenous enzymes during the extraction process (Foresti *et al*., 2014). The results with the NCS treatment showed that enzymatic action increased the surface area, diameter, and volume of the pores compared to native starch (Tab. 2). However, no significant increase in mesoporous properties was detected with the HMG treatment, possibly because the mechanical action during the size reduction facilitated the amyloglucosidase endo-corrosion phenomenon and led to a higher production of fragmented granules. Specifically, the granules pretreated with UTS and ANN-C exhibited more uniform porosity and larger pores that did not affect their native spherical or oval shape, as shown in the SEM microphotographs. Sharrifa *et al.* (2009) consider that temperatures close to gelatinization can swell the granule during pregelatinization, increasing the opening and size of the pores that could facilitate enzyme absorption and degrade the external part of the granule by exo-corrosion. As pore size grows due to exo-corrosion, it may also favor the phenomenon of endo-erosion toward the inside of the granule (Keeratiburana *et al.*, 2020). All the enzymatic actions occurred at the surface or internal level, leading to the production of mesoporous granules with better hydrophilic properties.

The enzymatic process significantly altered the interaction of polymer chains with water molecules (Zhong *et al.*, 2022). There was an increase in modified starches WSI and WAI with respect to the NCS samples (Tab. 1), possibly due to the breakdown of intermolecular bonds and the depolymerization of long-length chains, helping the release and availability of soluble polymer components during granules swelling process (Wang *et al.*, 2016; Jung *et al.*, 2017). The WSI values with respect to the control increased in the following order: HMG>UTS>ANN-C>ANN>NPS, while WAI values increased in the order: HMG>UTS>ANN-C>NPS>ANN. The increase of WSI and WAI in modified starches pretreated by HMG and UTS, possibly depends on granule size reduction by the HMG process or the increase of surface porosity, since those effects increase the surface area improving the interaction and penetration of water molecules, and other authors (Gao *et al.*, 2013; He *et al.*, 2014; Benavent-Gil & Rosell, 2017) report an increase in water solubility and absorption properties in corn starches subjected to a size reduction process. However, this contrasts with a decrease in WSI of modified starches that underwent annealing before the hydrolytic process (Gomes *et al.*, 2005). The decrease in solubility may be due to molecular reorganization between amylose and/or amylopectin helices during annealing, producing a more stable structure that prevents the leaching of soluble components of the granules (Dias *et al.*, 2010; Shariffa *et al.*, 2017).

The SP decreased after enzymatic hydrolysis (Tab. 1), and similar results were reported in the production of porous corn starches with amyloglucosidase and glycosyltransferases (Dura *et al.*, 2014; Benavent-Gil & Rosell, 2017). The decrease in SP has been attributed to the increase in crystal perfection, the intermolecular binding forces, and the decrease in WSI of polymer chains (Waduge *et al.*, 2006; Jayakody & Hoover, 2008; Shariffa *et al.*, 2017). The foregoing could explain the behavior in SP reduction in starches pretreated by UTS and ANN-C that showed higher relative crystallinity index and lower susceptibility to the enzymatic attack, as a consequence of strengthening between the amylose-amylose and amylose-amylopectin interactions. In relation to the pretreatment, SP values decreased in the following order: UTS<ANN-C<HMG<ANN<NPS. Several studies have reported the reduction of SP after annealing in starches from different starch sources associated with changes in amylose content and reordering of amylopectin double helices during cooling (Gomes *et al.*, 2005; Waduge *et al.*, 2006; Jayakody & Hoover, 2008; Dias *et al.*, 2010; Shariffa *et al.*, 2017).

Structural analysis of native and modified cassava starches

In FT-IR spectrum, characteristic peaks were observed in the 900 to 1200 cm^{-1} region (Fig. 3A), associated with crystalline and amorphous regions (Zhang *et al.*, 2012; Salcedo-Mendoza *et al.*, 2018; Figueroa-Flórez *et al.*, 2019; Zhang *et al.*, 2019). The absorption bands at 1130 and 1160 cm^{-1} attributed to the vibration of the C-O, C-C or O-H bonds present in amylose and amylopectin molecules, increased the intensity of the signal, possibly by the depolymerization and breakdown of glycosidic bonds with the enzymatic treatment (Salcedo-Mendoza *et al.*, 2018). In addition, a characteristic peak of the starch is band length at 995 cm^{-1} associated with the presence of water bound in the structure, whose band possibly moved to a greater availability of OH groups after depolymerization, favoring the capacity of the starch granule to retain and absorb water (Zhang *et al.*, 2012; Salcedo-Mendoza *et al.*, 2018). Absorption peaks at 1047 and 1022 cm^{-1} are closely related to the crystalline and amorphous structure (Ma *et al.*, 2018; Figueroa-Flórez *et al.*, 2019). The FT-IR spectra depend on the changes in the starch structure in a short-range order, defined as the double helix order, and that reflects the number of double helices ratio (ordered domains) with simple helices (amorphous domains) (Xu *et al.*, 2018). The determination of the order degree (OD) was made from the FT-IR spectra (Tab. 3), showing an increase after the enzymatic treatment (ANN-C>UTS>HMG>ANN>NPS) that are consistent results with those reported in previous studies on the enzymatic modification of cassava starch (Salcedo-Mendoza *et al.*, 2018; Figueroa-Flórez *et al.*, 2019). Modified starches pretreated by UTS and ANN-C showed the highest values of OD, possibly due to the release of free amylaceous materials and reorganization/recrystallization of amylose chains.

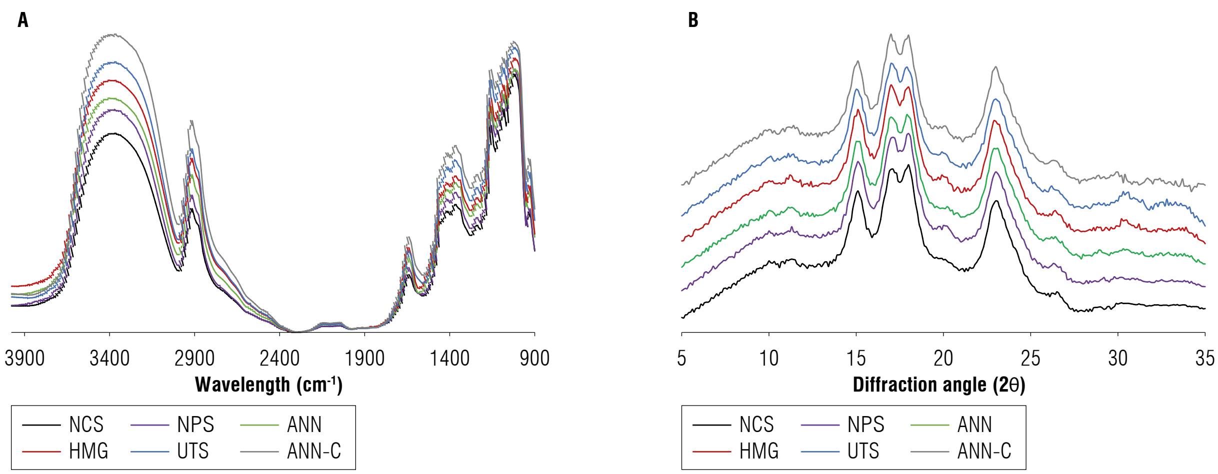

FIGURE 3. A) FTIR - infrared spectra and B) XRD - diffraction patterns in native and modified cassava starches. Native cassava starch (NCS); non-pretreated enzymatic modified starch (NPS); modified starch pretreated by annealing (ANN); modified starch pretreated by homogenization (HMG); modified starch pretreated by ultrasound (UTS), and modified starch pretreated by annealing and ultra-rapid freezing (ANN-C).

These results agree with the crystallinity index behavior estimated by XRD diffraction. In turn, Xu *et al.* (2018) linked the increase in the bands 1045/1024 ratio with the time and number of cycles of annealing treatment in bean starches and to the reorganization and a greater ordering of double helices of amylose and amylopectin in the crystalline domains of starch granules. By XRD diffraction, four crystallographic peaks were identified under angles of 15°, 17°, 17.8°, and 23° characteristic of tubular starches with diffraction pattern A-type (Fig. 3B) whose double amylopectin helices achieve a conformation in monoclinic form (Jayakody & Hoover, 2008). Starches showed higher intensity in peaks after physical and enzymatic treatments without evident changes in diffraction patterns. A similar behavior was found in previous studies possibly due to the decrease in amylose content in cassava starch granules (Salcedo-Mendoza *et al.*, 2018; Figueroa-Flórez *et al.*, 2019).

The crystallinity index increased in modified starches with respect to the control, in the following order: ANN-C>UTS>ANN>NPS>HMG (Tab. 3). The increase in CI of modified starches pretreated by UTS and ANN-C could be attributed initially to the removal of amorphous and unstable amylaceous materials by the action of the ultrasonic frequency, thermal energy, or enzymatic action (Li *et al.*, 2018) affecting the structural and morphological properties as seen in FT-IR spectra and microscopy analysis.

Other research also has reported an increase in the crystallinity index of starch granules with the annealing process without affecting the characteristic diffraction pattern, due to the hydration and mobility of glucan chains by the action of temperature accelerate interaction and reorganization phenomena of amylose or amylopectin double helices (Jayakody & Hoover, 2008; Xu *et al.*, 2018; Zhang *et al.*, 2019), as well as the formation of additional intra-helical hydrogen bonds that result in an increase in crystalline sheet thickness during cooling (Jayakody & Hoover, 2008; Seetapan *et al.*, 2016).

These results allow inferring that restructuring the short chains present in the amorphous and crystalline domains, together with the decrease of amylose content, altered the semicrystalline order of granules, and affected both the structural resistance of the granules to the enzymatic action and the behavior of thermal properties.

Thermal properties

In relation to gelatinization properties, significant changes in temperatures (T_O, T_P, and T_C) after the enzymatic process were estimated (Tab. 3), where the increase in T_O can be explained by the cleavage of glycosidic bonds that induce molecular and structural changes, affecting mainly the swelling process that regulates gelatinization (Chen *et al.*, 2011; Dura *et al.*, 2014). Likewise, recent investigations have explained that changes in transition temperatures are related to an increase of the crystallinity index, an indicator of structural stability and granular molecular resistance to gelatinization (Jung *et al.*, 2017; Zhao *et al.*, 2018; Xie *et al.*, 2019). These results are consistent with those found from the values of OD and CI determined by FT-IR and XRD diffraction. No significant differences were detected in T_O values between samples of hydrolyzed starches with the NPS, ANN, HMG and UTS treatments, except for the exception of the ANN-C treatment. The difference in the behavior of T_O through the ANN-C cycle may be due to the

TABLE 3. Crystallinity index and gelatinization properties in native and modified cassava starches.

Sample	CI	OD	To (°C)	Tp (°C)	Tc (°C)	(Tc-To) (°C)	ΔH (J/g)
NCS	0.487 ± 0.006^{a}	0.475 ± 0.000^{a}	64.49 ± 0.13^{a}	68.22 ± 0.22^{a}	86.72 ± 0.44^{a}	22.23 ± 0.20^{a}	19.34 ± 0.33^{a}
NPS	0.496 ± 0.007^{b}	0.483 ± 0.009^{b}	65.30 ± 0.11^{b}	70.14 ± 0.19^{b}	84.77 ± 0.27^{b}	19.45 ± 0.11^{b}	15.19 ± 0.41^{b}
ANN	0.491 ± 0.010^{b}	0.486 ± 0.004^{b}	70.27 ± 0.15^{bc}	73.17 ± 0.25^{c}	85.79 ± 0.76^{b}	15.52 ± 0.03^{c}	14.51 ± 0.71^{ab}
HMG	0.495 ± 0.000^{b}	0.472 ± 0.005^{a}	70.36 ± 0.09^{bc}	73.01 ± 0.17^{bc}	87.41 ± 0.52^{c}	17.05 ± 0.17^{d}	14.98 ± 0.50^{abc}
UTS	0.502 ± 0.004^{c}	0.497 ± 0.002^{c}	70.43 ± 0.16^{c}	73.28 ± 0.23^{c}	87.77 ± 0.53^{c}	17.34 ± 0.06^{e}	15.69 ± 0.54^{ac}
ANN-C	0.511 ± 0.008^{d}	0.506 ± 0.009^{c}	72.96 ± 0.10^{d}	75.38 ± 0.17^{d}	86.13 ± 0.72^{b}	13.16 ± 0.15^{f}	15.79 ± 0.63^{acd}

OD - order degree; CI - crystallinity index; To - onset temperature; Tp - peak temperature; Tc - conclusion temperature; ΔH - gelatinization enthalpy. Native cassava starch (NCS); non-pretreated enzymatic modified starch (NPS); modified starch pretreated by annealing (ANN); modified starch pretreated by homogenization (HMG); modified starch pretreated by ultrasound (UTS), and modified starch pretreated by annealing and ultra-rapid freezing (ANN-C). Different superscripted letters in the same column indicate significant differences for Tukey test ($P<0.05$).

initial endothermic transition caused in granules during the annealing process, where water diffuses freely in the amorphous and crystalline regions altering the semicrystalline order, together with the reorganization of short chains during ultra-rapid-freezing process (Waduge *et al.*, 2006; Jayakody & Hoover, 2008; Seetapan *et al.*, 2016; Zhao *et al.*, 2018). Authors argue that a greater reorganization of double helices during annealing resulting from an accentuation in the mobility of the glucan chain leads to the formation of intra-helical hydrogen bonds changing the thickness of the crystalline sheet and ultimately the melting temperature of double helices, delaying gelatinization (Kiseleva *et al.*, 2004). Analogous results have been found in the SP behavior (Tab. 1).

The temperature range (T_O-T_C) showed a marked difference with the implementation of pretreatments followed by the enzymatic process with respect to the NCS samples. This behavior is supported by the premise that enzymatic hydrolysis causes both morphological and structural changes that limit the thermal behavior of starch granules (Gao *et al.*, 2013; Li *et al.*, 2018). Likewise, the increase in gelatinization temperature is more pronounced for T_O (fusion of the weakest crystallites) and less for T_C (fusion of stable and more perfect crystals) during the annealing process (Kiseleva *et al.*, 2004; Jayakody & Hoover, 2008). For this reason, the decrease of T_O -T_C range with the ANN-C treatment, possibly due to a higher homogeneity, cooperative fusion, and more perfect crystals. This allows us to understand the most notorious changes on properties such as swelling, crystallinity, and susceptibility to enzymatic action after the hydrothermal pretreatment ANN-C. These same premises could explain the increase of ΔH in modified starches that is influenced by the hydration of the macromolecule and the interaction between polymer chains that alter the assembly of amorphous and crystalline lamellar structures (Jayakody & Hoover, 2008; Shariffa *et al.*, 2017; Xie *et al.*, 2019). However, the increase of ΔH in starches with different amylose contents after annealing can be correlated with an interaction of the short chains that can form new double helices or the granular conformation of the smectic type, whose reorganization will require higher values of energy to melt and start the gelatinization process (Kiseleva *et al.*, 2004; Waduge *et al.*, 2006; O'Brien & Wang, 2008; Dias *et al.*, 2010).

Conclusions

The integrated processes of enzymatic biocatalysis and physical-thermal pre-treatment introduced here allowed the development of modified cassava starches with microporous surfaces. The enzymatic process caused significant changes in the structural characteristics of such starches, altering their semicrystalline order and gelatinization. The HMG decreased the size of the granules, thus, affecting their swelling capacity and gelatinization properties that are defined by a flexible and unstable granular configuration of small and fragmented granules. In turn, UTS pretreatments and the ANN-C cycle caused changes in morphological properties and homogeneously increased the size and volume of the pores in the granular surface, thus decreasing the range and enthalpy of the gelatinization. The ANN-C cycle can be an affordable and effective pretreatment in the production of thermally stable microporous cassava starches. As a result, we recommend optimizing the hydrothermal annealing process in the production of porous cassava starches considering variables such as starch/water ratio, heating time, and temperature.

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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

JAFF, ECC, and ERS designed the experiments; JAF and EDAD carried out the experiments and data collection in laboratory experiments. JAFF, EDAD, and ECC contributed to the data analysis. JAFF, EDD, ECC, HJC, JGSM, and ERS wrote the article. All authors reviewed the final version of the manuscript.

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Influence of modified cassava starch on the physicochemical properties of a fermented soybean beverage

Influencia del almidón de yuca modificado sobre las propiedades fisicoquímicas de una bebida fermentada de soya

Julián David Rodriguez-Ruiz[1]*, Eduardo Rodriguez-Sandoval[2], and María Soledad Hernández[1]

ABSTRACT

Fermented soybean beverages are an alternative for improving intestinal health, and fermentation reduces the anti-nutritional factors of this legume. However, they do show high syneresis and low viscosity. Modified cassava starches could be added as a thickener and/or stabilizer to improve the quality of the product. The aim of this research was to assess the effect of adding modified cassava starch on the physicochemical properties of a fermented soybean beverage. Preliminary tests were carried out varying the concentration (0.8%, 1.0%, and 1.2%) of 3 types of modified cassava starch: octenyl succinic anhydride (OSA), acetylated distarch adipate (ADA) cross-linked starch, and substituted-crosslinked starch (mixed). A commercial culture of starter microorganisms and probiotics was used in the fermentation process. The statistical analysis was carried out with a two-factor (type of starch and concentration) and 3-level design; quality parameters such as pH, acidity, soluble solids, syneresis, and viscosity comparable to commercial fermented dairy beverages were evaluated. OSA starch had a lower syneresis and higher viscosity than the other starches for each concentration. Furthermore, the addition of 1.0% OSA and mixed starch, as well as 1.2% ADA starch, are comparable to the control commercial soybean beverage (SC).

Key words: syneresis, viscosity, physicochemical parameters, fermenting microorganisms, probiotics.

RESUMEN

Las bebidas fermentadas de soya son una alternativa para mejorar la salud intestinal y reducir los factores anti-nutricionales de esta leguminosa; no obstante, muestran alta sinéresis y baja viscosidad. Para mejorar estas características de calidad, se podrían adicionar almidones de yuca modificados empleados como espesante y/o estabilizante en el producto. El objetivo de este estudio fue evaluar el efecto de la adición de almidones de yuca modificados en las propiedades fisicoquímicas de una bebida fermentada de soya. Se realizaron pruebas preliminares variando la concentración (0.8%, 1.0% y 1.2%) de 3 tipos de almidón de yuca modificado: anhidrido octenil succinico (OSA), almidón entrecruzado de adipato de dialmidón acetilado (ADA) y almidón entrecruzado-sustituido (mixto). La fermentación se realizó a partir de un cultivo comercial de microorganismos iniciadores y cultivos probióticos. El análisis estadístico se realizó con un diseño de dos factores (tipo de almidón y concentración) y 3 niveles; se evaluaron parámetros de calidad como pH, acidez, sólidos solubles, sinéresis y viscosidad comparables a los de bebidas lácteas comerciales. El almidón OSA tuvo una sinéresis menor y una viscosidad mayor frente a los otros almidones para cada una de las concentraciones. Por otra parte, la adición del 1.0% del almidón OSA y mixto, así como del almidón ADA al 1.2%, son comparables con el control de bebida comercial de soya (SC).

Palabras clave: sinéresis, viscosidad, parámetros fisicoquímicos, microorganismos fermentadores, probióticos.

Introduction

Vegetable beverages have been developed as substitutes for dairy beverages; soybean beverages are important due to their high nutritional value and wide range of health benefits. However, the presence of antinutrients such as trypsin inhibitors raises concerns from a nutritional point of view. Trypsin inhibitors cause low protein efficiency rates and pancreatic hypertrophy (Aderibigbe *et al.*, 2021). Heat treatments more intensive than conventional pasteurization are needed to reduce or eliminate trypsin inhibitors due to their high thermal stability. Nevertheless, along with their reduction and degradation, the processed beverages show a reduction in soybean protein solubility kinetics that also serves as quality control for the presence of antinutritional factors in soybean-based products.

Emerging technologies such as high-pressure processing (HPP), electric pulses, and ultrasound for the inactivation of microorganisms and anti-nutritional factors are very

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[1] Institute of Food Sciences and Technology (ICTA), Faculty of Agricultural Sciences, Universidad Nacional de Colombia, Bogotá (Colombia).
[2] Department of Agricultural and Food Engineering. Faculty of Agricultural Sciences. Universidad Nacional de Colombia, Medellín (Colombia).
* Corresponding author: juldrodriguezrui@unal.edu.co

expensive (Guerrero-Beltrán *et al*., 2009; Yuan & Chang, 2010; Vanga *et al*., 2020; Aderibigbe *et al*., 2021). Hydrocolloids can be added to soybean beverages to improve their solubility, texture, viscosity, consistency, and syneresis (Yuan & Chang, 2010), and their effect on the gelling behavior of soybean beverage gels has been extensively studied; their thickening, stabilizing and/or gelling properties stand out. The function varies according to their molecular weight, structure, compatibility, gelling profile, dispersion, and hydration conditions that add value to food products (Rodríguez Sandoval *et al*., 2003).

Modified starches are widely used to improve the properties of food products, specifically to increase heat resistance, water retention capacity, viscosity, and to minimize the syneresis effect (Cho & Kang, 2022). We propose the use of three types of modified cassava starch as an alternative because of their low cost and function as a thickener and/or stabilizer in foods. They have various uses in the food industry.

Starch modifications affect the quality and the application of a food matrix. Thus, it is necessary to study the sensory properties, structure, and stability of the product during storage. Its rheological properties must also be known during production, *i.e.*, viscosity, shear strength, and physicochemical properties (Abbas *et al*., 2010). Some potato, cassava, and corn starches with high amylose content have been modified to achieve suitable physicochemical properties such as resistance to low or high storage temperatures, low pH, and opacity (Mahmood *et al*., 2017).

Three types of modified cassava starches were used in this study: octenyl succinic anhydride (OSA) with a substitution, crosslinked acetylated distarch adipate (ADA) starch and crosslinked-substituted starch (mixed). Different applications have been reported for these; for instance, the OSA modification changes its structure and emulsifying capacity (Gao *et al*., 2021). The latter depends on the degree of substitution, the size of the granules, the aggregation of the particles between the air/water interface, and the starch to oil ratio of the formulation in cassava, potato, rice, and wheat starches (Saari *et al*., 2016; Marefati *et al*., 2017; McNamee *et al*., 2018). OSA-modified starches have been used as carriers of bioactive food components (Wang *et al*., 2011) in dairy beverages using fermented whey (Imbachí-Narváez *et al*., 2018). They microencapsulate OSA and maltodextrins in the release of rose essential oils (Xiao *et al*., 2019; Partheniadis *et al*., 2020). Moreover, OSA starch is used in the cosmetics industry for its emulsifying capacity (León-Méndez *et al*., 2020). Modified acetylated distarch adipate (ADA) starch is used in foods as a bulking agent, stabilizer, and thickener (Zięba *et al*., 2014; Partheniadis *et al*., 2020). It shows improved stability, hardness, and adhesiveness of the gels formed from it as well as swelling power, solubility, pasting temperature, gelatinization, and retrogradation enthalpy (Prochaska *et al*., 2009). It also improves the resistance to mechanical processes, temperature, and acidic pH with greater stability during storage. Hence, it is important to evaluate the effect of adding three modified cassava starches - ADA, OSA, crosslinked-substituted starch at different concentrations on the physicochemical properties of a fermented soybean beverage.

Materials and methods

Materials

Soybeans were obtained from a local market in Bogotá. Modified cassava starches were donated by Poltec SAS (La Estrella, Antioquia, Colombia) as follow: starch with octenyl succinic anhydride substitution (OSA) (Gel®Lact), cross-linked acetylated distarch adipate (ADA) (Gel®Cream), and crosslinked-substituted starch, *i.e.*, mixed starch (Gel®Lact XP). For fermentation, freeze-dried starter culture was used for direct inoculation with selected *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus and* probiotic microorganisms *Lactobacillus acidophilus* and *Bifidobacterium animalis* subsp. *lactis* (SACCO Lyofast SYAB 1).

Preparation of fermented soybean beverages

Soybeans with optimal physical quality (without defects) were carefully selected and soaked overnight. They were washed several times and shelled and subjected to precooking at boiling temperature in a 0.02% (w/v) bicarbonate solution for 15 min (Zięba *et al*., 2014; Zheng *et al*., 2021). Wet grinding was carried out using a Blixer® equipment and water was slowly added according to one of the methods proposed by Zhang *et al*. (2012) with some modifications. Figure 1 shows the flow diagram of the preparation of the fermented soybean beverage.

The beverage was obtained by filtering and removing the solid residue (*Okara)* (Fig. 1). Sugar (5.5% w/w) and modified cassava starch were added according to the percentages used for each treatment with respect to the additional water, mixed and homogenized (APV Rannie, Lockerbie, United Kingdom) at 160 bars. The heat treatment was carried out at 85°C for 15 min according to the protocol proposed by Cui *et al*. (2021).

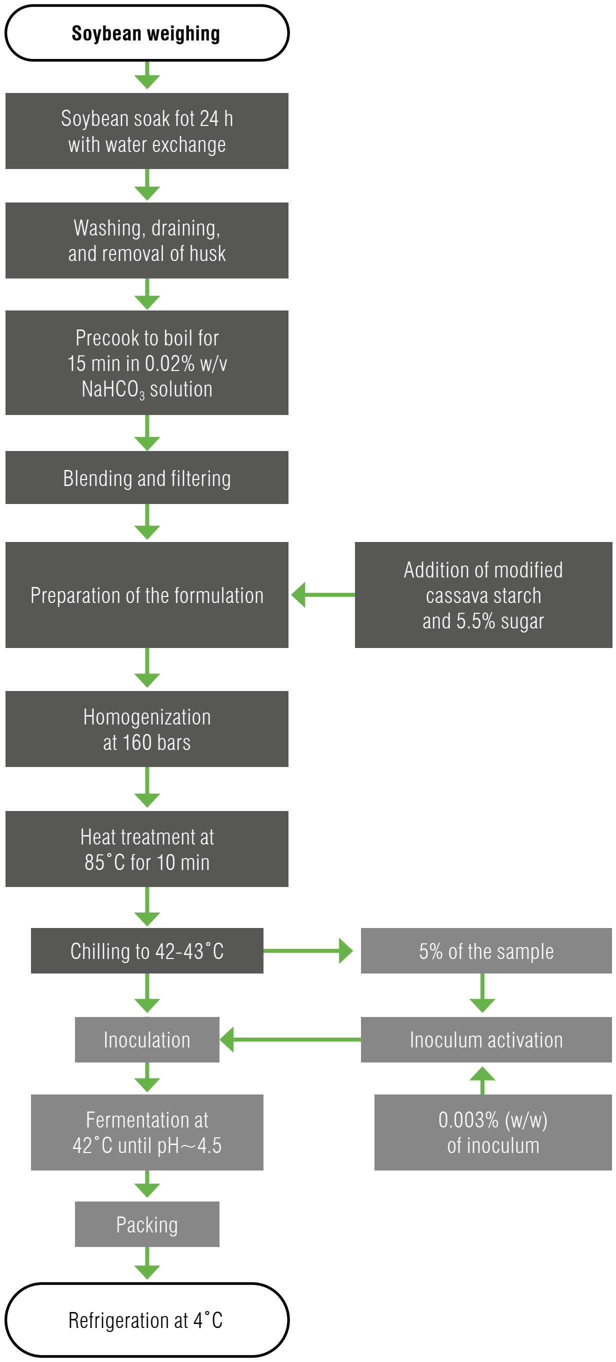


FIGURE 1. Elaboration of a fermented soybean beverage by wet grinding.

Soybean beverage fermenters

After the heat treatment, the beverage was cooled to 42°C, and a representative portion of commercial starter microorganisms and probiotics cultures (0.003% w/v) was inoculated into 100 ml of it (~4.97 ± 0.03 CFU/ml) and activated in an orbital shaker (Thermo Scientific, Solaris 4000). Ten ml were poured into flasks (200 ml) and incubated at 42°C in a Thermo Scientific 3911 incubator until reaching a pH of 4.5. It was then stored at 4°C for further analysis.

Physicochemical and rheological properties

A single concentration of cassava starch was considered for selecting the treatments. Three types of commercial controls were compared: soybean control (CS) (Toufree, Bogotá, Colombia), and two types of commercial fermented dairy beverages: CL1 (Yogo-yogo, Alpina, Sopó, Colombia), and CL2 (Pomar, Cajicá-Tabio, Colombia).

The physicochemical parameters of each treatment and control were evaluated. The quality parameters were pH, titratable acidity, apparent viscosity, syneresis, and total soluble solids. The pH was evaluated using a potentiometric method (AOAC 981.12) with a microprocessor-based pH meter (HANNA Instruments). Titratable acidity was determined according to the AOAC 947.05 using the volumetric titration method with a standardized 0.1 N NaOH solution. The results are expressed as a percentage of lactic acid. The soluble solids in the commercial beverage were determined according to the AOAC 22.019 using a refractometer calibrated at 20°C, and the result was expressed in Brix degrees. Syneresis was determined by centrifugation according to the methodology proposed by Joon *et al.* (2017). The test was performed in triplicate with a known weight of the sample for 10 min at 3500 rpm and 4°C. The supernatant was weighed and then syneresis was expressed as (g of supernatant/sample) x100 g of sample.

Apparent viscosity was established by a viscometer (Thermo Scientific® Haake Viscotester 550). A shear rate sweep from 0 to 80 s^{-1} (upward curve), followed by 80 to 0 s^{-1} (downward curve) was performed to assess the flow behavior of the commercial beverage and the treatments. Viscosity was measured in mPa s achieved at 80 rpm in triplicate. The power law was used to determine the rheological parameters of the fermented soybean beverage (Eqs. 1-2).

$$\tau = K\left(\frac{du}{dy}\right)^{n} \tag{1}$$

where τ is the shear stress, K is the consistency index, n is the flow behavior index, and du/dy is the change rate of the shear velocity. Likewise, the viscosity η can be predicted using Equation 2 by the same power law, where ε is the shear rate.

$$\eta = K\varepsilon^{n-1} \tag{2}$$

Each treatment was carried out in triplicate as well as the pH, titratable acidity, viscosity, syneresis, and contents of total soluble solids. Results were expressed as means with standard deviation.

Sensory evaluation

For the sensory test, the three types of starch (ADA, OSA, and mixed) were selected at 1.0% concentration and compared to a native cassava starch at the same concentration. Natural blackberry flavoring and blackberry red coloring (betanin E162) were added to all treatments. The sensory evaluation was carried out on the third day of preparation by 100 consumers. Participants were in an age range between 18 and 55 years old: 45 men and 55 women. Treatments were coded with three-digit random numbers (Kim *et al*., 2005). Participants were given instructions on how to perform the sensory test and a form where they were asked if they were regular consumers of non-dairy fermented beverages, allergic to soy-based products, or smokers; and if they had any disease that affected their senses. The sensory evaluation was made using a 1-7 hedonic scale categorized as follows: (1) dislike extremely; (2) dislike very much; (3) dislike; (4) neither like nor dislike; (5) like; (6) like very much; (7) like extremely. The evaluated parameters were general acceptability, texture, taste, smell, and color. Likewise, there was a blank space to indicate preference for any of the products and comments. The objective was to determine whether there are significant differences between the three modified starches and the native starch.

Statistical analysis

The experimental design was a 3^2 completely randomized factorial design with blocks (3 replicates per treatment); the factors were the type of starch (OSA, ADA, and mixed) and the concentrations of each modified cassava starch (0.8%, 1.0%, and 1.2%).

Levene's test was used to verify the homogenization of the data. This inferential test is applied to assess the equality of the variances of a variable calculated between groups or factors and to assess whether the variance in data is homogeneous under the null hypothesis. As an alternative hypothesis, if the data have a degree of significance of 0.05 or less, it is unlikely that the differences in the variations obtained in the samples come from a random sampling of a population with equal variances.

ANOVA was used to analyze the factors corresponding to each parameter using Model II to determine whether there was interaction. It was followed by the Tukey´s test with a significance level of 5%. The statistical analysis was carried out with Rstudio statistical software and the results were expressed as mean ± standard deviation.

The unifactorial test between the treatments and commercial controls was conducted by ANOVA with a significance of 5%. The statistical analysis was carried out with the Rstudio statistical software, and the treatments were selected according to the mean ± standard deviation of the commercial controls.

The analysis of the sensory test was carried out using the non-parametric Kruskal-Wallis test, considering that the obtained data are non-continuous. The comparison between samples was made by non-parametric multiple tests for each evaluated parameter.

Results and discussion

Table 1 shows the results of pH, acidity, soluble solids, syneresis, and viscosity for each treatment. The syneresis and apparent viscosity results were statistically significant ($P<0.05$). The other response variables were not significant; however, pH and soluble solids did not show a normal behavior according to the Shapiro normality test and Wilks and Bartlett's homoscedasticity test. There is no evidence of variance homogeneity ($P<0.05$); therefore, the pH was taken as a range between 4.0 and 4.6, and the soluble solids were between 8.8 and 10.5. These variables underwent a descriptive statistical analysis.

The Tukey's test was used to make multiple comparisons between the data collected on syneresis and viscosity (Tab. 1). First, the starch concentration had significant effects on syneresis (Fig. 3A) and presented higher syneresis with a 0.8% concentration (Fig. 2); second, there was no difference in syneresis for any starch at any concentration, except for 1.2%, at which it presented significant differences ($P<0.05$). From a more detailed analysis, 1.2% ADA starch had the lowest syneresis (27.08±1.72%) along with OSA starch at 1.2% (29.67±1.85%); they were considered similar as they did not present significant differences ($P<0.05$) but did have a significant difference compared to the treatment with 1.2% mixed starch (Tab. 1). There are other studies on milk beverages: Gomes *et al*. (2013) compares the physicochemical parameters of cow's milk, goat's milk, and a mixture of those two and obtained a syneresis similar to the one of this study. Likewise, Lobato-Calleros *et al.* (2014) use native starch and modified starches and pointed out a proportionally similar syneresis determined by the concentration. Furthermore, Imbachí-Narváez (2018) use ADA and OSA starches to make a fermented milk beverage; samples with OSA starch have less syneresis compared to those with ADA starch. According to the study, the syneresis parameter is higher than that of the last research and

TABLE 1. Physicochemical properties of the fermented soybean beverage according to the type of starch (ADA, OSA, mixed starch) and starch concentration (0.8%, 1.0%, and 1.2%).

Starch	Concentration (%)	pH	Acidity (%)	Soluble solids (°Brix)	Syneresis (%)	Viscosity (mPas) 80 s^{-1} at 10°C
ADA	0.8	4.45 ± 0.08^{a}	0.35 ± 0.03^{a}	9.11 ± 0.25^{a}	52.28 ± 2.91^{a}	107.54 ± 7.96^{f}
	1.0	4.53 ± 0.06^{ab}	0.34 ± 0.03^{a}	9.40 ± 0.43^{a}	33.82 ± 2.56^{b}	139.76 ± 9.77^{e}
	1.2	4.50 ± 0.07^{ab}	0.36 ± 0.03^{a}	9.93 ± 0.31^{a}	27.08 ± 1.72^{d}	185.64 ± 10.53^{c}
OSA	0.8	4.49 ± 0.04^{ab}	0.35 ± 0.03^{a}	9.51 ± 0.32^{a}	53.24 ± 2.85^{a}	160.00 ± 9.04^{d}
	1.0	4.53 ± 0.05^{ab}	0.37 ± 0.03^{a}	9.62 ± 0.18^{a}	34.27 ± 2.59^{b}	189.87 ± 11.81^{bc}
	1.2	4.53 ± 0.06^{ab}	0.37 ± 0.03^{a}	9.97 ± 0.33^{a}	29.67 ± 1.85^{cd}	241.73 ± 12.34^{a}
Mixed	0.8	4.52 ± 0.07^{ab}	0.35 ± 0.03^{a}	9.64 ± 0.50^{a}	53.25 ± 2.79^{a}	131.83 ± 11.18^{e}
	1.0	4.52 ± 0.06^{ab}	0.36 ± 0.01^{a}	9.61 ± 0.02^{a}	34.37 ± 2.85^{b}	180.39 ± 13.81^{c}
	1.2	4.57 ± 0.01^{b}	0.36 ± 0.01^{a}	9.93 ± 0.43^{a}	32.87 ± 2.82^{bc}	203.80 ± 6.95^{b}
CS		4.46 ± 0.00^{a}	0.38 ± 0.01^{a}	7.10 ± 0.05^{b}	37.58 ± 1.63^{b}	171.63 ± 1.74^{c}
CL1		4.29 ± 0.01^{c}	0.93 ± 0.02^{b}	16.33 ± 0.06^{c}	24.09 ± 2.18^{d}	162.93 ± 2.87^{d}
CL2		4.53 ± 0.01^{ab}	0.81 ± 0.01^{c}	7.27 ± 0.06^{b}	43.37 ± 2.75^{e}	211.38 ± 4.17^{b}

Treatments with acetylated distarch adipate (ADA), octenyl succinic anhydride (OSA), substituted cross-linked starch (mixed), fermented soymilk control (CS), and commercial fermented dairy beverages (CL1-Yogo-yogo, Alpina, Sopó, Colombia, and CL2-Pomar, Cajicá-Tabio, Colombia). Values with different lowercase superscript letters within a column are significantly different ($P<0.05$) from each other. Tukey's multiple comparison test was applied.

does not present a significant difference among starches; however, it depends on the concentration. We compared two matrices; therefore, starches tend to behave differently when the macromolecules in the food and their arrangement differ in proportions and conformations (Khurshida *et al*., 2021).

Different starches and hydrocolloids could be used as thickener to prepare soybean beverages (Santamaria *et al*., 2023). The results related to syneresis are lower as both starch and soybean concentrations increase (Peng & Guo, 2015; Huang *et al*., 2022) . Furthermore, the apparent viscosity is higher as both ingredients increase; however,

FIGURE 2. Effects of syneresis on treatments with the three types of starch at a concentration of 0.8%: treatments with acetylated distarch adipate (ADA), octenyl succinic anhydride (OSA), and substituted cross-linked starch (mixed).

the interface suspension between the aqueous phase and the starch is unstable (Huang *et al*., 2022). ADA and OSA starches have been applied to dairy beverages or plant-based substitutes; for instance, OSA starch has been compared with other modified starches at different concentrations (phosphorylated cross-linked starch and hydroxypropylated starch) applied to liquid kashk (sour yogurt) for 60 d. Despite this study indicating a preference for the treatment with hydroxypropylated starch at high concentration at a sensory level, the TPA analysis revealed that the treatment with OSA starch had a better consistency, texture, and softening (Hosseini & Ansari, 2019). In a formulation of a soybean and quinoa-based yogurt with added ADA starch as a thickener (Huang *et al*., 2022) and in an almond yogurt (Devnani *et al*., 2022), due to the three-dimensional network formed by ADA, the resulting gel prevents water loss and is more consistent. It forms a fairly strong viscoelastic fluid that stably joins different structures. In the same way, OSA starch has been used as an encapsulator of bioactive components and as an emulsifier in colloidal systems of oil in water (Wang *et al*., 2023). According to the above, ADA and OSA starches can be used for different food matrices and purposes to provide various texture, consistency, and viscosity characteristics, so the results may depend on the properties of the base system (Gao *et al*., 2021).

From another perspective, it must be taken into account that the syneresis, viscosity, and fluidity of the fermented soybean beverage are affected by factors such as homogenization by reducing the particle size, and heat treatment that allows the inactivation of microorganisms as well as the inactivation of proteins by changing their structural conformation (Zhang & Chang, 2022). The use of microbial strains and the fermentation process in which the gel is formed are related to the pH and isoelectric point of most proteins in a pH range between 4.4 and 4.6 (Verfaillie *et al*., 2023). These characteristics can be improved during and after fermentation by adding hydrocolloids.

Significant differences are evident in viscosity (Tab. 1), both for the type of modified starch, the concentration, and the interaction between factors (Fig. 3B). The treatment with 0.8% ADA starch presented lower syneresis with a shear rate of 80 s^{-1}, while the treatment with 1.2% OSA starch (241.73±12.34 mPas) resulted in the highest viscosity.

The commercial patterns have different variations in terms of syneresis and viscosity (Tab. 1). The commercial control CS (fermented soybean beverage) had acidity, syneresis, and viscosity values similar to data reported for the treatments at a concentration of 1.0% for each starch. Furthermore, the commercial controls CL1 and CL2 (dairy-fermented beverages) reported higher acidity than fermented soybean beverages and showed a greater variability in syneresis and viscosity. Both the syneresis and the viscosity were significantly different in the dairy controls (Tab. 1). Commercial beverages have standardized processing. The two dairy beverages use different hydrocolloids; thus, their physicochemical and rheological parameters change according to storage conditions and shelf-life, mainly due to post-fermentation processes that influence the decrease in pH and increase in acidity and affect the percentage of syneresis, viscosity, and texture. When relating the pH and acidity values evaluated in commercial dairy controls, the pH of CL2 is lower than CL1 and the acidity is higher, which directly affects the rheological properties. The decrease in pH down to the isoelectric point of milk proteins (pH 4.4~4.6) of the formed gel in fermentation is due to the

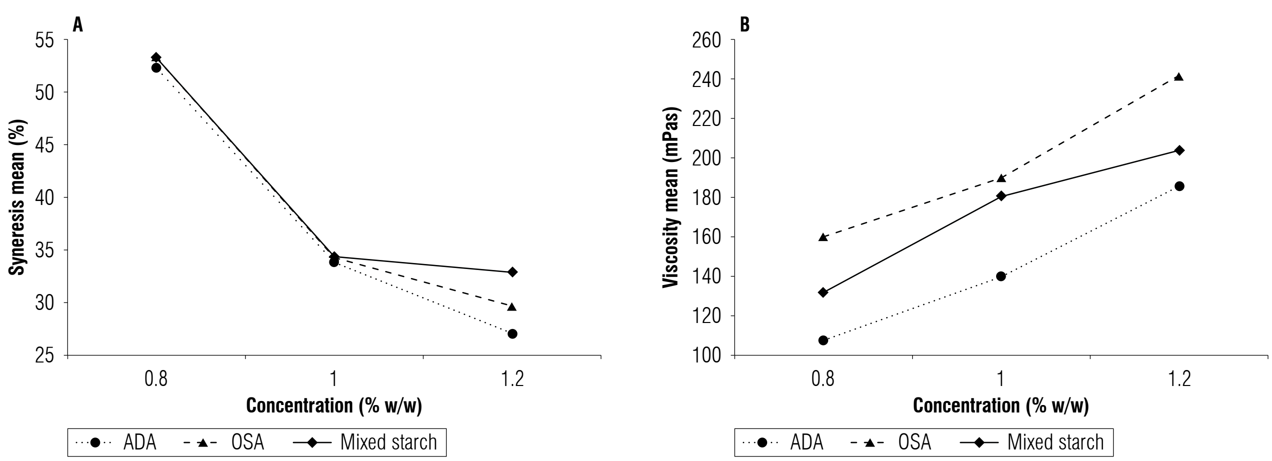


FIGURE 3. Main effects of the inclusion of samples with acetylated distarch adipate (ADA), octenyl succinic anhydride (OSA), substituted-crosslinked starch (mixed starch) and its concentration on the fermented soybean beverage found in the evaluation of syneresis (A) and viscosity (B).

effect of the acidic medium and the increase in lactic acid. Thus, it results in leaching of the water retained within the gel and phase separation and causes the increase in syneresis and decrease in viscosity (Gomes *et al.*, 2013; Peng & Guo, 2015; Yi *et al.*, 2020). These commercial beverages show variability in terms of rheological properties, and there is no single standard that characterizes them.

The soybean control (CS) is related to the values reported for the treatments with ADA, OSA, and mixed starch at 1.0%, also with 1.2% mixed starch. The CL1 control is related to ADA and OSA starch at 1.2%. Regarding viscosity, the CS is related to the OSA and mixed starch at 1.0% and ADA at 1.2%; while the CL1 control is related to the OSA 1.0% treatment, the CL2 control is more similar to the 1.2% mixed starch. The reported viscosity values are consistent for OSA starch (McNamee *et al.*, 2018) and ADA (Kapelko-Zeberska *et al.*, 2015). However, the rheological characteristics may vary for OSA according to the esterification grade of the hydroxyl groups at carbons 2, 3, and 6 in the starch glucose molecule (Sweedman *et al.*, 2013; Ovando-Martinez *et al.*, 2017). The increase in the number of OH groups substituted by OSA increases the native starch hydrophobicity and creates amphiphilic particles with different emulsifying degrees (Miao *et al.*, 2014). The use of different modified starches depends on their structural characteristics that affect to a lesser or greater extent, rheological characteristics such as the acetylation and cross-linking degree of the ADA starch, as well as other types of chemical modifications such as distarch phosphate (E1412), acetylated starch (E1420), hydroxypropyl starch (E1440), hydroxypropyl distarch phosphate (E1442). Depending on the degree of substitution, the above starches are characterized by high solubility, high-temperature impact resistance, low susceptibility to retrogradation and syneresis, and paste viscosity (Bravo-Núñez *et al.*, 2019). The ADA chemical modification in native starch affects the viscosity behavior according to the degree of its acetylation and cross-linking. A medium degree of acetylation (6%) results in high viscosity; but a low viscosity is caused by a high level of acetylation (12%) (Kapelko-Zeberska *et al.*, 2015). In addition, the viscosity in the preparation of retrograded ADA presents a wide range of degrees of acetylation together with an increase in starch cross-linking (Khurshida *et al.*, 2021).

Rheological properties

The apparent viscosity curve of a fermented soybean beverage is determined by adjusting the shear rate to between 0.01 and 80 s^{-1} (Fig. 4A). From our experimental data, an equation was obtained for each shear stress treatment as a function of the shear rate through the power law (Eq. 1), and rheological constants were predicted (Tab. 2). Under the same measurement conditions, the shear stress increased as a function of shear rate; the viscosity of the nine treatments was initially high, but it decreased rapidly as the shear rate increased (Fig. 4B).

Following the rheological model of the power law (Eq. 1), the consistency index (K), and the flow behavior index (n) were determined, and viscosity (Eq. 2) was predicted at 80 s^{-1} (Tab. 2).

TABLE 2. Predictive data according to the power law model with experimental data for the treatments and commercial controls.

Treatment	K (Pas^n)	N	R^2 (%)	η (mPas) at 80 s^{-1}
ADA 0.8%	0.501	0.641	98.85	103.94
OSA 0.8%	3.913	0.257	97.05	150.58
Mixed 0.8%	2.196	0.342	98.58	122.98
ADA 1.0%	3.516	0.259	96.49	136.94
OSA 1.0%	6.969	0.184	96.01	195.13
Mixed 1.0%	4.128	0.279	96.33	175.06
ADA 1.2%	2.640	0.390	99.07	182.44
OSA 1.2%	3.036	0.427	98.26	246.13
Mixed 1.2%	2.968	0.393	96.61	207.25
CS *	5.605	0.267	98.09	226.21
CL1	3.890	0.273	97.57	160.88
CL2	3.245	0.371	98.84	206.05

*The predictive model for CS was the 30 s^{-1}. Treatments with acetylated distarch adipate (ADA), octenyl succinic anhydride (OSA), substituted cross-linked starch (mixed), fermented soymilk control (CS), and commercial fermented dairy beverages (CL1-Yogo-yogo, Alpina, Sopó, Colombia, and CL2-Pomar, Cajicá-Tabio, Colombia).

The predictive model was applied to all samples with a shear rate in the 80 s^{-1} (Fig. 4A), except for CS, where 30 s^{-1} was used because the upper shear rates did not vary with the shear stress and kept constant, as shown in Figure 4A.

The samples with the OSA starch had a higher consistency index (K) for all concentrations evaluated with respect to the beverages with ADA and mixed starches. The samples with OSA starch have higher consistency, viscosity, creaminess and, therefore, a better texture. Similarly, mixed starch (crosslinked-substituted) has an intermediate viscosity and consistency index between the other two types of starches for each corresponding concentration. ADA starch exhibits a lower consistency index and, therefore, lower viscosity for each concentration. Moreover, all the samples presented a flow behavior index (n) less than 1 that is characteristic of pseudoplastic fluids (Chen *et al.*, 2023).

Empirical data were converted into functions of viscosity. Viscosity curves are presented (Fig. 4B) according to the predicted data for K and n; viscosity decreased as the shear rate increased.

The concentration of modified cassava starch affects the fluidity of the fermented beverage by having more molecules suspended in the aqueous medium, as well as the different molecular interactions that can occur between particles. These intermolecular interactions directly affect the fluidity and viscosity when applying shear stress that influences their shear thinning characteristics (Chen *et al.*, 2023). When comparing the shear stress of the nine treatments, an increase in the shear rate is evident and indicates that all the samples have pseudoplastic fluid behavior. This can be explained by the non-Newtonian behavior (shear-induced alignment) characteristic of lactic fermented beverages (Mitra *et al.*, 2022) that is consistent with the viscosity results.

The intermolecular interactions are given first by the soybean proteins that are mainly globulins such as glycinin and beta-conglycinin. The nonpolar residues of these proteins face inward (hydrophobic), and the polar residues face outward (hydrophilic) (Jia *et al.*, 2022). Furthermore, the starch has oil adsorption characteristics, and the aqueous phases improve with the modification octenyl succinic anhydride (OSA) because it acts as an emulsifier and stabilizer in the oil/water interface due to the particles and the stability of the drop (Marefati *et al.*, 2017; Moraes Filho *et al.*, 2019). This stability within the matrix is mainly due to soybean fatty acids and proteins with hydrophobic parts that prevent flocculation, retrogradation, and the formation of starch lumps by electrostatic force between the particles. Moreover, acetylated distarch adipate (ADA) starch forms a three-dimensional network that increases the elasticity of the stable liquid system during storage (Kapelko-Zeberska *et al.*, 2015; Partheniadis *et al.*, 2020), so it has broader water retention capacities in the formed gel. When the soybean beverage is acidified by fermentation, it generally drives out the water retained in the gel at a low pH, but the three-dimensional network formed by ADA starch can retain water during storage due to cross-linking, and this contributes to preserve the quality of the product in storage.

Sensory analysis

The general acceptance of all treatments with modified starch could be differentiated with respect to the native starch that had a lower acceptability score (Fig. 5B). Both the sample with OSA and the mixed starch differed completely in texture from the native starch. This attribute was judged correctly by consumers, who expressed that the sample with native starch had a very liquid, non-creamy texture, whereas the samples with OSA and mixed starch were very creamy, viscous, full-bodied, and had texture. Additionally, most of the consumers said that the samples with ADA starch were very fluid and not very consistent. There were no significant differences between the treatments for smell. The OSA and mixed starches differed in flavor from the native starch. The latter was judged as a rather acidic and

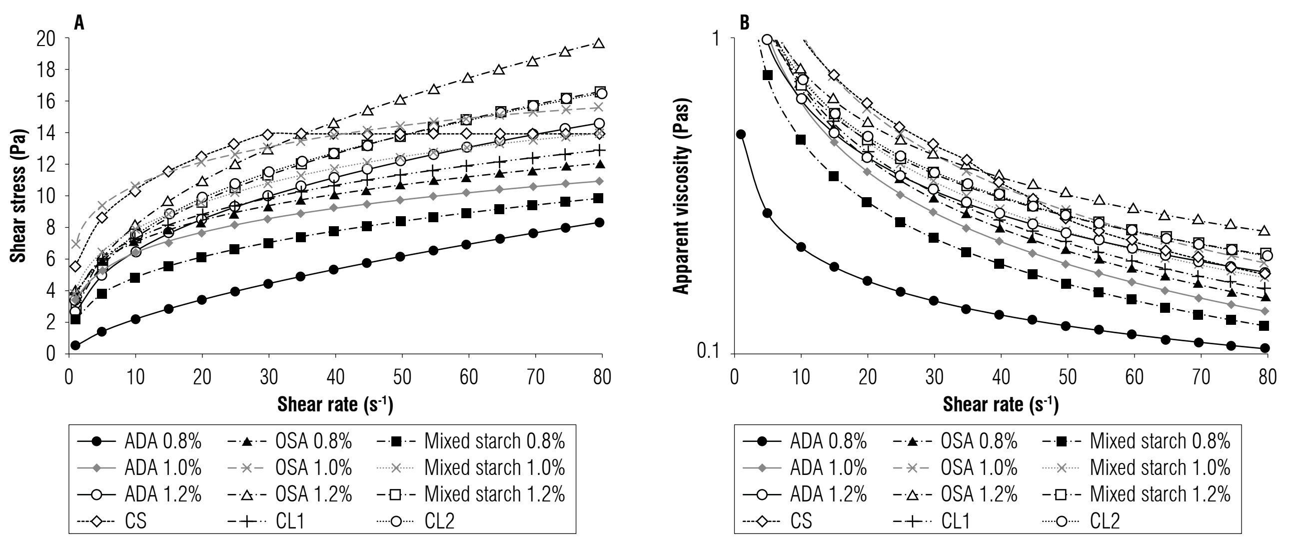


FIGURE 4. Treatments with acetylated distarch adipate (ADA), octenyl succinic anhydride (OSA), substituted cross-linked starch (mixed starch), fermented soymilk control (CS), and commercial fermented dairy beverages (CL1-Yogo-yogo, Alpina, Sopó, Colombia, and CL2-Pomar, Cajicá-Tabio, Colombia). A) Shear stress as a function of shear rate for treatments with 0.8% starch; B) Influence of shear rate on the viscosity curve for treatments with 1.0% starch.

fermented taste, related to unpleasant flavors, while the OSA and mixed starch samples were rated as pleasant with a dairy flavor or similar to dairy drinks or traditional yogurts. ADA starch had a neutral dairy connotation with a lack of flavor or increased sweetness, although the same amount of sweetener and strawberry flavoring were added to all treatments in order to mask the characteristic soybean flavor. There was no marked difference between samples regarding color. This is consistent because the same dye at the same concentration was applied to all the treatments.

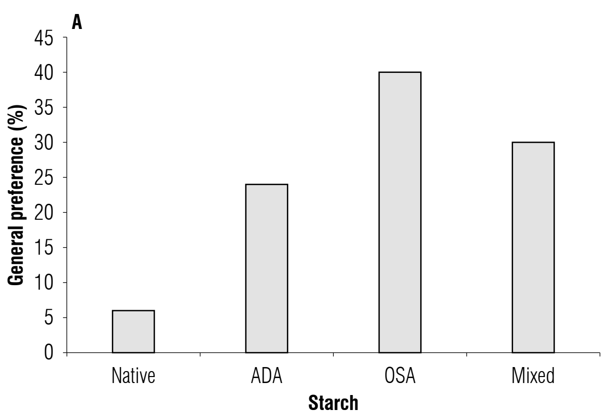
There was no difference between the treatments with modified starches for general acceptance in the test scores (Tab. 3). Even though most of the consumers preferred the sample with OSA starch (Fig. 5A-B), some rated the OSA starch beverage as a 7 (24 participants) on the hedonic scale; however, most of them gave it a 5 or 6 (27 and 29 participants). For the sample with mixed starch, the distribution was between 5, 6, and 7 (21, 32, and 28 participants). There were 43 participants who liked the product with ADA starch. Finally, general acceptance was rated 4 and 5 for the product with native starch.

When reporting the sensory analysis with parameters such as syneresis and viscosity (Tab. 2), the observations of the consumers agreed with the texture results of the OSA and the mixed starch samples. They referred to a beverage like yogurt with characteristics of creaminess, consistency, and viscosity. While the ADA sample had a fluid and low viscous connotation that agrees in a certain way by having a viscosity (Tab. 2) lower than the beverages with OSA and mixed starch. Equally, the sample with native starch was perceived as quite liquid, as if water had been added to the product that corresponds to the high syneresis reported (Tab. 2). The solid phase precipitated and separated from the liquid phase even though the sample had been homogenized before the sensory analysis. Some consumers stated that the sensation in the mouth was quite smooth to the palate; but, in general, they did not observe the presence of lumps or grit in the samples, so the particle size in all samples was similar and homogeneous. Finally, regarding the appearance, one of the physical characteristics observed in the samples with OSA starch (Fig. 2) was the fragility of the structure of the granules that leads to soft texture on the palate, just like the one with mixed starch. ADA starch has a granular morphology (Fig. 2), with a more compact and resistant appearance that may not be creamy or consistent from the sensory point of view. However, as previously analyzed, it may maintain itself longer without changing the syneresis, viscosity, and consistency during storage.

TABLE 3. Means of acceptability according to the score given by consumers.

Parameters	Native starch	ADA	OSA	Mixed starch
Acceptance	4.5 ^{a}	5.1 ^{b}	5.5 ^{b}	5.5 ^{b}
Texture	4.6 ^{a}	5.0 ^{ab}	5.2 ^{b}	5.3 ^{b}
Smell	4.6 ^{a}	5.1 ^{a}	5.2 ^{a}	5.1 ^{a}
Flavor	4.5 ^{a}	5.0 ^{a}	5.4 ^{b}	5.3 ^{b}
Color	5.4 ^{a}	5.5 ^{a}	5.4 ^{a}	5.6 ^{a}

Different superscript lowercase letters within the same row indicate statistical differences ($P<0.05$) according to the Kruskal-Wallis test. The standard error value was 0.1 (SE=0.1; n=100) for all parameters and treatments evaluated.



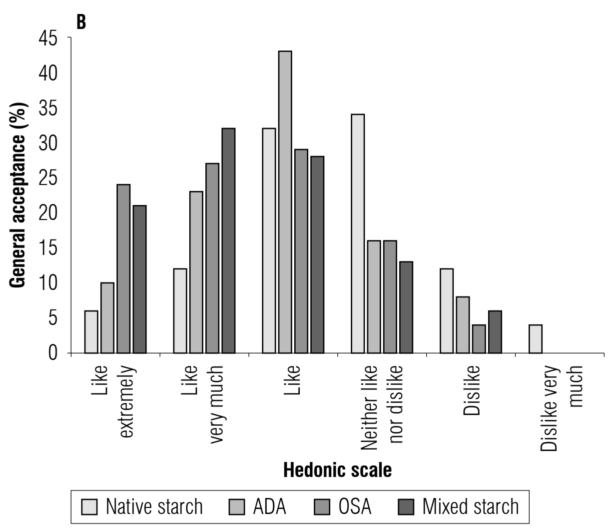


FIGURE 5. Treatments with native starch, acetylated distarch adipate (ADA), octenyl succinic anhydride (OSA), and substituted cross-linked starch (mixed starch). A) General preference for the treatments; B**)** general acceptance distribution according to the hedonic scale.

Conclusions

The addition of modified cassava starches at different concentrations does not directly influence the pH, acidity, and soluble solids of fermented soybean beverage. These parameters depend on the conditions of the fermentation process.

The treatments with less syneresis were ADA and OSA starch at 1.2% with no significant differences compared to the control dairy beverage (CL1). The treatment with the highest viscosity was the OSA 1.2% with a value of 241.73±12.34 mPas. Finally, the more similar treatments to the control soybean beverage (CS) for both syneresis and viscosity were OSA, mixed starch at 1.0%, and ADA at 1.2%.

The function for each treatment was predicted by the power law rheological model, and the fermented soybean beverage had a pseudoplastic behavior with a flow index of less than 1, similar to dairy products. Furthermore, the consistency index (K) depended on both the concentration and the type of modified starch.

The sensory analysis indicated a preference for the samples with OSA starch; however, the treatment with mixed starch could have similar properties and was also sensorially acceptable to consumers.

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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

JRR and MSH designed the experiments, JRR processed the fermented soybean beverage and carried out the physicochemical, microbiological, and sensory and laboratory evaluation and data analysis. JRR and ERS wrote the article. All authors reviewed the final version of the manuscript.

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Effect of apple peel extracts on storage quality of quince juice

Efecto de los extractos de cáscara de manzana en la calidad de almacenamiento del jugo de membrillo

Oday Hasan Ali AL-Jammaas[1*]

ABSTRACT

Enzymatic browning is one of the reactions influencing the quality of some fruit juices such as quince juice. Nowadays, there is an increased demand for application of antibrowning agents. This research was aimed at the effects of using each ethanolic and aqueous apple peel extracts (EAPE, AAPE) in two concentrations (0.3 and 3%) as inhibitor agents for polyphenol oxidase activity (PPO) of quince juice and their effects on the browning index (BI), total soluble solids (TSS), pH, total titratable acidity (TA), and total phenolic content (TPC) during storage at 4°C for 15 d. The effectiveness of 3% (w/v) alcoholic apple peel extract efficiently inactivated the activities of PPO compared with another three extracts during whole refrigerated storage time. The lowest inhibition percentage value of BI was found in 0.3% AAPE supplemented quince juices. EAPE and AAPE with concentrations of 3% had significantly larger pH values than that of EAPE and AAPE with concentration 0.3% and untreated quince juices at a final day of cold storage. TA values of juice samples untreated and treated with apple extracts did not show significant changes that ranged between 74-75 mg/100 ml at the beginning of storage and decreased with increased storage time, ranging between 0.68-0.72 mg/100 ml at the end of storage. The addition of apple peel extracts during the storage of quince juice had a positive effect on TSS values of the samples. Significantly larger TPC values ($P<0.05$) were obtained with 3% EAPE incorporated juice samples in comparison with other treatments.

Key words: enzymatic browning, fruit juices, alcoholic extracts, phenolics.

RESUMEN

El pardeamiento enzimático es una de las reacciones que influyen en la calidad de algunos jugos de frutas como el jugo de membrillo. Hoy en día, existe una mayor demanda de aplicación de agentes antipardeantes. Esta investigación tuvo como objetivo conocer los efectos del uso de cada extracto etanólico y acuosos de cáscara de manzana (EAPE, AAPE) en dos concentraciones (0.3 y 3%) como agentes inhibidores de la actividad polifenol oxidasa (PPO) del jugo de membrillo y sus efectos sobre el índice de pardeamiento (BI), sólidos solubles totales (TSS), pH, acidez titulable total (TA) y contenido fenólico total (TPC) durante el almacenamiento a 4°C durante 15 d. La efectividad del extracto alcohólico de cáscara de manzana al 3% (p/v) inactivó eficientemente las actividades de PPO en comparación con otros tres extractos durante todo el tiempo de almacenamiento refrigerado. El valor porcentual de inhibición más bajo de BI se encontró en jugos de membrillo suplementados con AAPE al 0.3%. EAPE y AAPE con concentración 3% tenían valores de pH significativamente mayores que los de EAPE y AAPE con concentración 0.3% y jugos de membrillo sin tratar en el último día de almacenamiento en frío. Los valores de TA de muestras de jugo no tratadas y tratadas con extractos de manzana no mostraron cambios significativos que oscilaron entre 74-75 mg/100 ml al comienzo del almacenamiento, y disminuyeron con el aumento del tiempo de almacenamiento, oscilando entre 0.68-0.72 mg/100 ml al final del almacenamiento. La adición de extractos de cáscara de manzana durante el almacenamiento del jugo de membrillo tuvo un efecto positivo en los valores de TSS de las muestras. Se obtuvieron valores de TPC significativamente mayores ($P<0.05$) con muestras de jugo incorporadas al 3% de EAPE en comparación con otros tratamientos.

Palabras clave: pardeamiento enzimático, jugos de frutas, extractos alcohólicos, fenólicos.

Introduction

Enzymatic browning is one of the problems that occurs during the processing and storage of some fruit juices such as apple and banana juices (Moon *et al.,* 2020). Generally, browning causes changes in loss of nutritional, functional, and sensory values as a result of darkening; and it leads to lowered quality of fruit juices. The browning phenomenon is an enzymatic process that occurs in juice of some fruits due to polyphenol oxidase (PPO) activity (enzyme commission-EC: 1.14.18.1) (Jia *et al.,* 2023). PPO refers to a copper containing enzyme that catalyzes in the presence

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[1] Department of Food Science, College of Agriculture and Forestry, University of Mosul, Mosul (Iraq).
[*] Corresponding author: dr.oday_aljammaas@uomosul.edu.iq

of oxygen with the hydroxylation of monophenols to diphenols and oxidation of dihydroxyphenols to quinone that are then subjected to more reactions, leading to a polymerizing pigment (Yıldız *et al.,* 2022). Quince (*Cydonia oblonga* Mill.) fruit has been used in juice processing in recent years (Llupa *et al.,* 2022). The quality of the quince juice is influenced by color-related changes during processing and storage. Because PPO is naturally found in quince fruit, the enzyme produces browning pigments through oxidation of phenolic compounds. So, inhibition of the PPO activity is very important. There are many synthetic substances used to inhibit PPO enzyme that eliminate fruit browning and their juices: sulphites (Muñoz-Pina *et al.,* 2022), sodium chloride (Alipoorfard *et al.,* 2020), and ascorbic acid (Xu *et al.,* 2022). However, these have negative effects on human health and have increased restrictions when used as browning inhibitors (Arnold & Gramza-Michałowska, 2022). Thus, a demand of consumers for substituting these antibrowning compounds with natural compounds has increased (Jirasuteeruk & Theerakulkait, 2020). The inhibitory effect of various natural agents on PPO and the degree of browning have been reported: these include onion extract (Bernaś & Jaworska, 2021), honey extract (de la Rosa *et al.,* 2011), green tea extract (Klimczak & Gliszczyńska-Świgło, 2017), shallot extract (Phaiphan *et al.,* 2019), and galla rhois (Lee *et al.,* 2022). The role and effectiveness of plant peel extracts depends on the polyphenol oxidase (PPO) source, the food matrix and the method and condition of the extraction. One of the apple juice processing wastes includes peels that contain numerous phenolic compounds. These compounds were identified as hydroxycinnamic acids, quercetin, catechin, and phloridzin (Bitalebi *et al*., 2019; Ahmad *et al*., 2020). The mechanism of reduced browning includes, probably, the action of these compounds as competitive inhibitors for PPO that have similar structures with enzyme substrates (Bobo *et al*., 2022). The aim of the present study was to compare the inhibitory action of alcoholic apple peel extract with aqueous extracts and their concentrations on PPO activity and browning in quince juices during cold storage.

Materials and methods

Raw plant material, chemicals, and reagents

A local variety of quince fruit (*Cydonia oblonga* Miller) and apple fruit (cv. Golden Delicious) were obtained from a supermarket in Mosul city, Iraq and stored in the refrigerator after being rinsed with water before being used in the experiments. Catechol, L-ascorbic acid, gallic acid were purchased from Sigma- Aldrich Chemical Co. Ltd (St. Louis, MO, USA). Folin–Ciocalteu reagent were obtained from Merck (Darmstadt, Germany). The common chemicals such as sodium hydroxide, ethyl alcohol (95%), as well as sodium carbonate were of analytical reagent grade, and solutions were prepared using distilled water.

Preparation of alcoholic and aqueous apple peel extract powders

The preparation was done following the procedure reported by Thanoun and Al-Jammaas (2022) with some modifications. Ripe apple fruits were washed with water; fruit peels were then manually removed and dried at 40°C until reaching a constant weight. An electrical mill was used to ground dried apple peels into fine powder. The powder was stored in a freezer at -18°C before extraction. The amount of apple peel powder was divided into two groups: the first group was extracted by 80% ethanol as alcoholic extraction for 72 h with shaking (mixing ratio 1:10 w/v). Afterwards, the obtained extract was concentrated by using a vacuum evaporator to remove ethanol by centrifuging for 15 min at 4°C and 5,000 rpm .The extract supernatant was dried in a lyophilizer and stored at -80°C until required as ethanolic apple peel extract (EAPE). The second group was extracted with distilled water as aqueous extraction, shaking for 72 h (1:20 w/v as mixing ratio). The aqueous extract was then centrifuged, dried as above, and used as aqueous apple peel extract (AAPE). For alcoholic and aqueous apple peel extracts, the percentages of extract yield were 4.64 and 1.56 g/100 g apple fruit, respectively.

Preparation of quince juice and its treatments

Hand peeled fruits were crushed using a blender (Waring commercial blender). After that, the crushed quince was filtered through two layers of muslin cloth. The quince juice samples were then divided into four groups: two of them were treated with alcoholic apple peel extract, and the other two were treated with aqueous apple peel extract. For each type of extract 0.3% and 3% concentrations were used as added concentrations in the quince juice. The control sample was quince juice without extract.

Analyses

Polyphenol oxidase (PPO) was extracted from the quince juice; its activity assay was based on the method of de la Rosa *et al.* (2011) with some modifications: firstly, the extraction of the enzyme was accomplished by mixing the same volumes of juice and phosphate buffer (0.05 M, pH=7), at 4°C for 60 min (obtained by using iced water bath) and then centrifuged at 500 rpm at 4°C for 10 min and filtered. The supernatant (enzyme extract) was used for PPO activity by mixing 0.5 ml of enzyme extract with 1 ml of catechol solution 0.1 M and 2 ml of phosphate buffer solution (0.05

M, pH 7.2). The absorbance values were recorded with a spectrophotometer at 420 nm every 30 s for 3 min at 30°C as incubation temperature. The activity of the PPO enzyme was obtained based on the slope of the linear portion of the curve plotted among absorbance values at 420 nm against time. One unit of enzyme activity was expressed as the change in absorbance at 420 nm per min per ml under the assay conditions. The percentage of polyphenol oxidase activity inhibition (PPO) was calculated using the following equation:

$$PPO\ (\%) = \frac{A - B}{A} \times 100 \tag{1}$$

where:

A = The enzyme activity value for quince juice sample without added extracts.

B = The enzyme activity value for quince juice sample with added extracts.

The browning index of quince juice samples was estimated at 420 nm with a UV-VIS spectrophotometer (Iqbal *et al.,* 2018; Iqbal *et al.,* 2020). The percentage of browning inhibition (BI) was computed using the following equation:

$$BI\ (\%) = \frac{A - B}{A} \times 100 \tag{2}$$

where:

A = The absorbance value at 420 nm for quince juice sample without added extracts.

B = The absorbance value at 420 nm for quince juice sample with added extracts.

Total phenolic content (TPC) of quince juice was measured according to the Folin-Ciocalteu reagent procedure reported by Trigo *et al.* (2020) with some modifications: 0.5 ml of quince juice ml was added to a test tube containing 0.5 ml of Folin-Ciocalteu reagent and after 4 min, 2 ml of Na_2CO_3 solution with 20% w/v was added. The total volume was adjusted to 5 ml with distilled water. The tubes were left for 120 min at room temperature in the dark. The absorbance value was analyzed at 760 nm in a UV-VIS spectrophotometer, and the results were expressed in mg gallic acid equivalents/100 ml through a gallic acid calibration curve. Total soluble solids (TSS) were determined as degrees Brix by using an Abbe refractometer (Atago, Tokyo, Japan). The pH values of the quince juice samples were determined using a WTW pH-meter at 25°C (Iqbal *et al.,* 2020). Titratable acidity content was performed as malic acid (%) after titration with sodium hydroxide standard solution (0.1 N) in the presence of the colored indicator (Saberi *et al.,* 2019).

Statistical analysis

A factorial completely randomized experimental design was conducted in triplicate (repeated three times) for research experiments. The two-way analysis of variance (ANOVA) was carried out to test the effects of peel extract method, peel extract concentration, storage time, and their interactions. Duncan's test was used to compare the means using the SAS program (Proc. GLM, SAS program, version 9.3, SAS, 2012). The differences at P<0.05 were considered statistically significant.

Results and discussion

Effect of apple peel extract addition on PPO enzyme inhibition in quince juice during storage

The inhibition of PPO activity (%) in quince juice treated with both ethanolic (EAPE) and aqueous (AAPE) apple peel extracts and stored for 15 d at 4°C is presented in Figure 1. The concentrations of each added extract were 0.3% and 3%. The enzyme inhibition percent differed according to the extract method, concentration added to the extract, and the storage time. The result showed that inactivation percent of PPO in the EAPE treated quince juice with 3% as concentration extract was a larger significant (P<0.05) compared to 0.3% treated juice samples.

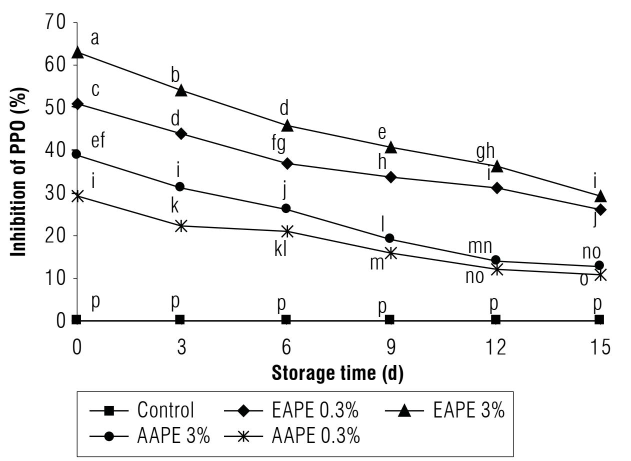

FIGURE 1. Effect of addition of alcoholic apple peel extracts to quince juice on inhibition percent of polyphenol oxidase (PPO). The same letters indicated no significant differences according to the Duncan test (P>0.05).

Based on 12th and 15th d of storage, the percent of inhibition of PPO enzyme in the 3% AAPE treated juice samples was insignificantly greater than juice samples treated with concentration of 0.3%. Quince juice samples treated with both concentrations of aqueous apple peel extract had significantly ($P<0.05$) lower PPO inhibition percentages (29% and 39%) than samples treated with same concentrations of alcoholic apple peel extract (51% and 63%) on 0 day of storage at 4°C.

According to Figure 1, the highest percent of inhibition of quince juice PPO enzyme was obtained with an alcoholic extract of apple peel, and this bioactivity could be related to its high content of phenolic compounds (Machado *et al.,* 2013). These results strongly supported the addition of the alcoholic, aqueous of natural extracts for effectiveness in inhibiting the juice PPO enzyme. In general, our results agree with other authors (Klimczak & Gliszczyńska-Świgło, 2017; Jirasuteeruk & Theerakulkait, 2020; Lee *et al.,* 2022) who studied the effects of shallot extract, mango peel extract, and galla rhois water extract on polyphenol oxidase activity for apple juice, potato puree, and apple juice.

Effect of apple peel extract supplementation on browning index inhibition in quince juice during cold storage

Changes in browning of quince juice in terms of browning index (BI) values are shown in Figure 2. Addition of extracts of apple peel with different concentrations caused significant variations in the BI values of quince juice. The treatment of quince juice with 3% alcoholic extract of apple peel showed more inhibitory effect on BI values of juice than that of the 0.3% of the same extract at the end of the storage. The BI (%) of water extracts of apple peel added juice samples was significantly decreased with the decrease of storage day ($P<0.05$): it was 15% and 9% on the 15th d of cold storage as compared to 0 d (34% and 28%) for extract concentrations of 3% and 0.3%, respectively. Results indicated that the browning (in terms of inhibition percentage of BI) of quince juice after being supplemented with alcoholic extracts had higher values in comparison with aqueous extracts (Fig. 2).

Samples treated with 3% EAPE showed the highest inhibition percentage of BI values of 52%, followed by 0.3% EAPE (40%), 3% AAPE (34%) and 0.3% AAPE (28%). Regardless of the natural anti-browning agent used, the findings described above are similar to that obtained by others (Altunkaya & Gökmen, 2008; Jirasuteeruk & Theerakulkait, 2020; Shomodder *et al.,* 2021).

Effect of supplementation of apple peel extract on phenolic content in quince juice during storage at 4°C

The results of the total phenolic contents (mg/100 ml) in quince juice treated with apple extracts during storage are shown in Figure 3. The thermally pasteurized quince juice contained 49.18 mg/100 ml phenolics, but this amount significantly decreased by 2.62% on the 15th d of storage at 4°C. Treatment of quince juice with EAPE and AAPE revealed significant differences compared to untreated juice samples at 0 and 15th d of storage. TPCs were increased throughout storage time. The higher added concentrations of extract had significantly more TPC than the lower concentrations. Due to the high content of extracted phenolics using the alcoholic method, the values of TPC in alcoholic extracts treated juices were found to be larger (53.05 and 54.63 mg/100 ml), compared to juice samples treated with aqueous extracts (50.43 and 51.74 mg/100 ml), at the end of the storage period (Fig. 3).

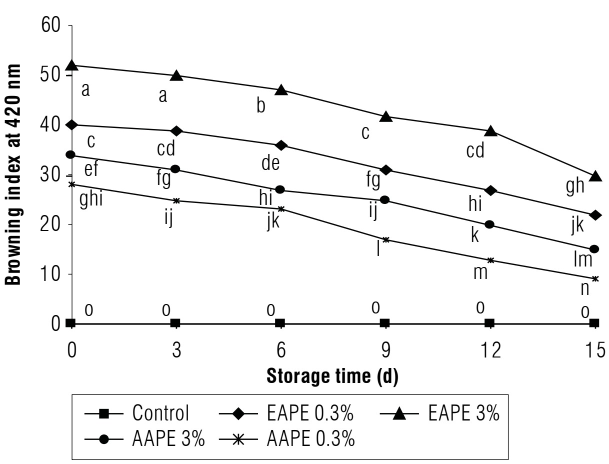

FIGURE 2. Effect of the addition of alcoholic apple peel extracts to quince juice on inhibition percent of browning index (BI). The same letters indicated no significant differences according to the Duncan test ($P>0.05$).

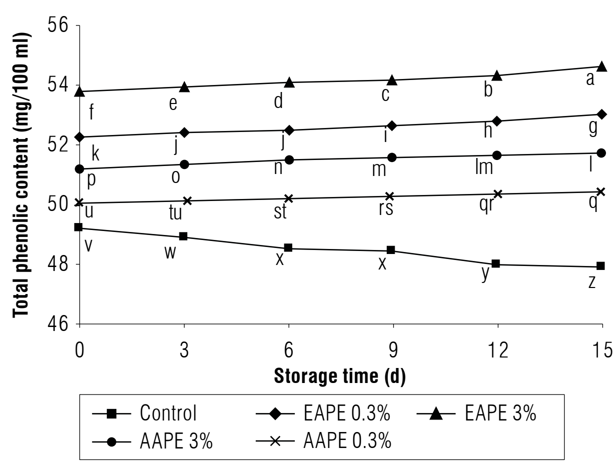

FIGURE 3. Effect of the addition of alcoholic apple peel extracts to quince juice on total phenolic content (mg/100 ml). The same letters indicated no significant differences according to the Duncan test ($P>0.05$).

Data for the effect of storage time on the amount of total phenolics in the juices showed significant increases in TPC with increased storage (15 d vs. 0 d). The results of TPC are in accordance with Altunkaya *et al.* (2013) who found significantly increases in the phenolic content of apple juice supplemented with pomegranate peel extracts. The results of this study are similar to other studies that showed an increase of phenolic values with an increment of natural extract concentrations in the fresh cut potato slices (Liu *et al.*, 2019) or apple juice (Phaiphan *et al.*, 2019) during storage.

Effect of addition of apple peel extract on TSS values in stored quince juice

The TSS changes of all samples during 15 d of storage at 4°C are shown in Figure 4. The significant changes in the TSS of quince juices were obtained with samples of juice treated with (0.3% and 3%) of alcoholic extracts and 3% of aqueous extracts. They were insignificant after treatment with 0.3% aqueous extracts compared with untreated juices at 0 d of storage. The addition of EAPE with 3% treated juices had TSS values significantly larger than that of EAPE with 0.3% treated samples during all storage periods. Treatment of quince juice with a higher concentration of AAPE was not significant differences compared to lower concentration of AAPE treated juice samples throughout the days of storage. The above results are consistent with those reported by Altunkaya *et al.* (2013), who studied the effect of pomegranate peel extract on the total soluble solids of apple juice. Another study also reported an increase of TSS when shallot extract was supplemented in apple juice at a concentration ranged between 0.5% and 2% (Phaiphan *et al.*, 2019).

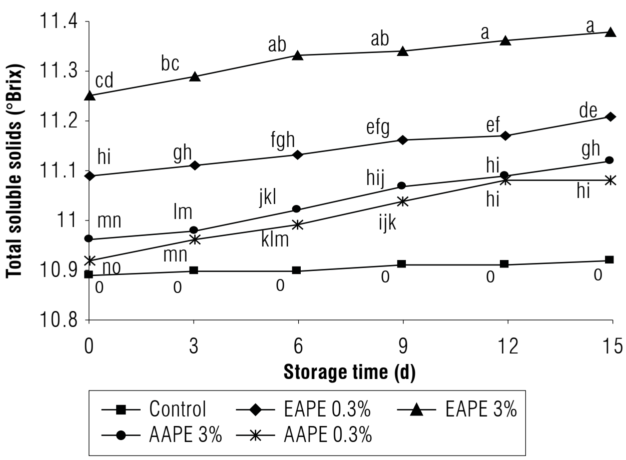

FIGURE 4. Effect of addition of alcoholic apple peel extracts to quince juice on total soluble solids (°Brix). The same letters indicated no significant differences according to the Duncan test ($P>0.05$).

Effect of apple peel extract addition on pH values of quince juice during storage at 4°C

Table 1 demonstrates the pH changes of the quince juice treated with two extracts of apple peel (differing in their extraction method and concentration).

The initial pH values of the untreated sample at 0 and 15 d were 4.63 and 4.70, respectively. Insignificant increment in pH values ($P>0.05$) were obtained immediately after peel extract treatment. From the same table, the EAPE 3% treated juices had a pH value of 4.74 that was significantly larger than EAPE 0.3% treated ones (4.71) after 15 d of storage at 4°C. Samples of quince juice supplemented with different concentrations of aqueous extracts of apple peel were significantly different in the pH at 4.69 and 4.72 at the last time of storage. EAPE and AAPE with concentration 3% had significantly higher pH values than that EAPE and AAPE with concentrations of 0.3% and untreated quince juices at a final day of cold storage (Tab. 1). These findings agreed with studies of aonla (or Indian Gooseberry *Emblica officinalis* Gaertn. Syn. *Phyllanthus emblica* L.) juice and guava slices in which the pH was increased significantly after plant extracts treatment (Mundhe *et al.*, 2016; Shomodder *et al.*, 2021).

TABLE 1. Effect of addition of alcoholic apple peel extracts to quince juice on pH values.

Treatments	pH values – Storage time (d)					
	0	3	6	9	12	15
EAPE, AAPE with 0% + quince juice	4.63±0.01 j	4.64±0.01 ij	4.66±0.01 gh	4.67±0.01 fg	4.69±0.01 de	4.70±0.01 cd
EAPE with 0.3% + quince juice	4.63±0.01 j	4.66±0.01 gh	4.66±0.01 gh	4.67±0.01 fg	4.68±0.01 ef	4.71±0.01 bc
EAPE with 3% + quince juice	4.64±0.01 ij	4.67±0.01 fg	4.68±0.01 ef	4.70±0.01 cd	4.72±0.01 b	4.74±0.01 a
AAPE with 0.3% + quince juice	4.63±0.01 j	4.65±0.01 hi	4.66±0.01 gh	4.68±0.01 ef	4.68±0.01 ef	4.69±0.01 de
AAPE with 3% + quince juice	4.64±0.01 ij	4.66±0.01 gh	4.66±0.01 gh	4.69±0.01 de	4.70±0.01 cd	4.72±0.01 b

The values in the above table represent means ± standard error and the same letters in each column and rows indicate no significant differences according to the Duncan test ($P>0.05$).

TABLE 2. Effect of addition of alcoholic apple peel extracts to quince juice on titratable acidity values (%).

Treatments	Titratable acidity values					
	Storage time (d)					
	0	3	6	9	12	15
EAPE, AAPE with 0% + quince juice	0.75±0.01 a	0.73±0.01 abc	0.72±0.01 bc	0.71±0.01 cd	0.70±0.01 de	0.69±0.01 e
EAPE with 0.3% + quince juice	0.74±0.01 ab	0.74±0.01 ab	0.74±0.01 ab	0.73±0.01 abc	0.73±0.01 abc	0.71±0.01 cd
EAPE with 3% + quince juice	0.74±0.01 ab	0.74±0.01 ab	0.73±0.01 abc	0.71±0.01 cd	0.70±0.01 de	0.68±0.01 e
AAPE with 0.3% + quince juice	0.75±0.01 a	0.74±0.01 ab	0.74±0.01 ab	0.73±0.01 abc	0.73±0.01 abc	0.72±0.01 bc
AAPE with 3% + quince juice	0.74±0.01 ab	0.74±0.01 ab	0.73±0.01 abc	0.72±0.01 bc	0.71±0.01 cd	0.69±0.01 e

The values in the above table represent mean ± standard error and the same letters in each column and rows indicate no significant differences according to the Duncan test ($P>0.05$).

Effect of apple peel extract supplementation on titratable acidity content in quince juice during storage

Table 2 illustrates the titratable acidity of the quince juice treated with EAPE, AAPE and untreated juices. Treatment of quince juice with EAPE and AAPE did not exhibit significant differences compared to untreated juice sample at 0 d of storage. The same previous treatment showed significant differences after 15 d of storage of juice samples at 4°C. A significant effect of the incorporation of natural extract from apple peel (alcoholic extraction method) into quince juice was shown after 12th d and continued to the end of storage. In the case of water extracts, apple added to quince juice samples during storage under used conditions, the changes in the acidity were not significant until the 15th d of storage. Depending on the concentration of added extracts, quince juices had lower acidity values. This result was similar to that obtained by Mundhe *et al.* (2016) who observed a reduction of titratable acidity of aonla juice with increasing concentrations of custard apple leaf extract. The EAPE added juice samples had minimum titratable acidity value (0.68), when compared to other treatments.

Similar results were reported by Shomodder *et al.* (2021) who studied the different characteristics including acidity of guava slices treated with different types of extracts during cold storage for 6 d.

Conclusions

PPO-catalyzed browning is considered a negative phenomenon in fruit juice processing. As far as we know, an application of apple peel extract as PPO inhibitor is the first experimental trial against quince juice browning. It was found that all extracts had the potentiality against browning-related PPO action. Addition of apple extracts to the quince juice improved many properties of the juice compared with extracts free from juice samples during storage for 15 d at 4°C; the increased percentages were (63, 26), (52, 30), (3.20, 4.31), (8.52, 9.98), and (0.22, 2.321), for PPO inhibition, browning index inhibition, TSS, TPC, and pH at 0 and 15 d of storage at 4°C for (3)% EAPE treated quince juice samples compared with control samples, respectively. The extracts used can be ranked as 3% EAPE > 0.3% EAPE > 3% AAPE > 0.3% AAPE. Finally, this study suggested that ethanolic and aqueous apple peel extracts could be utilized as a natural additive in the quince juice processing industry. The production of brown fruit juices caused by some enzymes may led to the appearance of toxic compounds, poor nutritional content, and lower levels of sensory properties. This is a very important processing challenge. To achieve improvement in this respect, it is necessary to investigate some biochemical properties related to safety and quality of these juices. Considering this matter from a future perspective, addition of plant peel extracts as antibrowning agents to the fruit juices should be given more attention.

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Conflict of interest

The author declares that there is no conflict of interests regarding the publication of this article.

Author contributions

OHA-J: conceptualization, formal analysis, research, methodology, resources, validation, visualization, writing of original draft, and editing. The author reviewed the final version of the manuscript.

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SCIENTIFIC NOTE

Effect of B vitamins on lettuce plants subjected to saline stress

Efecto de vitaminas B en plantas de lechuga sometidas a estrés salino

Akim Afonso Garcia[1], Eduardo Pradi Vendruscolo[1]*, Sebastião Ferreira de Lima[2], Cássio de Castro Seron[1], Murilo Battistuzzi Martins[1], and Gabriela Rodrigues Sant' Ana[1]

ABSTRACT

The use of water with a high concentration of salts has been increasingly frequent in vegetable production. This reduces the development and productivity of vegetables, raising the importance of the search for techniques to mitigate deleterious effects. In this sense, vitamins have the potential to improve conditions for plant development. The study was conducted to evaluate the effects of the application of B vitamins in lettuce plants submitted to irrigation with saline water. The treatments consisted of Control: irrigated with water and without application of vitamins; NaCl: irrigated with saline solution (50 mM NaCl, equivalent 5.18 dS m^{-1}) and without application of vitamins; NaCl+B1: irrigated with saline solution and application of vitamin thiamine (100 mg L^{-1}); NaCl+B3: irrigated with saline solution and application of vitamin niacin (100 mg L^{-1}). These treatments were applied to two lettuce cultivars, "Pira Roxa" and "Valentina". Both vitamins increased net photosynthesis when compared to the NaCl treatment. However, only the application of thiamine resulted in a mitigating effect on the losses of plant dry mass accumulation. Thus, the exogenous application of these vitamins alleviates the effects caused by salinity in lettuce plants, reducing stress on photosynthetic mechanisms and increasing photosynthetic activity. In addition, thiamine helps to reduce the deleterious effects of salinity on the accumulation of biomass.

Key words: biostimulants, wastewater, water management, regenerative agriculture, plant protection.

RESUMEN

El uso de agua con alta concentración de sales ha sido cada vez más frecuente en la producción de hortalizas. Esto reduce el desarrollo y la productividad de las hortalizas, lo que plantea la importancia de la búsqueda de técnicas para mitigar los efectos nocivos. En este sentido, las vitaminas son sustancias que tienen el potencial de mejorar las condiciones para el desarrollo de las plantas. El estudio se realizó para evaluar los efectos de la aplicación de vitaminas B en plantas de lechuga sometidas a riego con agua salina. Los tratamientos consistieron en: Testigo: irrigación con agua y sin aplicación de vitaminas; NaCl: irrigación con solución salina (50 mM NaCl, equivalente 5.18 dS m^{-1}) y sin aplicación de vitaminas; NaCl+B1: irrigación con solución salina y aplicación de vitamina tiamina (100 mg L^{-1}); NaCl+B3: irrigación con solución salina y aplicación de vitamina niacina (100 mg L^{-1}). Estos tratamientos se aplicaron a dos cultivares de lechuga, "Pira Roxa" y "Valentina". Ambas vitaminas incrementaron la fotosíntesis neta en comparación con el tratamiento NaCl. Sin embargo, sólo la aplicación de tiamina resultó en un efecto mitigador sobre las pérdidas de acumulación de masa seca de la planta. Así, la aplicación exógena de vitaminas alivia los efectos provocados por la salinidad en las plantas de lechuga, reduciendo el estrés sobre los mecanismos fotosintéticos y aumentando la actividad fotosintética. Además, la tiamina ayuda a reducir los efectos deletéreos de la salinidad sobre la acumulación de biomasa.

Palabras clave: bioestimulantes, aguas residuales, gestión del agua, agricultura regenerativa, protección vegetal.

Introduction

The growing demand for food in the world has led to an increase in the use of water bodies, depleting or contaminating rivers, lakes and surface waters, making access to drinking water more difficult, especially for human consumption (Santos & Spolador, 2022). This fact leads to restrictions on the use of drinking water in agriculture, increasing the use of water of inferior quality, such as saline water, in irrigation. Thus, the difficulty in accessing high quality water has accelerated the search for viable alternatives for food production (Zhang & Shen, 2019; Singh, 2021).

For the production of vegetables, the continuous use of soil and the high number of production cycles can lead to an increase in salt concentrations in the soil (Libutti & Monteleone, 2018). Also, wastewater from agro-industrial processes or low-quality water from other sources has been

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[1] Mato Grosso do Sul State University, Cassilândia University Unit, Cassilândia, MS (Brazil).
[2] Mato Grosso do Sul Federal University, Chapadão do Sul Campus, Chapadão do Sul, MS (Brazil).
* Corresponding author: agrovendruscolo@gmail.com

increasingly frequent in agriculture. This action results in significant productivity losses due to the presence of residues that can limit the development of plants, including lettuce (Adhikari *et al*., 2019; Zhang *et al*., 2021).

Irrigation with saline water is possible for many plant species, which have different levels of tolerance to this condition. However, many commercially important species have low salinity tolerance, and excess salts can lead to plant death (Hajihashemi *et al*., 2020). For these species, reduced fertility, changes in the physical and chemical characteristics of soils, in addition to the accumulation of Na both in the plants and in the soil, reduce germinative efficiency and promote physiological disorders, affecting the photosynthetic system, cell division and, consequently, the development of plant structures (Singh, 2021).

The impact of excessive use of soil and water resources can be reduced by applying alternative solutions that allow maintaining development without further damage to natural resources. The exogenous application of vitamins in plants can lead to increase in vigor, higher reproductive rates, and resistance to edaphoclimatic conditions (Contieri *et al*., 2018; Vendruscolo *et al*., 2019), among other physiological increments that allow cultivation under conditions of increased stress.

Thiamine, known as vitamin B1, has been used as a potential biostimulant in plant production (Vendruscolo *et al*., 2019; Vendruscolo & Seleguini, 2020; Jabeen *et al*., 2021). This vitamin acts directly on plant organelles as an enzymatic cofactor and indirectly on the respiratory and energy generation cycles of plants (Goyer, 2010; Taiz *et al*., 2017). The application of exogenous thiamine can stimulate the action of defense mechanisms in plants, in addition to promoting development and growth (Kaya *et al*., 2015).

Another substance with application to crop systems is niacin (vitamin B3), which is also found in several physiological systems of plants. It helps in vegetative growth and accumulation of reserves and minimizes adversities that the plant may undergo from stress (Taiz *et al*., 2017; Colla *et al*., 2021).

Based on the hypothesis that the application of the B vitamins, niacin and thiamine can change the characteristics of vegetables and mitigate the effects of abiotic stresses, the objective of the study was to evaluate the effects of the application of vitamins B on lettuce plants submitted to irrigation with saline water.

Materials and methods

Plant material and treatments

The experiment was conducted in a greenhouse covered with a 150-micron low-density polyethylene film and a thermoreflective screen (LuxNet®) with 42/50% shading under the film, in April 2021, in the experimental area of the State University of Mato Grosso do Sul, Cassilândia University Unit, Brazil.

The climate of the region, according to the Köppen classification, is tropical rainy (Aw), with rainy summers and dry winters (winter precipitation less than 60 mm), with annual precipitation of 1,520 mm and an average temperature of 24.1°C. During the experimental period, the climatic data were collected daily using equipment installed inside the greenhouse. (Fig. 1).

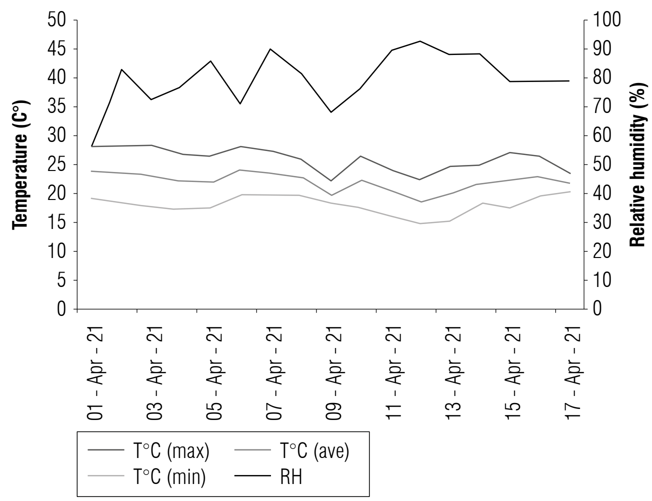

FIGURE 1. Maximum, average and minimum temperature and relative humidity during the experiment.

A completely randomized design was used, in a 2x4 factorial scheme, with four replicates. The treatments were composed of two lettuce cultivars (Valentina and Pira Roxa) and the four growing conditions, Control: irrigated with water and without application of vitamins; NaCl: irrigated with saline solution and without application of vitamins; NaCl+B1: irrigated with saline solution and application of vitamin thiamine; and NaCl+B3: irrigated with saline solution and application of vitamin niacin.

The lettuce seedlings were purchased from a certified producer (Agromudas, Jales, Brazil), ensuring phytosanitary quality and homogeneity in size. Before planting in the containers, the seedlings received a foliar application of water (control) and solutions of thiamine (100 mg L^{-1}) and niacin (100 mg L^{-1}), depending on the treatment. The vitamin solutions were prepared in water, and the treatments were applied with a foliar spray, using a plastic hand pump. One ml of solution was applied per plant and per treatment. After 24 h of application, the plants, containing four leaves and 15 cm high, were transferred to the containers with substrate. One treatment without application of vitamins was irrigated with fresh water (0.48 dS m^{-1}) (control), while another treatment without vitamins was irrigated with saline solution (50 mM NaCl, 5.18 dS m^{-1}), used as "stress control" (NaCl); the other treatments containing thiamine (NaCl+B1) and niacin (NaCl+B3) application were irrigated with saline solution for 15 d. Four replicates were used, consisting of two plants each.

For plant growth, recipients (0.35 dm^3) contained a mixture of Typic Quartzipsamments (pH 5.0, P 14.0 mg dm^{-3}, K 3.0 $mmol_c$ dm^{-3}, Ca 24.0 $mmol_c$ dm^{-3}, Mg 14.0 $mmol_c$ dm^{-3}, cation exchange capacity 58.0 $mmol_c$ dm^{-3}, base saturation 71.0%, organic matter 13.0 g dm^{-3}, 95 g kg^{-1} of clay, 50 g kg^{-1} of silt and 855 g kg^{-1} of sand), vermiculite and tanned bovine manure, in a proportion of 3:1:1 (v/v).

Gas exchange and plant weight accumulation

Fourteen days after the application of the vitamins, during the morning hours, the net photosynthesis (A; μmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s; mmol m^{-2} s^{-1}), intracellular CO_2 concentration (C_i; μmol mol^{-1}) and transpiration (E; mol H_2O m^{-2} s^{-1}) were evaluated, using a portable infrared gas exchange meter (LC_i, ADC Bioscientific, Hertfordshire, UK). Also, water use efficiency (A/E) and the instantaneous carboxylation efficiency (A/C_i) were calculated.

After obtaining the physiological characteristics, the aerial part of the plants was harvested and dried in a forced ventilation oven at 65°C, until a constant dry mass (SDW) was obtained. Also, the percentage loss of dry mass was calculated based on the control treatment.

Statistical analysis

Data were submitted to preliminary normality and homoscedasticity tests. Then, the means were submitted to analysis of variance (ANOVA) and the Scott-Knott test, at the 10% probability level. The analysis was performed using the SISVAR statistical software (Ferreira *et al.*, 2014).

Results

The treatments affected the net photosynthesis variable, with the highest average obtained in the control treatment, followed by the treatments composed of the application of vitamins B1 and B3, which differed significantly from the treatment composed only by irrigation with saline solution (Fig. 2A). Differences in the intracellular concentration of CO_2 only occurred between the cultivars, with Pira Roxa being superior in this variable. In addition, for both transpiration and stomatal conductance, the superiority of the control treatment over the others was observed, and of the cultivar Pira Roxa over Valentina, without considering the treatments (Fig. 2C-D).

For water use efficiency, higher values were found for treatments in which irrigation with saline solution was used and, among the cultivars, Valentina was superior (Fig. 3A). In addition, for the efficiency of instant carboxylation, the control treatment stood out, followed by the treatments composed by the application of vitamins, both superior to the treatment composed only by irrigation with saline solution, while among the cultivars Valentina stood out (Fig. 3B).

The cultivars had a similar response for the accumulation of dry mass, while, among the treatments, the superiority of the control was verified. However, among the treatments in which there was irrigation with saline solution, the superiority of the application of thiamine was observed (Fig. 4A), with lower percentage losses of dry mass (Fig. 4B).

Discussion

The presence of salt concentrations in the irrigation water affected most of the parameters evaluated compared to the control treatment (Figs. 2-4); the resulting osmotic stress caused decreases in leaf area, transpiration, osmotic potential, dry biomass, and photosynthetic rate (Coelho *et al.*, 2013; Silva Junior *et al.*, 2018); the application of vitamins attenuated some of these effects (Figs. 2-4).

Thiamine is responsible for signaling the presence of stress acting on plants; its natural levels in plant tissues decrease as stress continues (Goyer, 2010). Thiamine has key roles in plant metabolic processes, including carbon assimilation and respiratory processes (Fitzpatrick & Chapman, 2020). The exogenous application of thiamine acts as priming, potentiating the responses to stress through the production of secondary metabolites and favoring the plant self-protection (Goyer, 2010; Kaya *et al.*, 2015). The treatments with this vitamin showed lower losses of dry mass (Fig. 4), which may be an indication of the plant self-protection in response to stress. As well, the thiamine treatments with saline water irrigation led to a positive response for net photosynthesis (Fig. 2A).

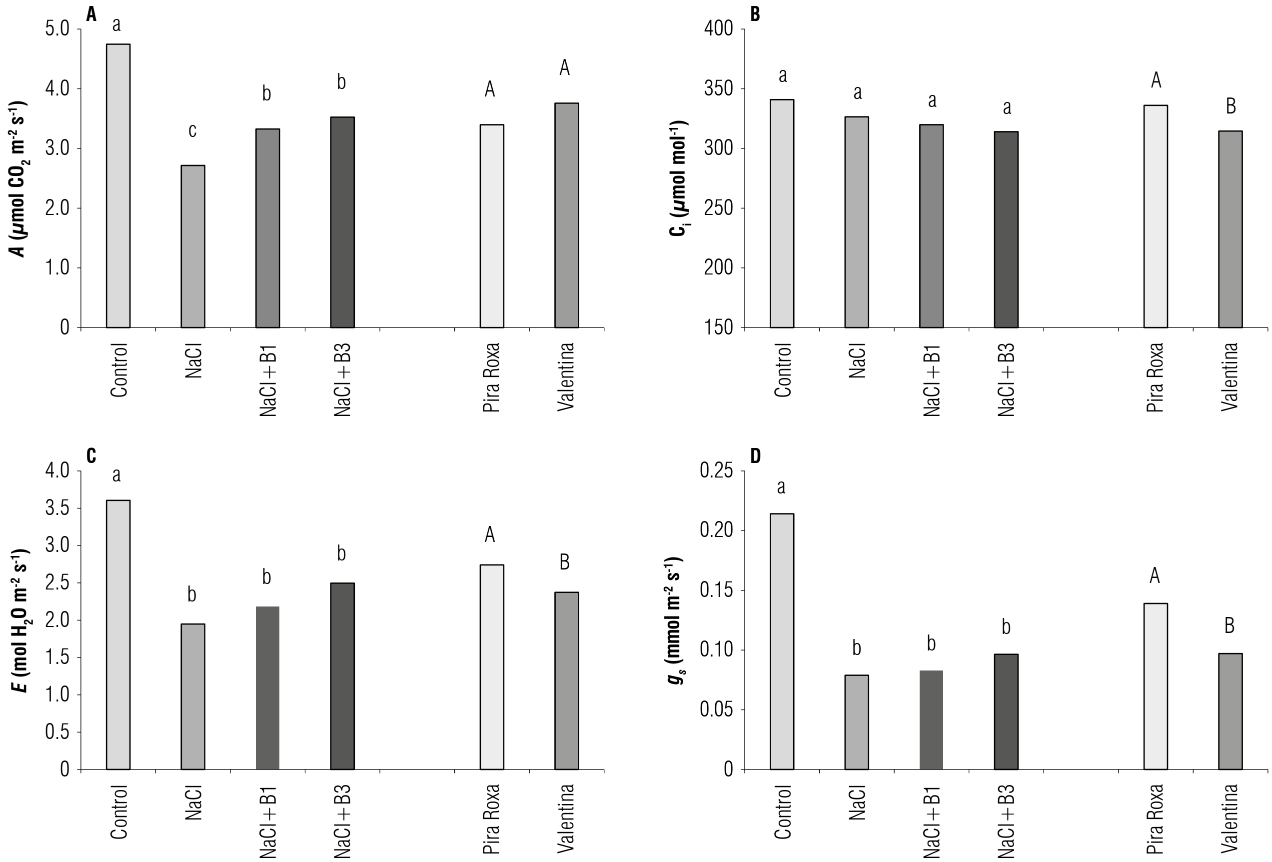

FIGURE 2. A) *A* - net photosynthesis; B) C_i - intracellular CO_2 concentration; C) *E*- transpiration, and D) g_s - stomatal conductance in lettuce plants submitted to foliar application with vitamins and irrigation with saline solution. Bars represent means; n=4. Control: irrigated with water and without application of vitamins; NaCl: irrigated with saline solution and without application of vitamins; NaCl+B1: irrigated with saline solution and application of vitamin thiamine; NaCl+B3: irrigated with saline solution and application of vitamin niacin. Lowercase letters compare treatment means and uppercase letters compare cultivar means.

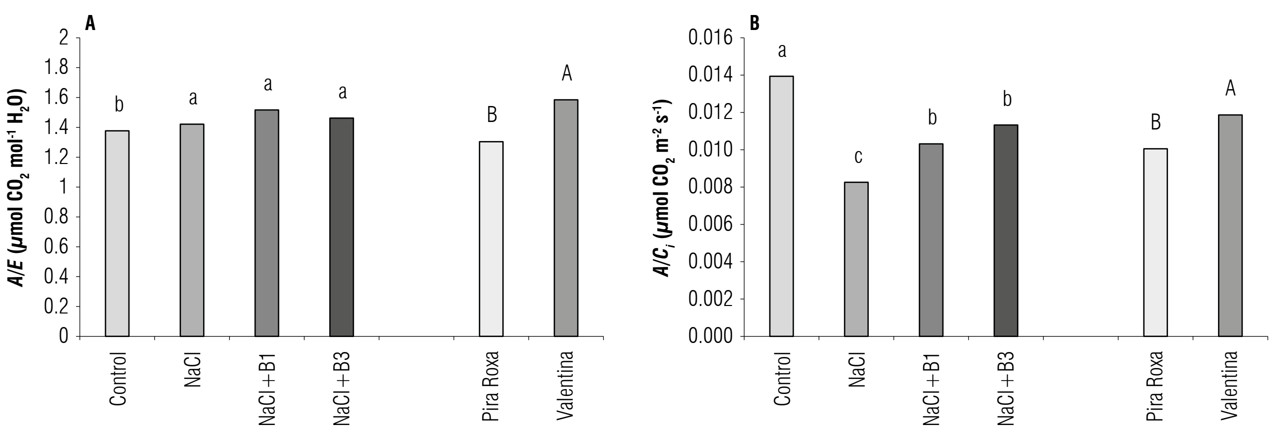

FIGURE 3. (A) Water use efficiency and (B) instantaneous carboxylation efficiency in lettuce plants submitted to foliar application with vitamins and irrigation with saline solution. Bars represent means; n=4. Control: irrigated with water and without application of vitamins; NaCl: irrigated with saline solution and without application of vitamins; NaCl+B1: irrigated with saline solution and application of vitamin thiamine; NaCl+B3: irrigated with saline solution and application of vitamin niacin. Lowercase letters compare treatment means and uppercase letters compare cultivar means.

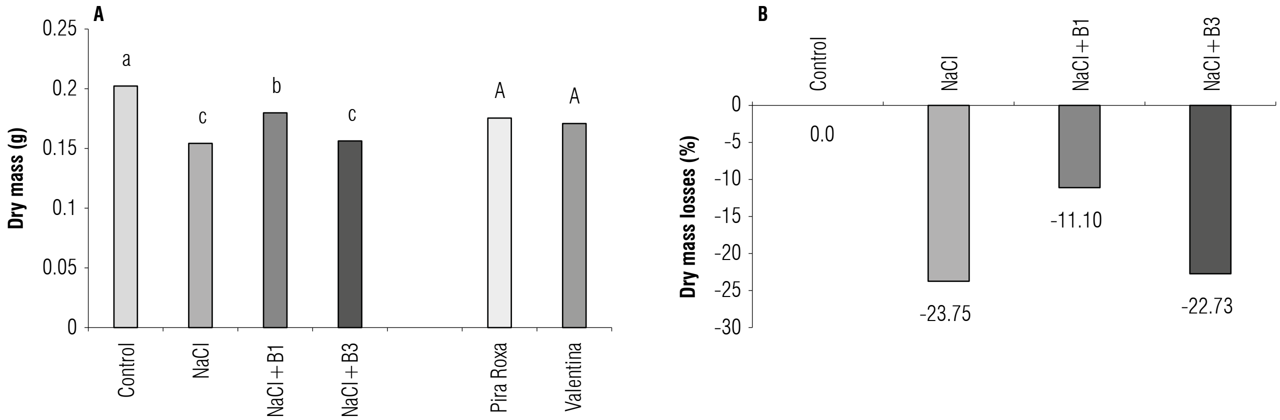

FIGURE 4. (A) Shoot dry mass and (B) average values of dry mass loss in lettuce plants submitted to foliar application with vitamins and irrigation with saline solution. Bars represent means; n=4. Control: irrigated with water and without application of vitamins; NaCl: irrigated with saline solution and without application of vitamins; NaCl+B1: irrigated with saline solution and application of vitamin thiamine; NaCl+B3: irrigated with saline solution and application of vitamin niacin. Lowercase letters compare treatment means and uppercase letters compare cultivar means.

When the plant is subjected to stress, the application of thiamine results in increases in glycine-betaine, the total levels of phenols, the activity of catalase and peroxidase enzymes and the levels of proline, which directly protect the plants by nullifying reactive oxygen species and helping to maintain photosynthetic pigments (Jabeen *et al*., 2021).

The application of thiamine also increases energy reserves (sugars) and nutritional reserves in plant tissues (Kaya *et al*., 2015). This increases plant resilience against the deleterious effects exerted by stress on the growing environment (Taiz *et al*., 2017).

The positive effect observed for the application of niacin on photosynthetic activity is related to its participation as a constituent of NAD^+ and $NADP^+$, acting directly in the transport of electrons in cellular and respiratory metabolism (Meyer-Ficca & Kirkland, 2016), with increases of the pigment photosynthetic agents and protection of the lipid layer in membranes of the leaf cells (Hussein *et al*., 2014). However, despite the positive effect reported in the literature, the application of niacin did little to alleviate the effects of salinity in the present study. This may be related to the composition of lettuce plants, which might have high levels of niacin. Thus, the exogenous application of this vitamin may not have been sufficient to result in significant changes in the plants. The application of niacin to a curly mustard crop at concentrations close to 485.20 mg L^{-1} did increase plant development (Vendruscolo *et al*., 2017).

Other authors observed that the application of thiamine at different concentrations effectively affects and favors the development of rice plants (Vendruscolo *et al*., 2019) and sweet corn (Vendruscolo *et al*., 2018), especially when the plants are subjected to abiotic stresses or field production conditions, where environmental factors act on the plants. Additionally, the application of niacin has a biostimulant effect, promoting the development and productivity of species such as corn (Colla *et al*., 2021) and beans (Abreu *et al.,* 2020) in the field.

Conclusions

The exogenous application of vitamins helps to mitigate the effects caused by irrigation with saline water on lettuce plants, reducing stress on photosynthetic mechanisms and increasing photosynthetic activity. In addition, thiamine helps to reduce the deleterious effects of salinity on the dry mass accumulation in plants.

Conflict of interest statement

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

Author's contributions

AAG and EPV designed and conducted the research; AAG, EPV, CCS, MBM, and GRS performed the field experiment; SFL and EPV performed the statistical analyses; AAG wrote the manuscript; and EPV, SFL, CCS, MBM, and GRS reviewed the manuscript. All authors reviewed the final version of the manuscript.

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SCIENTIFIC NOTE

Statistical model based on climatological variables for the prediction of pest and disease incidence in rose (*Rosa* spp.) crops

Modelo estadístico en función de variables climatológicas para la predicción de la incidencia de plagas y enfermedades en cultivos de rosa (*Rosa* spp.)

William Alberto Lombana-Peña[1]*, Oscar Eduardo Pedraza-Contreras[1], Ramiro Ordoñez-Córdoba[1], Omar Ariel Nova Manosalva[1], and Julián Andrés Salamanca Bernal[2]

ABSTRACT

In Colombia, floriculture is a very important section of the economy since it provides income to the country. Within this sector is the cultivation of roses (*Rosa* spp.), whose production and quality are affected by the presence of various pests and diseases. Among these pests are thrips *Frankliniella occidentalis* and mites *Tetranychus urticae*, and among the diseases are downy mildew *Peronospora sparsa*, powdery mildew *Podosphaera pannosa* and botrytis *Botrytis cinerea*. This problem generates large expenses in the purchase of agrochemical products for their control and management. This study analyzes the incidence of various pests and diseases in rose cultivation as a function of climatological variables (evaporation, temperature, relative humidity, and precipitation) in order to predict a future affectation. The analysis was carried out with R as programming language for the calculation of a multiple linear regression model. The results showed satisfactory prediction for the percentage incidence of each of the pests and diseases, since the difference between the predicted values and the values obtained by monitoring did not exceed 5% for the downy mildew, botrytis, mites, and thrips and 10% for the powdery mildew. The tool presented shows appropriate prediction for the possible behavior of the pests and diseases, and, thus, provides the opportunity to counteract their damage and estimate the investment required for their control. In this study, only the percentage incidence data of each of the pests and/or diseases was considered, as well as the value of the response variables in percentage incidence.

Key words: mites, botrytis, powdery mildew, downy mildew, pests, regression, thrips, diseases.

RESUMEN

En Colombia la floricultura es un sector importante para la economía por su valor en el momento de aportar ingresos al país. Dentro de este sector se encuentra el cultivo de rosa (*Rosa* spp.), cuya producción y calidad se ven afectados por la presencia de varias plagas y enfermedades. Dentro de las plagas se encuentran los trips *Frankliniella occidentalis* y los ácaros *Tetranychus urticae*, y dentro de las enfermedades el mildeo velloso originada por *Peronospora sparsa*, el mildeo polvoso originada por *Podosphaera pannosa* y la botrytis originada por *Botrytis cinerea*. Esta problemática genera grandes gastos en compra de productos agroquímicos para su control y manejo. En el presente estudio se analiza la incidencia de varias plagas y enfermedades en rosa en función de la expresión de variables climatológicas (evaporación, temperatura, humedad relativa y precipitación), con el fin de pronosticar una afectación futura. El análisis se realizó utilizando R como lenguaje de programación para el cálculo de un modelo de regresión lineal múltiple. Los resultados obtenidos fueron satisfactorios al momento de predecir el porcentaje de incidencia de cada una de las plagas y enfermedades, ya que la diferencia entre los valores predichos y los valores obtenidos por el monitoreo no superan el 5% para el caso de del mildeo velloso, botrytis, ácaros y trips, mientras que para el caso del mildeo polvoso la diferencia se mantiene por debajo del 10%. La herramienta presentada muestra un panorama apropiado de predicción, mostrando así el posible comportamiento de las plagas y enfermedades, igualmente, brinda la oportunidad de poder contrarrestar sus daños y estimar la inversión requerida para su control. En este estudio se tuvieron en cuenta únicamente los datos de porcentaje de incidencia de cada una de las plagas y/o enfermedades, así mismo se dieron los valores de las variables respuesta en porcentaje de incidencia.

Palabras clave: ácaros, botrytis, mildeo polvoso, mildeo velloso, plagas, regresión, trips, enfermedades.

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[1] Facultad de Ingeniería y Ciencias Básicas, Fundación Universitaria Los Libertadores, Bogotá (Colombia).
[2] Grupo de Física e Informática, FISINFOR, Universidad Distrital Francisco José de Caldas, Bogotá (Colombia).
* Corresponding author: wlombanape@libertadores.edu.co

Introduction

In Colombia, floriculture is one of the most important agro-industrial sectors, and it is oriented to cultivating, producing, and marketing flowers and ornamental plants. This sector has a high production for export, which gives international recognition to the country. Colombia is one of the countries with the highest exports in Latin America and, after Holland, the largest world exporter to the USA (Bautista Silva *et al.*, 2016). This situation contributes to the generation of employment and to foster the country's economic income. Colombia produces around 60 species and 1,400 varieties of flowers for export. The main species marketed are the carnation (since 1956), roses, chrysanthemums and alstroemeria, which reach 100 countries, mainly the USA, Japan, the United Kingdom, Canada, Korea, and Spain. Between January and May 2022, the country received approximately 1.050 billion $US from their commercialization (Colombiatrade, 2019).

In the agroindustrial sector, especially in cultivation of ornamental plants for export, different types of pests and diseases affect crops from the time of planting, growth and development, harvesting, cold chain, and vase life (Yanchapaxi *et al.*, 2010); this problem leads growers to face low productivity, decrease in quality, and high costs generated by the constant applications of agrochemical or biological products that help prevent and mitigate the effects caused by the biological targets (Rodríguez Ramírez & Torres, 1995; Álvarez Romero *et al.*, 2018). Additionally, on certain occasions, the application of these products does not give the expected results, thus reducing the export production and quality of roses (Gómez & Arbeláez, 2005).

Studies of the behavior of pests, such as mites *Tetranychus urtica,* and diseases in the rose crop (*Rosa* spp.), such as downy mildew *Peronospora sparsa*, powdery mildew *Podosphaera pannosa,* and botrytis *Botrytis cinerea* have shown a relationship of their incidence with the climatic conditions that occur during their growth (Perilla & Sanabria, 2007; Filgueira & Zambrano, 2014).

Modeling in agriculture becomes increasingly important not only for monitoring the variable state of the system but also in the decision-making and management processes of the system (Arredondo Hoyos & Castañeda-Sánchez, 2020). Some models are listed below.

According to Fatnassi *et al*. (2015), for each sampled monitoring site i, i = 1, ...,15, and observation time tj (in weeks) tj = j; j = 1,..., 5., let $H_{i,tj}$ denotes the weekly mean of humidity, $T_{i,tj}$ the weekly mean of temperature, $N^{L}_{i,tj}$ the number of thrips larvae, and $N^{A}_{i;tj}$ the number of thrips adults. This model takes into account several thrips states of development.

The Kiani and Mamedov (2017) experimental model for recognizing plant diseases consists of two processes; the first is detecting the healthy and disease infected area in plants based on color processing detection algorithm (CPDA). The second is decision making based on fuzzy logic classification algorithm (FLCA). Finding the binary image of the red and green pixel of the image will guide the program to the next step. The aim of this process is to find the type of disease on strawberry leaves, which may be iron deficiency or fungal infection (Kiani & Mamedov, 2017).

The objective of this study is to develop an innovative system to assess the risk of pests using a fuzzy logic approach (Tay *et al.*, 2021). The system is designed to provide farmers with an index representing an estimate of the risk of presence of Western Flower Thrips (WFT), *Frankliniella occidentalis,* in a greenhouse of roses. For this purpose, a modular knowledge-based decision support system has been designed.

González (2013) proposed the exponential increasing model, using the age of the crop in days of planting, the maximum, minimum and average temperature and the maximum, minimum and average relative humidity, 7 d before each evaluation.

The aim in this study was to obtain a mathematical model to predict the behavior and incidence of the most common pests and diseases in the rose (*Rosa* spp.) crop. This model considered local climatological variables of evaporation, precipitation, temperature, and relative humidity, which directly affect the presence of the biological targets (Rodríguez Ramírez & Torres, 1995). The prediction achieved with the model will contribute to preventive and/or curative actions to mitigate the effects caused by the biological targets.

Materials and methods

The main objective was to obtain an equation that allows predicting the incidence of each of the pests and diseases from the forecast of a climatological platform, to permit prompt action for preventive rather than curative control, thus, reducing costs in agrochemical purchases.

The instrument used in the study was database from the Betania farm located in the municipality of Tabio Cundinamarca - Colombia, belonging to C.I. SUNSHINE BOUQUET S.A.S. with the percentage of incidence of pests and diseases. The database contained the information of the predictor variables measured in an *in situ* meteorological station. The predictor variables were: evaporation, precipitation, temperature, and relative humidity, each taken as a weekly average. The weather station had evaporation and relative humidity sensors, with a rain gauge and thermometers to measure precipitation and temperatures, respectively. It was also connected to the Climate connector meteorological platform (Canal Clima S.A.S, 2023) (Canal Clima S.A.S., 2023) which shows the behavior in real time and a 14-d forecast for each of the aforementioned predictor variables.

Procedure

Direct monitoring

Direct monitoring sought to verify the presence or absence of insects, diseases and other factors that can limit or optimize the productivity of the crop.

Block monitoring was carried out by a trained monitor, who moves around the bed and directly observes the presence of pests, diseases and natural enemies in different parts of the bed, as well as the plant strata, with the help of implements such as magnifying glasses, sticks, bags to collect plant material, among others. This monitoring was carried out once a week, checking 100% of the beds on one side only; the following week the opposite side was covered and, thus, in 2 weeks, 100% of the area has been checked. Beds generally have an agronomic length of 32 linear meters, but there are some beds that, due to the topography of the site, are shorter (Tab. 1). Depending on the length of the bed, the product and the monitored object, the points where the work is to be done were defined, usually two fixed points at the beginning and end of the bed, and three additional points distributed along the bed for a minimum total of 5 points. In each monitored point, an area of approximately 1 m^2 and the three thirds of the plant (upper third, middle third, lower third) was checked.

TABLE 1. Characteristics of sampled beds.

Sampled beds	Bed area (m^2)	Total revised beds per week	Number of monitors	Number of frames per bed
32	25.6	7010	6	7

The presence of any pest or disease was recorded in the monitoring application. At the end of the check (only for some pests and diseases defined by the technical department), the beginning of the bed was marked with ribbons with the color corresponding to the biological target.

To ensure the process, the person in charge was checking 5 positive and 5 negative beds for each monitor and verifying what was reported in each table by the monitor. The quality level of direct monitoring had to be greater than 90%, obtained through the assurance.

Indirect monitoring

Indirect monitoring sought to detect the presence, absence or migration of pests or diseases out of the bed, through different strategies such as:

a) Wet chamber. This monitoring consists of an assembly of flowers of six each of the species and/or varieties (67 in total) in a space saturated with relative humidity, which makes it possible to identify the presence and reproduction of the disease. The reading in a humid chamber is carried out according to the frequency established by the work center, where the presence of the disease is evaluated in all samples, and those affected are eliminated. The results of the trace are recorded in a form;

b) Pheromone and kairomone traps. These traps (50 traps in 32 ha, 1.56 traps ha^{-1}) release a pheromone or sexual attractant, which attracts the adults of the species (moth, fly, or thrips) to the trap. Diffusers are designed to reproduce a proportion of the insect's own attractive chemical components, but with a higher emission rate. The detection and count of captured insects are carried out weekly to identify migrations or increased pest pressure on the farm;

c) ICA traps. These consist of placing kiosk traps at the four cardinal points of the farm determined by the ICA (4 traps for 32 ha, 0.125 traps ha^{-1}), used specifically for monitoring thrips and especially *Thrips palmi*, reading the trap tubes located there. The reading is carried out twice a week and is recorded in the ICA "form for the weekly report of detection of thrips". This information must be registered on the platform https://sisfito.ica.gov.co/

According to the schedule, the vials (sample of captured thrips) are sent to the ICA for analysis.

Monitoring plates

Internal plates

To quantify the presence of pests in the crop, the farms must place plaques at the end of or interspersed in the block, using colors depending on the biological target. The plates should be read weekly by the monitor in charge of recording, and immediately cleaned and prepared for re-reading. These readings must be entered into the Colibri app for indirect monitoring. This application is proprietary software developed by the company Sunshine Bouquet S.A.S.

External plates were placed as follows: plate No. 1 near the entrance of the farm and the following plates every 100 m according to the clockwise movement, until the periphery of the farm is covered (around the blocks). Each plate was located on a 2 m long pole, and this should have a height above ground level of 1.5 m, located at a maximum of 1.5 m from the greenhouses. The plate was identified with the abbreviation EXT and the number corresponding to its distribution. The identification had to be placed perpendicularly on the top of the mast.

The plates were read every 7 d on one side only (external side facing the periphery of the farm), and the number of individuals of each pest trapped per plate was counted. Once the reading of the plate was finished, it was removed and replaced by a clean plate. The information from the reading was recorded in the form (with the average number of individuals per plate/pest/week) and recorded electronically.

Result analysis

The technical department of each farm consolidated the indirect monitoring data for the incidences of each of the pests and diseases. The Farm Manager/Area Manager and Plant Health Manager analyzed the results and established the control strategies to be followed: Physical Strategy - Cultural Strategy - Biological Strategy - Chemical Strategy.

The first step in conducting this research was to collect data by monitoring the rose crop for pests and diseases. As a result of this monitoring, a report on the percentage of incidence of each of the pathogens on a weekly basis was obtained. This database was fed daily with data from the monitoring performed by the personnel in charge of this task at the farm. The percentage of incidence data is standard for the entire company. The reported percentage of incidence per pathogen is obtained by dividing the number of beds affected by the total number of beds monitored.

$$\% \text{ incidence} = \frac{\text{Number of beds affected}}{\text{Total number of bed}} \times 100 \qquad (1)$$

The information was also collected from the data obtained at the meteorological station for each of the predictor variables from week 01 of 2020 to week 24 of 2022.

The second step determined how to present the data to handle identical frequencies of analysis, considering that the pathogen incidence data were presented as a percentage on a weekly basis, and the data for the climatological predictor variables were presented on a daily basis. A weekly average of the predictor variables was chosen.

The third step cleaned the data obtained using the statistical program R. After this cleaning, correlation and multiple regression between the four predictor variables and a response variable for each pathogen was performed.

Model analysis

Pearson's correlation coefficient and multiple linear regression analysis with equations were used.

The following ranges were used for the correlation coefficient:

TABLE 2. Correlation coefficient ranges (adapted from Hernández-Lalinde *et al*., 2018).

Value of R	Relationship strength
-1.0 to -0.5 or 0.5 to 1.0	Strong
-0.5 to -0.3 or 0.3 to 0.5	Moderate
-0.3 to -0.1 or 0.1 to 0.3	Weak
-0.1 or 0.1	None or very weak

Using multiple linear regression in R, we obtained an expression of the form

$$\% \text{ Pathogen} = \beta_0 + \beta_1 X + \beta_2 Y + \beta_3 Z + \beta_4 W \qquad (2)$$

where:

% Pathogen = % incidence of the pathogen tested;

X = response variable "Average evaporation/week";

Y = response variable "Average temperature/week";

Z = response variable "Average relative humidity/week";

W = response variable "Average precipitation/week";

β_0 = Intercept; β_1, β_2, β_3, β_4 = slopes corresponding to variables X, Y, Z, and W, respectively.

Utilizing the *lm* function in the R software for the statistical analysis of the database, we performed the respective multiple linear regression to see the behavior of the response variables with regard to the predictor variables. The result of this function provides an equation that allows determining the prognosis or future behavior of each of the pathogens (Tab. 3).

Example equation for prediction of mites

$$\% \text{ Mites} = \begin{array}{c} 0.1318917 - 0.0161655\,X - 0.0020338Y \\ + 0.1211183\,Z - 0.0007188\,W \end{array} \qquad (3)$$

TABLE 3. Resulting multiple linear regression in the statistical software R. Example is given to predict percentage of mites.

Residuals:	**Min**	**1Q**	**Median**	**3Q**	**Max**
	-0.060106	-0.024621	-0.006072	0.024178	0.088272
Coefficients:		**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
	(Intercept)	0.1318917	0.0654840	2.014	0.0461 *
	Evaporation	-0.0161655	0.0070616	-2.289	0.0237 *
	Temperature	-0.0020338	0.0013004	-1.564	0.1204
	Relative humidity	0.1211183	0.0889850	1.361	0.1759
	Precipitation	-0.0007188	0.0007307	-0.984	0.3272

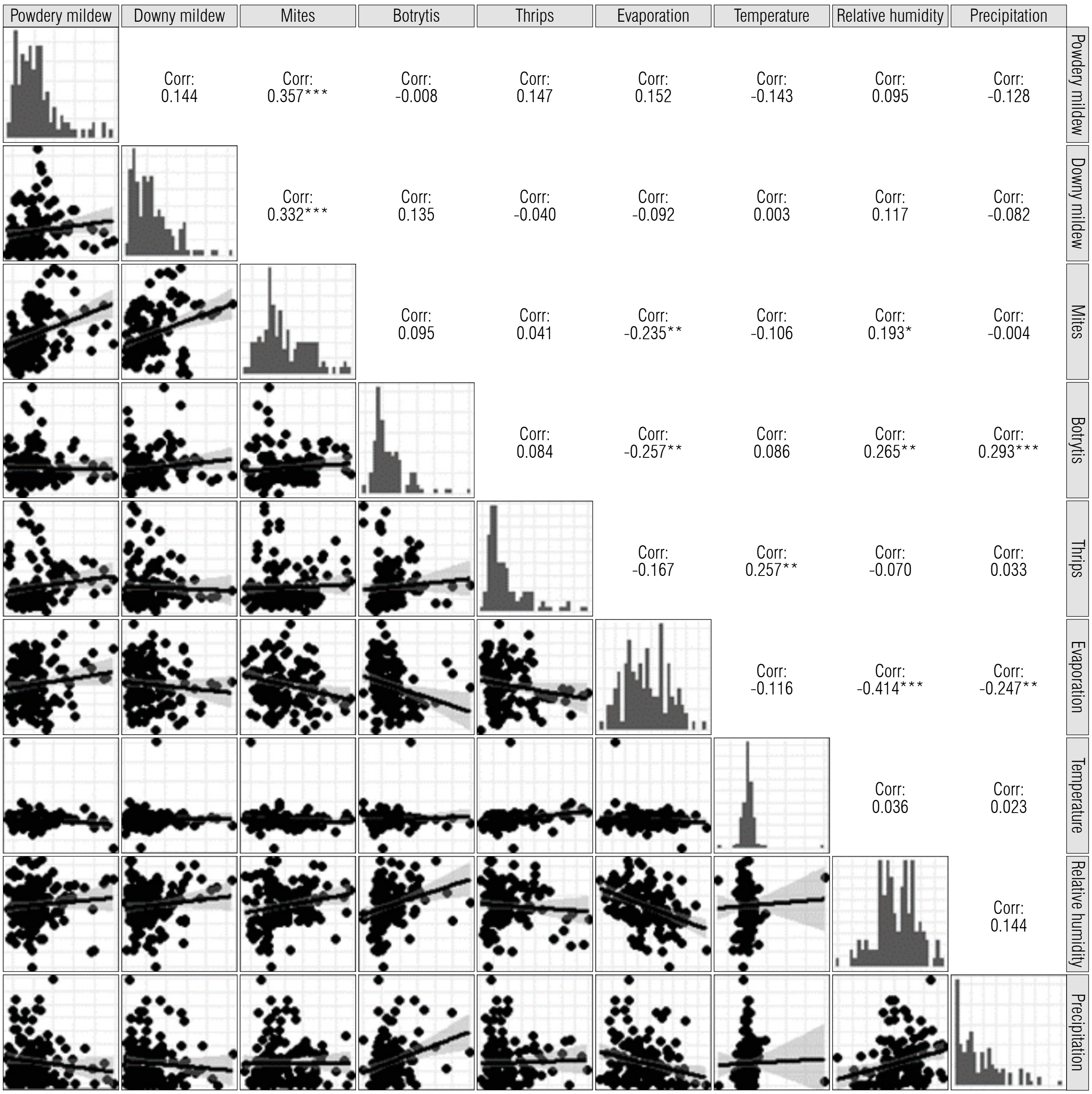

FIGURE 1. Correlation coefficients between each response variable vs. predictor variable, expressed in percent.

Results and discussion

Figure 1 shows each of the existing correlations between the predictor variables (evaporation, temperature, relative humidity, and precipitation) and the response variables (powdery mildew, downy mildew, mites, botrytis, and thrips), showing how these different variables interact with each other, either directly or indirectly or what type of correlation exists, whether strong, moderate, slight or null. The correlation is expressed in percent.

As a result, in Figure 1, the correlation coefficients between each response variable and predictor variable can be observed.

The powdery mildew has weak inverse correlation with the predictor variables temperature and precipitation and a weak direct correlation with the evaporation and relative humidity variables.

Downy mildew has a weak inverse correlation with the predictor variables evaporation and precipitation, a weak direct correlation with the predictor variable relative humidity, and a very weak correlation with the predictor variable temperature.

Mites have a weak inverse correlation with the predictor variables evaporation and temperature, a weak direct correlation with the predictor variable relative humidity and a very weak correlation with the predictor variable precipitation.

Botrytis has a moderate direct correlation with the predictor variables relative humidity and precipitation, a weak direct correlation with the temperature variable, and a moderate inverse correlation with the predictor variable evaporation.

Thrips have a weak inverse correlation with the predictor variables evaporation and relative humidity, a moderate direct correlation with the predictor variable temperature, and a very weak correlation with the predictor variable precipitation.

A histogram (Fig. 2) was used to graphically show the frequency of occurrence of each of the variables to determine which pest and disease occurs more frequently and in which interval.

Predictor equations obtained by means of the R statistical software

For a reliable statistical analysis, a relevant database is obtained to predict the behavior of the different pests and diseases that occur during the development of the rose crop. This analysis used the multiple linear regression method, which allows us to take more than one independent variable to see the behavior of the response variable; in this case, the independent variables are the climatic conditions, and the response variables are each of the pests and diseases that occur in the crop.

A database was used to record the climatic conditions and the presence of pests and diseases in the Betania farm.

By means of the statistical software R and the statistical method of multiple linear regression, the following equations are proposed as a contribution of this research with the objective of predicting the increase of diseases and pests in rose crops.

$$\%\ \text{Powdery mildew} = -0.05294 + 0.02177\ X - 0.002905\ Y + 0.3035\ Z - 0.001642\ W \tag{4}$$

$$\%\ \text{Downy mildew} = -2.313 \times 10^{3} - 4.160 \times 10d^{-3}X - 8.013 \times 10d^{-5}Y + 8.273 \times 10d^{-2}Z - 7.956 \times 10d^{-4}W \tag{5}$$

$$\%\ \text{Mites} = 0.1319 - 0.01617\ X - 0.002034\ Y + 0.1211\ Z - 0.0007188\ W \tag{6}$$

$$\%\ \text{Botrytis} = -\ 0.009652 - 0.002727\ X + 0.0002507\ Y + 0.03955\ Z + 0.0004993\ W \tag{7}$$

$$\%\ \text{Thrips} = 4.161 \times 10^{-2} - 6.906 \times 10^{-3} + 1.663 \times 10^{-3}\ Y - 7.192 \times 10^{-2}\ Z + 7.264 \times 10^{-5}\ W \tag{8}$$

These results were similar to the equation proposed (Eq. 9) in the exponential increasing model of González (2013), where the variables were the age of the crop in days of planting, the maximum, minimum and average temperature and the maximum, minimum and average relative humidity 7 d before each evaluation. This corroborates that there is a directly proportional relationship between pests and diseases with the behavior of climatological variables.

$$Y = 1 - \exp - (\exp(-24{,}2205) \times (\text{Age}^{4.083}) \times \exp(0.0492 \times \text{Tmax} - 0.1441 \times \text{Tmin} + 0.1328 \times \text{Tmed} + 0.0482 \times \text{Rhmax} - 0.0232 \times \text{Rhmin} + 0.0173 \times \text{Rhmed}) \times \text{Age}) \tag{9}$$

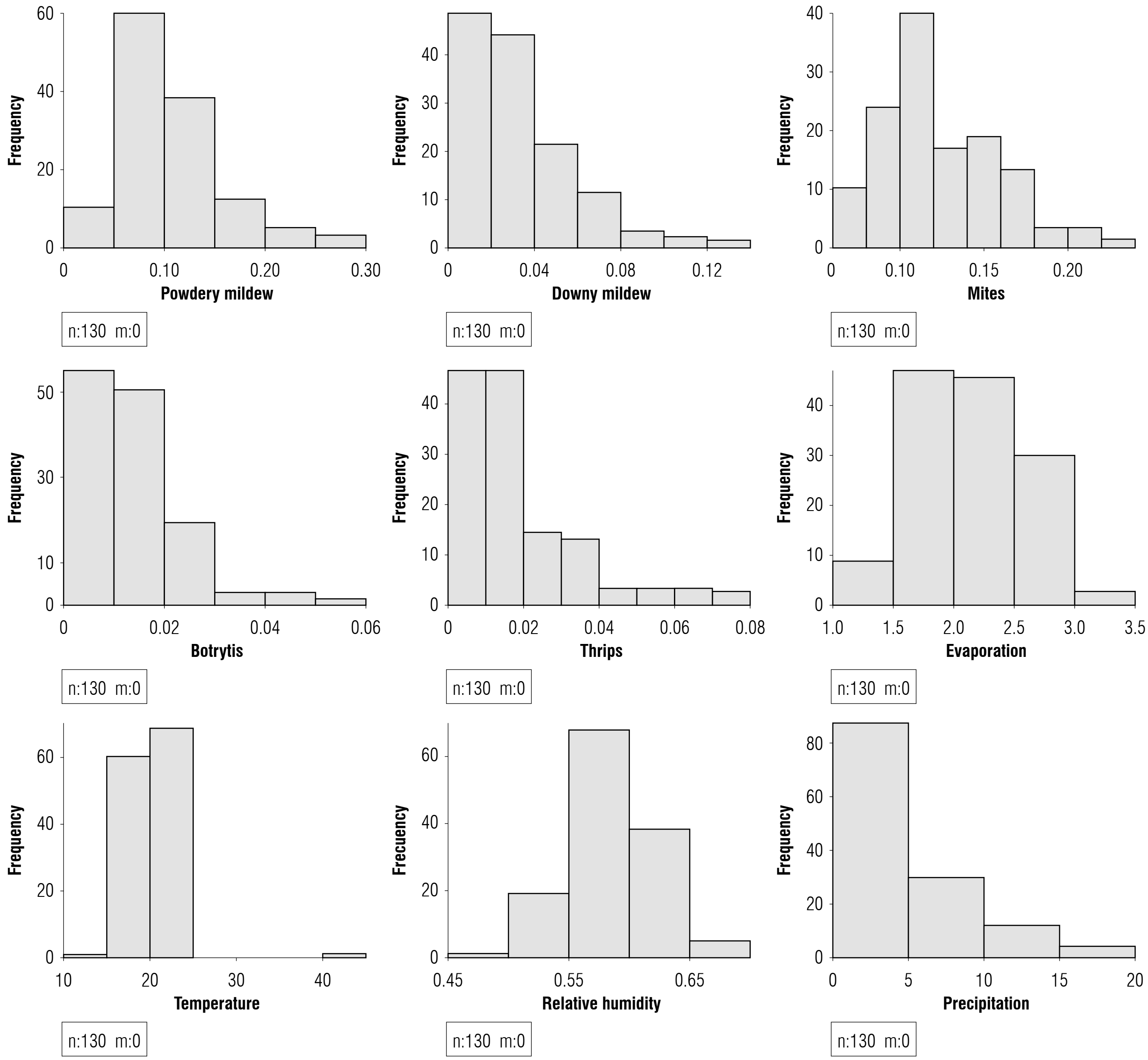

FIGURE 2. Incidence frequency values for each variable and its expression in different ranges.

where

Tmax = Maximum temperature;

Tmed = Medium temperature;

Tmin = Minimum temperature;

Rhmax = Maximum relative humidity;

Rhmed = Average relative air humidity;

Rhmin = Minimum relative air humidity.

In the different predictor equations, verifications were made by replacing the predictor variables with values already observed to predict the percentage of incidence of each of the pests and diseases and to compare with the values reported.

The monitoring obtained very close values, with differences of below 5% for the downy mildew, botrytis, mites and thrips, and below 10% for powdery mildew.

Table 4 shows the values of the predictor variables provided by the Canal Clima platform (CANAL CLIMA S.A.S., 2023), Table 5 shows the incidence percentages calculated with the predictor equations, and Table 6 shows the incidence percentages reported in the monitoring. These results show that the objective has been achieved, namely, to predict the incidence of a pest and disease based on the values of the predictor variables with an acceptable error percentage of +/- 5%.

The week number corresponds to the week of the year in which the evaluation was carried out.

TABLE 4. Values of the predictor variables provided by the Canal Clima platform.

Predictor variable values				
Week	Evaporation (X)	Temperature (Y)	Relative air humidity (Z)	Precipitation (W)
25	2.0	20.5	62.8	7.1
26	2.3	20.7	60.3	8.0
27	2.1	19.4	61.0	4.0
28	2.3	19.5	57.3	4.0

TABLE 5. Incidence percentages calculated with the predictor equations.

Response variable values					
Week	%Powdery mildew	%Downy mildew	%Mites	%Botrytis	%Thrips
25	19.0	5.2	7.7	2.5	-4.5
26	18.2	5.0	7.3	2.4	-4.3
27	18.4	5.0	7.4	2.4	-4.3
28	17.3	4.7	7.0	2.3	-4.1

TABLE 6. Incidence percentages reported in monitoring.

Monitoring values					
Week	%Powdery mildew	%Downy mildew	%Mites	%Botrytis	%Thrips
25	9.0	2.2	10.7	0.8	0.5
26	8.4	2.4	10.3	1.2	0.8
27	10.8	3.5	9.6	1.3	0.7
28	12.3	3.4	8.8	0.9	0.8

Although the correlations between pathogens and predictor variables are moderate or weak, the latter have a significant influence when predicting the percentage of incidence of each pathogen by replacing observed values of the predictor variables in the equations obtained.

Correlation tests are useful to identify the trend of the response variables as a function of each of the predictor variables, which does not necessarily mean that when two variables correlate with each other, it is due to a cause and effect relationship; these variables may be independently related to a third variable.

While this analysis obtained satisfactory predictions, the amount of information in the database should be increased in order to make a more accurate prediction, considering that the data used in this research were limited.

Agriculture has improved due to the use of intelligent agricultural machines for crops and livestock control, intelligent monitoring drones for pest control, as well as to observation sites that record agro-climatic variables to collect large amounts of data at the lowest costs. This has led to more information-oriented, potentially more productive, and efficient agricultural processes.

The above results indicate the need to deepen understanding of the incidence of diseases in the face of the climate changes that are manifesting themselves by constantly feeding the database and thus obtaining better predictions. Further studies to address the subject could be done using different methodologies, such as the application of Machine Learning and Deep Learning, thus creating an autonomous learning algorithm that can make it even more efficient in its predictions.

From the practical point of view, it is important to correct some aspects, to undertake improvements and to include new elements of interest for the solution to the problem addressed.

Conclusions and recommendations

The objective of obtaining equations that allow predicting the incidence of pathogens according to the predictor variables was achieved; this provides several benefits, including the ability to make timely decisions to counteract the effect or damage that pathogens may cause in the crop.

The incorporation of these equations into the agrochemical application program for pathogen control could clarify what type of agrochemical to use and which pest or disease to attack. This would also contribute to a rational use of the different agrochemicals, biological controls, or both, and would also yield cost savings in the purchase of agrochemicals.

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Conflict of interest statement

The authors declare that there is no conflict of interests with respect to the publication of this article.

Author's contributions

WALP and ROC formulated overall research objectives and goals, carried out the research process, specifically conducting data collection, and made the initial translation. OEPC, WALP, and ROC carried out activities to maintain research data for initial use and subsequent reuse and developed the methodology. OEPC applied statistical, mathematical, computational and other formal techniques to analyze or synthesize study data and implemented computer code and supporting software. OANM managed, coordinated, supervised, and led the planning and execution of the research activity, and verified the overall replication of the research results. OEPC, WALP, and ROC wrote the initial draft. OANM and JASB performed critical revision and comments. All authors reviewed the final version of the manuscript.

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SCIENTIFIC NOTE

Evaluation of the effect of quinoa germ on the chromatic properties of tomato sauce

Evaluación del efecto del germen de quinua en las propiedades cromáticas de la salsa de tomate

Alex Danny Chambi-Rodriguez[1*], Ana Mónica Torres-Jiménez[2], Carmen Rosa Apaza-Humerez[2], and Deysi Rosmery Salguero-Pacco[2]

ABSTRACT

Tomato sauce is one of the most consumed dish components in the world; and, for this reason, it is one of the most studied in the food industry. The objective of this research was to evaluate the effect of quinoa germ on the chromatic properties of tomato sauce. Tomato paste was prepared with additions of powdered quinoa germ using an analysis of variance of a single factor where the percentage of quinoa germ was the independent variable and water content, water activity (a_w), and chromatic properties were the response variables. The results showed that as the percentage of germ increased, values of water content and water activity lowered. However, the chromatic properties showed values with a tendency to increase. In conclusion, the quinoa germ directly impacted the variables studied.

Key words: colorimetry, *Chenopodium quinoa*, processed pasta.

RESUMEN

La salsa de tomate es uno de los alimentos más consumidos en el mundo, por lo tanto, es uno de los más estudiados en la industria alimenticia. En este sentido, el objetivo de la presente investigación fue evaluar el efecto del germen de quinua en las propiedades cromáticas de la salsa de tomate. Para esto, se procedió a elaborar pasta de tomate con adición de germen de quinua en polvo bajo la aplicación de un análisis de varianza de un solo factor donde el porcentaje de germen de quinua fue la variable independiente y como variables de respuesta se utilizaron la humedad, actividad de agua (a_w) y las propiedades cromáticas. Los resultados mostraron que a mayor porcentaje de adición de germen menor son los valores de humedad y actividad de agua. Sin embargo, las propiedades cromáticas presentaron valores con tendencia a incrementar. En conclusión, el germen de quinua impactó directamente en las variables estudiadas.

Palabras clave: colorimetría, *Chenopodium quinoa*, pasta elaborada.

Introduction

Tomato sauce is the most consumed tomato by-product in the world. The USA is one of the main exporters to the rest of North America, Japan, South Korea, and many European countries, making this market very dynamic (Valenzuela *et al.*, 2018; Subroto *et al.*, 2020). Commercial tomato sauce contains vinegar, sugar, salt and various spices, that are used to modify or intensify the flavor and aroma of various foods in the culinary world. From this viewpoint, consumers want sauces to have the characteristic red color, consistency, flavor, and aroma (Ahouagi *et al.*, 2021).

In the food industry and gastronomically, new product proposals are increasingly generated that satisfy the consumer gustatory sensibilities and that additionally provide functional properties through their ingredients that contribute to the health of the consumer (Garcia *et al.*, 2016; Ahouagi *et al.*, 2021; Uribe *et al.*, 2021). The addition of additional ingredients to tomato can affect multiple sensory, rheological, chromatic, and nutritional properties of tomato sauce (Mirzaei *et al.*, 2018). Since the consumer is the last commercial link that determines the quality of a product, the main analyses to be performed are color, texture, and flavor; and these will determine the acceptance of the product (Uribe *et al.*, 2021).

In the Andean region of South America, quinoa (*Chenopodium quinoa*) is considered a natural food resource of high nutritional value, recognized by the Food and Agriculture

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[1] Centro de Investigación en Ciencia de los Alimentos, Escuela Profesional de ingeniería de Industrias Alimentarias, Universidad Peruana Unión, Juliaca (Perú).

[2] Centro de Investigación en Tecnología de los Alimentos, Escuela Profesional de Ingeniería de Industrias Alimentarias, Universidad Peruana Unión, Juliaca (Perú).

* Corresponding author: adanny@upeu.edu.pe

Organization (FAO); for the FAO (2011), the quinoa germ (quinoa seed embryo) is a product high in fat (28.0% in the germ and 0.7% in the endosperm) and protein (48.5% in the germ and 3.5% in the endosperm). Quinoa germ is a source of nutrients and bioactive compounds that may provide various health benefits. First of all, it is an excellent source of vegetable protein that makes it an ideal ingredient for people who follow vegetarian or vegan diets. Quinoa germ contains high quality protein and all the essential amino acids for human health; in addition, quinoa germ is also rich in antioxidants and anti-inflammatory compounds (Vega *et al*., 2010). These compounds of quinoa germ can help prevent chronic diseases such as diabetes, hypertension, and cardiovascular disease. Antioxidants may also help to prevent cell damage and premature aging, and this may have long-term health benefits and when added to a sauce or dressing would modify its nutritional and physicochemical properties (Repo *et al*., 2003). The objective of the present research was to evaluate the effect of quinoa germ on the chromatic properties of tomato sauce.

Materials and methods

Samples of tomato sauces

Tomato sauce samples were prepared using a standard recipe as described by the following: 73.27% ripe tomato fruits, 25.39% onion, 0.64% celery, 0.60% garlic, and 0.07% pepper. This recipe was developed at the Universidad Peruana Union, Campus Juliaca. Finally, the powder of quinoa germ (quinoa seed embryo) was added to the tomato sauce in the percentages 2.5%, 5.0%, and 7.5%, based on a weight/weight mixture.

Moisture and water activity

The moisture content of the treatments was determined by weight loss by drying in a forced air oven (KERT LAB ODHG - 9030B) at 80°C until constant weight was obtained; these tests were analyzed in quintupled. Water activity 13.55±0.44°C was measured with AQUALAB (METER Group) at room temperature.

Image acquisition system

The image acquisition system for this research (Fig. 1) has the following characteristics: (1) the walls have a dimension of 30 cm, internal color matte black; (2) equilateral white light bulbs (10 cm edge); (3) a 13 megapixel image resolution camera; (4) a computer with an Intel Core i3 processor with 4G of RAM. The extracted samples were subjected to image acquisition in *.jpg format (Joint Photographic Experts Group).

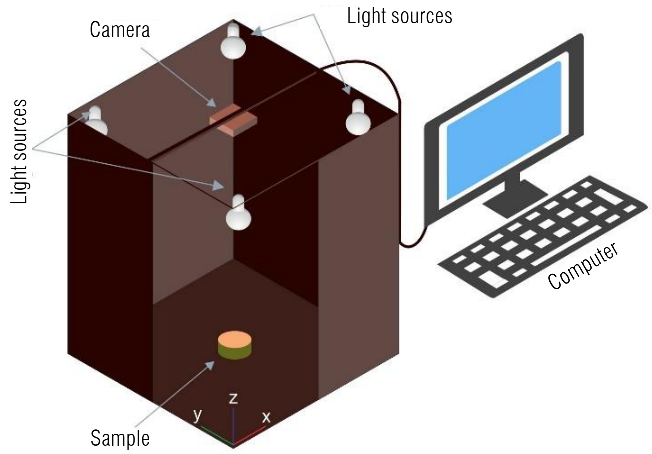

FIGURE 1. Image acquisition system.

Determination of color parameters

The color indicators L* (lightness) and the degree of redness (ratio s*/b*, where a* red – green and b* yellow – blue), the RGB information of the ROI's was extracted, from which the color was calculated in CIELab coordinates and these tests were analyzed in quintuplicate. This conversion is a mathematical process that allows colors to be represented in a three-dimensional space that is based on human perception. The Equations used to perform this conversion are as follows:

$$L^* = 116 \times f\left(\frac{Y}{Y_n}\right) - 16 \tag{1}$$

$$a^* = 500 \times \left[f\left(\frac{x}{x_n}\right) - f\left(\frac{Y}{Y_n}\right)\right] \tag{2}$$

$$b^* = 200 \times \left[f\left(\frac{Y}{Y_n}\right) - f\left(\frac{Z}{Z_n}\right)\right] \tag{3}$$

where L* is the luminosity, a* and b* are the chromaticity coordinates, Y is the relative luminance of the sample in RGB, Y_n is the relative luminance of a white reference color (for example, D65); X, Y, and Z are the trichromatic coordinates of the sample in RGB; X_n, Y_n, and Z_n are the trichromatic coordinates of a reference white color (for example, D65); $f_{(t)}$ is a nonlinear function used to adjust the response of the human eye to different wavelengths (Sharma *et al*., 2004).

From these coordinates, the purity (c*) was calculated from the following Equation:

$$c^* = \sqrt{(a^*)^2 + (b^*)^2} \tag{4}$$

Statistical analysis

An analysis of variance of a single factor (ANOVA) was applied at a significance level of P>0.05, adding quinoa germ flour at different concentrations (2.5%, 5.0%, or 7.5%) and applying a comparison by means (Tukey´s test) and a linear regression analysis (Montgomery *et al.*, 2021) to the data obtained through the equation:

$$y = mx + b \tag{5}$$

where y is the response variable (dependent); m is the slope; x is the predictor variable and b is the constant of the equation.

To adjust the models for each treatment, the criteria suggested by Chambi and Torres (2021) were applied, in which we have the coefficient of determination R^2.

$$R^2 = 1 - \frac{\Sigma\,(y_i - \hat{y}_i)^2}{\Sigma\,(y_i - \bar{y}_i)^2} \tag{6}$$

Results and discussion

Moisture content

The moisture content analysis (Tab. 1) showed that $T_{2.5\%}$ is significantly different from the other treatments and was superior at 83.824%.

TABLE 1. Descriptive statistics of the moisture content of tomato sauce treatments.

Treatments	Descriptive statistics		
	Mean ($\bar{x}$)	Standard deviation (σ)	Variance (σ^2)
$T_{2.5\%}$	83.824[a]	0.267	0.071
$T_{5.0\%}$	79.071[b]	0.222	0.049
$T_{7.5\%}$	78.963[b]	0.123	0.015

Note: means that do not have the same letters are significantly different at the 0.05 significance level.

The reduction in moisture content by increasing the content of quinoa germ in tomato sauce (Tab. 1) agrees with what was found by Tomas *et al.* (2018), who stated that the addition of dietary fibers can reduce up to 24.9% of the moisture content.

Water activity (a_w)

The distribution of water activity (a_w) data showed that the boxes corresponding to $T_{2.5\%}$ and $T_{5.0\%}$ overlapped each other, unlike $T_{7.5\%}$ with a lower range of data than those mentioned (Fig. 2).

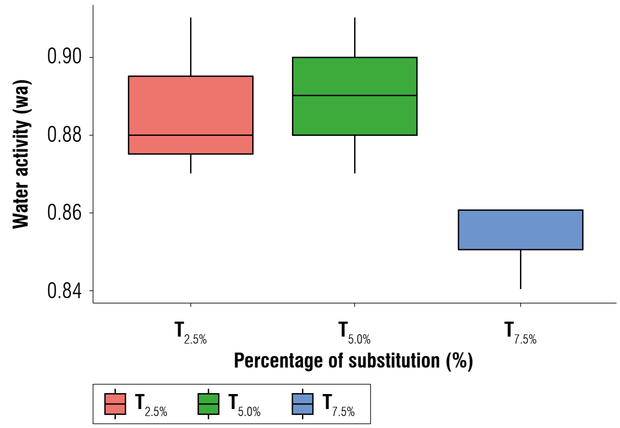

FIGURE 2. Water activity of tomato sauces with partial substitution by quinoa germ.

The water activity of tomato sauces is in a range of 0.95-0.98 of which treatments $T_{2.5\%}$ and $T_{5.0\%}$ are close to those already mentioned (Villalobos *et al.,* 2020). In $T_{7.5\%}$, the a_w content is lower than the other treatments because the germ content contains a higher proportion of protein, followed by lipids, dietary fiber, and minerals (Zhang *et al.*, 2023). These abundant proteins have polar hydrophilic parts and charged side chains, that convert them into a three-dimensional protein-water-carbohydrate structure that prevents free water from remaining, but rather binds it to the structure (Espinosa-Pardo *et al.*, 2020). The a_w for the corn germ studied by Tapia *et al.* (2001) had values close to 0.6. Likewise, the addition of ingredients to tomato paste like carbohydrates generates a significant decrease in water activity (Diantom *et al.,* 2017). But in contrast to this statement, Gerardi *et al.* (2018) found that the addition of anthocyanin extract does not modify water activity in tomato sauce reporting a value of 0.98 ± 0.02.

Chromatic properties

The chromatic properties (Tab. 2) of the treatments showed that $T_{7.5\%}$ is more luminous in contrast to the others. Likewise, it is observed that this has a linear behavior with a positive slope (m=213.820) with a coefficient of determination (R^2) of 0.928 with respect to the red/green coordinate (a*), indicating that all the samples tend towards red. $T_{2.5\%}$ was the sample of greatest intensity. In contrast to the luminosity this coordinate shows linear behavior with a negative slope (m=-590.850) and an R^2 of 0.972.

By adding additives to the sauce formulation, the brightness (L*) oscillated between the values of 42.73 and 52.19, demonstrating that there is an effect on the chromatic properties (Diantom *et al.,* 2017). In this research, a considerable spread was observed in the luminosity with values between 66,087 to 76,778 (Tab. 2). This is due to the color of the quinoa. The same happens with the two remaining properties (a* and b*). Likewise, Pereira *et al.* (2022) in a study on the substitution of tomato for avocado in obtaining sauce, demonstrated that the samples with avocado had high luminosity and yellowing.

TABLE 2. Chromatic properties of tomato sauces with a partial substitution of quinoa germ.

Treatments	L*	a*	b*
$T_{2.5\%}$	66.087 ± 3.940^a	44.926 ± 7.723^a	45.562 ± 2.006^a
$T_{5.0\%}$	73.996 ± 17.796^a	25.841 ± 4.643^b	40.800 ± 8.338^b
$T_{7.5\%}$	76.778 ± 24.956^a	15.383 ± 3.550^c	33.572 ± 8.694^b
Linear modeling and fitting criteria			
R^2	0.928	0.972	0.986
Slope (m)	213.820	-590.850	-239.800
Constant (b)	61.596	58.259	51.968

Note: All means are expressed as mean ± SD (n=5). The means that do not have the same letters are significantly different at the 0.05 significance level according to the Tukey test.

Regarding the calculation of purity (c*), the values decreased as the percentage of substitution increased in such a way that it is affected (Fig. 3).

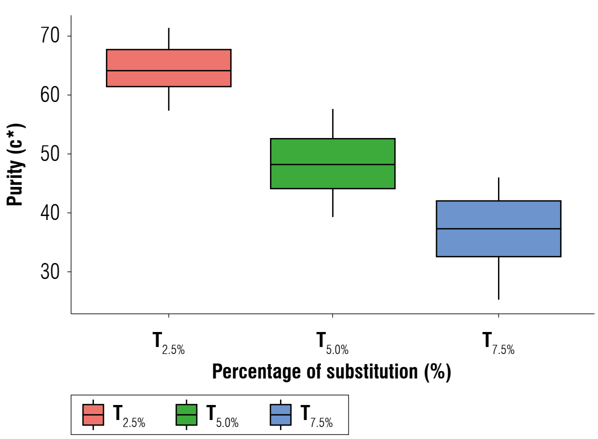

FIGURE 3. Color purity (c*) of tomato sauces with partial substitution by quinoa germ.

Color purity in tomato sauce is an important aspect that can influence sensory perception and acceptability of the product by consumers. The addition of other raw materials such as spices can affect the color purity of tomato sauce and its visual quality. Color purity refers to the intensity of the dominant color in a sample and is measured using a spectrophotometer. The addition of spices to tomato sauce can decrease color purity due to the presence of additional pigments in the spices that compete with the pigments in tomato (Botello *et al*., 2015). Additionally, the amount and type of spices added can influence the color purity of tomato sauce. The addition of turmeric to tomato sauce can significantly decrease color purity compared to other spices such as black pepper and cinnamon (Opara & Chohan, 2014). It is important to note that, although color purity is an important aspect of the visual quality of tomato sauce, it is not the only factor to consider. The addition of spices and other ingredients can enhance the flavor and complexity of tomato sauce and may compensate for the decrease in color purity.

Conclusions

The addition of quinoa germ had a direct effect on the analyses carried out. The more germ added, the greater was the reduction in water content. With respect to the water activity the same trend happens as with the water content, and, finally, the chromatic properties varied in all the substitutions, presenting a linear behavior with a negative slope and with a coefficient of determination (R^2) higher than 0.90.

The use of quinoa germ in the preparation of tomato sauce presents interesting chromatic properties, as well as an analysis of water activity and moisture. These aspects are relevant to assess the stability and quality of the product. By incorporating quinoa germ, a change in the chromatic properties of the sauce was observed, which can provide a distinctive visual appeal. Furthermore, the analysis of water activity and water content allowed for determining the water retention capacity and the stability of the product over time. These parameters are important to ensure proper texture and preservation of the quinoa germ-enriched tomato sauce.

Conflict of interest statement

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

Author's contributions

ADCR: conceptualization, methodology, formal analysis, research, writing of original draft editing. AMTJ: writing of original draft editing, research, validation. CRAH: writing of original draft editing. DRSP: data analysis. All authors reviewed the final version of the manuscript.

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Application and sensory evaluation of a hydroalcoholic extract of nasturtium (*Tropaeolum majus* L.) in a beverage from tropical fruits and vegetables

Aplicación y evaluación sensorial de un extracto hidroetanólico de capuchina (*Tropaeolum majus* L.) en una bebida de frutos y verduras tropicales

Camila Andrea Avila-Ortiz[1*], Andrés Giraldo-Toro[2], Valentina Guzmán[2], and María Soledad Hernández[1]

ABSTRACT

The nasturtium is diverse in bioactive compounds such as carotenoids, anthocyanins, and glucosinolates. Due to these attributes, it is important to apply them to novel foods, such as drinks. The objective of this study was to carry out a sensory evaluation for the viability of the addition of nasturtium extract to a beverage. Solvent extraction was performed at a solute-solvent ratio of 1:10 – 1:15 and ethanol purity of 45%, 70%, or 96%; antioxidant capacity was analyzed by the FRAP method and total phenolic compounds content by the Folin-Ciocalteu method. Additionally, three samples of the beverage that included nasturtium extract (extract content of 1000, 2500, or 5000 mg kg^{-1}) were evaluated through a 5-point hedonic test and a preference test for the attributes of flavor, aroma, consistency, and the product as a whole. The samples with the highest solute-solvent ratio presented the highest antioxidant capacity, while the samples with ethanol purity of 45% and 70% obtained the highest content of total phenolic compounds. The sample with 1000 mg kg^{-1} of the extract was the best qualified in all the sensory attributes evaluated.

Key words: phenolic compounds, fruit juices, hedonic scale, antioxidant capacity.

RESUMEN

La capuchina es diversa en compuestos bioactivos como carotenoides, antocianinas y glucosinolatos. Debido a estos atributos, es importante aplicarlos a nuevos alimentos, como las bebidas. El objetivo del estudio fue realizar una evaluación sensorial de la viabilidad de la adición del extracto de capuchina en una bebida. La extracción con solventes se realizó con una relación soluto-solvente de 1:10 – 1:15 y pureza de etanol de 45%, 70% o 96%; la capacidad antioxidante se analizó por el método FRAP y el contenido de compuestos fenólicos totales por el método de Folin-Ciocalteu. Adicionalmente, se evaluaron tres muestras de bebida que incluía extracto de capuchina (contenido de extracto 1000, 2500 o 5000 mg kg^{-1}) mediante una prueba hedónica de 5 puntos y una prueba de preferencia para los atributos de sabor, aroma, consistencia y el producto en general. Las muestras con mayor relación soluto-solvente presentaron mayor capacidad antioxidante, mientras que las muestras con pureza de etanol de 45% y 70% obtuvieron el mayor contenido de compuestos fenólicos totales. La muestra con 1000 mg kg^{-1} de extracto fue la mejor calificada en todos los atributos evaluados.

Palabras clave: compuestos fenólicos, jugo de frutas, escala hedónica, actividad antioxidante.

Introduction

Nasturtium (*Tropaeolum majus* L.) is a climbing plant of South American origin belonging to the Tropaeolaceae family (Brondani *et al*., 2016), characterized by its round, peltate leaves and orange, yellow, and red flowers (Hegnauer, 1973). Its application is mainly ornamental, although it has also been used in traditional oral medicine for treatment of respiratory and skin infections (Alonso & Desmarchelier, 2015). These applications are attributed to bioactive compounds found in the plant, such as carotenoids, anthocyanins, glucosinolates and phenolic compounds (quercitrin, flavanols, gallic acid, caffeic acid, coumaric acid, chlorogenic acid) (Martínez-Navarrete *et al*., 2008; Jakubczyk *et al*., 2018; Demasi *et al*., 2021). Due to the nasturtium attributes, the extraction of its bioactive compounds has been carried out through different methods, using solvents such as methanol (Navarro-González *et al*., 2015; Demasi *et al*., 2021) or acetone (Amiri, 2012), the Soxhlet method (Fernandes *et al*., 2017) and new technologies such as ultrasound-assisted extraction (Jha Kumar & Sit, 2022); the extraction of phenolic and antioxidant compounds has been carried out using ABTS, DPPH methods (Garzón & Wrolstad, 2009; Arellano *et al*., 2015), FRAP

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[1] Universidad Nacional de Colombia, Bogotá (Colombia).
[2] Pontificia Universidad Javeriana, Bogotá (Colombia).
[*] Corresponding author: caavilao@unal.edu.co

methods and HPLC (Garzón *et al*., 2015; Navarro-González *et al*., 2015). Despite this, there is still no application of these extracts or the plant in food products. Also, there are no antecedents or previous toxicological studies related to the intake of nasturtium. Therefore, the objective of this study was to do a sensory evaluation for the feasibility of adding nasturtium extract to a nectar-type drink, potentiating a novel beverage. It is important to clarify that this study is not trying to evaluate the interaction or antagonism between the components of the nectar drink and the extract.

Materials and methods

Plant material preparation

The nasturtium was harvested in the town of Cogua, in the department of Cundinamarca, Colombia and taken to the Pontificia Universidad Javeriana (Bogotá, Colombia), where it was separated into leaves, flowers, and calyx. Subsequently every part was freeze-dried in a FreeZone 4.5 LABCONO freeze-dryer at a temperature of -80°C and a pressure of 0.120 mbar and later ground in a KitchenAid grinder, to be packaged and stored at -20°C. For the extraction and evaluation methods, only the nasturtium leaves were used.

Extraction

Solvent extraction water-ethanol was performed at room temperature, under constant stirring for 48 h, employing 5 g of the nasturtium leaves. Then, the solvent was filtered and concentrated in an IKA brand RV 10 digital V rotary evaporator, at a bath temperature of 50°C, rotation speed of 55 rpm and absolute vacuum of 100 mbar. Two extraction factors were evaluated: solute-solvent ratio (1:10 and 1:15 w/w), and ethanol purity in the solvent mixture (45%, 70% or 96%), performing six treatments in total and three replicates for every treatment, with the same extraction conditions. For every treatment, a new extraction was developed. A two-way analysis of variance and the interaction was performed for the statistical analysis, in addition to Tukey's multiple comparison test.

Determination of antioxidant capacity and total phenolic compounds

The antioxidant capacity of the extract was determined by the FRAP spectrophotometric method following the method of Demasi *et al*. (2021), to include many compounds such as antioxidants, phenolic compounds, and vitamin C; the results were expressed in units of Trolox Equivalent Antioxidant Capacity - TEAC (µmol Trolox/g extract). The content of total phenolic compounds was analyzed by the Folin-Ciocalteu method (Navarro-González *et al*., 2015), and the results were expressed in mg gallic acid equivalents / g extract. Each of the six treatments was analyzed in triplicate.

Beverage development

A nectar-type drink of tropical fruits and vegetables was made: 25% w/w 'Valencia' orange juice (*Citrus sinensis* [L.] Osbeck), 23% w/w yacon pulp (*Smallanthus sonchifolius* [Pöpp. & Endl.] H. Rob.), 16% w/w 'Granny Smith' apple pulp (*Malus domestica* Borkh.), 10% w/w chayote pulp (*Sechium edule* [Jacq.] Swartz), 12% w/w of an infusion of mint (*Mentha spicata* L.) at 5% w/w and 11% w/w cucumber pulp (*Cucumis sativus* L.). A known concentration of the extract was added to this drink (see the sensory analysis method), together with additives such as xanthan gum (0.2%), maltodextrin (1%) and 1% of a commercial sweetener, Best4u (https://fitmarketbogota.co/products/endulzante-natural-250gr-best4u), which is a mixture of erythritol and stevia.

Sensory analysis

Three beverages were prepared with different extract content: 1000, 2500, or 5000 mg extract/kg beverage (the extract used in this experiment was obtained with ethanol with 70% of purity and relation solute-solvent 1:10; this extract had one of the highest total phenolic compounds compared to the other extracts evaluated). The beverages were analyzed through an affective test (hedonic scale of 5 points: 1: I dislike it very much, 2: I dislike it a little, 3; I neither like nor dislike it, 4: I like it a little, 5: I like it a lot) with 60 untrained consumers, evaluating three attributes (aroma, flavor, consistency) and the product in general. Each consumer was given 20 ml of each sample in glasses coded with 3 random digits, at a temperature of 10°C. In addition, a preference test was conducted, in which the evaluator was asked to place the sample code in front of the phrase that most identified it: "the one that I like the most – the one that follows me in taste – the one that I like the least."

For statistical analysis, an analysis of variance (ANOVA) was performed. When the results did not present normality, a non-parametric Kruskal-Wallis analysis was performed, with a confidence level of 95%.

Results and discussion

Antioxidant capacity and total phenolic compounds

Table 1 presents the six experimental samples and their antioxidant capacity and total phenolic compounds.

The experiments obtained values higher than those reported by others, with 12.95 mg gallic acid equivalent/g for the content of phenolic compounds reported by Navarro-González *et al.* (2015) and 9.51 TEAC and 14.2 TEAC for the antioxidant capacity reported by Navarro-González *et al.* (2015) and Demasi *et al.* (2021), respectively. Both studies carried out extractions with methanol-water, a solvent mostly used for the extraction of bioactive compounds; however, due to its toxicity when ingested (Alcalá Pedrajas, 2002), it is necessary to review other solvents with equal or better yields to be used in edible products. Therefore, extraction with food-grade ethanol is a good replacement for methanol.

Extracts 4 and 5 had higher antioxidant capacity (Tab. 1), both with the highest solute-solvent ratio evaluated (1:15). Similar results were obtained by Yang and Li (2022), in which a ratio of 1:20 (the largest evaluated in the study) was the one with the highest antioxidant capacity in an extraction with 80% methanol solvent from the *Angelica dahurica* (Fisch.) Benth. & Hook plant. Sample 1, with a ratio of 1:10 and 96% ethanol, obtained the lowest value for total phenolic compounds, while samples 2 (70% ethanol - ratio 1:10) and 6 (45% ethanol - ratio 1:15) obtained the highest value for phenolic compounds. Both samples had solvent mixtures (ethanol-water); this indicates that for optimal extraction of phenolic compounds it is necessary to consider solvent mixtures, taking advantage of the affinities of the compounds with different solvents used. In the review by Jha Kumar and Sit (2022), different solvents were used to extract polyphenols from different plants, and the authors mentioned that, to extract compounds flavonols and phenolic acids, it is necessary to use ethanol in concentrations of 10%-90%. In addition, various studies obtained results similar to the present study, obtaining extracts with higher contents of total phenolic compounds with the ethanol concentration of up to 80% (Celant *et al.*, 2016; Medina-Medrano *et al.*, 2018; Martínez-Patiño *et al.*, 2019). Samples with the highest antioxidant capacity were not the ones with the highest phenolic content (samples 4 and 5), which would indicate the extraction of other compounds with antioxidant capacity found in nasturtium leaves, such as carotenoids and glucosinolates (Brondani *et al.*, 2016; Hernández-Rodríguez *et al.*, 2020; Demasi *et al.*, 2021).

Sensory evaluation

The mean aroma for all samples was similar, this attribute being a non-determining factor for the evaluators (Fig. 1). Sample 3 was the one with the lowest rating for all the attributes, its means being significantly different from the others (ANOVA $P \leq 0.05$), showing a clear rejection by consumers. On the other hand, the 1000 mg kg^{-1} sample and the one with 2500 mg kg^{-1} had similar means in the attributes of taste, consistency, and the product in general, but for the attribute of consistency, the difference is greater compared to the others (Fig. 1).

Regarding the preference test, the sample with 1000 mg kg^{-1} was the highest qualified, followed by the sample with 2500 mg kg^{-1} and, finally, the sample with 5000 mg kg^{-1} (Fig. 2). Similar behavior was observed in the hedonic test, confirming that the drink with 1000 mg kg^{-1} of the extract was the most preferred by the evaluators. Also, the consumers had no adverse effects due to the intake of the samples.

TABLE 1. Antioxidant capacity average and content of total phenolic compounds average of the beverages evaluated.

Sample	Relation solute-solvent (w/w)	Purity of ethanol (% v/v)	Antioxidant capacity mean ± PSD [1,3]	Total phenolic compounds mean ± PSD [2,3]
1	1:10	96	19.86±0.35	2.60±0.50
2	1:10	70	24.49±0.35	39.58±0.50
3	1:10	45	17.76±0.35	16.91±0.50
4	1:15	96	36.30±0.35	21.87±0.50
5	1:15	70	32.21±0.35	33.75±0.50
6	1:15	45	30.17±0.35	40.75±0.50

[1]TEAC, [2]mg gallic acid equivalent/g extract, [3]PSD - Pooled Standard Deviation.

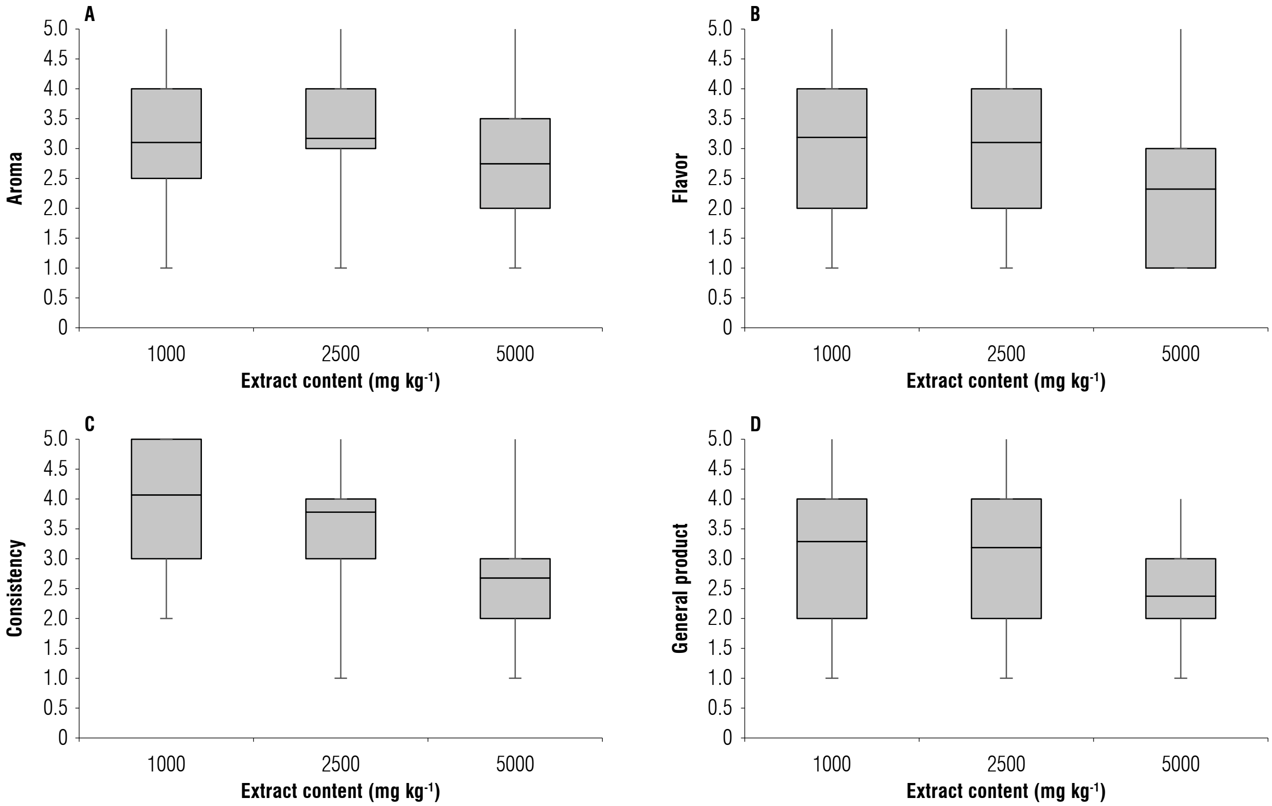

FIGURE 1. Sensory evaluation results for the three different samples of beverage. Different superscripts values indicate significant differences between them (ANOVA $P \leq 0.05$) in the attributes for aroma: 1000 mg kg$^{-1\ a,b}$ – 2500 mg/kg^{a} – 5000 mg/kg^{b} flavor; 1000 mg/kg^{a} – 2500 mg/kg^{a} – 5000 mg/kg^{b}; consistency: 1000 mg/kg^{a} – 2500 mg/kg^{a} – 5000 mg/kg^{b} and general product: 1000 mg/kg^{a} – 2500 mg/kg^{a} – 5000 mg/kg^{b}.

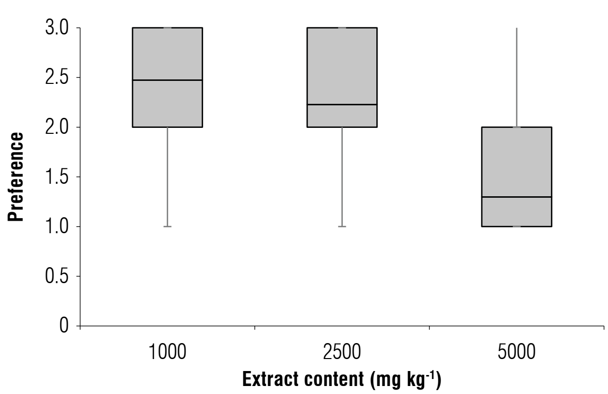

FIGURE 2. Preference test results for the three different samples of beverage. All samples have significant differences between them ($P \leq 0.05$).

Conclusions

Solvent extraction with ethanol-water mixtures (up to 80% v/v ethanol) and higher solute-solvent ratios allow the extraction of compounds that have antioxidant capacity: phenolic compounds, such as flavonols and phenolic acids, and glucosinolates and carotenoids. This study showed a promising application of nasturtium leaf extract in food products, more specifically in nectar-type drinks, with good reception by potential consumers.

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Conflict of interest statement

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

Author’s contributions

All authors conceptualized the study, CAAO developed the methodology, conducted the research, and wrote the manuscript. MSH administered the project, supervised the whole research, and reviewed the manuscript. AGT supervised the research. All authors reviewed the final version of the manuscript.

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SCIENTIFIC NOTE

Efficiency of capture of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) with mosquito killer light traps

Eficiencia de captura de *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) con trampas de luz mosquito killer

Enio Nunez[1*], Leandro Silva Barbosa[2], and Fernanda Avelino-Capistrano[2]

ABSTRACT

Tuta absoluta (Meyrick) is a cosmopolitan pest that affects leaves and fruits, causing damage to crops. Various control techniques are used to eliminate adults and larvae; however, none of them is completely effective. Thus, this study tested the use of a light trap with an ultraviolet lamp and an electrical device to control *T. absoluta* in a tomato greenhouse ('Grazianni') located in Paty do Alferes (Brazil) and recorded the best capture period. For this, three FIX model mosquito traps were placed inside the greenhouse (2.4 m high, 1.2 m apart) during one lunar cycle. The catch was evaluated in the following time periods: from 7:00 pm to 10:30 pm, from 10:30 pm to 02:00 am, and from 02:00 to 05:30 am. A total of 6,886 specimens were collected. The time interval from 7:00-10:30 pm resulted in the highest abundance captured (54.3%), similar to previous data on the flight period of *T. absoluta*. These results contribute to the Integrated Pest Management of the tomato crop, an alternative to the exclusive use of chemical control methods which fail to efficiently control the tomato moth.

Key words: flight period, greenhouse, Integrated Pest Management, tomato pest, ultraviolet lamps.

RESUMEN

Tuta absoluta (Meyrick) es una plaga cosmopolita que afecta hojas y frutos, causando daños a los cultivos. Se utilizan diversas técnicas de control para eliminar adultos y larvas; sin embargo, ninguno de ellos es completamente efectivo. Así, este estudio probó el uso de una trampa de luz con una lámpara ultravioleta y un dispositivo eléctrico para controlar *T. absoluta* en un invernadero de tomate ('Grazianni') ubicado en Paty do Alferes (Brasil), y registró el mejor período de captura. Para ello, se colocaron tres trampas para mosquitos modelo FIX dentro del invernadero (2.4 m de altura, 1.2 m de separación) durante un ciclo lunar. La captura se evaluó en los siguientes horarios: de 7:00 pm a 10:30 pm, de 10:30 pm a 02:00 am y de 02:00 a 05:30 am. Se recogieron un total de 6886 especímenes. El intervalo de tiempo de 7:00-10:30 pm resultó en la mayor abundancia capturada (54.3%), similar a datos previos sobre el período de vuelo de *T. absoluta*. Estos resultados contribuyen al Manejo Integrado de Plagas del cultivo de tomate, una alternativa al uso exclusivo de métodos de control químico que no logran controlar eficientemente a la polilla del tomate.

Palabras clave: período de vuelo, invernadero, Manejo Integrado de Plagas, plaga del tomate, lámparas ultravioletas.

Introduction

In the last twenty years there has been a wide proliferation of *T. absoluta* throughout the world, affecting tomato crops inside greenhouses. Although this pest originated in South America, it has settled around the Mediterranean and in many temperate European countries, including Austria (Gabl & Hausdorf, 2013) and Asian countries, including Nepal (IPPC, 2016), in addition to several African countries much further to the south of the Mediterranean Sea, including Benin (Karlsson *et al*., 2018). Pesticides have been used for more than a century and have played a relevant role in pest and plant disease control; however, they can lead to environmental imbalances and increase production costs. Therefore, Integrated Pest Management (IPM) is a strategy that must be adopted by all farmers at every technological level of production (Culliney, 2014).

One of the most usual methods for pest control is genetic resistance. Plants of the species *Lycopersicum hirsutum* var. *hirsutum* were used in breeding programs (Azevedo *et al*., 2003) to induce tomato leafminer –*Tuta absoluta* (Meyrick)– resistance. Nonetheless, tomato varieties with this kind of resistance are not yet available in the market.

Combined methods in Iran to control *T. absoluta* in tomato were implemented by Nazarpour *et al.* (2016). The authors used plant extracts with insecticidal action such as neem

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[1] Núcleo de Defesa Agropecuária de Vassouras, Secretaria de Estado de Agricultura, Pecuária, Pesca e Abastecimento do Rio de Janeiro, Niterói (Brazil).
[2] Curso de Ciências Biológicas, Escola de Saúde, Centro Universitário São José, Realengo (Brazil).
[*] Corresponding author: enio.nunez@agricultura.rj.gov.br

(*Azadirachta indica*), combined with insecticides, entomopathogenic fungi and generalist predators, and attained good results. For this, fungi and bacteria (among other microorganisms, such as virus, nematodes, etc.) are sprayed on biological targets. The fungus *Metarhizium anisopliae* parasitizes eggs and reduces the reproductive potential of *T. absoluta* females (Pires *et al*., 2009). *Beauveria bassiana* is another fungus that controls *T. absoluta* larvae, mainly the youngest ones (Silva *et al*., 2020). Insecticidal proteins, 100 ml/100 L, (*e.g.* Cry, Cyt and Vip) from bacteria *Bacillus thuringiensis* var. Kurstaki (BTk), were also applied to control lepidopteran caterpillars (Estruch *et al*., 1996).

Pheromones are also useful to monitor the presence or absence of a specific pest; however, they tend to capture only one of the adult genera of the pest and generally attract only males of the species. Cocco *et al*. (2013) verified that the use of pheromones may be a tool to control the mating of *T. absoluta* in greenhouses since pheromones cause disruption of mating signals. In relation to the use of pheromones, Caparros *et al.* (2012) reported that females can lay eggs by deuterotokous parthenogenesis under specific conditions. Lee *et al.* (2014) showed that the occurrence of polyandry explains the failure of control methods based on pheromones in open greenhouses in some tomato-growing areas.

There are some complementary methods to the use of pesticides in greenhouses. Biological methods, such as the use of parasitoid insects (Amaya, 1988) or predators (Frescata & Mexia, 1996) and predatory mites (Hussey & Scopes, 1985), which are registered in the Brazilian Ministry of Agriculture (MAPA), have not been used on a large scale by Brazilian farmers yet.

A greenhouse makes it difficult not only for most pest insects to enter but also for their natural enemies. Another physical method is the use of yellow traps covered with glue to attract winged insects and, consequently, kill them (Taha *et al*., 2012). IPM needs further improvements to avoid the spread of this pest to regions of the planet where it is still absent (Biondi *et al*., 2018).

The use of ultraviolet light traps to attract and capture Lepidoptera adults inside greenhouses may be a noteworthy method to reduce both pest spread, and the amount of pesticides in tomato crops.

In Brazil, a field experiment on tomato plants on stakes (Oliveira *et al*., 2008) confirmed that light traps with a combination of black light and "Blue Light Bulb" (ultraviolet) are far more attractive for adults of *T. absoluta* than white light traps.

Despite some studies addressing the presence, monitoring, and control of *T. absoluta* in greenhouses (Erler *et al*., 2010; Nannini *et al*., 2011), only a few refer to the use of light traps with ultraviolet lamps in these structures (Cocco *et al*., 2012; Mohammadipour *et al*., 2019). The results of Cocco *et al.* (2012) suggested that, at high infestations, the light traps were not efficient, while Mohammadipour *et al.* (2019) suggested quite the opposite.

Biological studies claim that the mating phase of *T. absoluta* occurs during the first hour of the photophase, and the mating pairs take from a few minutes to 6 h 40 min to uncouple (Lee *et al*., 2014). Moreover, they confirm the results of another experiment (Hickel & Vilela, 1991), in which the females mate several times, but only in the early hours of the morning.

This study aimed to test an industrial light trap with ultraviolet lamp and built-in electrical device for the control of adults of *T. absoluta* in greenhouses and, additionally, to determine the best night capture interval.

Materials and methods

The experiment site was Paty do Alferes (Rio de Janeiro, Brazil), with an altitude of 613 m, in Serra do Mar. It has a tropical climate with periodic rain and a dry winter (Aw) (Köppen, 1948). The average temperature is 20.0°C and there is significant rainfall, with an annual average of 1,637 mm (Paiva & Lopes, 2013).

The experiment took place in the locality known as Coqueiros, on a rural property called Bom Jardim in a greenhouse of 28 x 45 x 7 m area (22°22'19" S, 43°19'51" W), with tomatoes 'Grazianni' of average yield 5-7 kg/plant in 2,860 pots. The Grazianni type tomatoes are a hybrid type of indeterminate growth, with an oblong shape and an average fruit weight of approximately 190 g. The plants were sown on 10/10/2020 and planted permanently in pots on 11/23/2020.

Three mosquito killer traps FIX, composed of metal (built-in electrical device), polypropylene, electronic components and a LED lamp (Black Light 4 W F4T5 BL-UV) (Fig. 1), voltage 110 V, and dimensions of 27 x 12 cm, and a small brush which is part of the piece set were used to attract and trap *T. absoluta* moths. The experiment lasted a complete lunar cycle (begins and ends with a new moon), from 03/06/2021 until 04/04/2021.

The three light traps were set up at 2.40 m in height in line with the cultivation lines, 1.2 m apart and 11.5 m from the main entrance, in the anteroom of the greenhouse.

The on and off programming of the lighting traps proceeded as follows: the first trap was lit from 07:00 to 10:30 pm; the second from 10:30 pm to 02:00 am of the following day; and the third from 02:00 to 05:30 am. A single light source of attraction was used throughout the night; the three intervals of 3 h and a half each were designed to determine the best night capture interval, if any. To carry out the programming, a Weekly Digital Timer model YDT-MB, bivolt 127-220 v ~ 60 Hz, maximum load 10 A/2.200 W, minimum setting interval of 1 min, and operating temperature between -10ºC up to 50°C was used.

FIGURE 1. Mosquito light traps (FIX, Black Light 4 W F4T5 BL-UV) with *Tuta absoluta* (Meyrick) adults.

Light traps were revised daily in the morning. To separate the specimens attached to the metallic spiral, an appropriate brush belonging to the trap set was used. They were packed in transparent plastic bags and labeled to be counted later.

Phytosanitary treatments and fertilizers

The tomato seeds ('Grazianni') were planted in trays with substrate composed of *Sphagnum* spp. peat, expanded vermiculite, dolomitic limestone, agricultural plaster, and NPK fertilizer (traces). In the seedling nursery, the seeds received phytosanitary treatments with the fungicides metalaxyl-M + chlorothalonil (300 ml/100 L) and the insecticides: spinosad (10 ml/100 L), thiamethoxam (20 g/100 ml), abamectin (100 ml/100 L) and chlorantraniliprole (15 ml/100 L).

The seedlings treated were transplanted into pots and treated as follows: two sprayings with dicarboximide (240 g/100 L), copper oxychloride (200 g/100 L) fungicides with an interval of 10 d between them, and one drench with fungicide thiophanate-methyl (90 g/100 L). One irrigation was made with the insecticides thiamethoxam and chlorantraniliprole, one spraying with the insecticide cypermethrin + profenofos (15 ml/100 L) and one with the biological insecticide *Bacillus thuringiensis* var. Kurstaki (BTk). A drench with commercial compost and a spraying fertilizer rich in amino acids were also applied. Other treatments used were fertilization with calcium, spraying with the insecticide chlorantraniliprole, and watering with a copper-based commercial fertilizer. After 45 d, the biological insecticide (Btk) was sprayed on a weekly basis.

The data obtained were analyzed by the ANOVA test. Treatment means were compared by Tukey's test at 5% probability. For the analysis, the PAST 4.03 (Hammer *et al*., 2001) software was used.

Results and discussion

During the experiment, 6,886 specimens of *T. absoluta* were captured using a light trap, with different capture times (Tab. 1). The period of 7:00-10:30 pm was when most (54.3%) adults of *T. abso*luta were captured, resulting in a statistically significant difference in relation to other periods.

TABLE 1. Univariate analysis for the collection of *Tuta absoluta* (Meyrick) specimens, using light traps, with different capture periods.

	Capture periods		
	07:00 - 10:30 pm	10:30 pm - 02:00 am	02:00 - 05:30 am
N	30	30	30
Min	24	0	0
Max	384	249	248
Sum	3742	1378	1766
Mean	124.73a	45.93b	58.87b

Averages followed by different letters indicate significant statistical differences by the Tukey's test ($P \leq 0.05$).

The variation in the number of adults of *T. absoluta* collected within each capture period, during the performance of the study (Fig. 2), may be attributed to the period of flight activity of *T. absoluta*. This type of data can be very useful in establishing new population sampling protocols for this pest, thus, improving its control.

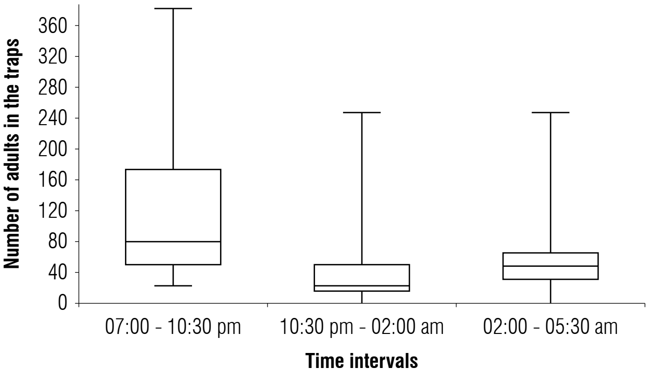

FIGURE 2. Comparison of distributions of *Tuta absoluta* (Meyrick) adults collected at three different capture times. The vertical line in the boxes shows the median, and boxes show 25-75% quartiles, and whiskers show minimal and maximal values.

A better understanding of the period of greatest activity of *T. absoluta* can improve IPM programs in tomato crops, helping both in the control and monitoring of the tomato moth population, allowing the use of resources according to the fluctuation of the pest.

Nowadays consumers are very concerned about excessive use of pesticides, and producers about the optimization of production expenses and satisfying the demands of the internal and external markets. Picanço *et al.* (1995) showed that the control of this pest is arduous, even with the intense use of chemical products. The results of this study are of great relevance for IPM programs in tomato crops, since the chemical control methods, applied exclusively, did not show efficiency for the control of *Tuta absoluta*.

Conclusions

The capture period that presented the greatest potential for capturing adults of *Tuta absoluta*, with mosquito FIX traps, was 7:00 pm to 10:30 pm. This information can be used to help control *Tuta absoluta* in pest integrated management programs and enhance organic crop 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

EN designed the experiments, carried out the field and laboratory experiments. LSB and FAC contributed to the data analysis. All authors wrote the manuscript and reviewed the final version of the manuscript.

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SCIENTIFIC NOTE

CH_4 and N_2O fluxes during paddy rice crop development, post-harvest, and fallow

Flujos de CH_4 y N_2O durante el desarrollo del cultivo de arroz, post-cosecha y barbecho

Magda Aparecida de Lima[1]*, Rosana Faria Vieira[1], Alfredo José Barreto Luiz[1], and José Abrahão Haddad Galvão[1]

ABSTRACT

Paddy fields are major sources of greenhouse gases, mainly methane (CH_4) and nitrous oxide (N_2O). Defining the sampling times for determining the average diurnal emission rates is an important step in optimizing field measurement, avoiding the influence of possible peaks. With this purpose, diurnal gas measurements (CH_4 and N_2O) were taken using the static chamber method during five 24 h-periods (campaigns), every 2 h, at three rice crop development stages (R2, C1 campaign; R5, C2 campaign, and R8, C3 campaign), and in post-harvest (PH, C4 campaign) and in fallow (FP, C5 campaign) periods. The CH_4 fluxes remained close to the average flux both at C1 (9.4 ± 1.0 mg CH_4 m^{-2} h^{-1}) and C2 (10.2 ± 1.4 mg CH_4 m^{-2} h^{-1}), allowing the gas sampling at any time of the day, except at 5:00 p.m. when a peak was observed at C1. As the CH_4 fluxes for C3, C4, and C5 were close to zero, no average value was identified. The average N_2O fluxes were low at C1 (1.0 ± 5.7 μg N_2O m^{-2} h^{-1}) and at C4 (6.7 ± 2.6 μg N_2O m^{-2} h^{-1}), increasing at C2 (26.9 ± 9.3 μg N_2O m^{-2} h^{-1}) and C3 (21.2 ± 7.2 μg N_2O m^{-2} h^{-1}) and reaching higher values during the C5 campaign (73.7 ± 33.3 μg N_2O m^{-2} h^{-1}). In general, considering the average flux values recorded in this study, the most appropriate times for sampling N_2O during the C1, C2, C3, and C4 campaigns would be from 9 p.m. to 1 a.m. and also around 11:00 a.m. Average N_2O flows in fallow would be more likely around 11:00 p.m. and 11 a.m.

Key words: greenhouse gases, diurnal flux variation, continuous water management.

RESUMEN

Los arrozales son fuentes importantes de gases de efecto invernadero, principalmente el metano (CH_4) y el óxido nitroso (N_2O). Definir los tiempos de muestreo para la determinación de las tasas de emisión diurna promedio es un paso importante en la optimización de la medición en campo ya que evita la influencia de posibles picos. Con este fin se realizaron mediciones diurnas de gases (CH_4 y N_2O) utilizando el método de cámara estática durante los periodos de muestreo (M) de 24 h, cada 2 h, en tres etapas de desarrollo del cultivo de arroz (R2, M1; R5, M2; y R8, M3), en los periodos post-cosecha (PH, M4) y barbecho (FP, M5). Los flujos de CH_4 permanecieron cercanos al flujo promedio en M1 (9.4 ± 1.0 mg CH_4 m^{-2} h^{-1}) y en M2 (10.2 ± 1.4 mg CH_4 m^{-2} h^{-1}), permitiendo la toma de muestras de gas en cualquier momento del día, excepto a las 5:00 p.m., cuando se observó un pico en M1. Como los flujos de CH_4 para M3, M4 y M5 fueron cercanos a cero, no se identificó un valor promedio. Los flujos de N_2O fueron bajos en M1 (1.0 ± 5.7 μg N_2O m^{-2} h^{-1}) y en M4 (6.7 ± 2.6 μg N_2O m^{-2} h^{-1}), aumentando en M2 (26.9 ± 9.3 μg N_2O m^{-2} h^{-1}) y M3 (21.2 ± 7.2 μg N_2O m^{-2} h^{-1}) y alcanzando valores más altos en M5 (73.7 ± 33.3 μg N_2O m^{-2} h^{-1}, en promedio). En general, considerando los valores de flujo promedio registrados en este estudio, los momentos más apropiados para el muestreo de N_2O en M1, M2, M3 y M4 serían de 9 p.m. a 1 a.m. y también alrededor de las 11:00 a.m. Los flujos promedio de N_2O en el período de barbecho serían más probables cerca de las 11:00 p.m. y 11:00 a.m.

Palabras clave: gases de efecto invernadero, variación diurna del flujo, manejo continuo del agua.

Introduction

Methane (CH_4) and nitrous oxide (N_2O) are the most important greenhouse gases (GHG), exceeding the 100-year global warming potential of carbon dioxide (CO_2) by factors of 28 and 265, respectively (Myhre *et al*., 2013). Flooded rice soils represent an important source of global CH_4 emissions (Shang *et al*., 2011). Moreover, it is increasingly recognized that rice-based cropping systems can emit substantial amounts of N_2O (Zou *et al*., 2009; Wang *et al*., 2011), although with a smaller contribution in terms of global warming potential (Linquist *et al*., 2012).

In Brazil, more than 80% of the rice production comes from wetland areas, where the basic cultivation system is irrigation by flooding (Marrenjo *et al.,* 2016). The anaerobic soil conditions in this type of management lead to CH_4 generation as the final product of the organic matter decomposition by methanogenic microorganisms (Dalal *et al*., 2008). Nitrous oxide, on the other hand, results from

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[1] Embrapa Meio Ambiente, Jaguariúna, São Paulo (Brasil).
* Corresponding author: magda.lima@embrapa.br

denitrification, the primary cause being the higher soil moisture content (Tan *et al*., 2018). However, the emission of N_2O from flooded rice may be lower than in other types of ecosystems since under prolonged soil flooding conditions the N_2O may be reduced to a great extent to N_2 (Zou *et al*., 2007).

Most of the studies assessing GHG emission rates in flooded rice systems are still based on manual chamber measurements, usually limited by low sampling frequencies, with only one or two measurements per day or week (Sander & Wassmann, 2014). However, previous studies have shown that, in flooded ecosystems, CH_4 fluxes fluctuate regularly at diurnal timescales (Ma *et al*., 2021). This may lead to overestimated or underestimated quantifications of the CH_4 and N_2O fluxes, depending on the time of day and stages of rice growth when the measurements are made. Therefore, knowledge of the diurnal patterns is very important to adequately determine the average gas emission values and to limit uncertainties concerning flux estimates on seasonal scales.

Experiments aiming to evaluate the diurnal variations in CH_4 and N_2O fluxes have been already carried out during different rice crop growth stages, but there were some inconsistencies regarding the time that best represented the average flux. According to Brye *et al.* (2017), the optimum time to determine mean CH_4 emissions is generally around late morning to mid-day. Weller *et al.* (2015) found that the best times to determine the mean diurnal CH_4 emissions were from 7:00-9:00 a.m. or 5:00-7:00 p.m. These authors also showed that the diurnal CH_4 emissions were affected by air, floodwater and soil temperatures. The importance of temperature in CH_4 and N_2O emissions has already been demonstrated by other authors (Das & Adya, 2012; Gaihre *et al*., 2016). Diurnal variations in N_2O emissions have also been found and may also differ according to the rice growth stages (Wang *et al*., 2017).

Data on the diurnal variation of greenhouse gases in rice production systems are scarce in Brazil and mostly related to CH_4 fluxes and to specific varieties (Costa *et al*., 2008; Lima *et al*., 2018). However, due to the variation that these results may have as a function of the climatic, soil type and handling conditions (Gaihre *et al*., 2014; Dai *et al*., 2019), more experiments should be carried out in different rice ecosystems to better interpret possible differences in emission patterns.

The CH_4 and N_2O fluxes in flooded rice fields were measured to identify diurnal patterns and to develop guidelines for timing flux measurements. In the Southeast region of Brazil, the rice crop is generally cultivated in one season, followed by a fallow period with no other commercial cultivation. The importance of measuring gas emissions in this period has been recognized by many authors (Weller *et al*., 2016; Maboni *et al*., 2021). Considering the rice cultivation and associated fallow period as a farming system, the objective of this study was to verify the diurnal CH_4 and N_2O flux variations during three stages of the rice development and during the post-harvest and fallow periods, to identify the average flux times. Also, the influence of the air, soil and water temperatures on CH_4 and N_2O emissions was investigated.

Materials and methods

Experimental site and field design

The study was carried out in 2018 in a rice paddy field in Pindamonhangaba, São Paulo State, Brazil (22º55’ S, 45º30’ W). The climate is of the Cwa type, according to the Köppen classification, with an average highest temperature above 22ºC and an average lowest temperature below 18ºC. The study area has been used for flooded rice cultivation for decades. The soil is classified as Haplic Gleysol (Embrapa, 2013), with 39.4 ± 2.5% of sand, 24.9 ± 2.4% of silt, and 35.6 ± 0.3% of clay, bulk density = 1.3 ± 0.3 g cm^{-3}, total porosity = 38.0 ± 1.6%, total carbon = 12.9 ± 0.8 g kg^{-1}, and total nitrogen = 1.4 ± 0.1 g kg^{-1}.

The experiment was carried out using continuous water management and the IAC-400 rice variety (average cycle = 115 d). The straw had been incorporated into the soil after the previous harvest. On January 12th, 2018, the area was flooded and drained 4 d later and maintained under this condition until sowing was carried out on January 25th, 2018. Eight lines were used, each 10.4 m long, with spacing of 0.2 m x 0.3 m. Emergence occurred on January 31st, 2018. When the plants reached approximately 8 cm in height, a water blade of 6 cm was established. Due to problems of unequal plant growth, more seedlings were transplanted on February 19th, 2018. The field was then flooded to an average water blade of 15 cm. The area was sprayed with the herbicides Basagran (500 ml ha^{-1}), Ally (0.66 ml ha^{-1}) and Ricer (40 ml ha^{-1}) on February 23rd, 2018, and with Nominee (30 ml ha^{-1}), Ricor (20 ml), Basagran (30 ml ha^{-1}), and oil (20 ml ha^{-1}) on March 23rd, 2018.

Fertilization was carried out twice, on March 5th and March 21st, 2018. On the first occasion (13 d after the V4 stage), 250 kg of NPK ha^{-1} were applied using the NPK formulation 10-10-10 and on the second occasion (one day before the panicle differentiation) the same amount of NPK ha^{-1} was applied using the formulation 20-05-20. Urea, simple superphosphate, and potassium chloride were used as sources of N, P, and K, respectively. Flowering occurred on April 17th, 2018 (77 d after emergence) and maturation occurred on May 23rd, 2018. Drainage was carried out on May 4th, 2018, and harvest was carried out on June 4th, 2018.

Measurement of the CH_4 and N_2O fluxes

The static closed chamber method was used for gas collection and flux calculation as described by Lima *et al.* (2018). Four chambers were positioned following a transect in an area of 88.8 m^2 containing the transplanted rice. Each measurement period (total of five) was considered as a 24 h-campaign. Gas sampling was carried out every 2 h at 0, 10, 20, and 30 min intervals, starting at 9:00 a.m. and ending at the same time on the next day, except for the C3, which started at 11:00 a.m. and ended at this same time on the next day, due to operational issues. The gas samples were analyzed using a Thermo Scientific chromatograph model Trace 1310, equipped with an automatic injector TriPlus RSH, and FID and ECD detectors.

Methane and nitrous oxide fluxes were calculated from the linear increase in gas concentration inside the chamber during the gas sampling, using the equation:

$$F = \frac{\Delta C}{\Delta t}\,\frac{PV}{RT}\,\frac{M}{A}$$

where F represents the flow of CH_4 (μmol m^{-2} h^{-1}) and N_2O (μmol m^{-2} h^{-1}), P is the mean atmospheric pressure in the chamber (assumed to be 1 atm), M is the gas molecular mass (CH_4 = 16.04 g mol^{-1}, N_2O = 44.013 g mol^{-1}), A is the chamber basal area (m^2); R is the universal gas constant (8.31441 J mol^{-1} K^{-1}), T is the temperature inside the chamber during degrees Kelvin sampling (K), V is the chamber volume (L), and $\Delta C/\Delta t$ is the change in gas concentration (ΔC) during the sampling time (Δt) (mg L^{-1} min^{-1}). The CH_4 and N_2O emission rate was converted to mg of CH_4 m^{-2} h^{-1} and µg of N_2O m^{-2} h^{-1}.

The 24 h gas sampling campaigns were carried out for the stages R2, R5, R8, and for the post-harvest and fallow (Tab. 1), defined in this study as C1, C2, C3, C4, and C5, respectively.

Environmental variables

The hourly data for the minimum and maximum temperatures (T_{max} and T_{min}) were obtained from an automatic weather station located about 6 km away. While sampling the gases, the air temperature (T_{air}) inside the chamber was measured and applied in the calculation of the gas fluxes. The maximum and minimum temperatures of the floodwater (T_{fw}) and of the soil (T_s) at a depth of 5 cm were also registered.

Statistical analysis

Measurements of the CH_4 and N_2O emissions were carried out using four chambers installed in the rice cultivation area, each of them considered a repetition. The mean standard error for the gas emissions was estimated for each sampling time for the five 24 h-campaigns using the PROC CORR procedure of the SAS program (SAS, 2011). Correlations between the CH_4 and N_2O fluxes and environmental parameters were estimated using the Pearson´s correlation method ($P \leq 0.05$).

Results and discussion

The results related to the temperatures measured during each campaign are shown in Table 2. It did not rain during the measurement days (Fig. 1).

TABLE 1. Dates of the 24 h gas measurement campaign in 2018.

Campaign	Rice growing stage	Date	Floodwater (cm)
C1	R2 – Booting (34 d after sowing)	March 28 - 29	9
C2	R5 – Milking grain (62 d after sowing)	April 25 - 26	12.5
C3	R8 – Mature grain (88 d after sowing)	May 21 - 22	-
C4	Post-harvest (44 d after harvest)	July 18 - 19	-
C5	Fallow (135 d after harvest)	October 17 - 18	-

TABLE 2. Maximum (T_{max}) and minimum (T_{min}) air temperatures, maximum ($T_{fw\ max}$) and minimum ($T_{fw\ min}$) floodwater temperatures and maximum ($T_{s\ max}$) and minimum ($T_{s\ min}$) soil temperatures at 5 cm depth, during each campaign.

Variables	Campaigns				
	C1	C2	C3	C4	C5
T_{max}	30.8	27.5	22.0	27.1	28.0
T_{min}	21.1	16.6	6.2	9.7	20.7
T_{fw_max}	33.7	24.8	-	-	-
T_{fw_min}	22.4	20.0	-	-	-
T_{s_max}	30.1	23.5	19.5	26.5	29.2
T_{s_min}	24.1	20.9	12.5	14.0	23.3

C1= R2 stage; C2= R5 stage; C3= R8 stage; C4= post-harvest (PH); C5= fallow period (FP).

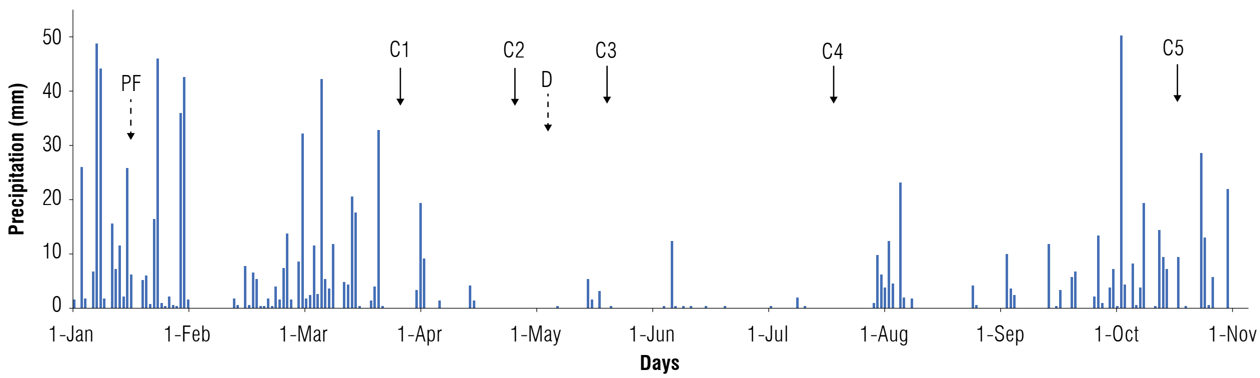

FIGURE 1. Distribution of precipitation throughout the experimental period. The solid arrows represent the campaign dates. Dotted arrows: PF = preliminary flooding, D = final drainage.

The methane fluxes during the C1 campaign were stable and near the average flux value (9.4 ± 1.0 mg CH_4 m^{-2} h^{-1}) (Fig. 2), except at 5:00 p.m. when a peak was observed with a value of 13.4 mg CH_4 m^{-2} h^{-1}. This late peak occurred within the range of time reported by Lima *et al.* (2018), between 2:00 p.m. and 5:00 p.m. These results also corroborate those obtained by Ma *et al.* (2021) where the CH_4 fluxes reached a peak at 2:00 – 4:00 p.m. during the reproductive stage. No significant correlations were observed between the CH_4 fluxes and T_{max}, T_{min}, T_s, and T_{fw}. These results are contrary to those obtained by other authors (Wassmann *et al.*, 2018; Dai *et al.*, 2019) and could indicate that other factors not evaluated in this study could be affecting the CH_4 emissions.

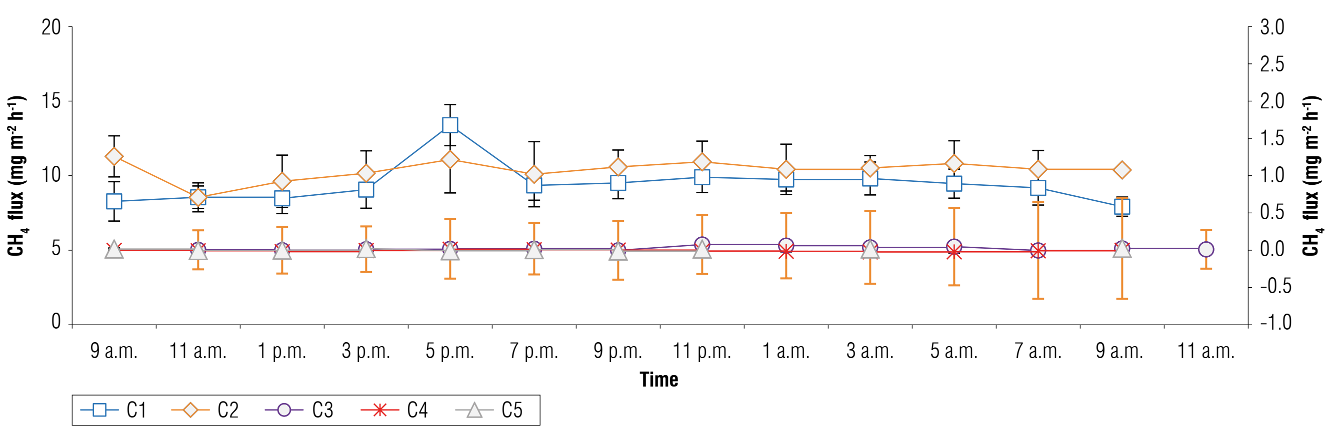

FIGURE 2. Diurnal CH_4 emission rates in the five 24-h campaigns. Bars indicate the standard deviation of the mean. The left Y axis presents the scale of the values for C1 and C2, while the right Y axis presents the scale of the values for C3, C4, and C5. C1=R2 stage; C2=R5 stage; C3=R8 stage; C4=post-harvest (PH); C5=fallow period (FP).

During the C2 campaign the CH_4 fluxes remained near the average value of 10.2 ± 1.4 mg CH_4 m^{-2} h^{-1} (Fig. 2) with no clear diurnal pattern. Similarly, Wassmann *et al.* (2018) showed that during wet seasons, the CH_4 fluxes of the reproductive and ripening stages remained within a stable range and no diurnal patterns were observed. According to Gaihre *et al.* (2014), the lack of a distinct diel variation may be due to a smaller change in soil temperature, which at C2 was within the range of 20.9°C and 23.5°C (Tab. 2). In this campaign, as in C1, no correlations were obtained between the CH_4 fluxes and the T_{max}, T_{min}, T_s, and T_{fw} values.

During the C3 campaign the CH_4 emission rates were very low, although in this campaign the soil was still soaked (Fig. 2). The low temperatures may also have affected the microbiological activity and consequently the CH_4 emissions during the C3 campaign, since temperature is a major factor regulating that activity (Schütz *et al.*, 1990). In addition, on reaching plant maturity, the CH_4 transport capacity decreases due to a probable collapse of the aerenchyma lacunae and resultant blockage of the aerenchyma channels (Aulakh *et al.*, 2000). Furthermore, the carbon supply from plant photosynthates is also reduced at the end of the growing season (Martínez-Eixarch *et al.*, 2018).

In the C4 and C5 campaigns, the CH_4 emissions were also low in all sampling times. Likewise, Maboni *et al.* (2021) reported low CH_4 fluxes, with values around zero, during the fallow period. During the C3, C4, and C5 campaigns, the CH_4 fluxes were not only very low but also stable throughout the 24 h period. In this case, the time of sampling would be irrelevant.

The N_2O fluxes were very low during the C1 campaign (Fig. 3), with an average estimated diurnal emission of 1.0 ± 5.7 μg N_2O m^{-2} h^{-1}. In general, N_2O emissions are negligible in continuously flooded rice. Once an anaerobic condition is installed in the soil, most of the N_2O produced in the soil is reduced to N_2 (Hou *et al.*, 2000; Liang *et al.*, 2013) and the diffusion of N_2O is hindered by the water layer (Hua *et al.*, 1997). Higher fluxes were observed during the C2 campaign, with an average of 26.9 ± 9.3 μg N_2O m^{-2} h^{-1}, and values close to this mean were observed at 5 p.m. and from 9 p.m. to 7 a.m. The N_2O fluxes were positively correlated with T_{max} and T_{min} and with the T_{fw} during the C1 and C2 campaigns, but not with T_s (Tab. 3). A positive correlation between the N_2O fluxes and the water temperature was also obtained by Denmead *et al.* (1979).

During the C3 campaign the N_2O fluxes varied from a maximum of 33.5 ± 6.9 μg N_2O m^{-2} h^{-1} registered at 9:00 a.m. to a minimum of 9.4 ± 2.1 μg N_2O m^{-2} h^{-1} at 11:00 a.m. The mean flux, estimated as 21.2 ± 7.2 μg N_2O m^{-2} h^{-1}, was observed at 7:00 a.m., 1:00 p.m. and 9:00 p.m. A similar result to this diurnal pattern was reported by Wang *et al.* (2017), who found maximum emission between 9:00 a.m. and 12:00 a.m. during the maturation stage. In this campaign (C3), the mean maximum and minimum temperatures were the lowest registered amongst all the campaigns (14.2°C and 12.1°C, respectively), as well as the soil temperature (mean of 16.3°C), which could have affected the activity of the denitrifying microorganisms, although no correlations were found between the N_2O emissions and temperature (Tab. 3). The soaked condition of the soil could have compensated the low temperature effect, creating favorable conditions for the occurrence of the fluxes registered. Future studies should test this hypothesis.

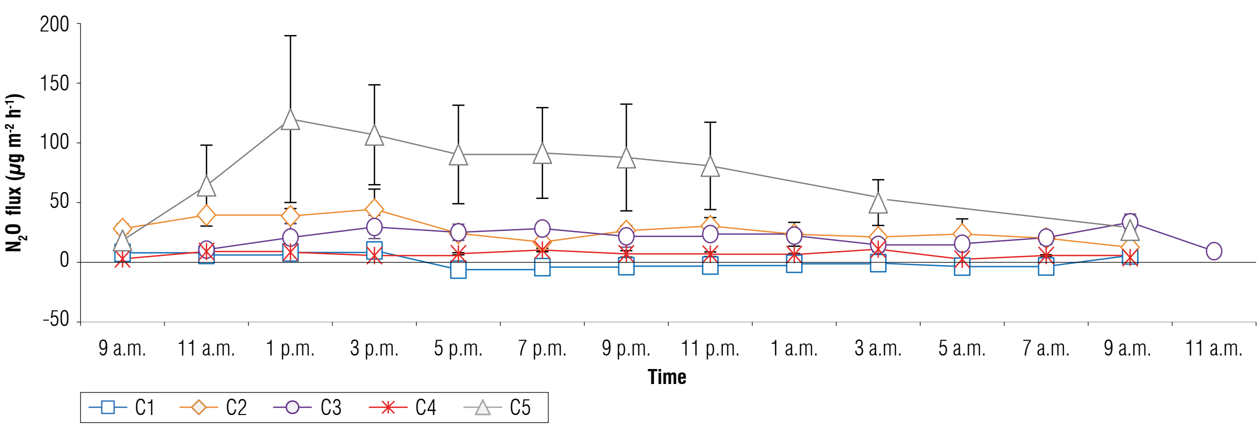

FIGURE 3. Diurnal N_2O emission rates at three stages of rice development, at post-harvest and at the fallow period. Bars indicate the standard deviation of the mean. C1=R2 stage; C2=R5 stage; C3=R8 stage; C4=post-harvest (PH); C5=fallow period (FP).

TABLE 3. Pearson´s correlation coefficients (r) between the N_2O fluxes and the maximum and minimum air temperatures (T_{max}, T_{min}), floodwater temperature (T_{fw}), and soil temperature (T_s).

Campaigns	T_{max}	T_{min}	T_{fw}	T_s
	r values			
C1	0.67***	0.59***	0.47***	0.22ns
C2	0.45***	0.44***	0.35**	0.10ns
C3	0.18ns	0.14ns	-	-0.33ns
C4	0.40***	0.33***	-	0.33**
C5	0.50***	0.51***	-	0.32**

C1=R2 stage; C2=R5 stage; C3=R8 stage; C4=post-harvest; C5=fallow period.
*** P-value ≤ 0.01, ** P-value ≤ 0.05, * P-value ≤ 0.10, ns - not significant.

During the C4 campaign, the N_2O fluxes were very low, possibly due to the low temperatures and dry soil conditions (Fig. 1). Positive, although low, correlations were obtained between the N_2O fluxes and $T_{max,}$ T_{min} and T_s, (Tab. 3). There was also a probable reduction in the available forms of nitrogen in the soil after harvest, affecting microbial activity. The average flux rate was estimated at 6.7 ± 2.61 μg N_2O m^{-2} h^{-1}, with values close to this occurring at 5:00 p.m. (7.2 μg N_2O m^{-2} h^{-1}), at 9:00 p.m. (7.1 μg N_2O m^{-2} h^{-1}), 11:00 p.m. (6.8 μg N_2O m^{-2} h^{-1}), 1:00 a.m. (6.5 μg N_2O m^{-2} h^{-1}) and 3:00 a.m. (6.6 μg N_2O m^{-2} h^{-1}) (Fig. 3).

During the C5 campaign, the average N_2O flux increased to 73.7 ± 33.3 μg N_2O m^{-2} h^{-1} with the values closest to this being obtained at 11:00 a.m. and at 11:00 p.m. The maximum flux was observed at 1:00 p.m. (119.9 μg N_2O m^{-2} h^{-1}) and the minimum at 9:00 a.m. This increase in the N_2O fluxes was probably due to an increment in organic residues, possibly originating from the decomposition of rice straw, from the humid soil conditions after abundant rain in the previous days and from higher air temperatures, as confirmed by the significant positive correlation between the N_2O fluxes and the mean maximum and minimum air temperatures (Tab. 3). The presence of C sources associated with high soil moisture is a condition that favors the activity of denitrifying microorganisms and, consequently, N_2O emissions (Pérez *et al*., 2010). Kajiura *et al.* (2018) showed that N_2O emissions during fallow periods (7-9 months) could be around 5-fold higher than those occurring during growing seasons.

Conclusions

Considering the average flux values recorded in this study, the sampling of CH_4 under the specific conditions of the study area and at the observed stages would be possible at any time of the day, except at 5:00 pm, during the C1 campaign. The most appropriate times for sampling the N_2O fluxes at the R2, R5 and R8 stages and PH would be from 9:00 p.m. to 1:00 a.m. and around 11:00 a.m. For the fallow period the most appropriate time would be at 11:00 p.m. and at 11:00 a.m. Stages R2 (C1) and R5 (C2) emitted more CH_4, while higher N_2O emissions were found in the fallow period (FP) (C5), with median flows at the R5 (C2) and R8 (C3) stages and lower flows at the R2 stage (C1) and at post-harvest (C4).

The results showed the importance of determining the best time for sampling gases, in order to avoid underestimates and overestimates of the mean fluxes. This trial should be repeated including other stages of plant growth to better characterize the diurnal CH_4 and N_2O flux patterns throughout the growing season. It must be emphasized that these results are specific for the area studied and could be influenced by the type of soil, field management, climate, rice cultivar, and environmental conditions.

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Conflict of interest statement

The authors declare there are no conflicts of interests regarding the publication of this article.

Author's contributions

MAL and AJBL designed the experiment, MAL and JAHG carried out the field experiments, AJBL contributed to the data analysis, MAL and RFV wrote the article. All authors reviewed the final version of the manuscript.

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Published dissertation or thesis references

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AGRONOMIA COLOMBIANA

P.O. Box 14490, Bogotá-Colombia

(571) 316 5355 / (571) 316 5000 ext. 10265

E-mail: agrocol_fabog@unal.edu.co

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