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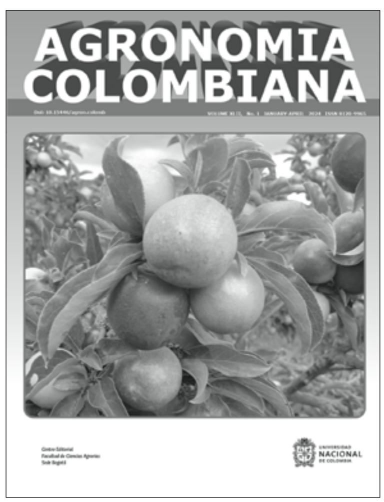

Our cover (photo courtesy of Mayerlin Orjuela Angulo)**:**

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FE DE ERRATAS

Los autores del artículo “Carbon-nitrogen ratio in soils with fertilizer applications and nutrient absorption in banana (*Musa* spp.) cv. Williams” reportan una fe de erratas para las ecuaciones de la Figura 1 del artículo publicado en Agronomía Colombiana, vol. 38 No. 2 (2020), pp. 253–260. Doi: 10.15446/agron.colomb.v38n2.78075

Las ecuaciones correctas de la Figura 1 deben ser las siguientes:

Ecuación para Ciclo 1: Y = 0.01526*X + 35.382

Ecuación para Ciclo 2: Y = 0.0172*X + 36.068

ERRATUM

The authors of the article “Carbon-nitrogen ratio in soils with fertilizer applications and nutrient absorption in banana (*Musa* spp.) cv. Williams” report an erratum in the equations of Figure 1 of the article published in Agronomía Colombiana, vol. 38 No. 2 (2020), pp. 253–260. Doi: 10.15446/agron.colomb.v38n2.78075

The correct equations in Figure 1 should be as follows:

Equation for Cycle 1: Y = 0.01526*X + 35.382

Equation for Cycle 2: Y = 0.0172*X + 36.068

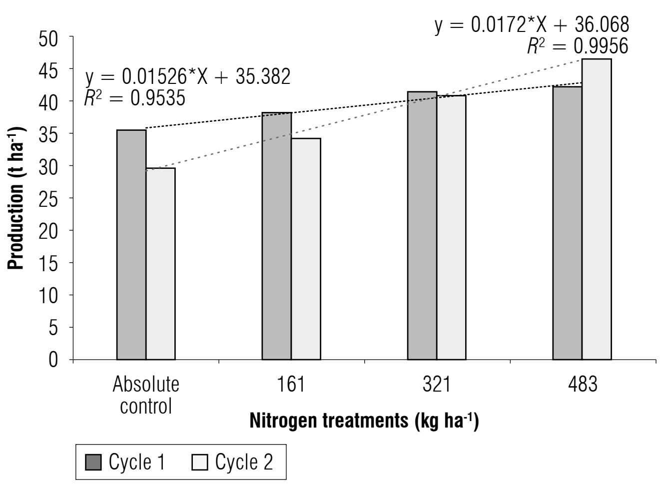

FIGURE 1. Production in t ha^{-1} in the treatments and productive cycle.

STANISLAV MAGNITSKIY
Editor-in-Chief / Editor en Jefe
Revista Agronomía Colombiana

April 30, 2024

Evaluation of drought-tolerant rice (*Oryza sativa* L.) genotypes under drought and irrigated conditions in Bhairahawa, Nepal

Evaluación de genotipos de arroz (*Oryza sativa* L.) tolerantes a la sequía en condiciones de sequía y riego en Bhairahawa, Nepal

Himani Chand[1*], Mukti Ram Poudel[1], Preeti Kayastha[1], Barsha Kc[1], Biddhya Pandey[1], Janak Bhandari[1], Bimal Roka Magar[1], Prakash Baduwal[1], Pawan Lamichhane[1], Pragyan Bhattarai[1], and Netra Prasad Pokharel[1]

ABSTRACT

Rice production can be severely affected by drought stress and this could cause massive economic losses every year. Global climate change is steadily becoming an important issue. This research was conducted in order to identify drought-tolerant rice genotypes using stress tolerance indices. Employing a randomized complete block design, a total of nine rice genotypes were assessed under irrigated and drought-stress conditions from June to November 2022 at the Institute of Agriculture and Animal Science (IAAS), Paklihawa, Nepal. In particular, the stress susceptibility index (SSI), mean productivity (MP), and geometric mean productivity (GMP) revealed strong and highly significant positive correlations to agricultural yields under both irrigated and drought stress conditions. The stress tolerance index (STI) and yield stability index (YSI) showed strong and highly significant positive correlations to yield under drought conditions, while the tolerance index (TOL) and yield index (YI) showed strong and negative significant associations to yield under stress conditions. The highest STI, GMP, and MP were observed in the IR16L1713 genotype followed by IR17L1387, establishing these two as the steadiest and most efficient genotypes among nine genotypes of rice. These genotypes have the potential to be selected for maximum outputs under both irrigated and drought-stress situations. A biplot analysis showed a positive association of MP, GMP, and YI to rice yields in an irrigated environment and a negative correlation of SSI, STI, and TOL, with a reduction percentage in a drought-stressed environment. Therefore, these stress indicators can be used to evaluate rice genotypes under both normal and drought stress settings.

Key words: drought stress, stress tolerance indices, yield, stability.

RESUMEN

La producción de arroz podría verse gravemente afectada por el estrés provocado por la sequía, lo que podría causar enormes pérdidas económicas cada año. El problema del cambio climático global se está convirtiendo cada vez más en una cuestión importante. Esta investigación se llevó a cabo con el fin de identificar los genotipos de arroz tolerantes a la sequía utilizando índices de tolerancia al estrés. Empleando un diseño de bloques completos aleatorizados, se evaluaron un total de nueve genotipos de arroz en condiciones de riego y estrés por sequía de junio a noviembre de 2022 en el Instituto de Agricultura y Ciencia Animal (IAAS), Paklihawa, Nepal. En particular, el índice de susceptibilidad al estrés (ISE), la productividad media (PM) y la productividad media geométrica (PMG) revelaron correlaciones positivas fuertes y altamente significativas con el rendimiento tanto en condiciones de riego como de estrés por sequía. Asimismo, el índice de tolerancia al estrés (ITS) y el índice de estabilidad del rendimiento (IER) mostraron correlaciones positivas fuertes y altamente significativas con el rendimiento en condiciones de sequía, mientras que el índice de tolerancia (TOL) y el índice de rendimiento (IR) mostraron asociaciones significativas fuertes y negativas con el rendimiento en condiciones de estrés. Los mayores ITS, PMG y PM se observaron en IR16L1713, seguido por IR17L1387, estableciéndolos como los genotipos más estables y eficientes entre nueve genotipos de arroz. Estos genotipos tienen el potencial de ser seleccionados para una producción abundante tanto en condiciones de riego como de estrés por sequía. Un análisis biplot mostró una asociación positiva de PM, PMG e IR con el rendimiento en un ambiente irrigado y una correlación negativa de ISE, ITS y TOL con reducciones en el porcentaje en un ambiente de estrés por sequía. Por lo tanto, estos indicadores de estrés se pueden utilizar para evaluar genotipos de arroz tanto en condiciones normales como de estrés por sequía.

Palabras clave: estrés por sequía, índices de tolerancia al estrés, rendimiento, estabilidad.

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Introduction

Rice (*Oryza sativa* L.) is the world's second most important crop after wheat; it belongs to the Poaceae family and feeds over half of the world's total population (Fukagawa & Ziska, 2019). It represents 18.6% of cereal output and 11.14% of worldwide cereal commerce (FAO, 2020). Worldwide it was cultivated on 165.25 million ha in 2021, yielding 502.98 million t with a productivity of 3.04 metric t ha^{-1} (Statista, 2023). Global rice production was expected to reach 515 million t by 2022. This corresponds to a 0.23% rise in world output compared to the previous year (Rice, 2023). The area, productivity, and output of rice in Nepal are 1.48 million ha, 3.46 t ha^{-1}, and 5.13 million t, respectively. Rainfed rice farming accounts for 57% of the acreage utilized for rice agriculture in Nepal (MOALD, 2021). Despite the addition of 4,000 ha for rice cultivation, the total amount of rice produced dropped from 5.55 million t in 2020 to 5.13 million t in 2021, an 8.74% decrease in output (MOALD, 2021). Drought-prone zones are estimated to cover 0.8 million ha of agricultural land (MoAD, 2015/16). The current output is insufficient to fulfill the demands of the rising population and assure food security in Nepal (Shrestha *et al.*, 2021).

Rice represents more than 21% and 76% of human caloric and the calorific requirements of Southeast Asian people (Mohidem *et al.*, 2022). According to the OECD-FAO Agricultural Outlook 2023-2032, global rice intake is estimated to increase by 0.9% per year over the decade, compared to 1.1% annually in the past decade. The demand for edible rice in Nepal is estimated to be 4.270 to 4.818 million metric t by 2030 and between 4.784 and 6.238 million metric t by 2050 (Timsina *et al.*, 2023). Nepal should increase rice production by between 42-85% by 2050 to meet future consumption requirements (Timsina *et al.*, 2023). This highlights numerous improvement strategies for increasing both yield and productivity as a 21st-century urgency (Poudel & Poudel, 2020). This could be achieved via vigorous selection in the field to produce high-yielding, climate-adapted, and abiotic stress-tolerant rice cultivars (Riaz *et al.*, 2021).

The ideal precipitation and temperature for cultivation of rice is 1300-1500 mm at between 25°C to 35°C (Hussain *et al.*, 2019; Rice Knowledge Bank, 2023). Temperature beyond 35°C during growth and reproductive stages is harmful (Xie *et al.*, 2023). Under extreme drought stress, yield losses in rice vary from 65% to 85% as compared to regular irrigation conditions (Kumar *et al.*, 2008). Combined heat and drought reduce rice output by 0.7% (0.4% to 1.0%) on average, while cold-wet circumstances have no effect. Moisture stress may lower biomass by limiting photosynthesis, mostly via decreased stomatal conductance (Heino *et al.*, 2023).

The number of tillers was decreased by water stress during the vegetative stage, while the quantity and weight of grains is lowered by stress during the reproductive and grain-filling stages (Bhattarai *et al.*, 2024). According to Bouman and Tuong (2001), the quantity of tillers and panicles per hill decreases when conditions are too dry, either before or during plowing. Since late-maturity cultivars drastically hinder panicle growth, they are not appropriate when late-season dry conditions were the primary cause of poor production. When drought stress is experienced during blooming, the total quantity of grains per panicle is significantly decreased. Drought during blooming causes crop growth to decrease. During blooming, 46% of the grain is empty because of drought stress, compared to 22% under control circumstances of adequate irrigation. At the grain filling stage, the 1000-grain weight is 17% lower in the drought stress group compared to the control group. Therefore, during the blooming stage, drought stress reduces yield mostly due to a drop in the 1000-grain weight and an increase in empty grain relative to filled grain, as well as causing a decrease in the total number of grains per panicle (Sarvestani *et al.*, 2008). Drought has a greater impact on pre-dawn mitochondrial respiration, the highest speed of RuBisCO carboxylation, and the maximum rate of electron transport (Perdomo *et al.*, 2016). Drought-induced malfunctioning of important physiological systems includes reduced photosynthetic activity, lower water use efficiency, reduced transpiration rate, inadequate stomatal conductance, lower CO_2 fixation, unbalanced water relations, and membrane degradation (Dash *et al.*, 2018; Panda *et al.*, 2021; Zhu *et al.*, 2020).

Drought tolerance indices can be computed using a variety of metrics, including the stress tolerance index (STI), the tolerance index (TOL), the stress susceptibility index (SSI), and the drought tolerant efficiency (DTE), which may be used to detect superior varieties of cultivars in harsh environmental settings (Kandel *et al.*, 2022). TOL or the tolerance index is the difference between yield in a stress condition (Ys) and yield in a non-stress condition (Yns) as well as mean productivity as the average of Ys and Yp (Lamba *et al.*, 2023). The stress tolerance index (STI) is used to discover high producing genotypes under stress conditions (Anwaar *et al.*, 2020). The concept of SSI is introduced by Mokhtari *et al.* (2022). The combination of high relative production and yield stability may be a significant preference benchmark for characterizing genotypic overall performance under varying degrees of drought stress (Bhattarai *et al*., 2024; Paudel *et al*., 2021). The purpose of our research was to discover rice cultivars with robust yield potential and stability under drought-stressed circumstances by studying drought tolerance indicators throughout key developmental stages under the rainfed scenario of western Terai conditions of Nepal.

Materials and methods

Site selection and experimental materials

This research was carried out on the Agronomy farm of the Institute of Agriculture and Animal Science (IAAS, Nepal), Paklihawa campus from June to November 2022. The study area is situated at 79 m a.s.l. with the coordinates 27˚30'N and 83˚27'E. The National Rice Research Program (NRRP), Hardinath provided the nine rice genotypes indicated in Table 1. These genotypes were chosen as stable genotypes among several available genotypes and with superior yield relative to the others. Common availability of these genotypes also means that these can be studied relatively easy.

TABLE 1. Rice genotypes utilized in the study.

Treatments	Genotypes	Source
T1	IR17L1408	Coordinated varietal trial (CVT)
T2	IR16L1713	Coordinated varietal trial (CVT)
T3	IR16L1704	Coordinated varietal trial (CVT)
T4	IR16L1831	Coordinated farmers field trial (CFFT)
T5	IR17L1323	Coordinated farmers field trial (CFFT)
T6	IR17L1387	Coordinated farmers field trial (CFFT)
T7	Sukhdhan 3	Coordinated farmers field trial (CFFT)
T8	IR16L1801	Coordinated farmers field trial (CFFT)
T9	Vandana	Breeders seed

Experimental details

The experiment was carried out with three replicates in a randomized complete block design. There were nine plots in each replicate measuring 5 m × 2.5 m in size.

Agro-meteorological data

Figure 1 shows the agro-meteorological records during the experiment from June 7, 2022 to November 2, 2022. The average temperature during the trial period was almost constant, but at the end of the trial the minimum temperature dropped, resulting in a drop of the average temperature. The precipitation during the trial period increased and reached a maximum at the middle of the trial and decreased at harvest.

Crop management

The field was ploughed and a nursery bed was prepared. On June 7, 2022, rice seeds were soaked overnight and were planted on the next day. After 4-5 weeks the seedlings were transplanted to the main rice field at a spacing of 20 cm × 20 cm. The number of seedlings per hill was controlled at 2 seedlings. The farmyard manure (FYM) was applied at the rate of 5 t ha^{-1} and a recommended dose of NPK, *i.e.* 100:40:30 kg ha^{-1} (Nepalese government recommended dose) was used on individual plots (5 m × 2.5 m.) Half a dosage of nitrogen was administered as a baseline dose, with the remainder applied in two separate doses, the first at 20-25 d after transplant (DAT) and the second at 40-60 DAT. Weeding was performed twice, first at 20 DAT and again at 40 DAT following the initial weeding. Harvesting took place on November 2, 2022.

Preparation of drought conditions

The soil moisture level was maintained by preparing a structure of bamboo poles covered with plastic to protect

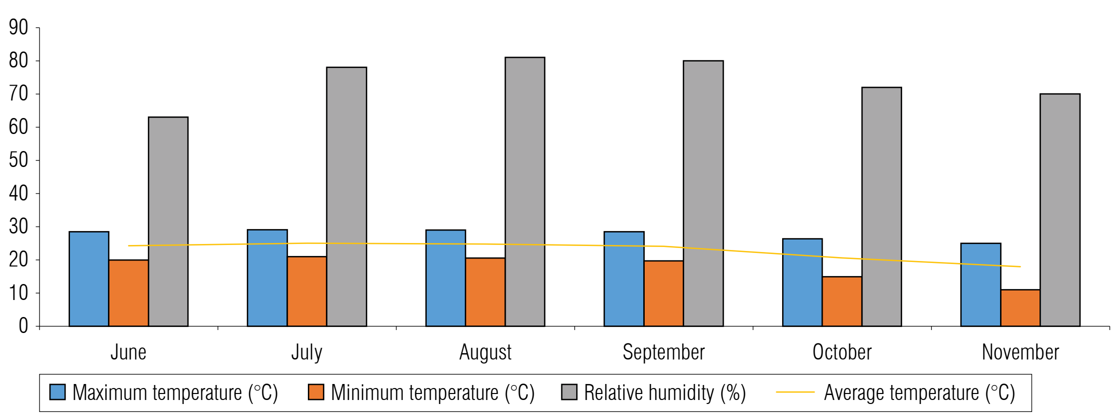

FIGURE 1. Monthly meteorological conditions at Bhairahawa, Nepal from June to November 2022. Source: Weather-2-Visit (https://www.weather-2visit.com/asia/nepal/bhairahawa.htm).

rain in the rice fields. Two to three irrigations were applied to the field after transplanting. Each artificial irrigation was applied during the vegetative stage while drought conditions were maintained during the reproductive stage. Irrigation was used when the water level in the vegetative stage fell below 5 cm. This was regularly monitored during the entire experiment. Irrigation used underground water from bores. The total amount of water applied to each field was about 0.42 acre-foot equal to 518 m^3.

The normally irrigated condition in the field was prepared by applying artificial irrigation during different growth stages (vegetative and reproductive stages). The moisture status in the field was regularly observed and irrigation was applied when the water level fell below 5 cm during the vegetative stage. The level of water in the rice field was maintained at 5 cm throughout the growth phase (vegetative stage).

Traits under study

Ten plants were randomly chosen for data collection. We noted the days to 50% flowering (DTF), plant height (PH), panicle length (PL), panicle weight (PW), and the number of grains per panicle (NGPP), and we reported these data as yield-attributing characteristics. We used an average area of 1 m^2 per plot to represent the entire plot. Additional data collected were the effective panicle per m^2 (EP/m^2), grain yield (GY), and 1000 grain weight (TGW).

Statistical analysis

We performed an analysis of variance (ANOVA) for mean comparison and least significant difference (LSD) at 5% level of probability using the R software (R 4.3.1). We identified resilient and susceptible genotypes using principal component analysis (PCA), biplot diagrams, and correlation between stress tolerance indices with yield utilizing IBM SPSS Statistics (version 25).

Results and discussion

Yield performance

Under drought stress conditions PL, TGW, and GY of different rice genotypes (Tab. 2) showed significant variation, as seen in Table 3, while DTF, TGW, and GY showed significant differences (*P*<0.05) among different rice genotypes in irrigated conditions (Tab. 4). IR17L1387 (4.37 t ha^{-1}) had a superior yield in irrigated conditions, followed by IR16L1713 (4.30 t ha^{-1}) and IR16L1831 (4.01 t ha^{-1}), and Vandana (2.79 t ha^{-1}) showed the lowest yield. Additionally, IR16L1801 (3.35 t ha^{-1}) had the highest yield under drought stress conditions followed by IR16L1713 (3.20 t ha^{-1}) and Sukhdhan 3 (3.14 t ha^{-1}). The high yield of rice genotypes is associated with panicle length (PL), panicle weight (PW), number of grains per panicle (NGPP), panicle yield per m^2 (EP/m^2), and thousand-grain weight (TGW). Plant height (PH) is a critical factor that determines or enhances yield-contributing characteristics and ultimately shapes the grain yield (Reddy & Reddy, 1997). Diverse genetically controlled variables are a product of intricate characters, the majority are governed by genotype genetic composition and are normally defined by the number of nodes and the internode lengths (Rahman *et al*., 2018).

The average yields under drought stress and irrigated conditions were 2.65 t ha^{-1} and 3.60 t ha^{-1}. The average grain yield dropped by 26.5%. The mean reduction in effective shoots per m^2 was 52.21%. The decline in effective shoot formation under low soil moisture conditions might be attributed to the restricted availability of assimilates under water stress conditions. It may also occur due to insufficient

TABLE 2. Eight indices of stress tolerance used in evaluating the rice genotypes.

Drought tolerance indices	Formula equation	References
Tolerance index (TOL)	$TOL = Y_{ns} - Y_s$	(Anwaar *et al*., 2020; Hossain *et al*., 1990; Rosielle & Hamblin, 1981)
Mean productivity index	$MP = (Y_{ns} + Y_s)/2$	(Adhikari *et al*., 2019; Hossain *et al*., 1990; Rosielle & Hamblin, 1981)
Geometrical mean productivity	$GMP = \sqrt{} (Y_{ns} \times Y_s)$	(Fernández, 1992)
Stress tolerance index	$STI = Y_{ns} \times Y_s/(Y_{ns}^2)$	(Anwaar *et al*., 2020; Hao *et al*., 2011)
Yield stability index	$YSI = Y_s/Y_{ns}$	(Bouslama & Schapaugh Jr., 1984)
Stress susceptibility index	$SSI = 1- (Y_s/Y_{ns})/SI$ where $SI = 1-(Y_s/Y_{ns})$	(Fischer & Maurer, 1978)
Yield index	$YI = Y_s/ Y_{s-}$	(Khan & Kabir, 2015)
Reduction percentage	$Red = (Y_{ns}-Y_s)/ Y_{ns})*100$	(Bennani *et al*., 2017)

water uptake for the adequate mineral nutrition and the inhibition of cell division of meristematic tissue (Bhattarai *et al.*, 2024). The lack of availability of soil water at grain filling and other development stages is detrimental for the panicle initiation stage and affects days to flowering, panicle length, panicle weight, effective tillers, number of tillers per hill, thousand-grain weight, grains per panicle, etc. (Paudel *et al.,* 2021). This might be due to a considerable decrease in photosynthetic rate that results in less assimilate generation for panicle development and filling of rice grains. As a result, rice yield is substantially reduced (Moonmoon & Islam, 2017). Drought stress at various phases of development may reduce assimilate transfer to grains, lowering grain weight and increasing the quantity of empty grains. Drought stress at crucial developmental phases reduces rice yield (Cattivelli *et al.*, 2008; Pantuwan *et al.*, 2002).

Stress tolerance index (STI)

Table 5 shows the stress index of nine distinct rice genotypes. The average grain yield for all cultivars differed significantly under stressed and non-stressed conditions. The average yield performance under non-stressful conditions was superior to that under stressful conditions. Under

TABLE 3. Yield attributing characteristics of different rice genotypes under a drought stress environment.

Genotypes	DTF (d)	PH (cm)	PL (cm)	PW (g)	NGPP	EP/m^2	TGW (g)	GY (t ha^{-1})
IR17L1408	73.33±6.11	73.21±10.90	20.53^{b}c±0.73	22.86±2.72	87.33±7.74	160.33±20.50	26.33^{bcd}±2.33	2.02^{ab}±0.42
IR16L1713	74.67±5.03	82.47±5.11	20.80^{c}±0.59	19.37±1.86	76.93±7.60	275.00±56.10	25.03^{a}±2.54	3.20^{ab}±0.79
IR16L1704	71.00±9.16	76.42±7.13	19.27^{c}±0.44	20.00±2.60	104.58±14.30	214.67±50.70	23.57^{de}±0.81	2.15^{b}±0.53
IR16L1831	73.67±3.79	81.73±3.28	19.10^{bc}±0.79	19.43±1.33	68.50±3.74	177.00±57.70	30.83^{cde}±0.58	2.22^{a}±0.38
IR17L1323	75.33±4.93	90.64±2.95	22.76^{a}±1.21	24.03±2.76	98.63±13.30	223.33±46.10	26.03^{bcde}±0.03	3.00^{a}±0.16
IR17L1387	78.67±3.06	83.76±3.91	21.46^{bc}±0.58	20.47±1.03	74.73±5.46	186.33±8.00	29.47^{cde}±0.76	2.97^{b}±0.36
Sukhdhan 3	71.67±2.31	84.76±3.83	21.71^{ab}±0.18	20.57±1.24	94.83±3.53	218.33±12.40	21.93^{e}±1.21	3.14^{a}±0.33
IR16L1801	75.67±4.04	84.27±5.63	21.12^{ab}±0.74	20.57±1.29	82.70±12.10	240.67±19.80	28.00^{abc}±1.16	3.35^{a}±0.12
Vandana	69.67±3.22	85.20±3.51	20.01^{ab}±0.84	21.07±1.80	92.77±12.30	112.00±40.40	24.90^{ab}±0.96	1.59^{a}±0.68
LSD (P<0.05)	8.33	16.46	1.84*	6.07	30.35	108.17	4.27**	1.26*
CV %	6.52	11.53	5.12	16.75	20.21	31.11	9.41	27.56
Mean	73.74	82.50	20.75	20.93	86.78	200.85	26.23	2.65

Note: DTF: days to flowering in 50% plants; PH: plant height; PL: panicle length; PW: panicle weight; NGPP: number of grains per panicle; EP/m^2: effective panicle per m^2; TGW: thousand-grain weight; GY: grain yield; LSD: least significant difference; CV: coefficient of variation; * and ** indicate significant differences at 5% and 1% level. Means followed by the same letters within the same columns are not significantly different based on LSD at P<0.05.

TABLE 4. Yield-attributing characteristics of different rice genotypes under an irrigated environment.

Genotypes	DTF (d)	PH (cm)	PL (cm)	PW (g)	NGPP	EP/m^2	TGW (g)	GY (t ha^{-1})
IR17L1408	72.33^{ab}±3.53	81.39±10.90	19.93^{bc}±0.73	22.00±2.72	87.07±7.74	256.00±20.50	28.33^{ab}±2.33	3.15^{cd}±0.45
IR16L1713	72.67^{ab}±2.91	76.73±5.11	19.24^{d}±0.59	16.67±1.86	62.70±7.60	239.00±56.10	25.83^{bcd}±2.54	4.30^{ab}±0.30
IR16L1704	71.00^{ab}±5.29	82.56±7.13	19.94^{abc}±0.44	22.00±2.60	86.33±14.30	276.00±50.70	22.67^{ef}±0.81	3.90^{abc}±0.41
IR16L1831	71.67^{bc}±2.19	82.05±3.28	18.91^{de}±0.79	23.33±1.33	75.63±3.74	234.00±57.70	32.63^{e}±0.58	4.01^{a}±0.17
IR17L1323	79.00^{a}±2.85	84.45±2.95	21.75^{a}±1.21	25.33±2.76	88.90±13.30	209.33±46.10	22.67^{e}±0.03	3.13^{cd}±0.75
IR17L1387	73.67^{ab}±1.76	86.81±3.91	21.24^{a}±0.58	22.67±1.03	85.33±5.46	258.67±8.00	27.33^{ab}±0.76	2.79^{d} ±0.18
Sukhdhan 3	70.00^{bcde}±1.33	68.02±3.83	20.58^{ab}±0.18	24.27±1.24	107.23±3.53	170.33±12.40	24.17^{cde}±1.21	3.18^{bcd}±0.33
IR16L1801	78.3^{a}±2.33	85.29±5.63	21.70^{ab}±0.74	24.00±1.29	87.40±12.10	207.00±19.80	26.00^{abc}±1.16	3.60^{abcd}±0.61
Vandana	69.33^{c}±1.86	95.08±3.51	19.86^{abc}±0.84	22.00±1.80	86.40±12.30	184.00±40.40	24.00^{abcd}±0.96	4.37^{a}±0.34
LSD (P<0.05)	7.32*	16.46	1.84*	6.07	30.35	108.17	4.27**	0.85**
CV%	6.52	11.53	5.12	16.75	20.21	31.11	9.41	13.69
Mean	73.11	82.49	20.35	22.47	85.22	226.04	25.96	3.60

Note: DTF: days to flowering in 50% plants; PH: plant height; PL: panicle length; PW: panicle weight; NGPP: number of grains per panicle; EP/m^2: effective panicle per m^2; TGW: thousand grain weight; GY: grain yield; LSD: least significant difference; CV: coefficient of variation; * and ** indicate significant differences at 5% and 1% level. Means followed by the same letters within the same columns are not significantly different based on LSD at P<0.05.

stress and non-stress environments, the average grain yield varied from 1.59-3.35 t ha^{-1} to 2.79-4.37 t ha^{-1}, respectively.

The highest TOL was observed in IR16L1831 followed by IR16L1704 and IR17L1387 while the least TOL value was recorded for Sukhdhan 3 followed by IR17L1323. A lower TOL (stress tolerance) rating indicates that a certain cultivar is highly stress tolerant. Table 6 indicates that TOL has a negative connection to yield under stress situations. As a result, these genotypes produced abundant grain yield under non-stress settings but not such high yield in stress situations. As a result, they may be regarded as stress-vulnerable genotypes. Sukhdhan 3 and IR17L1323 performed poorly under irrigated and drought stress environments. Modest TOL resulted from a modest difference among yields in the two situations. IR16L1704 had the highest SSI while Sukhdhan 3 had the lowest SSI. So, IR16L1704 was the most susceptible genotype and Sukhdhan 3 was the least susceptible genotype to drought stress. An SSI value greater than one indicates intensified susceptibility, whereas SSI less than one shows below-average susceptibility. Low TOL and SSI do not mean that these were highly producing genotypes. Crop yield should also be considered when selecting stress resistant genotypes (Thapa *et al*., 2022). The highest STI, MP, and GMP were seen in IR16L1713, followed by IR17L1387, indicating that these genotypes were the most stable and productive of the nine rice genotypes tested. Kamrani *et al*. (2017) also determine genotypes with maximum MP, GMP, and STI as top yielding genotypes.

Correlation among yield and drought stress indices

We computed the association between Y_{ns}, Y_s, and the other stress tolerant indices shown in Table 6 to discover the most acceptable stress tolerance selection criterion. Our study found a link between crop output under irrigated conditions and drought stress. This suggested that genotypes with large grain yields under normal irrigated settings were more likely to have higher grain yields than under drought stress conditions. A similar finding is shown by Kandel *et al*. (2022), who shows a link between grain productivity in irrigated fields and heat stress associations. Similarly, under drought stress conditions, SSI showed a positive and significant connection with yield, indicating that an increase in SSI would result in a significant increase in production. Adhikari *et al*. (2019) find a significant association between SSI and yield under stress. YSI also showed a significant and positive connection with yield under stress, but a positive and non-significant correlation with yield under irrigation (Tab. 6). As a result, choosing genotypes with higher SSI and YSI values under drought stress conditions will aid in the identification of superior varieties. Adhikari *et al*. (2019) and Kandel *et al*. (2022) also report that the genotypes with higher SSI and YSI would have better results under drought stress conditions.

STI had a negative and non-significant correlation with yield under irrigated circumstances, but it had a positive and significant correlation with yield under stress conditions (Tab. 6). YI, on the other hand, showed a positive and

TABLE 5. Yield (kg ha^{-1}) of rice genotypes with stress tolerance indices under irrigated and drought stress environments.

Genotypes	Y_{ns}	Drought tolerance indices								
		Y_s	TOL	STI	MP	GMP	YSI	SSI	YI	Red%
IR17L1408	$3153.33^{cd}±452$	$2202.33^{ab}±421$	951.00±337	0.56±0.17	$2677.83^{bc}±403$	$2623.72^{bc}±406$	$0.70^{ab}±$	$1.12^{ab}±0.32$	$0.83^{ab}±0.16$	29.77±8.58
IR16L1713	$4296.67^{ab}±301$	$3203.67^{ab}±790$	1093.00±864	1.07±0.25	$3750.17^{a}±413$	$3704.07^{a}±509$	$0.74^{ab}±0.09$	$0.97^{ab}±0.74$	$1.21^{a}±0.30$	25.83±19.70
IR16L1704	$3900.00^{abc}±413$	$2154.67^{ab}±527$	1745.33±496	0.66±.019	$3027.33^{ab}±403$	$2867.69^{abc}±437$	$0.55^{b}±0.19$	$1.70^{a}±0.45$	$0.81^{ab}±0.20$	45.11±11.80
IR16L1831	$4007.67^{a}±171$	$2226.00^{a}±379$	1781.67±231	0.68±0.17	$3116.83^{ab}±270$	$2893.46^{abc}±287$	$0.56^{b}±0.12$	$1.65^{a}±0.23$	$0.84^{ab}±0.14$	43.84±5.98
IR17L1323	$3136.67^{cd}±74.50$	$2995.00^{a}±16$	141.67±69.30	0.72±0.02	$3065.83^{ab}±41.2$	$3064.65^{ab}±40.4$	$0.96^{a}±0.06$	$1.17^{b}±0.08$	$1.13^{a}±0.01$	4.42±2.06
IR17L1387	2790.33^{d} ±177	$2969.00^{b}±358$	1404.33±513	0.99±0.06	$3671.17^{a}±111$	$3533.18^{a}±184$	$0.69^{ab}±0.02$	$1.15^{ab}±0.60$	$1.12^{a}±0.14$	30.55±15.80
Sukhdhan 3	$3176.67^{bcd}±328$	$3144.00^{a}±329$	32.67±8.19	0.79±0.17	$3160.33^{ab}±328$	$3160.28^{ab}±328$	$0.99^{a}±0.16$	$0.04^{b}±0.01$	$1.19^{a}±0.12$	1.05±0.30
IR16L1801	$3600.00^{abcd}±61.10$	$3346.67^{a}±116$	253.33±135	0.93±0.04	$3473.33^{a}±63.6$	$3469.65^{ab}±65.1$	$0.93^{a}±0.04$	$0.26^{b}±0.14$	$1.26^{a}±0.04$	6.97±3.64
Vandana	$4373.33^{a}±337$	$1589.07^{a}±682$	1201.27±824	0.33±0.24	$2189.70^{c}±346$	$2063.65^{c}±447$	$0.59^{b}±0.18$	$1.56^{a}±0.68$	$0.60^{b}±0.26$	41.32±18.00
LSD ($P<0.05$)	853.73**	1263.10*	1477.61	0.44	785.05*	902.15*	0.34*	1.29*	0.48*	34.30
CV%	13.69	27.56	89.30	34.02	14.51	17.13	26.57	77.93	27.56	77.93
Grand mean	3603.85	2647.82	956.03	0.75	3125.84	3042.26	0.75	0.96	1.00	25.43

Note: Y_{ns}: yield under irrigated condition; Y_s: yield under drought stress environment; TOL: tolerance index; SSI: stress susceptibility index; MP: mean productivity; GMP: geometric mean productivity; STI: stress tolerance index; YSI: yield stability index; YI: yield index; Red%: reduction percentage; LSD: least significant difference; CV: coefficient of variation; * and ** indicate significance at 5% and 1% levels. Means followed by the same letters within the same columns are not significantly different based on LSD at $P<$ 0.05.

non-significant association with yield while irrigated but showed a negative and significant correlation with yield when stressed. As a result, all these characteristics should be considered while choosing high producing genotypes under both environments.

The biplot analysis was done to find out the most stable and least stable genotypes under drought stress and irrigated condition in the field. Figure 2 illustrates the ranking of genotypes according to mean performance and stability. The average environment coordinate (AEC) axis, which is defined by the average PC1 and PC2 scores of all the environments, is the line that goes through the biplot origin and the average environment. The yield stability of genotypes was assessed using the AEC approach, which uses the average principal components in all conditions. IR16L1831 exhibited the highest yield and excellent stability under both drought stress and irrigated conditions, as depicted in Figure 2. The Vandana variety demonstrated the highest average yield but with low stability. Additionally, IR16L1704 and IR17L1408 exhibited moderate yield but low stability. The genotype with moderate yield and highest stability was Sukhadhan 3. IR17L1387 and Vandana are the least stable genotypes according to their yield as revealed by biplot analysis.

TABLE 6. Correlation of rice yield with stress tolerance indices under irrigated and drought stress conditions.

	Y_{ns}	Y_s	TOL	SSI	MP	GMP	STI	YSI	YI	Red
Y_{ns}	1									
Y_s	0.295	1								
TOL	0.446*	-0.724**	1							
SSI	0.626**	0.918**	-0.408*	1						
MP	0.745**	0.857**	-0.265	0.978**	1					
GMP	0.621**	0.930**	-0.423*	0.993**	0.984**	1				
STI	-0.253	0.836**	-0.966**	0.555**	0.448*	0.584**	1			
YSI	0.295	1.000**	-0.724**	0.918**	0.857**	0.930**	0.836**	1		
YI	0.253	-0.836**	0.966**	-0.555**	-0.448*	-0.584**	-1.000**	-0.836**	1	
Red	0.253	-0.836**	0.966**	-0.555**	-0.448*	-0.584**	-1.000**	-0.836**	1.000**	1

Note: Y_{ns}: yield under irrigated condition; Y_s: yield under drought stress environment; TOL: tolerance index; SSI: stress susceptibility index; MP: mean productivity; GMP: geometric mean productivity; STI: stress tolerance index; YSI: yield stability index; YI: yield index; Red: reduction percentage. * and ** indicate significance at 5% and 1% level, respectively.

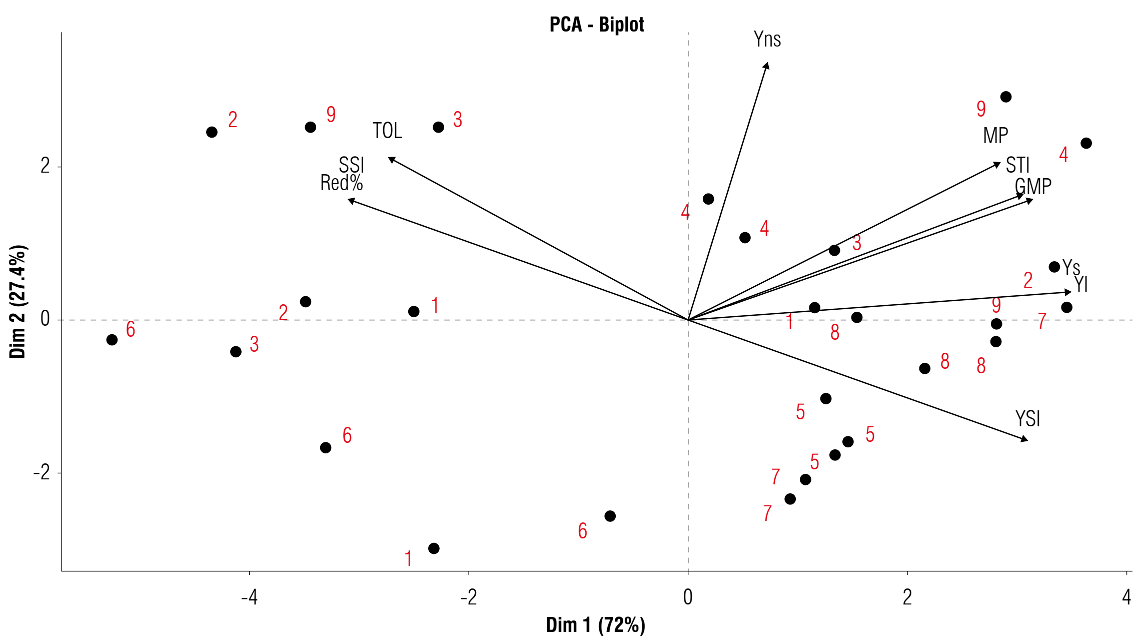

FIGURE 2. Biplot based on PC1 and PC2 using the values from principal component analysis.

Conclusion

This study showed that drought stress during the critical growth period of rice has a significant impact on the yield of different rice cultivars. It also suggests that choosing cultivars on the basis of moisture tolerance indices might be a viable way to discover improved drought resistant cultivars with enhanced production potential and stability. Based on data examined from numerous drought tolerance index factors, we determined that the rice cultivars IR16L1713 and IR17L1387 had a high degree of drought resistance since they had low SSI and TOL values with high STI values compared to other genotypes. The stable yield of Sukhadhan in both irrigated and drought stress environments indicated that it was the most stable rice genotype compared to other studied cultivars. These cultivars produced the highest yield in irrigated areas and performed well under drought stress conditions. As a result, in rainfed and drought-prone locations, these drought-tolerant rice cultivars could be superior to traditional, basic, and untested cultivars.

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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

Methodology, investigation, software, validation, data curation: H. C., M. R. P., P. K., B. K., B. P., J. B., B .R. M., P. B., P. L., P. B., and N. P. P., writing, original draft preparation: N.P.P., conceptualization, review, editing, and supervision: M. R. P. The final version of the manuscript was read and approved by all authors.

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Physiological adaptations of the Japanese plum tree for agricultural productivity: A promising crop for high altitude tropics

Adaptaciones fisiológicas del ciruelo japonés para productividad agrícola: un cultivo promisorio para el trópico alto

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ABSTRACT

The Japanese plum tree is of great importance in the productive development of Colombian fruit growers due to its nutritional contribution to human health, its great adaptability in the tropical highlands, and its good yields. This review presents the current investigative state of physiology of this plant and its management in tropical highlands, including aspects such as its ecophysiology, forced production, gas exchange, flowering, pollination, and fruit development. In Colombia, Japanese plum production systems are adapted between 1670 and 2900 m a.s.l., average solar brightness of 1400 h per year, 12 h photoperiods, temperatures between 14 and 20°C during the day and 6 and 8°C during the night, and rainfall between 700 and 1600 mm per year. Under these conditions, management can be implemented to produce cyclical crops of the Japanese plum. This management consists of the selection of varieties with low chilling requirement, chemical defoliation, proper fertilization, fruit and green pruning, and the application of chemical substances that promote the breaking of flower buds. Flowering and pollination require a high specificity so that they do not present incompatibility. The growth and development of the fruit requires 1538 degree days until harvest. This review indicates the great adaptability, management, and production of Japanese plum in the Colombian high tropics.

Key words: *Prunus salicina* Lindl., ecophysiology, stone fruit, dormancy, flowering, harvest.

RESUMEN

El ciruelo japonés es de gran importancia en el desarrollo productivo de los fruticultores colombianos, debido a su aporte nutricional a la salud humana, su gran adaptabilidad a la altitud tropical y sus buenos rendimientos. Esta revisión presenta el estado investigativo actual de la fisiología de esta planta y su manejo en tierras altas tropicales, incluyendo aspectos como su ecofisiología, producción forzada, intercambio gaseoso, floración, polinización y desarrollo de frutos. En Colombia, los sistemas de producción de ciruela japonesa están adaptados entre 1670 y 2900 m s.n.m., brillo solar promedio de 1400 h anuales, fotoperiodos de 12 h, temperaturas entre 14 y 20°C durante el día y 6 y 8°C durante la noche, y precipitaciones entre 700 y 1600 mm anuales. Debido a estas condiciones, se puede implementar un manejo para producir cultivos cíclicos de ciruela japonesa. Este manejo consiste en la selección de variedades con bajo requerimiento de frío, defoliación química, adecuada fertilización, poda de fructificación y poda en verde, y la aplicación de sustancias químicas que favorezcan la brotación de las yemas florales. La floración y polinización requieren una alta especificidad para que no presenten incompatibilidad. El crecimiento y desarrollo del fruto requiere 1538 grados día hasta la cosecha. Esta revisión indica la gran adaptabilidad, manejo y producción de la ciruela japonesa en el trópico alto colombiano.

Palabras clave: *Prunus salicina* Lindl., ecofisiología, fruta de hueso, dormancia, floración, cosecha.

Introduction

The Japanese plum is a deciduous tree belonging to the family Rosaceae, genus *Prunus*. Ancestral plum plants have been domesticated independently on three continents, mainly in temperate zones, with three large centers of domestication: (1) Europe for *P. domestica*, (2) China for *P. salicina*, and (3) North America for the species of section *P. americana* (Ramming & Cociu, 1991; Topp *et al.*, 2012). *P. salicina* originated in China and was introduced to Japan approximately 200 to 400 years ago, where the domestication and diversification of the crop began and most of the varieties that are grown worldwide today were obtained (Okie & Ramming, 1999; Ruiz *et al.*, 2018). Modern Japanese plum cultivars are from *P. salicina*, but they also include other species resulting from genetic improvement and later use of their cultivars as parents, namely *P. simonii* Carr., *P. cerasifera* Ehrh., and *P. angustifolia* Marsh. (Fanning *et al.*, 2014).

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In 2021, the production of plums and sloe in the world reached 12,014,482 t; China was the main producer with 55.15% participation (6,626,317.1 t), Colombia contributed with 0.15% participation (18,460 t) (FAO, 2023). In Colombia, the production of deciduous fruit trees in the high-altitude tropics began in the 1980s with the introduction of varieties from Germany and Brazil, via alliances between private producers and government entities (Patiño & Miranda, 2013). In the case of the plum tree, a drupaceous plant, planting and production has focused on the Japanese plum tree of the species *P. salicina* (Fischer, 2013). This is because the cultivation of some genotypes of plum trees of Japanese heritage, unlike European plums (*Prunus domestica* L.), is favored in tropical and subtropical areas due to their warmer summers and less accumulation of chilling hours (Looney & Jackson, 2010). The production and consumption of this plum tree have been increasing in recent years, promoting employment and productive development in rural Colombian communities. Between 2012 and 2021, the quantity of plums harvested in Colombia increased by 66.7%, reaching a production of 18,459 t in 1387 ha (13.30 t ha^{-1}) for 2021 (FAO, 2023). Boyacá is the department with the largest production, contributing 78% of the total (Agronet, 2021), since it has comparative advantages in the planting of deciduous crops, including its climate, soils, regimes of rainfall, favorable accumulation of cold hours in some varieties and the fruit vocation of the producers (Puentes Montañéz, 2006). This was due to two key factors: (1) the organoleptic and nutritional quality it possesses compared to other imported varieties (Puentes *et al*., 2008) and (2) the agronomic management that allows the producer to obtain two harvests in one year according to the ecophysiology (Castro & Puentes, 2012).

The physiological behavior of deciduous trees in the high tropics is affected by climatic conditions since, due to their origin and adaptation in temperate zones (Erez, 2000; Fischer, 2013), the ecophysiological requirements of the species and the agronomic management of the crop must be fulfilled in a differential way (Gutiérrez-Villamil *et al*., 2022). In addition, climate change is a current risk for the production of deciduous fruit trees, including plum, as changes in temperature can affect their physiology, phenology and production (Egea *et al*., 2022). Considering the great importance and the productive and nutritious potential of the plum tree for growing and adaptation in the tropical highlands, the objective of this review was to present the current state of research in physiology adaptation and management, including aspects such as variety selection, ecophysiology, forced production management, gas exchange, flowering, pollination and fruit development of the Japanese plum. This information serves as a basis for maximizing the productive potential of this deciduous fruit tree under tropical altitudinal conditions.

Varieties in Colombia

Temperate deciduous fruit crops, such as Japanese plum, are limited in tropical zones mainly by the lack of sufficient low temperatures to meet chilling hour (CH) requirements and overcome bud dormancy (Erez, 2000). Therefore, varieties with low chilling requirements are favored. Additionally, global warming has become a new challenge for the physiology of deciduous species (Luedeling, 2012), since an increase of 3.2°C in the global average temperature is expected by the end of the 21st century, compared to the year 1960 (IPCC, 2021). This could decrease the accumulation of CH in deciduous species, affect bud break and reduce pollinator activity (Egea *et al*., 2022), as has been observed in cultivated apple trees in the subtropical highlands of India (Nautiyal *et al*., 2020). Added to this, the highland areas of the tropical Andes will be more affected by climate change than the low-lying valley areas (Fischer, Parra-Coronado *et al*., 2022b). Therefore, implementing varieties with a low CH requirement could meet the physiological requirements of deciduous fruit trees and reduce the negative effects of the increase in temperature (Gutiérrez-Villamil *et al*., 2022).

In Colombia, the varieties of Japanese plum that are used for the production of fresh fruit are characterized by having a low CH requirement, which is why they adapt and are planted in high-altitude areas (Tab. 1). This allows good dormancy behavior and high yields. The 'Horvin' variety is a plum tree with skin and red pulp that is widely accepted in markets due to its excellent organoleptic and nutritional characteristics (Álvarez-Herrera *et al*., 2021). In addition, it is the most cultivated variety in the main Colombian producing regions (Miranda & Carranza, 2013), since it has an early harvest and two harvests a year can be obtained due to its low chilling requirement (Castro & Puentes, 2012; Fischer, 2013).

TABLE 1. Physiological characteristics of Japanese plum (*P. salicina*) varieties cultivated in the Colombian high tropics.

Variety	Adaptation altitude (m a.s.l.)	Rootstock	CH accumulation <7.2°C	Heat accumulation (GDD)	Pollinating varieties	Reference
'Horvin'	2400 – 2800	'DBC' y 'Ecuatoriano'	300 – 400	1528 between fruit set and fruit harvest	Self-compatible	Fischer (2000); Campos (2013)
'Amarillo Japonés' or 'Ogden'	2400 – 2700	'DBC'	650 – 100	-	'Santa Rosa', 'Horvin', and 'Methley'	Campos (2013)
'Santa Rosa'	2200 – 2600	'DBC' y 'Ecuatoriano'	600 – 700	6591–9099	Self-compatible, 'Beauty', 'Horvin', and 'Mirabolano'	Ormistas (1993); Agustí (2010); Ruiz *et al.* (2018)
'Beauty'	2500 – 2900	'Ecuatoriano'	750 – 800	6727–7183	'Santa Rosa'	Campos (2013); Agustí (2010); Ruiz *et al.* (2018)
'Ecuatoriano'	2200 – 2700	'DBC'	400 – 450	-	'Santa Rosa', 'Horvin' and 'Beauty'	Ormistas (1993)
'Methley'	2200 – 2800	'DBC', 'Mirabolano' and 'Ecuatoriano'	235 – 700	-	'Santa Rosa', 'Satsuma' and 'Golden Japan'	Ormistas (1993); Campos (2013); González-Pérez *et al.* (2018)

DBC: 'Durazno blanco común'; CH: Chilling hours; GDD: Growing degree days.

Ecophysiology

Altitude, light, and solar radiation

The producing areas of the tropics are characterized by constant photoperiod conditions throughout the year and do not present marked temperature seasons (Fischer, 2013). However, altitude is one of the key factors in the production of fruit trees in the tropics, since it determines most climatic parameters, such as temperature, radiation, pressure, atmospheric CO_2, and their effect on the different phenological stages of fruit trees (Fischer, Parra-Coronado *et al.*, 2022). In the tropics, especially in the Cundinamarca and Boyaca departments of Colombia, Japanese plums adapt to high altitudes, between 1,800 and 2,800 m a.s.l. (Tab. 1), which are characterized by low temperatures, increased radiation (UV, visible and infrared light), a lower concentration of CO_2 and O_2, and more intense winds (Fischer, Parra-Coronado *et al.*, 2022). However, commercial peach and Japanese plum crops have been established in the mountains of Norte de Santander (Colombia), specifically in the Catatumbo River basin at an altitude of 1670 m a.s.l. (Quevedo-García *et al.*, 2017). The varieties of Japanese plum trees grown there have not been reported. Fruit plants at higher tropical altitudes, as in the case of cape gooseberry, have been shown to increase the number of stomata to compensate for the lesser concentration of O_2 and CO_2 at these high elevations (Fischer *et al.*, 2023). Also, at these elevations, humidity is typically low and diurnal temperature variation is large, even at the equator (George & Erez, 2000).

Solar radiation is the source of energy for photosynthesis and therefore for the growth and development of all fruit trees and is determined by intensity, quality and duration. In general, deciduous fruit trees grow in tropical areas with an average of 1400 h of sunlight per year (Fischer, 2013). In addition, the tropics have a constant photoperiod throughout the year (about 12 h), so deciduous fruit trees have photosynthetically active leaves that last for more than 11 months a year, unlike in subtropical and temperate areas where leaves are only active for 8 to 9 months and 6 to 7 months, respectively (George & Erez, 2000). Likewise, the high UV radiation to which deciduous fruit trees are subjected can influence the physiology of the plant. Positively, visible and UV light regulate anthocyanin synthesis in Japanese plums in the photosynthesis-respiration interaction by controlling malate metabolism through malate dehydrogenase and the ethylene ATP signaling pathway (Zhang *et al.*, 2021). Adversely, the high UV radiation in the tropical highlands causes sunburn on the fruits, which greatly affects the marketability of the fruit and, therefore, the productivity of the crop (Fischer, Orduz-Rodríguez *et al.*, 2022). In plum and peach trees, open vase formation is commonly used, which allows a higher radiation level and encourages sunbursts (Fischer, 1992a). In this regard, Makeredza *et al.* (2018) reported that excess radiation causes an increase in fruits affected by sunburn in Japanese plums, mainly those located in the upper part of the canopy.

Temperature and chilling requirement

Deciduous fruit trees need to accumulate a specific amount of cold during winter to overcome dormancy and then experience warm temperatures to finally flower in spring (Erez, 2000). This factor conditions the adaptation of species and cultivars in the regions and is the main drawback for its extension to lower altitudes. In the tropics, there are fewer seasonal temperature fluctuations than in temperate regions, with a relatively constant temperature throughout the year (Fischer, 2000). Temperatures, where highly productive crops of Japanese plum are found (high-altitude tropical climate), range between 14 and 20°C during the day, and 6 and 8°C at night (Fischer, 2013). In the tropical mountains of Morocco, the Japanese plum tree 'Angelino' grown under heat stress (+2.5°C in average daily temperature) had a decrease in leaf area, number of leaves per fruit, stomatal area index, conductance stomata, chlorophyll concentration, and the yield and fresh weight of the fruits (Hamdani *et al*., 2023). Therefore, the Japanese plum tree is a species with considerable risk due to the temperature that will be caused by climate change in tropical areas.

Bud dormancy is a physiological period within the development cycle of deciduous fruit trees that, through genetic control, allows the tree to accumulate reserves, mainly carbohydrates, to eliminate sensitive organs (leaves), develop organs to protect the meristems and resist excessively chilling conditions during the winter season (Falavigna *et al*., 2019). Low temperatures are the most important factor that induces dormancy and requires the plant accumulate the necessary amount of chilling, which is known as endodormancy. In the tropics, most deciduous fruit trees, such as Japanese plum, exhibit symptoms of chilling failure, commonly leading to poor and patchy bud break, reduced and delayed foliage development, poor flowering, abnormal flower development, deficient fruit set and/or early cessation of growth (Dennis Jr., 2000; Ramírez & Kallarackal, 2014), so special management must be implemented to compensate for chilling hours (CH). Each species and variety of deciduous fruit tree has some specificity to the requirements of CH to break dormancy. In the Japanese plum grown in tropical areas, the accumulation of CH depends on the variety (Tab. 1), with the variety 'Horvin' requiring the least CH, which means it adapts well to the high tropics. In temperate zones, 1 CH is described, in many cases, as 1 h <7.2°C. However, for the tropics, the best way to quantify CH is with the Utah model of Richardson *et al*. (1974) because it also includes the effect of hours that exceed 7.2°C; thus, in the Utah model, a temperature between 2.5 and 9.1°C for 1 h is 1 chilling unit, and a temperature between 9.2 and 12.4°C for 1 h is 0.5 chilling units. These are temperatures that often occur in the tropical highlands and greatly influence the reproductive physiology of deciduous trees. Likewise, this model includes negative weights for temperatures above 15.9°C. In addition, it has been shown that the dormancy of *P. salicina* buds is controlled by the Dormancy Associated MADS-box (*PsDAM6*) genes, and their expression is lower in varieties with low CH requirements than in those with high CH requirements (Fang *et al*., 2022).

Although it is true that plants require a certain amount of chilling during endodormancy, exposure to and accumulation of warm temperatures are needed to grow after the release of dormancy (accumulation of heat), so it is necessary to quantify the thermal time through the growing degree days (GDD) (Fadón *et al*., 2020). In the tropics, depending on management, deciduous fruit trees can suppress or evade endodormancy (Fischer *et al*., 2011), so GDD accumulation has not been studied much in varieties planted during dormancy (Tab. 1). However, Orjuela-Angulo, Parra-Coronado *et al*. (2022) reported that the 'Horvin' variety planted in Colombia has a base temperature of 2.9°C and requires 1,528 GDD between fruit set and fruit harvest. Further studies are required to report on the warm temperature requirements of tropical varieties when endodormancy is not suppressed. In addition, as reported by Erez (1986), in continuous production systems in the tropics, in order to suppress deciduous trees that are not the intended crop, soil temperatures should not drop below 10°C, as this prevents the cultivated crop roots from reducing their activity and facilitating the entrance of roots from other trees.

Water and soil

The absorption of water is an essential process for plants, derived from their need to lose water to the atmosphere through the stomata and thus regulate their temperature. Deciduous fruit trees in Colombia receive rainfall between 1,000 and 2,000 mm per year (Fischer, 2013). To manage the production of two harvests per year in some plum varieties, it is recommended to carry it out in areas with rainfall between 1,400 and 1,600 mm per year (Gutiérrez-Villamil *et al*., 2022). However, it is important to have a good water resource and drip irrigation systems (Castro & Puentes, 2012). For the 'Methley' variety grafted on 'Mirabolano', the combination of a flow rate of 0.85 L h^{-1} (daily irrigation) and the implementation of manure cover maintained soil moisture and improved the yield and quality of the fruits (Eduardo del Angel *et al*., 2001). In subtropical semi-arid regions, it is recommended to manage two drippers per tree with flow rates of 4 L h^{-1} and not to implement controlled deficit irrigation during the year, since this reduces gas exchange, tree water status, growth, yield and quality of fruits in Japanese plum varieties (Hajlaoui *et al*., 2022). In addition, there are modeling reports on the redistribution of soil water in *P. salicina* crops through the HYDRUS-2D model, which provides information on the amount of water required according to seasonality, cultivar, and soil water balance (Jovanovic *et al*., 2023). A 50% deficit irrigation regime based on the daily water requirements of the crop (ETc) in different varieties of *P. salicina* (including 'Santa Rosa') caused a decrease in the yield and fresh weight of the fruit, leaf area, stomatal density, chlorophyll concentration, and stomatal conductance (Hamdani *et al*., 2022; Hamdani, Hssaini, Bouda, Charafi *et al*., 2023).

Soil conditions for deciduous fruit trees are mainly loamy soils with a good organic matter content, good drainage, and deep soils with a low water table (1.20 m), due to their susceptibility to root asphyxiation (Castro & Puentes, 2012). In Japanese plum var. 'Horvin' cultivated in Colombia, the mass and length of the fruits highly correlated with soil chemical properties, such as pH, cation exchange capacity, total organic carbon, contents of phosphorus, calcium, magnesium, potassium, zinc, copper, boron, and sulfur, and with soil physical properties, such as bulk density, which indicates that good root development requires good oxygenation (Orjuela-Angulo, Dussán-Sarria *et al*., 2022). Managing the soil with mulches between rows in Japanese plum crops significantly improved the maximum organic carbon content and available nutrient content in soil, nutrient content of leaves, yield (Rakesh *et al*., 2020), and soil moisture content (Jovanović *et al*., 2023). The cover with mulches should not be placed below the tree, since there may be competition for nutrients from fertilization and water, affecting the optimal development of the plum tree.

Nutrition and fertilization

As in most fruit trees, mineral nutrition is one of the fundamental aspects in the production of deciduous fruit trees since it provides the necessary elements for proper growth and development. To effectively manage the fertilization of deciduous fruit trees, the effect of soil pH must be managed as a priority since the availability of nutrients for the plant depends on it, and the decisions regarding their application, whether conventionally, foliar or in fertigation, are taken based on the foliar analyses that are carried out in each season (Klein & Weinbaum, 2000). In seedlings of Japanese plum var. 'Horvin' at the nursery stage of growth, the concentrations of foliar N were highest, followed by K, and finally P and Mg (Orduz-Ríos *et al*., 2020). Likewise, the plum tree is one of the deciduous fruit species with the lowest demand for nitrogen (N), however, Japanese plum trees require a greater amount of N than European plum trees (Agustí, 2010). In tropical areas, management of forced production greatly affects fertilization, which will be discussed in a later section of this review. In general terms, it is recommended to apply N before flowering, and K and P at the end of dormancy (Agustí, 2010). Additionally, when analyzing the relationship between the mineral elements and the severity of cracking of the *P. salicina* fruits, it was found that when the organic matter content, the total porosity, the Ca^{2+} content in the soil and leaves, and the apparent density decreased, the cracking of the Japanese plum fruits was milder; meanwhile, the higher the Mn content in the soil and leaves, the more severe the fruit cracking, possibly due to competition with Ca or Mn toxicity (Ma *et al*., 2023).

Gas exchange and leaf traits

There has been little study of the quantification of the net photosynthetic rate (A_n), stomatal conductance (g_s), transpiration (E), and dark respiration (R_d) in Japanese plum, especially in the tropics. Due to the aforementioned ecophysiological conditions in the tropical highlands, the leaves of deciduous fruit trees are photosynthetically active for longer periods and with a higher incidence of UV radiation, which could alter these variables. Ziska *et al*. (1990) reported that Japanese plum var. 'Santa Rosa' reached A_n, g_s, and R_d values of 20 to 25 μmol CO_2 m^{-2} s^{-1}, 200 to 250 mmol H_2O m^{-2} s^{-1}, and 0.5 μmol CO_2 m^{-2} s^{-1} at a saturation of 350 mg kg^{-1} of internal CO_2, respectively. However, under saline stress, stomatal and metabolic limitations in photosynthesis occurred through a decrease in g_s, RuBisCO activity, chlorophyll content and in an increase in R_d (Ziska *et al*., 1990). Similar results were obtained in a study carried out in China, where Japanese plum leaves had values of 11.8 μmol CO_2 m^{-2} s^{-1}, 0.24 mmol H_2O m^{-2} s^{-1}, and 6.7 μmol H_2O m^{-2} s^{-1} for A_n, g_s, and E, respectively, which decreased with the application of saline stress accompanied by low water use efficiency (WUE) (Wang *et al*., 2016). Likewise, under drought stress, drastic decreases in A_n, g_s, and WUE were observed in three Japanese plum cultivars (Hajlaoui *et al*., 2022). Therefore, *P. salicina* trees are very sensitive to osmotic stress due to their low WUE and limiting photosynthetic mechanisms.

Chloroplasts perform multiple biological functions within plants, such as photosynthesis and the synthesis of organic compounds. To reveal their formation and evolution, Fang *et al*. (2021) sequenced the complete chloroplast genome of *P. salicina*, identifying a circular structure of 157,921 bp, containing a large single-copy (LSC) region of 86,184 bp, a small-copy (SSC) region of 19,031 bp, and 110 unique genes. Compared to other species of the genus *Prunus*, *P. salicina* shows interesting similarities and differences in phylogenetic evolution with *P. armeniaca* and *P. mume* (Xue *et al*., 2019). In the Japanese plum trees 'Horvin' grown in tropical highlands, the relative chlorophyll content in SPAD units had values of 23.3 and 47.7 in young and fully developed leaves, respectively (Orduz-Ríos *et al*., 2020).

In *P. salicina* cv. 'Horvin', the increase in width and length of the leaf are continuous and adjust to a potential model. These length and width parameters are very useful to estimate the leaf area through a simple or bivariate regression model (Vera Rodríguez & Pérez Chasoy, 2021), resulting in a non-destructive determination of the leaf area as a basis for a large number of physiology studies of this species. These same authors also found a positive correlation between the leaf width and length with the fruit diameter (Vera Rodríguez & Pérez Chasoy, 2021), indicating that larger leaves can perform greater photosynthesis to provide carbohydrates to the fruits as the main sinks.

González-Pérez, Quevedo-Nolasco *et al*. (2018) reported correlation between intercepted photosynthetically active radiation (iPAR) and phenological phases of *P. salicina* cv. Methley, indicating that the leaf area increased during the growth and development of fruits. The greatest leaf area was obtained during the second fruit growth stage and physiological maturity. There was a significant positive correlation of the leaf area with iPAR and the vegetative and fruit growth. These authors also reported that efficient radiation use was highest during the vegetative and reproductive stage but decreased when the fruit developed (González-Pérez, Quevedo-Nolasco *et al*., 2018). An efficient way to capture greater radiation in the plum is by training in open vase. This strategy places the main branches almost horizontally and within the center of the free tree, thus allowing the tree to capture greater PAR that it will then use in photosynthesis.

Agronomic management in forced production

As previously mentioned, the agroclimatic conditions of the tropics alter the physiology of dormancy in deciduous trees, and so specific cultural management is carried out to promote fruit production. Within this management, sprouting can be encouraged to the point of obtaining two harvests in a year or cyclical harvests, as has been reported in peach (Fischer, 1993; Fischer *et al*., 2011), apple (Fischer, 1992b; Gutiérrez-Villamil *et al*., 2022), and Japanese plum (Castro & Puentes, 2012). This phenomenon occurs because ecodormancy (imposed by the environment) is used to provide the preconditions for floral initiation and differentiation in predormancy (George & Erez, 2000), and, thus, suppresses deep rest. As a result, a new growth cycle can be forcibly induced by agronomic techniques, before the plants eventually enter endodormancy (Westwood, 1993).

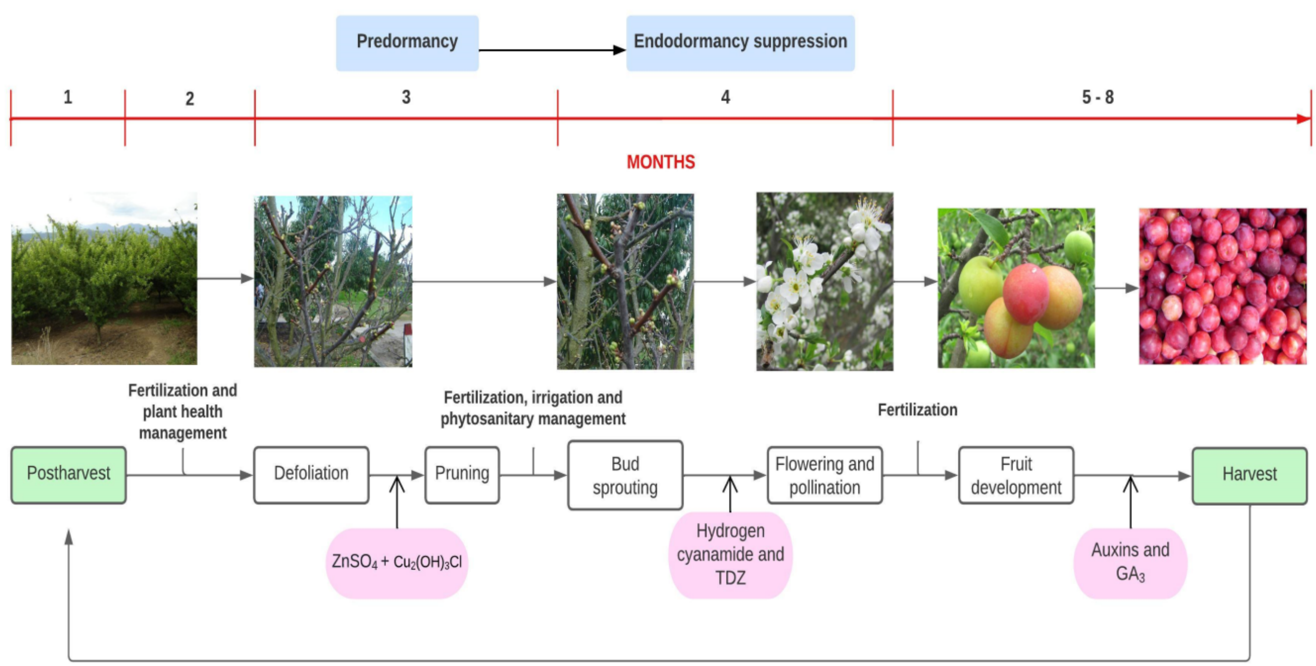

FIGURE 1. Diagram of agronomic management for obtaining two harvests per year in the Japanese plum (*P. salicina*) cultivated in tropical highlands. TDZ – thidiazuron. Adapted from Fischer *et al.* (2010) and Castro & Puentes (2012), with permissions of Revista Colombiana de Ciencias Hortícolas and Produmedios. Photos: G. Fischer.

In summary, the continuous harvest technique is based on suppressing the deep dormancy of deciduous fruit trees but not on breaking dormancy (Gutiérrez-Villamil *et al*., 2022).

In all cases, according to the authors of this review, Japanese plum producers in the tropics should monitor their trees for normal growth and development because, in the cycle of two harvests per year, the time available to rest and accumulate a sufficient amount of reserve carbohydrates before the new flowering is highly reduced. This could cause a setback in the normal development of the tree, which would mean working with only three crops in two years.

As a first step, continuous harvests in Japanese plum should be carried out in varieties that have a low chilling requirement, since they present an early harvest and accumulate more CH in a shorter time. 'Horvin' is the variety with the lowest CH requirement in the tropics (Tab. 1) and the time between harvests is 7 to 8 months (Puentes Montañéz, 2006), which makes it a suitable variety for cycling. Once the variety has been chosen, areas with a bimodal climate (two rainy seasons a year) must be selected to avoid a long chilling season and provide a good amount of water to the plum trees for fruiting (Fischer, 1993). Examples of these zones are the municipalities of Duitama and Paipa (Boyacá, Colombia), which have bimodal rainfall during the year, unlike the municipality of Nuevo Colon (Boyacá, Colombia), which has a monomodal rainfall regime (Fischer, 2013).

After the first harvest, Japanese plum trees should be fertilized with an N-rich formula until bud swelling (Fig. 1) to increase reserve accumulation in the trunk and branches. This improves bud swelling, bud break and fruit set, altering the perception of dormancy in the plants. Subsequently, an application is made again after fruit set and during its development but not as close to harvest, as otherwise the postharvest handling could be affected (Agustí, 2010; Castro & Puentes, 2012).

Defoliation is a process that occurs naturally in deciduous fruit trees in temperate zones. However, in the tropics, this process does not occur in the same way, so chemical or manual treatments are needed for 100% defoliation (Fischer, 2013). Removing the leaves of the trees is the main technique used to suppress endodormancy, since it decreases the concentration of abscisic acid (which comes from the leaves) in the buds and increases the activity of gibberellins and cytokinins (Ramírez & Kallarackal, 2014). In addition, early defoliation has been shown genetically to accelerate auxin translocation from buds, which accelerates dormancy release and promotes bud break (Wei *et al*., 2022). Castro and Puentes (2012) recommend that, for early production of the Japanese plum, it should be defoliated with copper oxychloride (110 g 20 L^{-1}) + zinc sulfate (200 g 20 L^{-1}). In addition, these authors suggest that diseases that affect flowering, such as flower blight (*Monilia* sp.), should be controlled.

Water stress followed by a period of rain or irrigation is a technique that promotes bud breaking in apple and peach trees (Fischer, 1993). However, no effect on the suppression of dormancy has been reported in Japanese plum trees planted in the tropics of stress. Faust (2000) states that the water deficit, on occasion, is not necessary to force flowering in deciduous trees, since a good supply of N, defoliation and the application of chilling compensators can achieve sprouting. On the contrary, Samperio *et al*. (2015) found that by applying controlled deficit irrigation after harvest in the Japanese plum 'Red Beauty', the water potential of the stem decreased, which enabled not only saving of water but also control of vegetative growth (considered as total pruning), maintenance of the yield and quality of the fruits, and increase in the final profitability of the producer. However, studies are needed to evaluate the effect of water deficit on floral sprouting and yield of Japanese plums grown in tropical areas.

Fruit pruning is carried out on deciduous trees to regulate the fruit load and the balance of vegetative-reproductive branches (taking into account the angle of their position) and to enable the differentiation of flower buds and the translocation of nutrients from the leaves (Fischer, 2013). In Japanese plum trees in the tropics, the formation of shoots (productive or mixed branches) is sought cycle after cycle (Castro & Puentes, 2012); therefore, this practice must be carried out to obtain high yields. Green pruning is implemented to eliminate the "suckers" that are produced inside the tree and hinder the penetration of solar radiation; this pruning improves the differentiation of branches and generates better color development in plum fruits (Castro & Puentes, 2012).

The aforementioned techniques will cause bud swelling, which is the optimum time for the application of rest-breaking chemicals. However, these chemicals will not act as chilling compensators as the tree will not enter endodormancy and, therefore, they will not work as rest-breaking substances (Gutiérrez-Villamil *et al*., 2022). The application of hydrogen cyanamide (Dormex®) at 1% increased the flowering percentage, fruit set, number of fruits per tree and yield, and reduced the days until harvest in the Japanese plums 'Horvin', 'Methley', 'Santa Rosa', 'Sangre Toro', 'Santa Rosa', and 'Ogden' cultivated in the municipality of Nuevo Colón (Buitrago *et al*., 1992). Thidiazuron (TDZ) is a substance with cytokinin activity used to increase bud-breaking. In the Japanese plum var. 'Shiro', the application of TDZ at 50, 100, and 200 mg L^{-1} increased the flowering percentage and showed the same performance as with the application of Dormex® (5 ml L^{-1} a.i. hydrogen cyanamide); however, TDZ at 100 ml L^{-1} increased the diameter of the ovary, which can increase the size of the fruits (Alvarado-Raya *et al*., 2000). Similar results were obtained in the variety 'Santa Rosa' by Almaguer-Vargas *et al*. (2000). Contrary to these reports, the exogenous application of gibberellins (GA_3 > 50 mg L^{-1}) to the buds inhibited flowering but increased the fruit quality parameters such as weight, diameter, and soluble sugar contents in cultivars of *P. salicina* (González-Rossia *et al*., 2006), so the use of GA_3 to inhibit flowering appears to be an indirect technique to reduce fruit load and a useful method to control the cost of manual fruit thinning in Japanese plum. Thus, the application of Dormex® or TDZ promotes bud break in Japanese plums grown in the tropics, making this an essential tool for the production of two crops per year and to suppress dormancy (Fig. 1).

Flowering, pollination, and fruit development

When the technique of suppressing dormancy through defoliation and the application of chemical substances that induce flowering is used, the flower buds swell and show light brown scales (Fischer, 2013). When this method is not used, flowering depends, ultimately, on whether the bud has received enough CH to satisfy its low-temperature requirement, which can be complemented by the application of chilling compensators. Once the bud opens, the individual flowers appear separated on short stems, showing first the green sepals, then the white petals, forming a globe, where gradually the anthers become evident followed by the pistil, which is the flower. It is fully open in anthesis (Hartmann & Neumüller, 2009).

In general, the flowering of Japanese plum occurs soon and the flowering period is the shortest of all stone fruit trees. The inflorescence is an umbel that generally contains one to three flowers, which are smaller than those of other *Prunus* species, with a diameter of 5 to 25 mm and white petals that open flat on the cup-shaped corolla. The flowers are hermaphrodite, with a single pistil, and have 20 to 30 stamens that are enclosed in five petals and five sepals (Guerra & Rodrigo, 2015). It should be noted that the percentage of flowers that become fruits is much lower in the Japanese plum (5% to 14%) than in other *Prunus* species (Guerra & Rodrigo, 2015), since a mature tree can produce up to 100,000 flowers, of which only 1% to 5% must be set for an economically profitable harvest and, in many cases, chemical or manual thinning is necessary (Fischer, 2013). The application of GA_3 (50 mg L^{-1}) 14 d after anthesis, when the buds are swelling, causes flower abortion in the Japanese plum tree, decreasing fruit yield per tree, but increasing size and fruit quality characteristics (Erogul & Sen, 2015), which is why it is considered an effective technique to control the thinning of flowers and fruits in this species.

Pollination in the Japanese plum tree must be managed very carefully, since fertility is cultivar-specific and, in most cases, there is incompatibility between varieties (Looney & Jackson, 2010). Most Japanese plums, like other *Prunus* fruit tree species, are self-incompatible and require cross-pollination to ensure fruit set, *i.e*., the plant rejects its pollen. Pollen recognition or rejection is determined by the genotype and the incompatibility reaction is genetically controlled by a polymorphic (S) locus, which encodes two linked genes that establish the alleles of the pistil and pollen (Guerra & Rodrigo, 2015). Therefore, knowledge of the pollination requirements of the cultivars is essential for solving low-yield problems related to the lack of pollination and for the planting design of new orchards with an adequate proportion of pollinating agents. In the tropics, there is recognition of some varieties that are self-compatible with each other or that require specific cross-pollination of other genotypes (Tab. 1). It is important to recognize pollen compatibility for each variety within the Japanese plum crop since the pollen source directly affects fruit set, growth, gene expression, and fruit quality (Deng *et al*., 2022).

The identification of pollinating agents is important in order to increase their presence and occurrence during the flowering stage and thus increase the percentage of fruit set in deciduous fruit trees. Vaca-Uribe *et al*. (2021) observed in four orchards (apple, pear, peach, and plum) located in the municipality of Nuevo Colon, Boyacá (Colombia) the presence of 453 flower visitors from 35 taxa, of which the Japanese plum had the highest richness of floral visitors (71.4% of the total flower visitor species) of all deciduous trees, Hymenoptera being the most abundant order of flower visitors. Also, it was found that the plum tree shares floral visitors with 44 species of plants, including weeds, other deciduous fruit trees, and native species of the area. Of these floral visitors, the *Apis mellifera* species is particularly important, so care of this species is necessary to increase the flow of pollinating agents. In addition, during the bud break and flowering of the Japanese plum, the number of floral visitor species increased considerably compared to when it was not in the flowering stage, indicating that plum blossom events could be influencing the mobilization of the flower visitor populations in the environment (Vaca-Uribe *et al*., 2021). Therefore, it is recommended to promote the production of these insects to increase biodiversity and fruit set. In this sense, Castro and Puentes (2012) recommend implementing three boxes of beehives per ha for good peach and plum pollination.

Stone fruit trees (*Prunus* spp.), including Japanese plums, exhibit a typical double sigmoid growth pattern during fruit development and ripening (Casierra-Posada *et al*., 2004; González-Pérez, Becerril-Román, *et al*., 2018). Within this period of development, four distinct stages (S1-S4) are recognized. The first stage (S1) is characterized by a rapid increase in cell division and elongation, S2 by only slightly increased fruit size but an endocarp hardened to a solid bone (bone hardening), S3 by a rapid cell division that results in a significant increase in the size of the fruit, and S4 by the ripening of the fruit or climacteric stage (Casierra-Posada *et al*., 2004). During fruit development, the regulation of sugar transport from the source to the sink cells is a key factor in ensuring the growth, yield, and quality of the fruits. SWEET proteins (sugars will eventually be exported to transporters) are essential for the transport of sugars. The relative expression of 15 SWEET genes has been identified during the development and ripening of *P. salicina* fruits (*PsSWEET1-15*) and correlated with fructose and sucrose content, suggesting their possible functions in the transport and accumulation of these two sugars in the fruits of Japanese plums (Jiang *et al*., 2023).

The Japanese plum 'Horvin', cultivated in the tropics, requires a period of 1,538 GDD and 81 d from fruit set to the optimum harvest point (Orjuela-Angulo, Parra-Coronado *et al*., 2022). This information is very important in order to identify the appropriate moments to carry out good agricultural practices more effectively in tasks such as irrigation, fertilization and phytosanitary management, among others. Fruit development is the longest of the other phenological stages of the Japanese plum and one of the most important in the management of fruit load (thinning) since it can affect productivity. In varieties of *P. salicina*, the trees with a higher fruit load (without thinning) decreased the individual dry mass of the fruits in comparison with trees with a low load (thinning); this effect was associated with a limitation in the source-sink relationship, since the source presented limitations in the S1 growth stage and the sink in the S3 stage (Basile *et al*., 2002). This shows the importance of agronomic management of Japanese plum thinning in fruit quality for market requirements.

Different preharvest treatments have been reported to increase the quality of Japanese plum fruits. The exogenous application of different sources of auxins when the fruits had a size of 22 mm caused an appreciable and significant increase in fruit size and, therefore, in yield, due to an increase in the size of fruit cells; however, auxins did not affect the quality of the fruits or the yield of the following season (Stern *et al*., 2007), which is a promising result for agronomic management in tropical conditions (Fig. 1). Additionally, the application of GA_3 (50 mg L^{-1}) before harvest increased the weight, size, firmness, and total soluble solid contents, and reduced mass loss in the fruits of two Japanese plum varieties during storage, transportation, and commercialization (Harman & Sen, 2016).

Reducing tree fruit load by decreasing the number of fruits can improve fruit size and weight by inducing a better distribution and balance of assimilates between the vegetative and reproductive growth, as reported by Pavanello *et al*. (2018) for 'Katinka' plum. Kang *et al*. (2023) quantify the carbohydrate/nitrogen (C/N) relationship as the ratio between the non-structural carbohydrates produced by leaves and the nitrogen absorbed by the roots, which strongly depends on the growth period and phenology of the tree. Variation in the leaf/fruit ratio has an impact on the C/N ratio in the leaves of plum trees. In 'African Rose' plum trees, leaf C/N ratio and yield were increased by hand thinning compared to thinning by ethephon, naphthalene acetic acid, 6-benzyladenine, or control (Maged *et al*., 2020).

The color of the fruits is a determining aspect of the quality of the Japanese plum and each cultivar synthesizes different pigments in the peel, which is why there are yellow ('Ogden') and red ('Horvin') Japanese plums (Fischer, 2013). As mentioned in the ecophysiology section of this review, visible light (including UV radiation) is responsible for regulating the synthesis of anthocyanins in Japanese plum (Zhang *et al*., 2021), which is promoted by the expression of the *PsbZIP1* and *PsbZIP10* genes (Shen *et al*., 2023), so this process can be favored by the high levels of UV radiation in the high tropics. González *et al*. (2016) mention that the color in Japanese plum trees changes from the S2 growth stage until the end of S4, due to increased expression of the leucoanthocyanidin dioxygenase (LDOX) gene, which regulates flavonoid biosynthesis in the fruits. Likewise, the significant contribution of odorants to the characteristic flavor of the fruits directly affects their quality. In this regard, in the Japanese plum 'Horvin', the analysis of volatile compounds led to the identification of 148 components, including 58 esters, 23 terpenoids, 14 aldehydes, 11 alcohols, 10 ketones, 9 alkanes, 7 acids, 4 lactones, 3 phenols, and another 9 compounds of different structures (Pino & Quijano, 2012). Additionally, organic acids are key components to determine the flavor of the fruits. Yu *et al.* (2023) identified malic acid as the predominant organic acid during the development and ripening of Japanese plum fruits and the potential genes involved in the synthesis of this acid, such as 11 enzyme-coding genes, 21 transporter genes, and 5 *MYB* transcription factor.

Conclusion and future perspectives

Production of Japanese plum in the tropical highlands is affected by differences and particularities in ecophysiology with respect to the temperate zone; agronomic management is required to achieve the optimal productive potential of this fruit tree (Fig. 2). In Colombia, Japanese plum production systems are adapted to altitudes ranging between 2,000 and 2,900 m a.s.l., average solar brightness of 1,400 h per year, photoperiods of 12 h, temperatures between 14 and 20°C during the day and 6 and 8°C at night, rainfall between 700 and 1,600 mm per year, and loamy soils with low apparent density and good fertility. Due to these conditions, varieties with low CH requirements, preferably less than 800 CH, should be selected to achieve good production.

When Japanese plum varieties that accumulate few CH are selected, such as the 'Horvin' variety (300 – 400 CH), in an area with bimodal rainfall distribution, two harvests a year can be obtained by applying special agronomic management and achieving endodormancy suppression (Fig. 2). This management consists of chemical defoliation (copper oxychloride + zinc sulfate), good nitrogen fertilization during plum phenology, fruit and green pruning, and the application of chemical substances that promote breaking of flower buds (Dormex® and TDZ). However, it is necessary to investigate in depth the application of a water deficit in the Japanese plum cultivated in the tropics to induce flowering.

In the tropics, Japanese plum varieties require high specificity and study to achieve fertility of the flowers with pollen that does not present incompatibility. The pollinating agents in the Japanese plum tree interact with multiple native species of the producing areas, including weeds, so it is recommended to carry out sustainable management in the control of weeds to avoid affecting the biodiversity

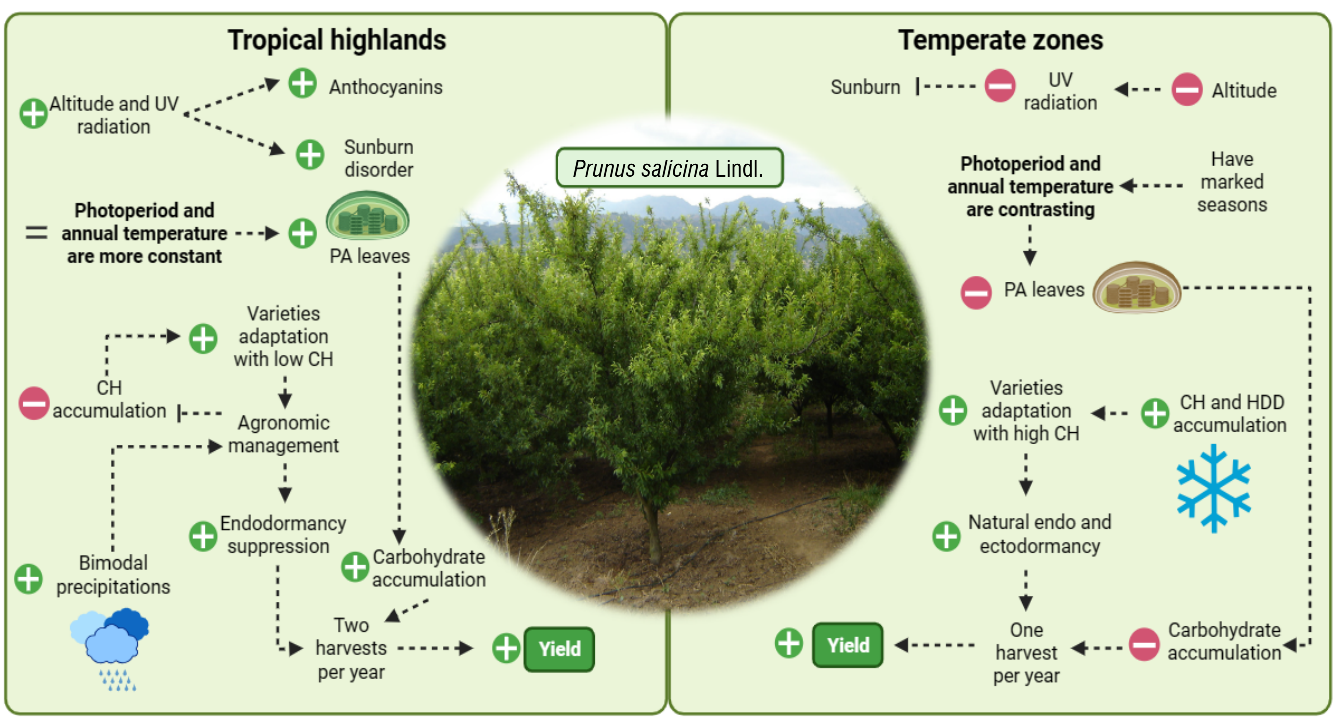

FIGURE 2. Comparison of ecophysiological and management components in the production of Japanese plum (*P. salicina*) cultivated in tropical and temperate zones. PA: photosynthetically active, CH: chilling hours, HDD: heating degree day. The + (green) and - (red) symbols correspond to an increase or decrease effect, respectively. Photo: G. Fischer.

of pollinators and, therefore, reducing the fruit set. The growth and development of the Japanese plum fruits conform to a double sigmoid model, where some varieties take 3 months from fruit set to harvest. The application of growth regulators in pre-harvest seems to show good results in some varieties of *P. salicina*, but there is no information on the effect of these regulators in Colombian varieties and conditions; therefore, it is suggested this promising research topic be investigated.

Conflict of interest statement

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

Author´s contributions

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

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Effects of cadmium on the physiology of *Solanum lycopersicum* L. grown in alternative hydroponic media

Efectos del cadmio en la fisiología de *Solanum lycopersicum* L. cultivados en medios hidropónicos alternativos

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ABSTRACT

Cadmium (Cd) is one of the most toxic metals for the physiology of plants. Proper nutrient management through wastewater reuse can be an efficient strategy to mitigate its effects. In this research, the effects of cadmium were evaluated in the hydroponic cultivation of *Solanum lycopersicum* L. We conducted two experiments: one using mining wastewater with concentrations of 0, 5, 10, and 15 mg L^{-1} of Cd^{2+} (Experiment 1) and another using deionized water with concentrations of 0, 2.5, 5, 10, and 15 mg L^{-1} of Cd^{2+} (Experiment 2). Cadmium stress in plants reduced leaf area, chlorophyll content, and concentrations of potassium (K) and manganese (Mn), and increased concentrations of sulfur (S), phosphorus (P), iron (Fe), and copper (Cu). The employment of mining wastewater improved the plant's response to Cd stress by reducing the translocation of Cd and increasing the contents of P, S, calcium (Ca) and magnesium (Mg) in leaves. At the same time, the use of deionized water decreased the contents of Cu in leaves. These nutrition-related effects influenced leaf area and chlorophyll content, as both indicators showed less impairment in the experiment with wastewater. These results provide additional value to the reuse of wastewater in agriculture.

Key words: heavy metal, tomato, wastewater, hydroponics.

RESUMEN

El cadmio (Cd) es uno de los metales más tóxicos para los procesos fisiológicos de las plantas. El manejo adecuado de nutrientes a través de la reutilización de las aguas residuales puede ser una estrategia eficiente para minimizar sus efectos. En el presente estudio se evaluaron estos efectos en el cultivo hidropónico de *Solanum lycopersicum* L. Se realizaron dos experimentos: uno con aguas residuales mineras y concentraciones de 0, 5, 10 y 15 mg L^{-1} de Cd^{2+} (Experimento 1) y otro con agua desionizada y concentraciones de 0, 2.5, 5, 10 y 15 mg L^{-1} de Cd^{2+} (Experimento 2). El estrés por Cd en plantas redujo el área foliar, el contenido de clorofila y las concentraciones de potasio (K) y manganeso (Mn) y aumentó las concentraciones de azufre (S), fosforo (P), hierro (Fe) y cobre (Cu). El uso de aguas residuales mineras mejoró la respuesta de las plantas al estrés por Cd al reducir su translocación y aumentar los contenidos de P, S, calcio (Ca) y magnesio (Mg) en las hojas. Al mismo tiempo, el uso de agua desionizada disminuyó el contenido de Cu en las hojas. Estos efectos relacionados con la nutrición influyeron en el área foliar y el contenido de clorofila, ya que ambos indicadores mostraron un menor deterioro en el experimento con aguas residuales. Estos resultados proporcionan un valor adicional a la reutilización de aguas residuales en la agricultura.

Palabras clave: metal pesado, tomate, aguas residuales, hidroponía.

Introduction

Due to industrial and anthropogenic activities, concentrations of toxic metals in water, soil, sediments, and other ecosystems have significantly increased in recent decades. Among them, cadmium (Cd) has drawn the attention of soil and plant sciences due to its high toxicity, mobility, and bioaccumulation potential (Abdel-Satar *et a*l., 2017; Bala Murugan *et al*., 2019; El Rasafi *et al*., 2022).

In the 1940s, Cd pollution in the Jinzu River and in rice cultivation became evident when over 100 people in Japan died from a disease named Itai-Itai (Ogawa *et al*., 2004). Additionally, scientific evidence of Cd contamination in water,

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sediments, and plant tissue has been reported in various regions worldwide. Examples include lakes in northeastern Wisconsin (USA), Central Ontario (Canada), northeastern Sweden, southeastern Norway (Spry & Wiener, 1991), the Huelva River (Spain) (Martorrell *et al*., 2009), and soils and vegetables in Ethiopia (Duressa *et al*., 2015) and India (Gimba *et al*., 2015).

This metal is recognized as one of the most toxic for plant physiological processes (Li *et al*., 2023). In many crops, including the tomato, Cd has been reported to inhibit growth, photosynthesis, transpiration, and the formation of photosynthetic pigments. It also causes chlorosis, nutritional imbalances, oxidative stress, and modifies the activity of enzymes involved in organic acid metabolism (Hédiji *et al*., 2015). To mitigate its effects, proper nutrient management is known to be an effective alternative (Samet *et al*., 2017) and can be achieved through the reuse of previously treated wastewater.

In addition to Cd pollution, another issue currently affecting agriculture is the high levels of water deficit reached in many regions of the world (Muller, 2017). As a solution, different countries have proposed the reuse of wastewater, recognized since ancient times as its high nutritional value (Ramírez *et al*., 2021; Samet *et al*., 2017).

Despite the above research, studies to thoroughly understand the effects of Cd on physiological and nutritional indicators of plants, as well as the role of nutrients in tolerance to this type of stress, are insufficient. Because of this and the need to reuse wastewater, the objective of the following research was to evaluate the effect of Cd on growth indicators, photosynthetic activity, and the nutrient composition of tomato plants grown in two different hydroponic media: wastewater and deionized water.

Materials and methods

Growth conditions of tomato plants

We conducted two experiments in a growth chamber under controlled light conditions (12/12 h light/darkness), temperature (18/25°C light/darkness), and light intensity (800 µmol m^{-2} s^{-1}). In both experiments, the tomato seeds germinated and grew in vermiculite. After 7 d, we transplanted the plants into a hydroponic system in plastic trays measuring 30 x 60 x 80 cm. We maintained the system continuous aeration. The Hoagland and Arnon (1950) nutrient solution (Tab. 1) was used that had been modified at the Laboratory of Plant Physiology of the National Institute of Agricultural Sciences (Cuba). The pH was adjusted to 6.5 and the nutrient solution was renewed every 8 d.

The main difference between both experiments was the type of water used in the hydroponic solution. In experiment 1, water was collected from the Biajaca River, where residues from the Castellanos Mines in the province of Pinar del Río, Cuba, are discharged. In contrast, deionized water was used for experiment 2.

After 15 d of germination, treatments were applied following a completely randomized design with 20 plants per treatment. In experiment 1, the treatments were as follows: T1 - deionized water, T2 - wastewater, T3 - wastewater with 5 mg L^{-1} of Cd, T4 - wastewater with 10 mg L^{-1} of Cd, and T5 - wastewater with 15 mg L^{-1} of Cd. In experiment 2, the

TABLE 1. Composition of the modified Hoagland and Arnon (1950) solution.

Salt	Content (mM)	Element	Content (mg L^{-1})
$Ca(NO_3)_2$	2.5	Ca	103
KH_2PO_4	0.5	N	105
KNO_3	2.5	K	118
$MgSO_4$	1.0	S	33
$ZnSO_4$	0.00039	Mg	25
$MnSO_4$	0.0046	P	15
$CuSO_4$	0.00016	Fe	10
H_3BO_3	0.0234	B	0.25
H_2MoO_4	0.000051	Mn	0.25
Fe- EDTA	0.179	Zn	0.025
		Cu	0.01
		Mo	0.0052
		Cl	0.50

treatments were as follow: T1 - deionized water, T2 - deionized water with 2.5 mg·L^{-1} of Cd, T3 - deionized water with 5 mg L^{-1} of Cd, T4 - deionized water with 10 mg L^{-1} of Cd, and T5 - deionized water with 15 mg L^{-1} of Cd. Cadmium chloride ($CdCl_2$) was used as the metal carrier salt.

The wastewater used was classified as sulfated bicarbonate of chlorinated sodium calcium magnesium (SO_4 = HCO_3^- > Cl-Na = Ca > Mg). The electrical conductivity (270 µS cm^{-1}), alkalinity (1.03 meq L^{-1}) and the concentrations of N, K, Ca, Mg, Fe, Mn, Zn, B, Mo, Cu, Co, Ni, Na, Al, Cd, Pb, As, Cr, Li, Be, Sr, Sb, Se, V, Tl, Rb, Bi, Cl^-, NO_3^-, SO_4^{2-}, Br^- were below the permissible limits established by FAO (Hernandez-Baranda *et al.*, 2018). The initial pH was 4.0, which did not fall within the range established by the FAO. However, the water was neutralized to a pH of 7.0 before being used. The chemical composition of the water utilized is presented in Hernandez-Baranda *et al.* (2018). After 25 d of treatments, evaluations were performed at a rate of eight plants per treatment, as detailed below.

Leaf area

The leaf surface area was calculated through image analysis. Initially, all leaves of each plant were scanned using a Canon MF4800 scanner in jpg format. Subsequently, the images were processed in Adobe Photoshop SC5 image analysis software, and the area in cm^2 was determined.

Chlorotic leaf area

For the damaged leaf area, the same procedure was followed as above, but only the damaged surface or leaves with obvious symptoms of chlorosis were calculated.

Chlorophyll content

Six well-developed leaves of the upper third of the plants were measured with a Portable MINOLTA Chlorophyll Meter SPAD 502 Plus.

Plant chemical analysis

A destructive sampling was carried out, where the organs were separated into root, stem, and leaves. Subsequently, they were dried in an oven at 80°C until reaching a constant mass, and the samples were ground. The content of Cd, P, K, S, Ca, Mg, Cu, Mn, and Fe in each organ was determined by ICP-OES after microwave-assisted acid digestion. The procedure for this analysis was established at the Ionomics Laboratory of CEBAS-CSIC (Hernandez-Baranda *et al.*, 2018).

Statistical analysis

Results underwent one-way ANOVA (factor with five levels). Indicators with differences were further analyzed using Duncan's Multiple Range Comparison test ($P \leq 0.05$). Additionally, an independent sample T-test compared Cd concentrations in root, stem, and leaves (5, 10, and 15 mg L^{-1}) between the two experiments.

Results and discussion

Effects of Cd on leaves of tomato plants

The presence of Cd in the environment, even at its lowest concentrations, caused a significant reduction in leaf area and led to chlorosis-related damage, affecting over 40% of leaf tissue in both experiments (Fig. 1). These damages

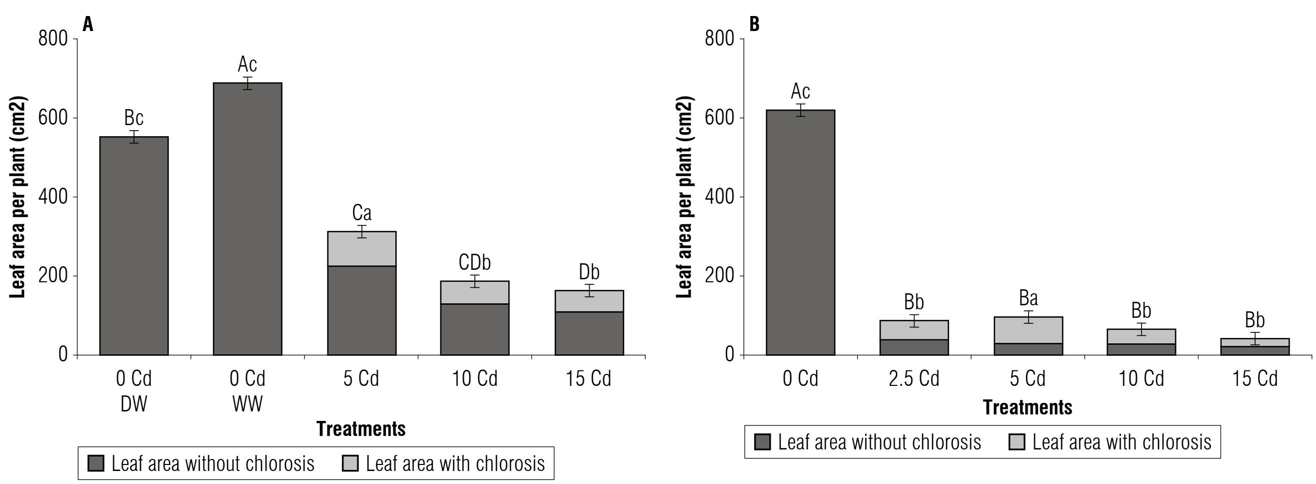

FIGURE 1. Leaf area of tomato plants after 25 d exposure to different concentrations (0, 2.5, 5, 10, and 15 mg L^{-1}) of Cd^{2+}. The data are shown as mean value + standard error of the mean (n=6). Capital letters compare the total leaf area and the lowercase letters that leaf area with chlorosis according to the Duncan's multiple range comparison test, $P \leq 0.05$. DW - deionized water, WW - wastewater. A - experiment 1, B - experiment 2.

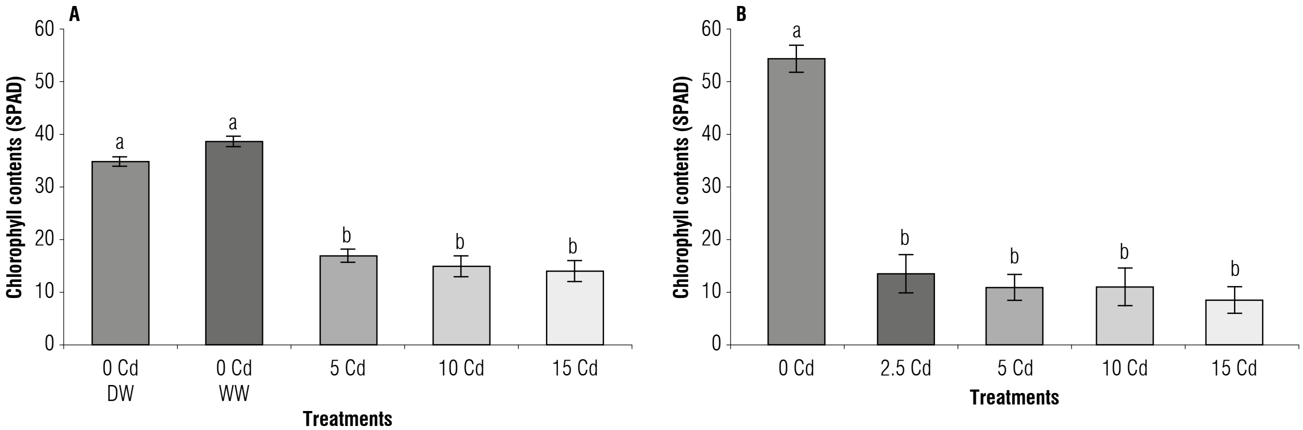

FIGURE 2. Chlorophyll content (SPAD units) in leaves of tomato plants after 25 d exposure to different concentrations (0, 2.5, 5, 10, and 15 mg L^{-1}) of Cd^{2+}. The data are shown as mean value + standard error of the mean (n=6). Lowercase letters compare the chlorophyll contents according to the Duncan's multiple range comparison test, $P \leq 0.05$. DW - deionized water, WW - wastewater. A - experiment 1, B - experiment 2.

were mainly evident in young leaves. Notably, reductions in leaf area were less severe in experiment 1 (67%, 81%, and 84% in T3, T4, and T5) compared to experiment 2 (85%, 89%, and 93% in T3, T4, and T5). Initial Cd doses also led to less chlorosis damage in experiment 1, suggesting wastewater cultivation positively mitigated Cd toxicity in this indicator.

Leaf tissue damage is evident in chlorophyll content estimates (Fig. 2). Cadmium toxicity caused reductions exceeding 50% compared to control treatments. Similar results were obtained in other crops exposed to high concentrations of Cd, such as peas (Agrawal & Mishra, 2009), soybeans (Xue *et al*., 2014), lettuce (Monteiro *et al*., 2009) and potatoes (Xu *et al*., 2013). Notably, similar to leaf area, experiment 1 showed less pronounced reductions in chlorophyll content (around 57%) compared to experiment 2 (around 73%). This indicates that wastewater cultivation had a beneficial impact on mitigating Cd toxicity in this indicator.

Effects of Cd on the concentration of mineral nutrients in roots, stems, and leaves

Cd toxicity altered normal nutrient concentrations in the organs of tomato plants. Control treatment levels of nutrients closely matched standard nutrient concentrations in plant tissues (Azcón-Bieto & Talón, 2013).

Cadmium

Different authors have studied plant response mechanisms to Cd toxicity, including exclusion via root accumulation and detoxification in leaf organelles. Figure 3 indicates that roots accumulated the highest Cd levels, consistent with exclusion behavior (Seregin & Kozhevnikova, 2004). This doesn't imply a singular tolerance mechanism, rather it suggests a combination of mechanisms. Accumulation of Cd in roots is proposed as a significant contributor in this study. Similar results were reported by Hernández *et al*. (1998) in pea plants (*Pisum sativum* L.) exposed to concentrations of 10 mM and 50 mM of Cd in a hydroponic system. They described that the reason for this higher accumulation in roots is that the metal is primarily concentrated in the cell wall, in the soluble fraction, associated with molecules of molecular weight higher than 6-8 kDa, possibly corresponding to phytochelatins (Hernández *et al*., 1998).

Nevertheless, in leaf tissue, plants from both experiments accumulated Cd concentrations similar to known hyperaccumulator plants of Cd, such as *Viola baoshanensis*, *Arabis paniculata*, *Potentilla griffithii* (Liu *et al*., 2004; Qiu *et al*., 2011; Zeng *et al*., 2009). The Cd levels (4.7 mg g^{-1}) are comparable to hydroponically grown tomato varieties (Sagardoy Calderón, 2011), three orders of magnitude higher than soil-grown varieties identified as tolerant (4.3 mg kg^{-1} and 13.4 µg g^{-1}) (Andal, 2016; Sbartaï *et al*., 2017). This highlights greater Cd availability and absorption in hydroponic systems.

Figure 3 shows that as Cd levels in the solution increased, its content in leaves and stems also increased. However, root behavior differs; in some cases, plants exposed to higher toxicity accumulated less Cd than in previous treatments. This suggests greater Cd accumulation in roots at lower toxicity levels, with consistent patterns at higher levels. Notably, at these higher levels, Cd translocation to aerial organs increased.

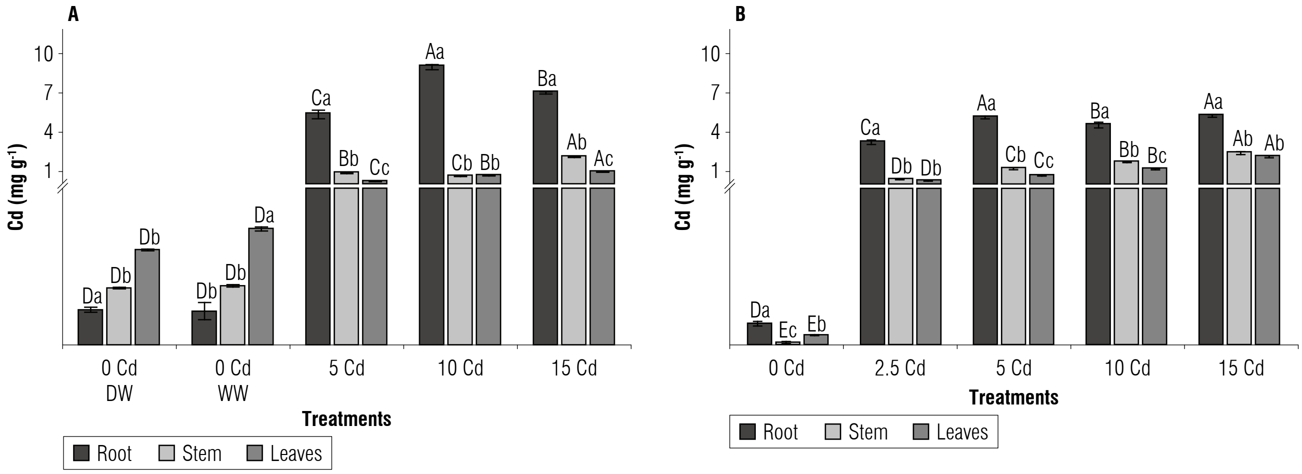

FIGURE 3. Concentration (mg g^{-1}) of Cd in root, stem, and leaves of tomato plants after 25 d exposure to different concentrations (0, 2.5, 5, 10, and 15 mg L^{-1}) of Cd^{2+}. The data are shown as mean value + standard error of the mean (n=6). Capital letters compare between treatments in the same organ, and lowercase letters compare between organs in the same treatment according to the Duncan's multiple range comparison test, $P \leq 0.05$. DW - deionized water, WW - wastewater. A - experiment 1, B - experiment 2.

TABLE 2. Concentration (mg g^{-1}) of Cd in roots, stems, and leaves of tomato plants grown in different treatments of Cd (5, 10, and 15 mg L^{-1}). $P<0.05$ indicates statistical differences between the experiment with wastewater (1) and the experiment with deionized water (2).

Exp	Roots			Steams			Leaves		
	5 Cd	10 Cd	15 Cd	5 Cd	10 Cd	15 Cd	5 Cd	10 Cd	15 Cd
1	5.45	9.08	7.10	0.96	0.71	2.18	0.30	0.76	1.04
2	5.23	4.65	5.36	1.31	1.80	2.51	0.77	1.28	2.22
P	0.55	0.0	0.00	0.00	0.00	0.02	0.00	0.00	0.00

Exp: experiment, *P*: P-value.

Table 2 compares Cd concentrations in the most stressed treatments between the two experiments. Experiment 1 shows higher Cd concentration in roots, while experiment 2 exhibits higher Cd concentration in aerial tissues (stem and leaves). This suggests increased Cd translocation to aerial organs in experiment 2, where nutrient availability in the growth medium is lower.

Two genotypes of pea and wheat with different levels of Cd tolerance have been identified, and the authors attributed their higher tolerance to a reduction in Cd translocation to leaves (Ci *et al*., 2011; Rahman *et al*., 2017). Therefore, we suggest that, due to reduced translocation, plants cultivated with wastewater have developed more efficient Cd response mechanisms than those grown solely with nutrient solution.

One of the tolerance strategies of plants to Cd stress is the immobilization of the ion in the roots through chelation with sulfur-rich proteins (Nocito *et al*., 2011). This coincides with the fact that the higher concentration of sulfate in wastewater is one of the most pronounced differences compared to deionized water. Consequently, plants from experiment 1 grew with a higher available sulfur content. Both criteria suggest that sulfur may be responsible for the observed lower translocation of Cd in experiment 1 and its higher accumulation in the roots in both experiments.

Sulfur

Sulfur concentrations in the root (Fig. 4 A and B) corroborate the assumption of the relationship between the content of this element and the immobilization of Cd. In treatments 3 and 4 of experiment 1 and treatments 2 and 3 of experiment 2, the sulfur concentrations in the root were increased when the Cd content in this organ increased. Subsequent treatments also found a total correspondence between the behavior of these elements (S and Cd), decreasing both in treatment 5 of experiment 1 and in treatment 4 of experiment 2.

In leaves, which are the primary organ for sulfur accumulation, discordant results were observed between the experiments. Unlike experiment 1, in experiment 2, there was no consistent correspondence between Cd and S concentrations. Up to and including treatment 3, both elements increased, but in treatments 4 and 5, S concentration decreased, reaching levels similar to the control and lower than experiment 1. Conversely, Cd concentration increased to higher levels than in experiment 1. Thus, the greater Cd accumulation in experiment 2 leaves is unrelated to S concentration. However, in experiment 1, where sulfate concentration in the solution is higher, a relationship between them exists.

Plant exposure to Cd could increase sulfate assimilation (Nussbaum *et al*., 1988) and this coincides with the increase in S observed in the Cd concentration levels of both experiments (Fig. 4). Notably, the S increase in leaves across all stress treatments in experiment 1, and in treatments 2 and 3 of experiment 2, exceeded concentrations (0.1%) considered adequate for S in plant organs (Azcón-Bieto & Talón, 2013).

Various authors have linked sufficient S availability to the biosynthesis of Cd detoxifying agents, mitigating their effects (Anjum *et al*., 2012; Hassan *et al*., 2006). Given this evidence, the greater impact on leaf tissue in experiment 2 is attributed to lower S availability in the hydroponic medium, leading to reduced accumulation in the leaves.

Potassium and manganese

Analysis of K behavior in both experiments revealed progressive decreases in concentrations across all organs with increasing Cd in solution (Fig. 5). In the most toxic treatment of experiment 1, K concentrations decreased by 51%, 55%, and 48% in the root, stem, and leaves. In experiment 2, the decreases were 42%, 57%, and 60% in the same organs.

Regarding the decrease of the concentration of K, the leaf was the organ least affected in experiment 1 and, in turn, the most affected in experiment 2, due to the higher concentration of Cd found in the leaf tissues of this last experiment. The decrease caused was of such magnitude that, according to the sufficiency interval, 3-6% (Azcón-Bieto

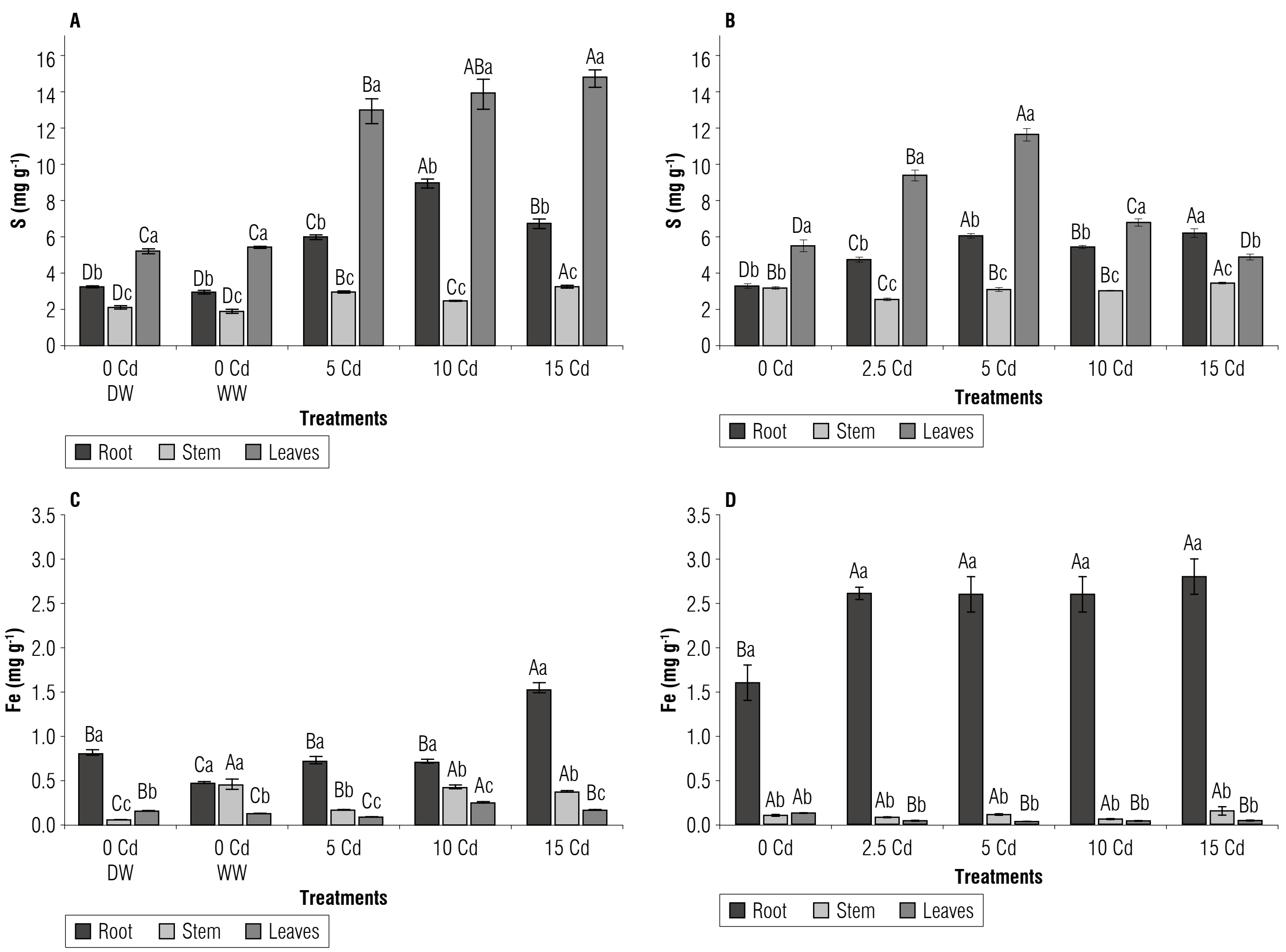

FIGURE 4. Concentration (mg g^{-1}) of S (A and B) and Fe (C and D) in root, stem, and leaves of tomato plants after 25 d exposure to different concentrations (0, 2.5, 5, 10 and 15 mg L^{-1}) of Cd^{2+}. The data are shown as mean value + standard error of the mean (n=6). Capital letters compare between treatments in the same organ, and lowercase letters compare between organs in the same treatment according to the Duncan's multiple range comparison test, $P \leq 0.05$. DW - deionized water, WW - wastewater. A - experiment 1, B - experiment 2.

& Talón, 2013), the plants were grown under 15 mg L^{-1} of Cd in experiment 1 showed a deficiency of K. For experiment 2, the deficiency of K was marked from the treatment with 5 mg L^{-1} of Cd. Due to the vital role of K in plant cells, plants with a significant deficiency of this nutrient end up showing a reduction in growth, especially in the aerial part (Azcón-Bieto & Talón, 2013), similar to the changes observed in the present study.

Cd toxicity led to a decline in Mn concentration throughout the plants as well (Fig. 5 C and D). The root was the most affected organ in both experiments, showing a difference of over 30% compared to the stem and leaves. This impact is attributed to the chemical similarity between these elements, facilitating the entry and transport of Cd through Mn-specific transporters (Conn & Gilliham, 2010). In this scenario, Cd displaces Mn in the binding sites of the transporter protein, consequently reducing its uptake into the plants. There is an antagonistic relationship between Cd and Mn in root tissue in pea plants (*Pisum sativum* L.) grown in hydroponics (Hernández *et al.*,1998).

The concentration of Mn in the root exhibited reductions of 56%, 68%, and 71% (Experiment 1) and 88%, 90%, and 91% (Experiment 2) in the treatments with 5, 10, and 15 mg L^{-1} of Cd (Fig. 5 C and D). The most significant impacts occurred in experiment 2, where, unlike experiment 1, the root was not the organ with the highest accumulation of Mn.

Phosphorus and copper

Phosphorus and Cu, unlike K and Mn, exhibited a positive correlation with Cd (Fig. 6). Similar outcomes are reported by Nogueirol *et al.* (2016) in two tomato varieties exposed to varying Cd levels (0, 3, 6, and 12 mg kg^{-1}). They observe antagonistic associations with Mn and synergism with P. Positive correlations between Cd and Cu are also identified in various rice cultivars (Liu *et al.*, 2003).

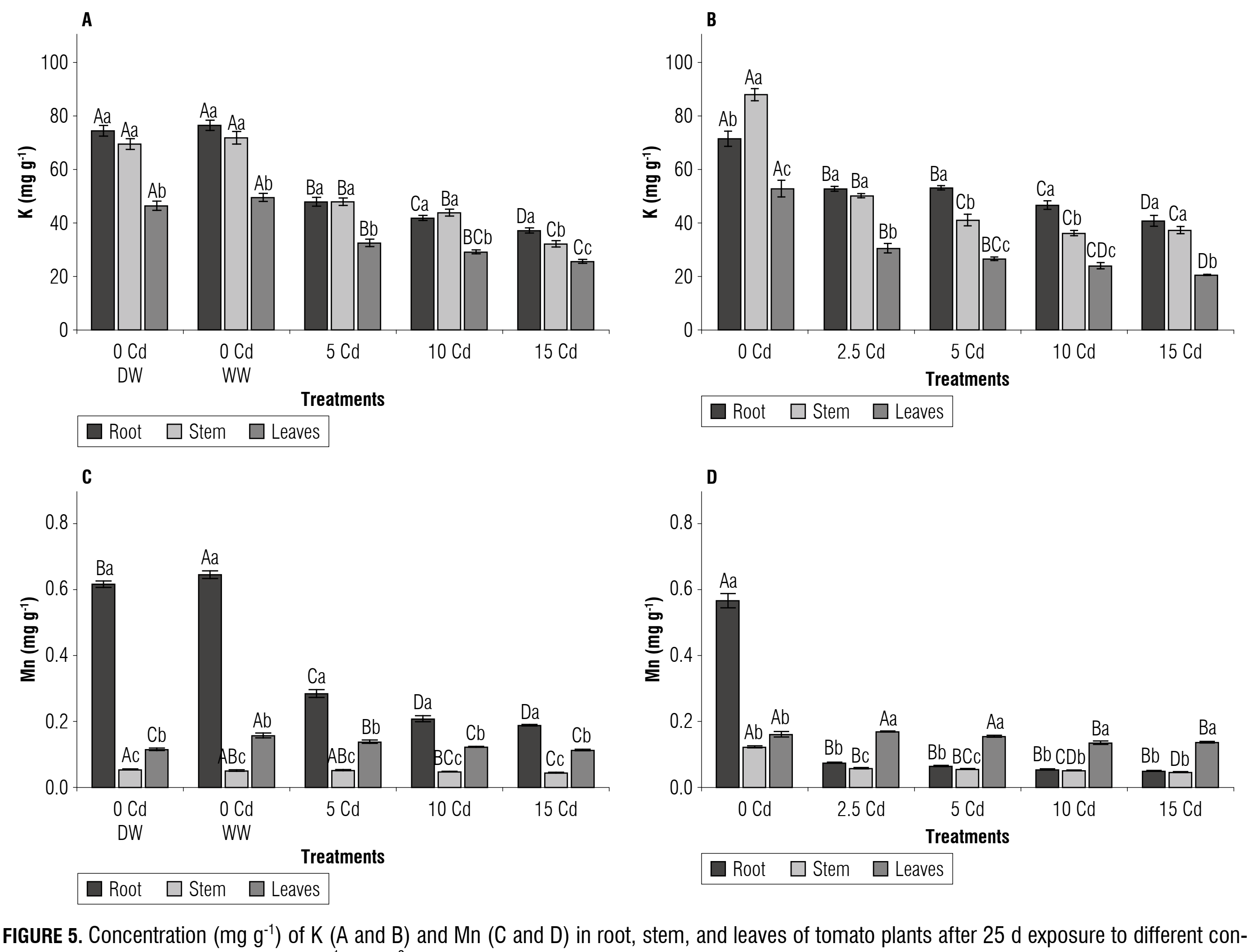

FIGURE 5. Concentration (mg g^{-1}) of K (A and B) and Mn (C and D) in root, stem, and leaves of tomato plants after 25 d exposure to different concentrations (0, 2.5, 5, 10 and 15 mg L^{-1}) of Cd^{2+}. The data are shown as mean value + standard error of the mean (n=6). Capital letters compare between treatments in the same organ, and lowercase letters compare between organs in the same treatment according to the Duncan's multiple range comparison test, $P \leq 0.05$. DW - deionized water, WW - wastewater. A - experiment 1, B - experiment 2.

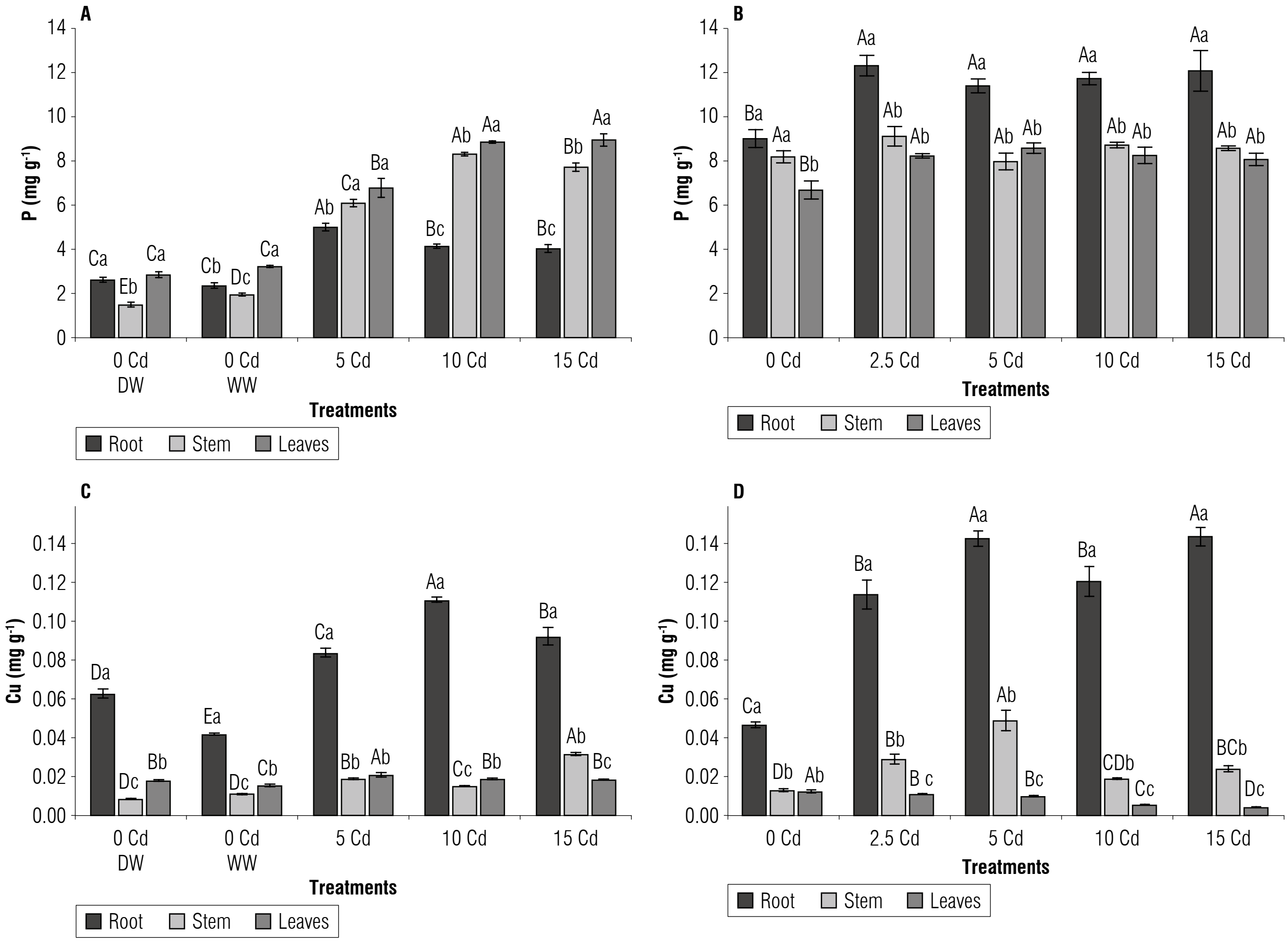

FIGURE 6. Concentrations (mg g^{-1}) of P (A and B) and Cu (C and D) in root, stem, and leaves of tomato plants after 25 d exposure to different concentrations (0, 2.5, 5, 10 and 15 mg L^{-1}) of Cd^{2+}. The data are shown as mean value + standard error of the mean (n=6). Capital letters compare between treatments in the same organ, and lowercase letters compare between organs in the same treatment to the Duncan's multiple range comparison test, $P \leq 0.05$. DW - deionized water, WW - wastewater. A - experiment 1, B - experiment 2.

As a common result between both experiments, the increase in toxicity caused an increase in P content in all organs, except in the stem of experiment 2. It's noteworthy that the increase in leaves in both experiments surpassed the sufficiency range (0.3-0.6%) typical for tomato plants at the same phenological stage as in this study (Azcón-Bieto & Talón, 2013). Some authors have noted the onset of nutritional stress in tomato plants when P levels exceed 1.0% (Benton Jones Jr, 1998) and 0.6% (Peñalosa *et al.*, 1989), particularly in hydroponic crops.

P distribution varied between experiments; in experiment 1, leaf tissue had the highest P accumulation, while in experiment 2, it was the root that had the highest P accumulation. In both experiments, the organs with the highest accumulation were the most affected by Cd toxicity. Notably, prior to treatment application, experiment 2 exhibited higher P concentration in leaves compared to experiment 1, and after Cd exposure, experiment 1 showed a higher P concentration in leaves. This suggests that plants grown in wastewater displayed greater P translocation, intensified with increasing Cd doses, thus recognized as another response mechanism that contributed to mitigating the effects of Cd on the leaves of experiment 1.

Similar to manganese in experiment 1, the root was the organ with the highest copper accumulation (Fig. 6) and was the most affected by Cd toxicity. Cu levels in the roots were approximately twice as high in stressed treatments as in the control. In the leaves, the content of Cu only decreased in experiment 2 and that is attributed to greater leaf damage seen in this experiment that can affect the accumulation of this element in the leaf tissue.

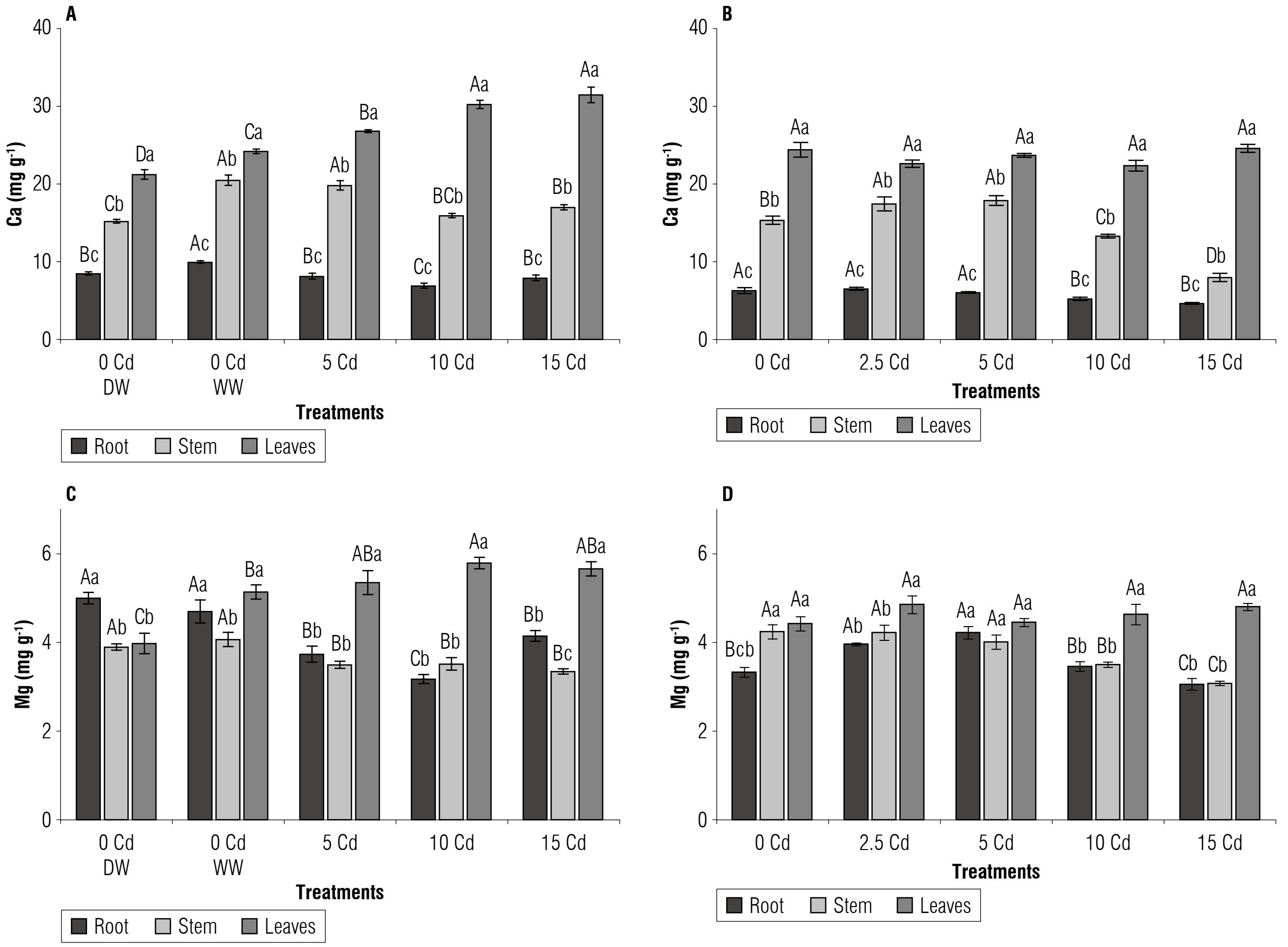

FIGURE 7. Concentration (mg g^{-1}) of Ca (A and B) and Mg (C and D) in root, stem, and leaves of tomato plants after 25 d exposure to different concentrations (0, 2.5, 5, 10 and 15 mg L^{-1}) of Cd^{2+}. The data are shown as mean value + standard error of the mean (n=6). Capital letters compare between treatments in the same organ, and lowercase letters compare between organs in the same treatment according to the Duncan's multiple range comparison test, $P\leq0.05$. DW - deionized water, WW - wastewater. A - experiment 1, B - experiment 2.

Calcium and magnesium

As Cd toxicity levels increased, the concentrations of Ca and Mg decreased in the root and stem in both experiments, while they only increased in the leaves of experiment 1 (Fig. 7).

Ca, like S, is another predominant element in wastewater that distinguishes the two hydroponic media. Previous studies suggest that adequate Ca availability inhibits Cd accumulation in plants like *Arabidopsis thaliana* and *Trifolium repens* (Suzuki *et al*., 2005; Wang *et al*., 2009). Therefore, the higher Ca content present in the leaves of experiment 1 could be another possible cause of the observed lower translocation of Cd in this experiment.

Previous research has also found a positive correlation between Cd and Mg in the leaves of rice plants (Liu *et al*., 2003). Additionally, Küpper *et al*. (2002) identified, in a Cd toxicity study, that mesophyll cells show higher concentrations of Mg than normal, interpreting it as a defense mechanism against the substitution of Mg by Cd in chlorophyll molecules. This mechanism is presumed to be related to the findings in experiment 1 as well.

Iron

In both experiments, Cd presence elevated Fe concentrations in the roots, making it the organ with the highest Fe accumulation (Fig. 4 C and D). Similar increases in root Fe content were noted in previous studies on tomatoes (Sagardoy Calderón, 2011) and rice (Liu *et al*., 2003) under Cd treatments.

Cd had different effects in the leaves. In experiment 1, Fe concentration increased in the treatment with 10 mg L^{-1} of Cd and then decreased in the high-stress treatment. In experiment 2, it decreased from the first level of Cd, persisting in subsequent treatments. It's noteworthy that the 5 mg L^{-1} Cd treatment in both experiments accumulated a lower Fe concentration in the leaves and simultaneously exhibited a larger leaf area with chlorosis (Fig. 1). A similar effect on chlorophyll content in sugar beet was observed in the lower stress Cd treatment, attributed to induced Fe deficiency at low Cd levels (Larbi *et al*., 2002).

Cd toxicity can occur due to the exchange of Fe for Cd (Kabata-Pendias *et al*., 2010). Based on the results presented and the type of damage observed in the leaves, it is considered that the observed chlorosis is not only a result of Cd toxicity but also characteristic of Fe deficiency. However, in experiment 1, the relationship between Fe content and chlorosis seems to contradict this, as the treatments with higher stress showed 49% leaf damage and the Fe contents in the leaves are slightly higher than the controls (Fig. 4 C and D).

The iron chlorosis paradox, where Fe-deficient chlorotic leaves often exhibit higher Fe concentrations than green leaves, suggests that accumulated Fe might be in an unavailable form for the plant (Römheld, 2000). Phosphates and a high pH of the apoplast might cause the precipitation of Fe outside the cell, preventing its utilization (Römheld, 2000). This aligns with elevated P levels in leaves, indicating that Fe precipitation as phosphates could contribute to the apparent contradiction in experiment 1.

In experiment 2, Fe levels in the leaves of stressed treatments were below concentrations considered adequate in plants (100 mg kg^{-1}) (Azcón-Bieto & Talón, 2013). Given the crucial role of Fe in chlorophyll biosynthesis and photosynthetic electron transport, its deficiency in leaves could, along with other factors, contribute to the observed decline in chlorophyll content.

Conclusion

The stress caused by Cd, even at low doses (2.5 and 5 mg L^{-1}), resulted in a significant reduction in chlorophyll content and leaf area, and caused chlorosis damage affecting more than 40% of leaf tissue in both experiments. The presence of Cd was observed to increase the concentrations of P, Fe, Cu, and S, while decreasing the concentrations of K and Mn. Roots and leaves were the organs most affected for K, with roots being the most affected organ for Mn, P, and Cu. The greater availability and concentration of essential nutrients such as S, Ca, and Mg in the hydroponic medium from the Biajaca river enhanced the response of tomato plants to Cd stress by reducing the translocation of this element to the leaves and minimizing the impact on leaf area and chlorophyll content. Additionally, in the experiment with wastewater, there was greater translocation to the leaves of S, P, Ca, Mg, and Fe, with evidence of lesser reductions in the concentrations of K and Mn. These results provided information on the effects of Cd on leaf area and nutrient homeostasis in tomato plants, highlighting the potential of using mining wastewater as a strategy to mitigate these effects. However, it is crucial to know the composition of such waters beforehand to ensure that they receive appropriate treatment.

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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

YHB contributed to the research process in the experiment conception and design; data acquisition; analysis and interpretation of results, article writing and review. ZNCP, YMH, OCR participated in the data acquisition, analysis, interpretation of results, and article review. MPI, JLMO, IEM, and MME participated in the analysis, interpretation of results, and article writing and review. PRH contributed to the research process in experiment conception and design; data acquisition; analysis, interpretation of results, and article writing and review. Al authors reviewed the final version of the manuscript.

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Can biostimulants and grafting alleviate salinity stress in purple passion fruit (*Passiflora edulis* f. *edulis* Sims)?

¿Pueden los bioestimulantes y la injertación mitigar el estrés por salinidad en gulupa (*Passiflora edulis* f. *edulis* Sims)?

Ana María Orjuela-Rodriguez[1], Juan Esteban Castilla-Baquero[1], and Helber Enrique Balaguera-López[1*]

ABSTRACT

Purple passion fruit crops are affected by salinity conditions in productive systems. The aim of this research was to evaluate the effect of the application of *Ascophyllum nodosum* extract on salinity stress in purple passion fruit plants at the vegetative stage of growth with and without grafting. Eight treatments were evaluated corresponding to the combination of grafting or non-grafting on the *Passiflora maliformis* rootstock, the presence or absence of salt stress, and the application or not of the *A. nodosum* biostimulant. Physiological and growth parameters were evaluated. Salinity significantly decreased (P<0.05) growth and stomatal conductance (g_s), increased contents of photosynthetic pigments and did not affect the chlorophyll *a* fluorescence. The grafted plants presented a positive response (P<0.05) in chlorophyll relative contents (~63 SPAD units) and shoot length (~106 cm); lower g_s (~163 mmol H_2O $m^{-2}s^{-1}$), number of leaves (~43 leaves) and root weight (3.5 g of dry weight), and no change in chlorophyll *a* fluorescence. The biostimulant mitigated the salinity effect on g_s and photosynthetic pigments. In the case of salinity, non-grafted purple passion fruit may present a better performance at the vegetative stage, and the biostimulant can have a slight mitigation effect on salt stress. However, if it is essential to use grafted plants for sanitary reasons, the evaluated salinity does not affect them drastically.

Key words: *Passiflora maliformis*, *Ascophyllum nodosum*, water status, chlorophyll fluorescence.

RESUMEN

En los sistemas productivos de gulupa se presentan condiciones de salinidad que afectan el cultivo. El objetivo de este trabajo fue evaluar el efecto de la aplicación de un extracto de *Ascophyllum nodosum* sobre el estrés salino en plantas de gulupa en etapa vegetativa de crecimiento con y sin injertación. Se evaluaron ocho tratamientos correspondientes a la combinación de la injertación o no sobre el patrón de *Passiflora maliformis*, la presencia o no de estrés salino, y la aplicación o no del bioestimulante *A. nodosum*. Se evaluaron parámetros fisiológicos y de crecimiento. La salinidad disminuyó significativamente (P<0,05) el crecimiento, conductancia estomática (g_s), incrementó los pigmentos fotosintéticos y no afectó la fluorescencia de clorofila *a*. Las plantas injertadas obtuvieron una respuesta positiva (P<0,05) en contenido de clorofila (~63 unidades SPAD) y longitud de la parte aérea (~106 cm), presentaron menor g_s (~163 mmol H_2O $m^{-2}s^{-1}$), número de hojas (~43 hojas) y peso de raíces (3,5 g de peso seco), pero no afectaron la fluorescencia de la clorofila *a*. El bioestimulante mitigó el efecto de la salinidad en g_s y pigmentos fotosintéticos. En el caso de salinidad, las plantas de gulupa sin injertar pueden presentar un mejor desempeño en etapa vegetativa, y el bioestimulante puede presentar un leve efecto en la mitigación del estrés salino. Sin embargo, si es indispensable utilizar plantas injertadas por causas sanitarias, la salinidad evaluada no las afecta drásticamente.

Palabras clave: *Passiflora maliformis*, *Ascophyllum nodosum*, estatus hídrico, fluorescencia de la clorofila.

Introduction

The purple passion fruit (*Passiflora edulis* f. *edulis* Sims), is a fruit crop from Brazil, belonging to the Passifloraceae family and cultivated in various Latin American countries due to their edaphoclimatic conditions (Armas Costa *et al.*, 2022). In Colombia, there are suitable conditions for cultivation of this fruit crop at elevations between 1,400 to 2,200 m a.s.l., temperatures from 15°C to 20°C, and minimum annual rainfall of 900 mm (Rodríguez-Polanco *et al.*, 2022). In 2022, Colombia had a national production of 32,353.78 t of the fruits harvested from the area of 2,059.47 ha (Agronet, 2023), with exports of 14600 t, representing a commercial value of USD 45.8 million FOB (Free on board). In 2023, this crop was the fifth most highly exported fruit from Colombia (ANALDEX, 2023). Currently, it is an important product in the international market due to its nutraceutical properties, presenting significant contents of vitamins A, B3, B12, and C, ascorbic acid, minerals, carbohydrates, proteins, and antioxidants (Armas Costa *et al.*, 2022).

Changes in climatic conditions resulting from climate change have a strong impact on the increase of soil salinity

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(Corwin, 2021). Soil salinization is the accumulation of salts in soil due to mineral deposition, capillary rise of groundwater, or agricultural and industrial practices (Eswar *et al*., 2021; Okur & Örçen, 2020). This condition has an adverse impact on the productivity and quality of purple passion fruit, which can be sensitive to salinity (Moura *et al*., 2019). In addition, purple passion fruit is cultivated in greenhouses with plastic coverings and in semi-covered conditions, using a fertigation system (Ocampo *et al*., 2020). These cultivation techniques increase salinity problems in this production system. Among the physiological impacts that are generated in plants under saline stress are osmotic stress, ionic stress, nutritional imbalance, and oxidative stress (Okon, 2019; Ullah *et al*., 2021). Collectively, these effects lead to a decrease in the hormone contents of auxins, gibberellins, cytokinins and to an increase in abscisic acid and ethylene, which reduces cell expansion, increases leaf abscission and stomatal closure, reduces nitrogen assimilation, decreases biomass of shoots and roots, and affects growth, development, and yields (Ahmad *et al*., 2022; Okon, 2019; Ullah *et al*., 2021).

Soil is saline if its electrical conductivity (EC) is greater than 4.0 dS m^{-1}, which is equivalent to 40 mM of NaCl. Na^+ is the most common ion that generates soil salinity, along with, but to a lesser extent, other ions such as Cl^- and SO_4^{-2} (Ahmad *et al*., 2022; Ullah *et al*., 2021). It has been established that plant tolerance to soil salinity decreases when EC exceeds 2.0 dS m^{-1} (Ahmad *et al*., 2022; Ullah *et al*., 2021). In *Passiflora*, saline stress with an EC greater than 1.4 dS m^{-1} in irrigation water is a limiting factor during the formation of seedlings and decreases growth, photochemical efficiency, photosynthetic pigment contents, and biomass production (Andrade *et al*., 2022; Bezerra *et al*., 2016; Nascimento *et al*., 2017).

Various strategies have been evaluated to mitigate the adverse effects generated by salinity in crops (Khalid *et al*., 2023). Among these strategies is the use of biostimulants, which are substances other than nutrients that benefit plants and can increase yield and quality of crops, photosynthetic activity, availability and absorption of soil nutrients, and tolerance to biotic and abiotic stress factors. Biostimulants can be classified depending on their mode of action and active component (Ahmad *et al*., 2022; Rakkammal *et al*., 2023). These biostimulants can be obtained from plants and/or algae extracts, including, in the case of vascular plants, leaves, roots, stems, and other organs (Ahmad *et al*., 2022). One kind of biostimulant is produced in algae, which are autotrophic organisms that can synthesize a large number of secondary metabolites. The extract of the *Ascophyllum nodosum* algae has been reported as a promoter of growth and yield increments in crops. Its action is based on triggering specific metabolic pathways in treated plants and providing organic compounds that may have various effects on plant metabolism (Ahmad *et al*., 2022; Carillo *et al*., 2020). *Ascophyllum nodosum* acts through various metabolic pathways that enable the activation of mechanisms of tolerance to salinity. These can include increases in enzymatic and non-enzymatic antioxidants, essential amino acids, K/Na ratio, beneficial mineral elements, and growth parameters (Carillo *et al*., 2020).

The use of biostimulants in various crops generates favorable effects on salinity tolerance. This includes the use of biostimulants elaborated from marine plants and algae on *Lactuca sativa* cultivated under saline conditions (40 mM NaCl), where increases of 9% and 18% of fresh weight under saline and non-saline conditions were obtained, respectively (Rouphael *et al*., 2022). Additionally, the application of hydrolyzed proteins together with *Ascophyllum nodosum* in *Solanum lycopersicum* plants generated a 31.8% increase in fruit yield under saline conditions and 16.9% increase under non-saline conditions (Ikuyinminu *et al*., 2022). There are some studies on physiological responses and impacts under saline stress in various passion fruit crops (Lima *et al*., 2023; Moura *et al*., 2020; Souza *et al*., 2018), but these studies are scarce for purple passion fruit.

Grafting is a rapid and non-chemical alternative to overcome the adverse effect of salinity (Mozafarian *et al*., 2023). In this respect, autografted *P. edulis* under salt stress conditions develops vital mechanisms that attenuate the effects of salinity (Moura *et al*., 2020). The purple passion fruit is commercially grafted on a rootstock of *P. maliformis* as a strategy to tolerate *Fusarium oxysporum* f. sp. *passiflorae* (Forero *et al*., 2015; López *et al*., 2023). It has been reported that water deficit reduces the growth of purple passion fruit grafted on *P. maliformis* (Jiménez-Bohorquez *et al*., 2024), but the effect of grafting on the growth of purple passion fruit under saline stress is almost unknown.

Given the above, the objective of the present study was to evaluate the effect of the application of an *Ascophyllum nodosum* extract on saline stress in grafted and non-grafted purple passion fruit plants (*Passiflora edulis* f. *edulis* Sims) at the vegetative stage of growth, considering variables of growth and physiology.

Materials and methods

Establishment of the experiment and plant material

The experiment was conducted at the greenhouses of the Faculty of Agricultural Sciences of the Universidad

Nacional de Colombia, Bogota (4°35'56" N, 74°04'51" W) at an altitude of 2650 m a.s.l., with temperature and average relative humidity of 19.8°C and 66.4%, respectively. The plant material consisted of 3-month-old purple passion fruit seedlings propagated by seeds (*Passiflora edulis* f. *edulis* Sims). Additionally, purple passion fruit plants were grafted as a terminal graft on *Passiflora maliformis* rootstock (propagated by seeds), both species being 3 months of age and grafted 45 d after germination. *Passiflora maliformis* is the main rootstock used in Colombia for the control of *Fusarium oxysporum* f. sp. *passiflorae* (Forero *et al*., 2015; Rodríguez *et al*., 2020). The plants were transplanted into polyethylene pots with dimensions of 20 cm x 20 cm x 20 cm containing a mixture of soil and Pindstrup® peat in a ratio 2:3 (v/v). The properties of these substrates are presented in Table 1.

Water was supplied to the plants using the gravimetric method adapted from Segura-Castruita *et al*. (2011), and fertilization was carried out with Nutriponic® (Walco S.A.S, Bogotá, Colombia) in a 5% v/v solution twice per week before the start of treatments, with a dose per plant of 15 ml during the first 10 d after transplant (DAT), 32 ml per plant between 10 and 20 DAT and 50 ml up to 30 DAT. Nutriponic® has the following composition: 40.3 g L^{-1} of NO_3^-, 4.0 g L^{-1} of NH_4^+, 20.4 g L^{-1} of P_2O_5, 50.6 g L^{-1} of K_2O, 28.8 g L^{-1} of Ca, 11.4 g L^{-1} of Mg, 1 g L^{-1} of S, 1120 mg L^{-1} of Fe, 112 mg L^{-1} of Mn, 12 mg L^{-1} of Cu, 26.4 mg L^{-1} of Zn, 106 mg L^{-1} of B, 1.2 mg L^{-1} of Mo, 0.36 mg L^{-1} of Co. After the start of the treatments, fertilizations were carried out twice a week with Nutriponic® and each plant was additionally fertilized with 50 ml of $MgSO_4$ and KNO_3 solution, where 47.8 g and 93.6 g were diluted, respectively, in 1 L of water.

Experimental design and treatments

A completely randomized design was used, with eight treatments (Tab. 2) that corresponded to the combination of the two levels of grafting (without grafting and with grafting), two levels of saline stress (without stress, application of saline solution at 5.0 dS m^{-1}), and the application or non-application of biostimulant (based on *Ascophyllum nodosum* at a dose of 1.5 g L^{-1}). The *Ascophyllum nodosum* extract corresponded to a product with registration number 451-F-AGR-P of the Acadian Plant Health and QSI Ecuador S.A. (Ecuador). Each treatment had 5 replicates, giving a total of 40 experimental units, each composed of one plant.

The saline stress consisted of subjecting plants to a constant stress period at 36 DAT and applying a dose of NaCl to maintain a constant electrical conductivity of 5.0 dS m^{-1} in

TABLE 1. Physicochemical characteristics of soil and composition of peat and biostimulant.

Parameter	Soil	Pindstrup® peat *	Biostimulant
Dry matter	-	55-75 g L^{-1}	-
Texture	Sandy loam	-	-
C organic	7.83%	-	-
Organic matter	-	70%	50%**
ECEC	7.48 meq 100 g^{-1}	-	-
pH	5.31	5.5-6	-
EC	-	1.0 mS cm^{-1}	-
N-total	0.66%	120 g	1%
N-ammonium	-	50 g	-
N-nitric	-	70 g	-
P	12 mg kg^{-1}	140 g	1%
K	0.83 meq 100 g^{-1}	240 g	20%
Ca	4.7 meq 100 g^{-1}	-	1%
S	90.1 mg kg^{-1}	-	-
Mg	0.99 meq 100 g^{-1}	23 g	1%
B	0.73 mg kg^{-1}		-
Cu	0.70 mg kg^{-1}		-
Mn	1.08 mg kg^{-1}	Traces	0.9%
Fe	10 mg kg^{-1}		0.01%
Zn	0.46 mg kg^{-1}		0.01%
Na	0.23 meq 100g^{-1}	-	-
Al	0.73 meq 100g^{-1}	-	-

*Nutrient content in 300 L of peat. **100% Pure and natural seaweed extract (*Ascophyllum nodosum*).

the solution. The application of the *Ascophyllum nodosum* extract began at 14 DAT. This consisted of 4 applications as a preventive measure before the start of saline stress, followed by continued application every 7 d from 32 DAT to the end of the experiment, with a dose of 1.5 g L^{-1}, 60 ml per plant in drench + foliar application with the adjuvant Mixel Top® until reaching total coverage.

TABLE 2. Description of treatments.

Treatment	Description
1	Non-grafted - salinity + *A. nodosum*
2 (control)	Non-grafted - salinity - *A. nodosum*
3	Non-grafted + salinity + *A. nodosum*
4	Non-grafted + salinity - *A. nodosum*
5	Grafted - salinity + *A. nodosum*
6	Grafted - salinity - *A. nodosum*
7	Grafted + salinity + *A. nodosum*
8	Grafted + salinity - *A. nodosum*

The growth variables and physiological variables were measured at the end of the experiment, that is 53 d after the salinity treatments began.

Growth variables

The aerial part length, the number of leaves, the fresh weights of roots, aerial part and whole plant were measured. Additionally, the leaf area (LA) was calculated using the foliar area meter LI-3100 (LI-COR Inc., Lincoln, NE, USA).

Physiological variables

Stomatal conductance

Stomatal conductance (mmol H_2O m^{-2} s^{-1}) was measured with a leaf porometer (SC-1, Decagon Devices Inc., Pullman, WA, USA). The measurements were made between 9:00 am and 10:00 am on leaves of the middle part, which were different from those used for fluorescence and chlorophyll contents.

Contents of chlorophylls and carotenoids

The relative chlorophyll content was measured with a chlorophyll meter (SPAD 502 plus, Konica Minolta, Japan) on 10 completely expanded leaves per plant from the middle strata. The extraction and quantification of total carotenoids and total chlorophylls were performed with acetone according to López-Gómez *et al.* (2015) and Wellburn (1994), using 300 mg of leaf tissue (M) and a final volume of 20 ml (V). Absorbance (A) reading was done with a spectrophotometer (Spectronic BioMate 3 UV–vis, Thermo, Madison, WI, USA) at wavelengths of 647 and 663 nm. To calculate the content of chlorophyll *a* (Chl a), chlorophyll *b* (Chl b), and total chlorophyll (Chl Total), Equations 1, 2, and 3 were applied, respectively.

$$\text{Chl a (mg g}^{-1}\text{)} = \frac{[(12.25 \times A_{663}) - (2.79 \times A_{647})] \times V}{1000 \times M} \quad (1)$$

$$\text{Chl b (mg g}^{-1}\text{)} = \frac{[(21.5 \times A_{647}) - (5.1 \times A_{663})] \times V}{1000 \times M} \quad (2)$$

$$\text{Chl Total (mg g}^{-1}\text{)} = \frac{[(7.15 \times A_{647}) + (18.7 \times A_{663})] \times V}{1000 \times M} \quad (3)$$

The results were expressed in mg g^{-1} of fresh weight (FW). For the quantification of total carotenoids, a calibration curve was carried out with different concentrations of β-carotene (Sigma-Aldrich Co., Nueva York, USA) and was measured in absorbance at 450 nm. Then, Equation 4 was used to establish the total carotenoid content.

$$\text{Total carotenoids (μg g}^{-1}\text{ FW)} = \frac{(A_{450} - b) \times V}{m \times M} \quad (4)$$

Chlorophyll a fluorescence

Chlorophyll *a* fluorescence was determined with the fluorometer JUNIOR-PAM (Walz®, Germany), on a fully expanded leaf from the middle third of the plant, subjected to 30 min of darkness, evaluating the electron transport rate (ETR), the photochemical quenching (QP) and non-photochemical quenching (QNP), and the maximum quantum efficiency of the photosystem II (*Fv/Fm*).

Statistical analysis

The data obtained were evaluated for normality and homoscedasticity through the Shapiro-Wilk and Levene tests, respectively. The statistical analysis was carried out using one-way analysis of variance and the treatment comparisons were carried out using the LSD test (P<0.05). All analyses were performed in the R 4.3.1 software.

Results and discussion

Growth parameters

Grafted plants without biostimulant applications and under salinity presented a greater length (P<0.05), while non-grafted plants without salinity and with and without *A. nodosum* generated the lowest response (Fig. 1A). On the other hand, non-grafted plants under salinity with biostimulants obtained a greater number of leaves (P<0.05), while the opposite trend occurred with plants with biostimulants and without salinity.

Regarding leaf area, non-grafted plants under salinity and without the application of *A. nodosum* presented a greater leaf area (P<0.05), while grafted plants with salinity and without application of *A. nodosum* presented a lower leaf expansion. Intermediate results were obtained for the control plants (Fig. 2).

For the weight of the aerial part, grafted plants without salinity and with the application of *A. nodosum* presented the greatest response (P<0.05), in contrast to the non-grafted plants with salinity and the application of *A. nodosum*, which obtained lower fresh weight of the aerial part. Intermediate results were obtained for the control plants (Fig. 3B). Regarding root fresh weight, the plants with the highest fresh weight (P<0.05) were those not grafted, regardless of the presence of salinity or *A. nodosum*, while those grafted had lower root fresh weights (P<0.05) (Fig. 3A). On the other hand, the non-grafted plants with salinity and without application of a biostimulant had a higher root dry weight (P<0.05) than the plants under the same treatment with grafting (Fig. 3C).

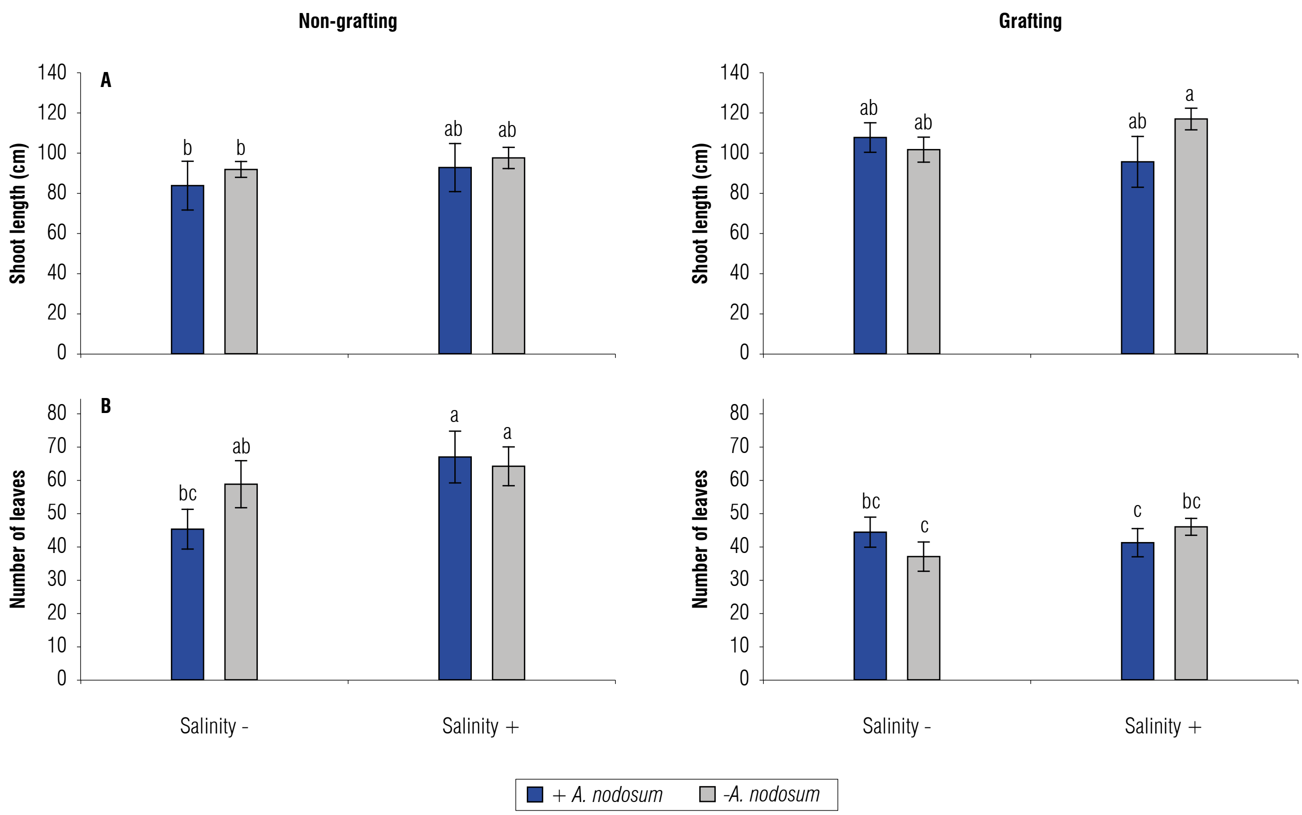

FIGURE 1. Effects of grafting and application of *Ascophyllum nodosum* on purple passion fruit plants subjected to salinity on (A) shoot length and (B) number of leaves. Averages followed by different letters indicate statistical differences according to the LSD test (P<0.05). Vertical bars in each column indicate the standard error (n=5).

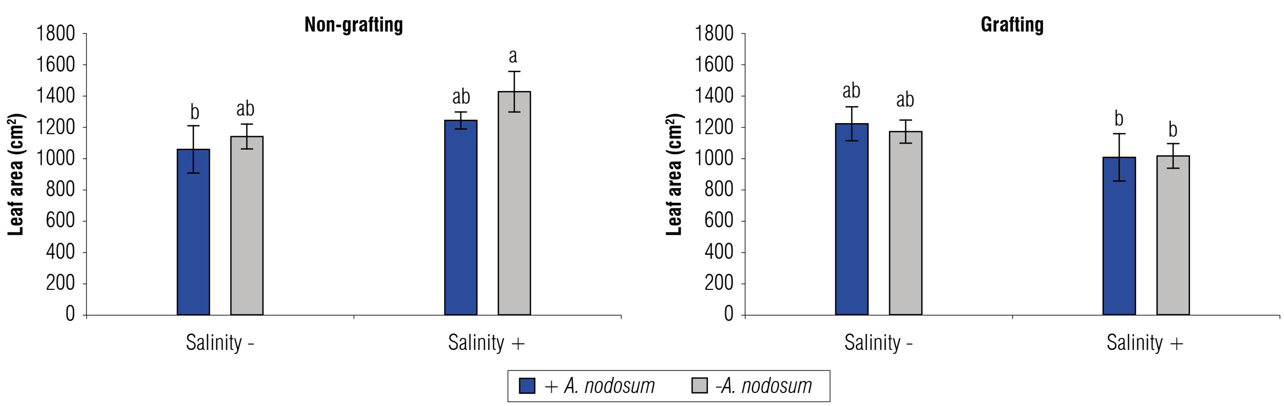

FIGURE 2. Effects of grafting and application of *Ascophyllum nodosum* on leaf area in purple passion fruit plants subjected to salinity. Averages followed by different letters indicate statistical differences according to the LSD test (P<0.05). Vertical bars in each column indicate the standard error (n=5).

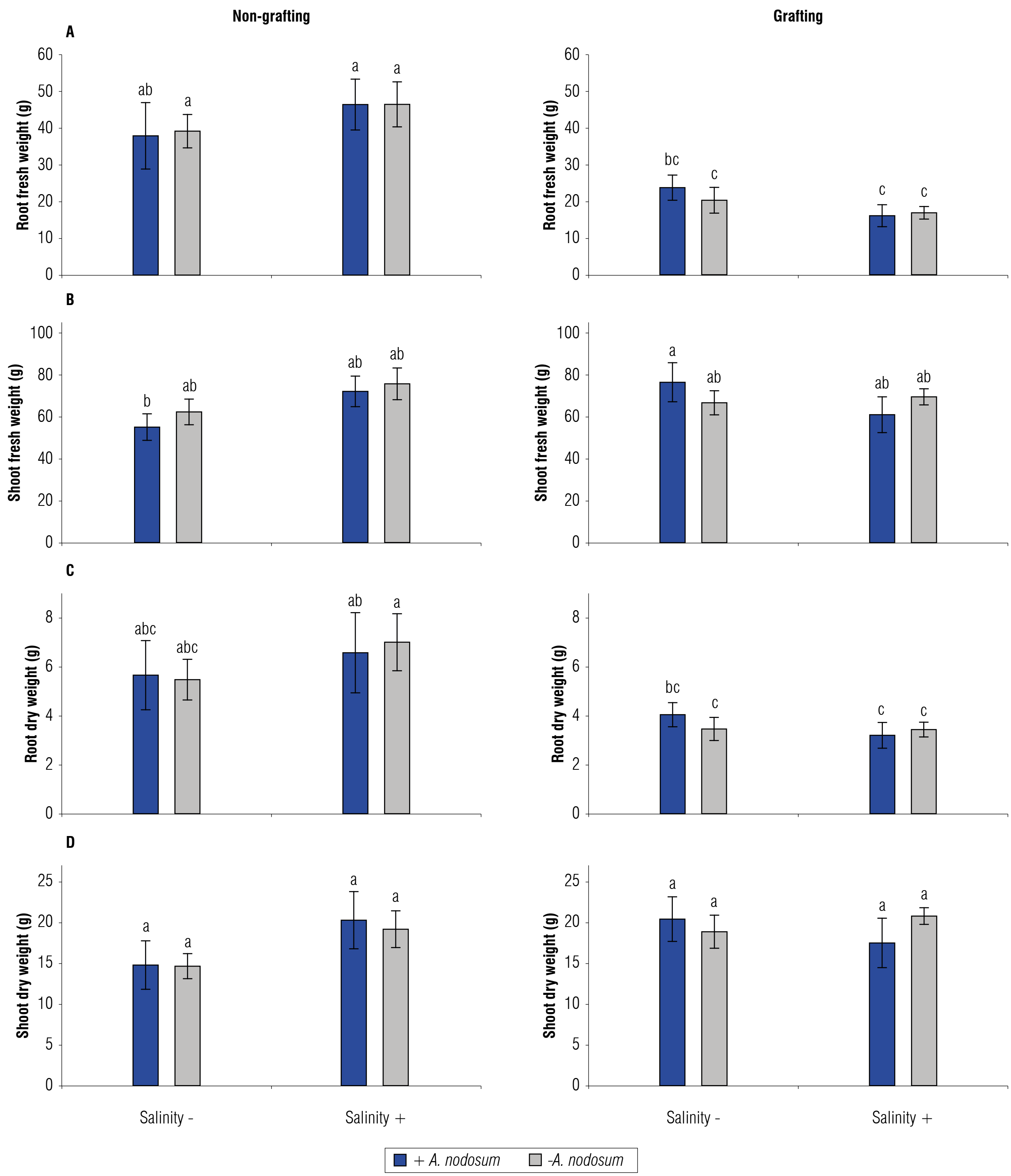

FIGURE 3. Effect of grafting and application of *Ascophyllum nodosum* in purple passion fruit plants subjected to salinity on (A) root fresh weight, (B) shoot fresh weight, (C) root dry weight, and (D) shoot dry weight. Averages followed by different letters indicate statistical differences according to the LSD test ($P<0.05$). Vertical bars in each column indicate the standard error (n=5).

A notable effect of grafting on shoot length was found. Apparently, the pattern of the *P. maliformis* generates a positive response in growth even in salinity conditions and without *A. nodosum* application. This is crucial to consider, as this rootstock also presents resistance to *Fusarium oxysporum* f. sp. *passiflorae,* and it was for this reason that it started to be used commercially as a rootstock in Colombia (Forero *et al*., 2015; López *et al*., 2020). However, the mechanism by which *P. maliformis* generates this response is unknown. These results differ from those obtained by Moura *et al*. (2020), where no significant differences were established for this variable in grafted plants of different passion fruit species under saline stress. However, a reduction in plant height was present at a concentration of 4.5 dS m^{-1}, indicating a differential response to salinity between passion fruit (*P. edulis*) and purple passion fruit (*P. edulis* f. *edulis* Sims).

Regarding the number of leaves, it was established that salinity, together with *A. nodosum,* increases the number of leaves in non-grafted plants, and that the non-grafted plants presented a greater number of leaves than the grafted plants (Fig. 1B). This response indicates a noticeable effect of the rootstock, leading to fewer leaves, which is an aspect that should be studied in greater depth. It has been found earlier that salinity in non-tolerant plants impacts plant growth by altering transpiration and stomatal conductance and generating high concentrations of Na and Cl that accumulate in the cytosol, affecting the expansion of mature leaves. Moreover, there is early senescence, abscission and reduction in formation of young leaves and lateral buds, which reduces the number of leaves (Carillo *et al*., 2020; Okon, 2019). This occurred in the purple passion fruit plants grafted on *P. maliformis*, while *P. edulis* plants have shown tolerance under electrical conductivity up to 4.7 dS m^{-1} in irrigation (Moura *et al*., 2016). Moura *et al*. (2020), for autografted *P. edulis* plants, found an increase in the number of leaves under salinity since the graft attenuated the salinity effects. On the other hand, Bonomelli *et al*. (2018) established that the application of *A. nodosum* on avocado plants under salt stress increased the plant height and number of leaves, in contrast to treatments with salinity. *A. nodosum* may promote plant growth under salinity by stimulating antioxidant responses and increasing the K/Na ratio in plants (Carillo *et al*., 2020).

Plants grafted under salinity presented the lowest leaf area, which correlates with the number of leaves (Fig. 2), but a higher shoot length (Fig. 1A), indicating greater internode length. The leaf area is decreased in non-tolerant plants to reduce water loss through transpiration and also to reduce the consumption of photosynthates, metabolic production (Moura *et al*., 2020), and accumulation of toxic ions in shoots (Lima *et al*., 2020). This coincides with what was reported by Bezerra *et al*. (2019), where passion fruit plants subjected to salinity presented a reduction in leaf area. Meanwhile, the larger leaf area found in non-grafted plants under salinity (Fig. 2) may be due to a possible beneficial effect of Na, a result not found in grafted plants. It is recommended to study this in more detail. In this case, *A. nodosum* had no mitigating effect on salinity, nor did it stimulate leaf area in plants without salinity.

While the highest shoot fresh weight was obtained with the application of *A. nodosum* in grafted plants without salinity (Fig. 3B), the fresh and dry weight of the root was higher in non-grafted plants than in grafted plants (Fig. 3A, 3C). This indicates a greater growth potential in purple passion fruit roots compared to *P. maliformis*, a result that warrants further attention in future studies. On the other hand, salt stress generates lesser growth and, therefore, reduced shoot and root weight (Rakkammal *et al*., 2023) due to the osmotic stress, stimulation of synthesis and accumulation of aminocyclopropane 1-carboxylic acid generated from the accumulation and absorption of toxic ions and the decrease in the foliar content of cytokinins and indole-3-acetic acid (Okon, 2019). Moura *et al*. (2019) found no significant differences in the dry weights of the aerial part and the root in *P. edulis* plants under salt stress. Likewise, Sá *et al*. (2018) found an increase in the fresh weight of the aerial part in passion fruit plants subjected to salt stress with biostimulant application.

Stomatal conductance

The grafted plants subjected to salinity and application of *A. nodosum* presented a lower stomatal conductance g_s ($P<0.05$) than non-grafted plants under biostimulant treatment without salinity (control), which had higher stomatal conductance (Fig. 4B). Low water availability and osmotic stress generate stomata closure, preventing water loss through transpiration (Jiménez-Bohórquez *et al*., 2024; Lozano-Montaña *et al*., 2021). This occurred in grafted plants under salinity, gradually increasing their temperature (Andrade *et al*., 2022; Okon, 2019). Similarly, Lima *et al*. (2020) and Lima *et al*. (2023) found a reduction in stomatal conductance in *P. edulis* plants subjected to salt stress. In contrast, Al-Ghamdi and Elansary (2018) obtained an increase in stomatal conductance in asparagus plants subjected to salt stress with applications of *A. nodosum* due to an increase in phenolic content, antioxidant activities, chlorophyll, sugars, and proline. The grafted purple passion fruit showed the lowest stomatal conductance, possibly because the rootstock (*P. maliformis*) induces some effect on the stomatal closure of the graft to avoid excessive water loss due to transpiration, potentially increasing ABA levels. This response is evident in plants with and without salinity.

Photosynthetic pigments

Grafted plants had higher relative chlorophyll contents (P<0.05) than non-grafted plants with and without salinity or *A. nodosum* (Fig. 4). Regarding photosynthetic pigments, higher concentrations of Chl a, Chl b, total Chl and total carotenoids (P<0.05) were observed in grafted and non-grafted plants subjected to salinity and with applications of *A. nodosum* (Fig. 5A).

Under salt stress, a negative effect on photosynthetic pigments is expected (Arif *et al*., 2020; Okon, 2019); however, our results show the opposite. Chlorophyll content can be considered a biochemical marker of tolerance, with plants that maintain or increase their chlorophyll content under high salinity concentrations being tolerant (Moura *et al*., 2020). This possibly occurred in non-grafted plants, whereas in grafted plants, increasing chlorophyll content may be a "concentration effect" because these plants have a lesser leaf area (Fig. 2). The biostimulant *A. nodosum* contains high levels of K, Ca, and proline, and generates greater activity of antioxidant enzymes, leading to reduced osmotic stress and salt stress through the elimination of reactive oxygen species (ROS), thus, allowing the plants to improve their physiological response and resistance to salinity (Rakkammal *et al*., 2023).

Moura *et al*. (2020) reported that grafted *P. edulis* plants subjected to salt stress had a reduction in chlorophyll contents. Likewise, Andrade *et al*. (2022) found a reduction in chlorophyll *a* concentration and an increase in chlorophyll *b* and total carotenoids in passion fruit plants subjected to salt stress. Furthermore, Al-Ghamdi and Elansary (2018) obtained an increase in photosynthetic pigments in asparagus plants subjected to salt stress with *A. nodosum*.

Chlorophyll *a* fluorescence

Regarding the electron transport rate (ETR), non-photochemical quenching (QNP), and maximum quantum efficiency of photosystem II (*Fv/Fm*), there were no significant differences between the treatments (Fig. 6), while the photochemical quenching (QP) presented a greater value (P<0.05) in the grafted plants without salinity and

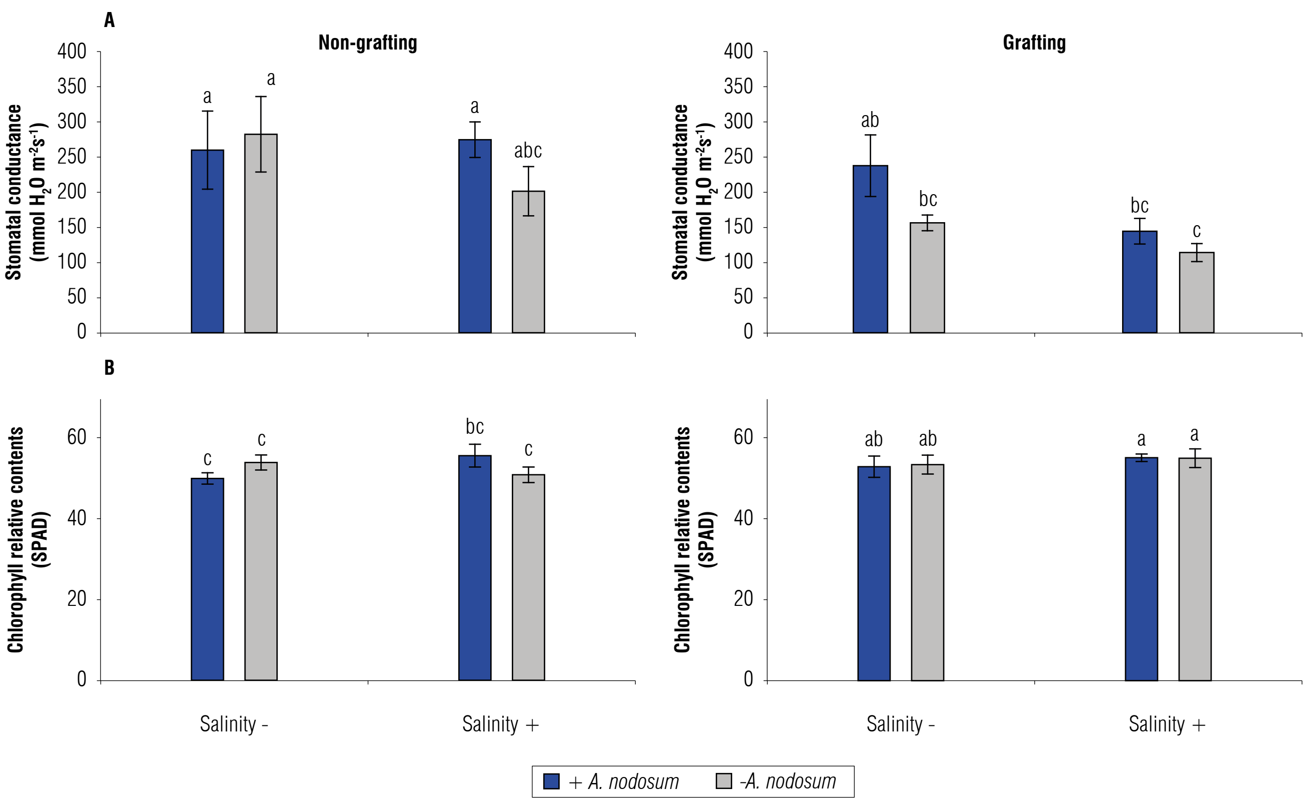

FIGURE 4. Effect of grafting and application of *Ascophyllum nodosum* in purple passion fruit plants subjected to salinity on (A) stomatal conductance (g_s), and (B) chlorophyll relative contents (SPAD units). Averages followed by different letters indicate statistical differences according to the LSD test (P<0.05). Vertical bars in each column indicate the standard error (n=5).

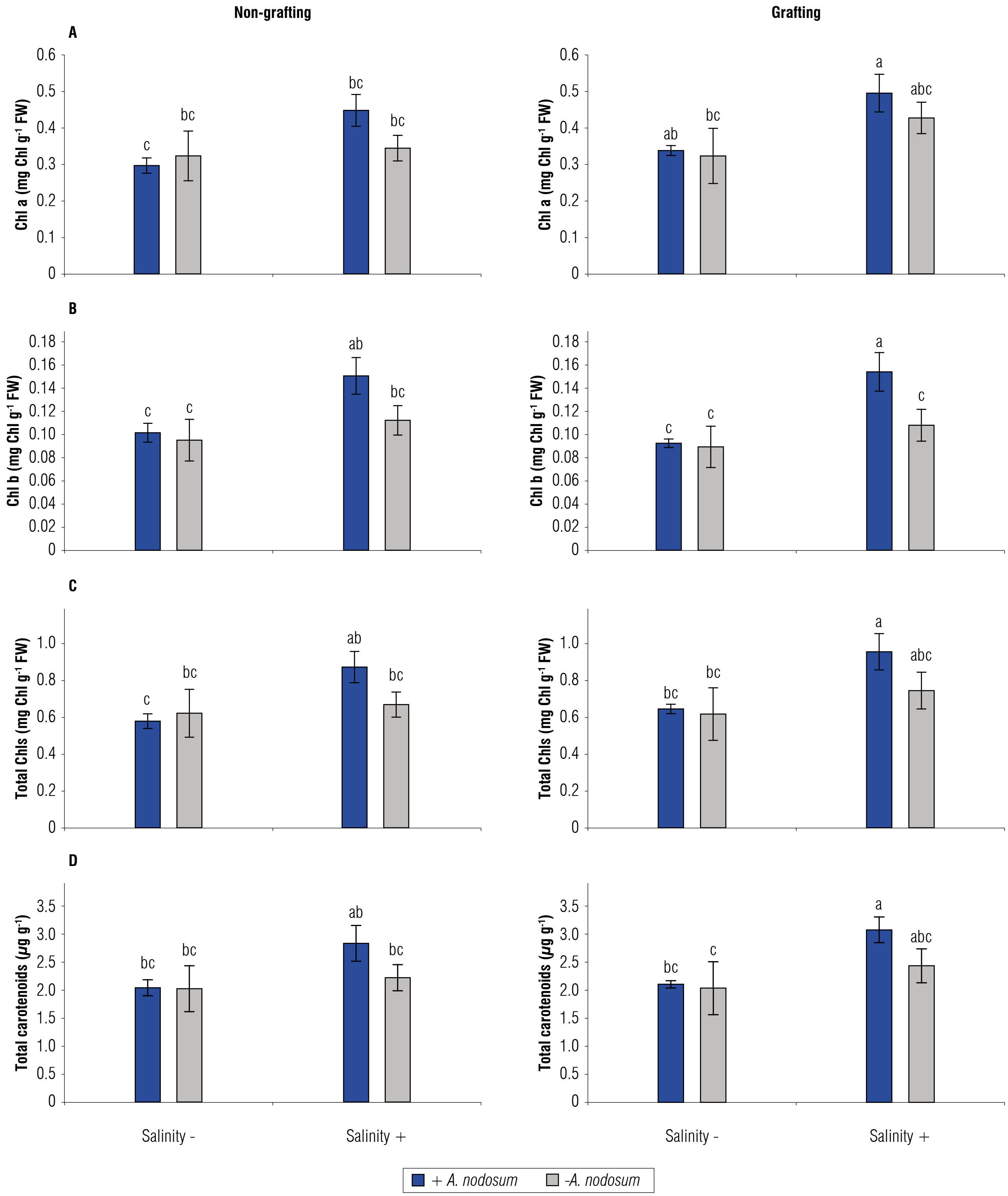

FIGURE 5. Effect of grafting and application of *Ascophyllum nodosum* in purple passion fruit plants subjected to salinity on (A) chlorophyll *a* (Chl a), (B) chlorophyll *b* (Chl b), (C) total chlorophylls (Total Chls), and (D) total carotenoids. Averages followed by different letters indicate statistical differences according to the LSD test ($P<0.05$). Vertical bars in each column indicate the standard error (n=5).

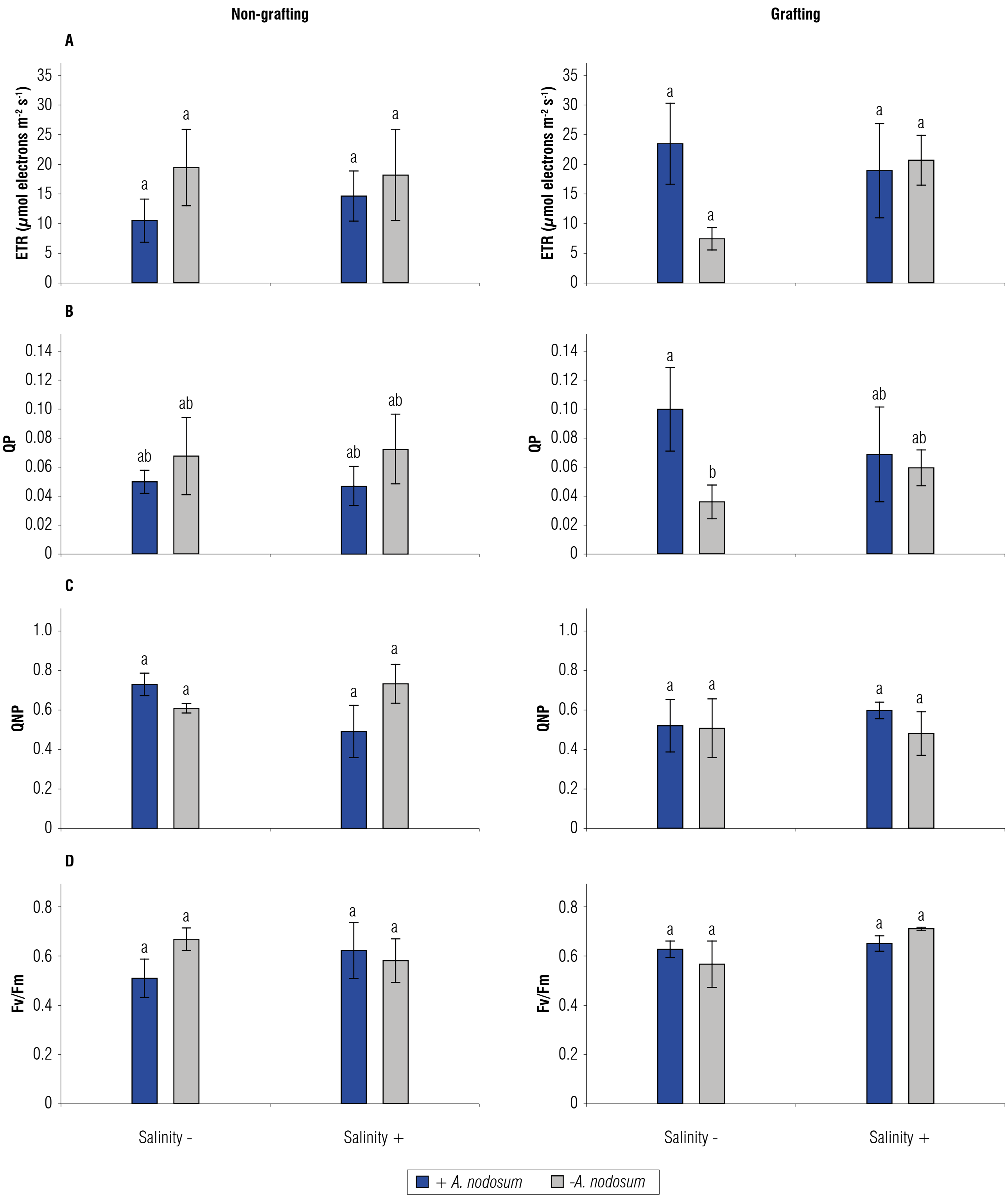

FIGURE 6. Effect of grafting and application of *Ascophyllum nodosum* in purple passion fruit plants subjected to salinity on (A) electron transport rate (ETR), (B) photochemical quenching (QP), (C) non-photochemical quenching (QNP), and (D) the maximum quantum efficiency of the photosystem II (*Fv/Fm*). Averages followed by different letters indicate statistical differences according to the LSD test ($P<0.05$). Vertical bars in each column indicate the standard error (n=5).

with applications of *A. nodosum* than in the grafted plants without salinity and without *A. nodosum*. The control plants showed an intermediate response (Fig. 6B).

The reductions in the fluorescence parameters occur due to damage in the PSII of the plants. In this case, the salt stress applied to purple passion fruit does not alter PSII, indicating adequate functioning of PSII and stability of the photosynthetic apparatus under salt stress (Guedes *et al*., 2023). Interestingly, in grafted plants, the biostimulant tended to increase QP, which may be due to antioxidant mechanisms induced by *A. nodosum*. This is consistent with Guedes *et al.* (2023), there was an increase in QP and ETR and a non-significant increase in *Fv/Fm* in passion fruit plants subjected to salt stress with the application of fertilizers.

Conclusions

These results suggest that purple passion fruit without grafting exhibit tolerance to salt stress (5.0 dS m^{-1}). This plant showed good performance in growth parameters such as leaf number, leaf area, shoot and root weights, and g_s (Fig. 7). Meanwhile, the grafted plants with *A. nodosum*

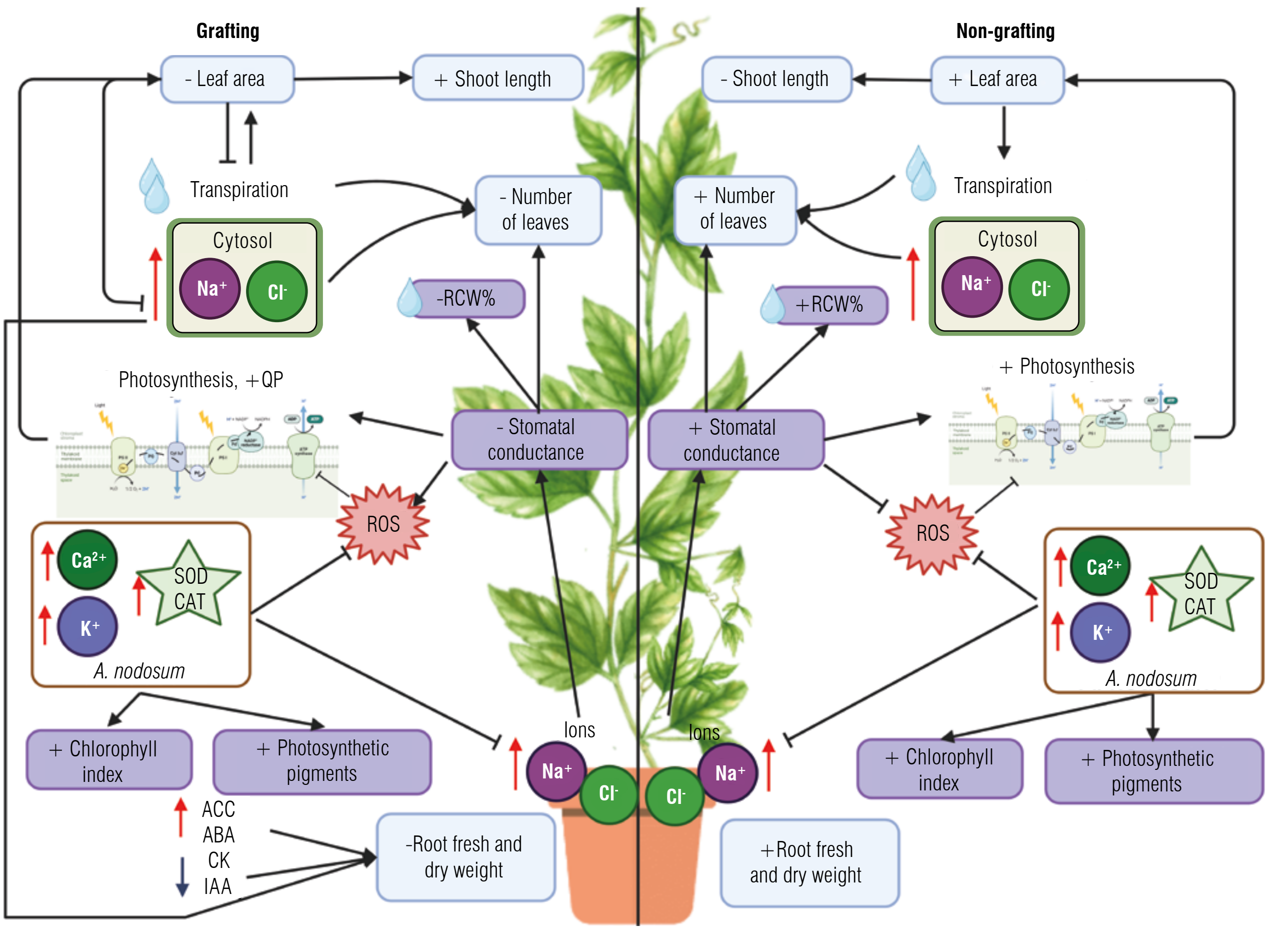

FIGURE 7. Model of the effect of grafting and application of *Ascophyllum nodosum* on purple passion fruit plants subjected to salinity. Left) Plants grafted on *P. maliformis*, Right) plants without grafting. In both cases, salinity initially generated osmotic stress, reducing water availability due to a decreased osmotic potential in soil solution. This caused a lower stomatal conductance in the grafted plants compared to the non-grafted plants, leading to a gradual increase in temperature in non-grafted plants. However, non-grafted plants presented a higher relative water content (RCW) and a lower inhibition of photosynthesis than the grafted plants. Subsequently, ionic stress was generated that harmed leaf expansion, increased leaf abscission, and impaired photosynthesis, especially in grafted plants. This stress indirectly benefits the plants by reducing water loss through transpiration. Lesser leaf emission is also related to the stimulation of synthesis of abscisic acid (ABA), and aminocyclopropane carboxylic acid (ACC) and to the decrease in the foliar content of cytokinins (CK) and indole acetic acid (IAA). Oxidative stress may be induced by the increased reactive oxygen species (ROS) production, impacting the permeability of the plasma membrane and photosynthesis. Finally, photosynthetic pigment contents were reduced, possibly due to the nutritional imbalance. The biostimulant *A. nodosum*, with its proline content and greater antioxidant enzyme activity, likely reduced osmotic and salt stress by eliminating ROS, thereby enhancing physiological response and resistance to salinity in non-grafted plants. In grafted plants, *A. nodosum* increased the contents of chlorophyll and photosynthetic pigments. SOD – superoxide dismutase, CAT – catalase, QP - photochemical quenching.

applications had significantly higher concentrations of photosynthetic pigments and QP. However, if grafting is required for sanitary reasons (e.g., management of *Fusarium oxysporum* f. sp. *passiflorae*), the evaluated salinity does not affect them drastically. Further research is recommended to confirm and establish the tolerance pathways of this species to salt stress. Additionally, evaluating different doses of *A. nodosum* on *P. edulis* under field conditions and varying salinity conditions will be necessary.

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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

AMOR: conceptualization, research, writing of original draft, visualization, writing, and editing. JECB: conceptualization, research, writing of original draft. HEBL: conceptualization, visualization, writing, and editing. All authors have read and approved the final version of the manuscript.

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Evaluation of top-dressing fertilization with nitrogen for the morphophysiology of wheat crops

Evaluación de la fertilización de cobertura con nitrogeno sobre la morfofisiología de cultivos de trigo

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ABSTRACT

The aim of this study was to evaluate the influence of different management of nitrogen fertilization in top dressing on the morphophysiology of spring wheat cultivars in a low-altitude subtropical environment of Brazil. The experiment was carried out in 2022 in a greenhouse. The experiment had a randomized block design. We evaluated 10 wheat cultivars with two different applications of nitrogen with three replicates. The variables evaluated included the number of fertile tillers, plant height, length of the main spike and tiller, number of spikelets in the main spike and tiller, number of grains in the main spike and tiller, number of grains per plant and mass of grains in the main spike and tiller. For wheat cultivated in a low-altitude subtropical environment, nitrogen installments have no influence on the number of fertile tillers, plant height, or crop yield components. It was possible to infer that the difference obtained in the tillering pattern of the cultivars tested was due to their genetic potential.

Key words: *Triticum aestivum* L., plant physiology, tillering, plant height.

RESUMEN

El objetivo de este estudio fue evaluar la influencia de diferentes manejos de fertilizantes nitrogenados de superficie sobre la morfofisiología de cultivos de trigo de primavera en un ambiente subtropical de baja altitud de Brasil. El experimento se llevó a cabo en 2022 en un invernadero. El diseño experimental utilizado fue de bloques al azar, 10 cultivos de trigo y dos momentos de aplicación de nitrógeno fueron evaluados, con tres repeticiones. Las variables evaluadas incluyeron número de macollos fértiles, altura de la planta, longitud de la espiga principal y del macollo, número de espiguillas en la espiga principal y del macollo, número de granos en la espiga principal y en el macollo, número de granos por planta y peso de granos en la espiga principal y el macollo. Cuando se cultiva trigo en un ambiente subtropical de baja altitud, los aportes de nitrógeno no influyen en el número de macollos fértiles, la altura de la planta o los componentes del rendimiento del cultivo. Se pudo inferir que la diferencia obtenida en el patrón de macollamiento de los cultivos ensayados se debió a su potencial genético.

Palabras clave: *Triticum aestivum* L., fisiología de plantas, macollamiento, altura de planta.

Introduction

Wheat (*Triticum aestivum* L.) is an annual grass grown in southern Brazil mainly during the winter period during the months of June, July, August, and September. It is an important crop of sustainable agricultural production systems as an alternative to winter crop rotation in production systems (Barro *et al*., 2017).

The use of management practices that optimize resources contributes to increasing wheat yields in Brazil. Among the determining factors in establishing the crop productive potential are genetics and fertilization; these boost grain yields, since wheat is one of the main cereal crops that provides protein, carbohydrates, minerals, and vitamins for most of the world population (Conab, 2017). Wheat grain is used to make flour and is used in different ways depending on the quality of the grain, grain yield, endosperm content, the proportion of protein in the flour and the quality of the protein; all of these determine the quality of the wheat cultivar (Ahmed & Fayyaz-ul-Hassan, 2015). The genetic improvement of any crop is a fundamental tool, as it provides the launch of cultivars with different characteristics such as high production potential, wide adaptation to contrasting soils and environments, and tolerance to disease.

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To increase productivity in wheat, it is necessary to increase the yield potential in tillage conditions, where cultivars need to interact with different environmental and management situations; it is necessary to identify cultivars that respond to specific environmental stimuli (Benin *et al*., 2012; Scheeren, 1999). In order to obtain high yields, the following aspects are important: management adjustments and rational use of available resources among which nitrogen fertilization and the choice of cultivars best adapted to the growth environment stand out as important aspects (Ecco *et al*., 2020; Silva *et al.,* 2024). From a nutritional point of view, nitrogen (N) is one of the most important elements, fundamental for plant metabolism and development, acting in the expansion and cell division of the photosynthetic area, in addition to being an essential component of proteins, nucleic acids, and chlorophyll (Silva *et al.,* 2024; Taiz *et al*., 2017).

One way in which various nitrogen management strategies can help to achieve high grain yields is by increasing the number of tillers per plant (Ecco *et al*., 2020). This means that the final number of fertile tillers is related to the number of ears and an increase in grains per area, leading to higher yields from wheat in a subtropical environment. The emergence and survival of tillers is associated with factors such as plant population density, N availability, temperature, salinity, and other factors (Shang *et al*., 2021).

In order to obtain high productive potential of wheat crops, monitoring soil fertility and plant nutrition are also essential issues, considering that N is the nutrient that has greater requirement and uptake by wheat plants. This element also has a strong influence on the productivity of wheat (Prando *et al*., 2013). Deficiencies in N can cause a reduction in evapotranspiration and water use efficiency and a decrease in leaf size related to solar radiation use efficiency that causes a drop in the photosynthetic rate, as well as a limitation in the number of tillers that also reduce the number of stalks and ears per area and, consequently, wheat yield (Ferreti & Fernandes, 2011). The use of N fertilization contributes to an increase in tillering, as well as an increase in the growth of the wheat canopy (Pietro-Souza *et al*., 2013). Therefore, mineral N fertilization represents a significant cost in wheat yield (Cui *et al*., 2014). Moreover, good N agronomic practices that help farmers increase productivity, reduce production costs, and diversify wheat production should be studied to guarantee the sustainability and viability of wheat crops, as well as lowering Brazilian independence from the import of this cereal (Kaneko *et al*., 2010; Pires *et al*., 2019).

Studies evaluating the morphophysiology of wheat cultivars associated with N management are limited to cultivars that have only been launched in the Brazilian market for a short time. It is necessary to verify whether and which of the moments of N fertilization in top dressing provide better tillering conditions and, consequently, greater grain yields. The objective of this study was to evaluate the influence of N fertilizer management strategies on the morphophysiology of spring wheat cultivars in a low-altitude subtropical environment.

Materials and methods

The experiment was carried out in the 2022 crop season in a greenhouse at the Phytotechnics Department of the Universidade Federal de Santa Maria - RS. The climate, according to Köppen classification system, is Cfa, humid subtropical, with warm summers during the months of December, January, February, and March, and no defined dry season. The crop was sown on June 22, 2022, within the recommended sowing period according to the Agricultural Risk Zoning (Zoneamento Agrícola de Risco Climático - ZARC) (Ministério da Agricultura e Pecuária, 2021). We planted the crop in 20 L pots with 16 plants per pot, filled with a soil classified as Arenic Profondic Rhodic Acrisol (ISSSWG RB, 1998). The pots were placed on benches under a Van der Hoeven greenhouse made with a galvanized iron and polycarbonate structure with temperatures ranging from 10°C to 32°C and irrigation according to the crop's demand.

The experiment used a randomized block design, in a bifactorial arrangement 10x2, consisting of ten wheat cultivars and two periods of N application with three replicates. The following cultivars were used: TBIO Audaz, TBIO Aton, TBIO Toruk, TBIO Ello, TBIO Calibre, TBIO Fusão, TBIO Ponteiro, TBIO Duque, TBIO Astro and TBIO Trunfo (Tab. 1). The trial consisted of 60 experimental units made up of 20 L white pots distributed on 3 benches, each with 20 pots, representing each block. The population used in the trial was 16 plants per pot, and the adjustment was made based on the cultural value of the seeds, so that the recommended population for wheat was 300 plants m^{-2}.

To fertilize the soil in pots, we sampled and analyzed the soil and corrected the pH before the pots were filled using limestone in coverage and applied two months before sowing, based on the recommendations of the fertilization and liming manual for the states of Rio Grande do Sul and Santa Catarina (Comissão de Química e Fertilidade do Solo

TABLE 1. Description of cultivars tested for cycle and industrial quality.

Cultivars	Cycle	Classification
TBIO Audaz	Precocious	Improver
TBIO Aton	Medium	Bread/improver
TBIO Toruk	Medium	Bread/improver
TBIO Ello	Medium	Bread
TBIO Calibre	Super precocious	Bread/improver
TBIO Fusão	Precocious	Improver
TBIO Ponteiro	Mid-late	Bread
TBIO Duque	Precocious	Bread/whitener
TBIO Astro	Super precocious	Improver
TBIO Trunfo	Precocious	Bread

Commercial cultivars were obtained from the holder BIOTRIGO Genética (Brazil).

– RS/SC, 2016). Base fertilizer was applied on the surface at a dose of 200 kg ha^{-1} of 5-20-20 (N-P_2O_5-K_2O) formulation, equivalent to 1.2 g of fertilizer per pot.

When N top dressing fertilizer was applied to meet the N crop requirement during its production cycle, N was spread with two different methods. The first was to apply 50% of the recommended dose in installments, when the wheat was at the beginning of tillering (V3/V4 vegetative stage) and the remaining 50% was spread at the beginning of elongation (V7/V8 vegetative stage) according to the Zadoks Decimal Scale by Zadoks (1974). The second strategy for applying N was to apply 100% of the recommended dose at the beginning of tillering (vegetative stage V3/V4). For crop management, physical weed control was carried out; and, for pests and diseases affecting the crop, we followed the technical recommendations for wheat (Comissão Brasileira de Pesquisa de Trigo e Triticale, 2022).

On June 28, 2022 the seedling emergence rate was assessed in each pot and thinning was performed where necessary. Four plants in the useful area of the experimental unit were marked with colored wire. Beginning from July 15, 2022 some of the cultivars began to be tillered and weekly evaluations began: we counted the number of tillers and checking the plant height of the four plants previously marked in each pot.

Before the plants were harvested, we collected the four marked plants to assess the number of fertile shoots and other crop yield components. We harvested the crop manually on October 26, 2022, when the plants were mature, taking into account the difference in cycle between the cultivars. Later, in the laboratory of the Research Group on Ecophysiology and Management of Annual Crops (Grupo de Pesquisa em Ecofisiologia e Manejo de Culturas Anuais - GEMCA), we evaluated the yield components and characters related to grain yield using a measuring tape and precision scale as follow: number of fertile tillers = tillers that had spikelets with grain formation (NFT), plant height (PH), main spike length (SL_main), tiller spike length (SL_til.), number of spikelets on the main spike (NSS_main), number of spikelets on the tiller spike (NSS_til.), number of grains in the main spike (NGS_main), number of grains in the tiller spike (NGS_til.), number of grains per plant (NG), main spike grain mass fresh (MGS_main) and tiller spike grain mass fresh (MGS_til.).

Statistical analysis

We subjected the characters evaluated to a two-way analysis of variance; and for the crop yield components we grouped the means of the treatments using the Scott-Knott test with a significance level of 5% probability of error. For these analyses, we used the Sisvar statistical software and the Microsoft Office Excel® software to insert the data and draw up the tables.

Results and discussion

The different moments of N application did not differ statistically (P>0.05) for the factor number of fertile tillers (Tab. 2). Similar results were also identified for plant height and some yield components, such as SL, NSS, NGS, and MGS. For the environmental conditions of the experiment, the N installment did not influence the number of fertile tillers, plant height, and crop yield components. Due to adequate irrigation and temperature control in the greenhouse, the difference in the tillering pattern of the evaluated cultivars could be due to their genetic potential, which corroborates the data obtained by Orso *et al.* (2014). The wide variety of cultivars currently available on the market allows the producer to choose the genetic material most adapted to

TABLE 2. Average number of fertile tillers per plant produced by different nitrogen managements applied to the wheat crop, Santa Maria/RS, Brazil.

Nitrogen management	Number of fertile tillers
50% of the recommended N dose applied at vegetative stage V3/V4 (beginning of tillering) and the remaining 50% applied at vegetative stage V7/V8 (full tillering)	2.28 ns*
100% of the recommended N dose applied at vegetative stage V3/V4 (beginning of tillering)	2.40
Overall average	2.34
CV (%)	29.93

CV – coefficient of variation. * ns – not significant using the Scott-Knott test at 5% significance level.

the production system, level of technology, and investment capacity (Pires *et al*., 2005).

In relation to the analysis of variance of the characters evaluated (Tab. 3), we found a low coefficient of variation (CV %) for PH, SL_main, and NSS_main indicating good experimental precision (CV less than 10%), demonstrating the reliability of the tests (Pimentel-Gomes, 1990). The other characters, NFT, SL_til., NSS_til., NGS_main, NGS_til., MGS_main, and MGS_til., had a high coefficient of variation, which may indicate difficulties in measuring these characters. This indicates a lower reliability of results, therefore, in future experiments, a greater number of plants per experimental unit or a greater number of replicates should be used.

The average MGS_til. of each cultivar were presented separately for each type of N fertilizer management (Tab. 4).

Only for the cultivar TBIO Audaz did we observe a significant difference between management A in relation to nitrogen management B. For this cultivar, the application of 50% of the N dose recommended at the beginning of tillering and 50% of the recommended dose applied at the beginning of elongation provided higher MGS_til. values compared to management B, where 100% recommended dose of N was applied at the beginning of tillering of the crop. Similar results are reported by Costa *et al.* (2013) and by Mattuela *et al.* (2018).

TABLE 4. Fresh grain weight of tiller spike (MGS_til.) averages for each cultivar presented separately for the two types of nitrogen fertilizer management, Santa Maria/RS, Brazil.

Cultivar	N fertilizer management	MGS_til.
TBIO Audaz	A	0.87 a
	B	0.26 b
TBIO Aton	A	0.41 a
	B	0.47 a
TBIO Toruk	A	0.43 a
	B	0.64 a
TBIO Ello	A	0.53 a
	B	0.81 a
TBIO Calibre	A	0.57 a
	B	0.62 a
TBIO Fusão	A	0.6 a
	B	0.83 a
TBIO Ponteiro	A	0.55 a
	B	0.54 a
TBIO Duque	A	0.65 a
	B	0.51 a
TBIO Astro	A	0.55 a
	B	0.61 a
TBIO Trunfo	A	0.65 a
	B	0.55 a

CV – coefficient of experimental variation in %. N fertilizer management A - 50% of the recommended N dose applied at vegetative stage V3/V4 (beginning of tillering) and the remaining 50% applied at vegetative stage V7/V8 (full tillering), B - 100% of the recommended N dose applied at vegetative stage V3/V4 (beginning of tillering). * Averages followed by the same letter in the column do not differ by the Scott-Knott test at a 5% significance level.

TABLE 3. Mean squares of the analysis of variance (ANOVA) for productive and morphological characters evaluated in ten wheat cultivars, Santa Maria/RS, Brazil.

SV	DF	NFT	PH	SL_main	SL_til.	NSS_main	NSS_til.
Cultivar	9	0.73	81.75	2.89	1.59	3.28	1.50
Nitrogen	1	0.23	1.58	1.70	0.33	0.65	4.11
Cult X Nitrog	9	0.22	8.60	0.73	0.52	3.37	2.97
Error	40	0.49	22.13	0.87	0.99	2.53	2.99
Mean	-	2.34	62.80	8.71	7.40	15.43	11.15
CV (%)	-	29.93	7.49	10.69	13.45	10.31	15.51

SV	NGS_main	NGS_til	MGS_main	MGS_til.
Cultivar	41.59	41.57	0.13	0.03
Nitrogen	4.00	0.02	0.12	0.00
Cult X Nitrog	51.25	34.48	0.04	0.10
Error	35.95	28.79	0.05	0.04
Mean	36.98	16.66	1.53	0.58
CV (%)	16.22	32.20	15.23	36.17

CV – coefficient of variation. NFT - number of fertile tillers, PH -plant height, SL _ main - main spike length, SL _ til. -tiller spike length, NSS _ main - number of spikelets on the main spike, NSS _ til. - number of spikelets on the tiller spike, NGS _ main - number of grains in the main spike, NGS _ til. - number of grains in the tiller spike, NG - number of grains per plant, MGS _ main - main spike grain mass, and MGS _ til. - tiller spike grain mass.

The results of the averages of the plant traits (Tab. 5) show that a balance is needed between NGS and MGS, both in the main ear and in the tillers. Despite having a considerable number of grains per ear, they may not have a high mass. The quantity of grains produced per area verified in the NGS_main does not directly express high MGS_main; productivity will end up being influenced by other factors. The MGS_main was segregated into two groups according to the average grain weight. In the group with the highest MGS_main averages made up of TBIO Toruk, TBIO Calibre, TBIO Fusão, TBIO Duque and TBIO Trunfo there was no interaction with NGS_main where cultivars that showed the highest value for this variable did not necessarily show the highest MGS_main values.

The TBIO Duque cultivar had the highest NGS_main, and MGS_main also fell into the group with the highest MGS_main averages. Meanwhile, TBIO Audaz and TBIO Astro have the lowest NGS and MG values for the main ear. For NGS_til., TBIO Toruk has the lowest averages and TBIO Ello has the highest averages, but they do not make up the highest MGS_til. Direct selection using the number of grains per ear, considering the thousand-grain mass, is the best strategy for obtaining superior genotypes in terms of grain yield (Vesohoski *et al*., 2011). Indirect selection for grain yield, when it comes to ear weight, considering the number of grains per ear and/or grain mass, is the best indirect selection strategy for choosing superior genotypes (Caierão *et al*., 2001).

For the SL, NSS and NGS characters, despite there being no statistical difference for the cultivar factor, the greater length of ears does not imply a greater number of spikelets per ear or number of grains per ear (Tab. 5) in absolute values. An example of this is the performance of the TBIO Ponteiro cultivar that, despite having the highest SL_main and the highest NSS_main, has the lowest NGS_main. Meanwhile, although TBIO Calibre has one of the lowest SL_main and NSS_main values, it had one of the highest NGS_main values. This differs from Silva *et al.* (2005), who observe that the higher the number of grains per spike (NGS), the greater spike length (SL) and the greater the number of spikelets per spike (NSS).

One of the reasons for the low average yield of spring wheat crops is attributed to the small share of fertile tillers in the final yield (Mundstock, 1999). This can be seen by observing the lower contribution of the tillers to the yield components when compared to the main ear (Tab. 5). There was no statistical difference for the cultivar factor in NFT, but TBIO Ello was superior in the final number of fertile tillers, and TBIO Duque was inferior for the same variable (Tab. 6). However, TBIO Duque stands out in terms of NGS and MGS when compared to TBIO Ello and the other cultivars analyzed. In this case we can infer that the greater number of fertile tillers did not interfere with higher grain yields, but we can consider it a safety mechanism; in years with lower than ideal production, the compensation of fertile tillers can help maintain grain yields.

TABLE 5. Plant characters, SL_main, SL_til., NSS_main, NSS_til., NGS_main, NGS_til., MGS_main and MGS_til., associated with grain yield in ten wheat cultivars, Santa Maria/RS, Brazil.

Cultivar	SL_main (cm)	SL_til. (cm)	NSS_main	NSS_til.	NGS_main	NGS_til.	MGS_main (g)	MGS_til. (g)
TBIO Audaz	8.76 b*	7.09 ns	15.79 ns	10.98 ns	32.29 ns	14.42 ns	1.51 b	0.56 ns
TBIO Aton	7.97 b	6.93	14.88	10.63	38.38	14.64	1.38 b	0.44
TBIO Toruk	9.47 a	7.68	15.92	11.58	37.79	13.69	1.73 a	0.53
TBIO Ello	8.10 b	7.03	14.75	10.61	36.13	21.58	1.33 b	0.67
TBIO Calibre	8.25 b	7.15	14.79	11.00	38.33	15.23	1.66 a	0.59
TBIO Fusão	8.94 a	7.78	16.25	11.39	37.67	17.20	1.65 a	0.71
TBIO Ponteiro	9.12 a	7.98	15.63	12.03	36.25	17.20	1.41 b	0.55
TBIO Duque	8.44 b	6.80	15.13	10.72	40.17	16.06	1.65 a	0.58
TBIO Astro	7.98 b	7.18	14.46	10.83	32.96	20.73	1.36 b	0.58
TBIO Trunfo	10.05 a	8.35	16.70	11.73	39.79	15.86	1.60 a	0.60
Mean	8.71	7.40	15.43	11.15	36.98	16.66	1.53	0.58
CV (%)	10.69	13.45	10.31	15.51	16.22	32.20	15.23	36.17

CV - coefficient of experimental variation in %. * Averages followed by the same letter in the column do not differ by the Scott-Knott test at a 5% significance level. ns – not significant according to the Scott-Knott test at 5% significance level. SL _ main - main spike length, SL _ til. - tiller spike length, NSS _ main - number of spikelets on the main spike, NSS _ til. - number of spikelets on the tiller spike, NGS _ main - number of grains in the main spike, NGS _ til. - number of grains in the tiller spike, MGS _ main - main spike grain mass, and MGS _ til. - tiller spike grain mass.

These contrasts with Camponogara *et al.* (2016). They show that treatments with greater tillering also have greater significance for yield. This leads to an indirect relationship between these characters, possibly meaning that the greater magnitude of yield in these treatments can be explained by greater tillering. However, when NFT is compared with the variables MGS_main and MGS_til., there is no association between these variables in relation to the weight of grains produced in the main ear of the plant. However, in relation to MGS_til., there is a greater weight of grains produced by the cultivars TBIO Ello, TBIO Calibre and TBIO Fusão, which have higher absolute values of NFT, despite the absence of a statistical difference for NFT and MGS_til. Factors such as the incidence of solar radiation and competition for nutrients result in a higher population of fertile tillers per unit area (Ozturk *et al.*, 2006).

The application of N in top dressing is not capable of increasing the number of tillers (Ecco *et al.*, 2020; Penckowski *et al.*, 2009). So, this is probably a genetic characteristic of the cultivar linked to population management and the thermal conditions arising during its development. In production terms, genotypes with lower tillering potential are subject to greater dependence on sowing density and this characteristic is also related to tiller senescence (Valério *et al.*, 2008). Plants should ideally have two or three tillers in addition to the main stem, to minimize possible environmental damage (Common & Klinck, 1981). The number of tillers is determined based on the population of plants in the crop, where the number of tillers changes to compensate for the lack or excess of plants (Orso *et al.*, 2014).

Despite the fact that there was statistical differentiation for the cultivar factor, according to the PH character (Tab. 6), the averages were segregated into two groups. The group with the highest average PH is made up of the cultivars TBIO Audaz, TBIO Ello, TBIO Calibre and TBIO Duque, while the others had lower values. We can infer that the higher the PH, the lower the NFT, except for the TBIO Astro cultivar that, despite having a lower NFT, also has a low PH average. However, the higher the PH, the greater the possibility of the crop lodging in the face of more intense winds; lodging is one of the factors that most limits the maximization of wheat grain production (Costa *et al.*, 2013).

When growing wheat in a low-altitude subtropical environment, N installments have no influence on the number of fertile tillers, plant height, or crop yield components. If environmental conditions are favorable, nitrogen can be applied in a single application. For the experimental condition, the higher number of fertile tillers is not related to an increase in grain yield. However, this can be considered an important agronomic trait for regulating the number of spikes per area when there is a lower crop density after the crop is established.

TABLE 6. Plant height (PH) and number of fertile tillers (NFT) per plant of ten wheat cultivars, Santa Maria/RS, Brazil.

Cultivar	PH (cm)	NFT
TBIO Audaz	65.13 A*	2.08 ns
TBIO Aton	57.08 B	2.38
TBIO Toruk	59.00 B	2.58
TBIO Ello	64.25 A	3.04
TBIO Calibre	68.25 A	2.50
TBIO Fusão	62.08 B	2.50
TBIO Ponteiro	60.46 B	2.38
TBIO Duque	63.46 A	1.88
TBIO Astro	60.42 B	2.00
TBIO Trunfo	67.92 A	2.04
Mean	62.80	2.33
CV (%)	7.49	29.93

CV – coefficient of experimental variation in %. * Averages followed by the same letter in the column do not differ by the Scott-Knott test at a 5% significance level; ns – not significant according to the Scott-Knott test at 5% significance level.

In addition to the data generated in a controlled environment (greenhouse), it is important to carry out studies in field conditions in order to have a greater number of potentially troubling environmental characteristics such as temperature fluctuations and stress due to excess or deficit water. In this context, we suggest a more in-depth study on the interference of N fertilization management on the tillering and height of wheat plants. The study should also be further developed in a greenhouse that includes more genotypes and nitrogen top dressing, including different N sources, doses, and times of application in order to obtain a significant interaction between N fertilization and the analyzed characters of the number of fertile tillers, plant height, and yield components.

Conclusions

The nitrogen installments had no influence on the number of fertile tillers, plant height or crop yield components.

The difference obtained in the tillering pattern of the cultivars tested could be a function of their genetic potential.

For the experimental condition, an elevated number of fertile tillers was not related to an increase in grain yield.

Conflict of interest statement

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

Author's contributions

FMM, EDS, and DNF designed the experiments, DNF obtained financial support for the project that gave rise to this publication. FMM and RIR carried out the experiments in a greenhouse; FMM, RIR, EDS, and ACP assisted in data collection and processing. EDS contributed to the data analysis and FMM and EDS wrote the article. All authors reviewed the final version of the manuscript.

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Effects of phosphine and plant extracts on flower thrips mortality and the quality of cut flowers

Efectos de la fosfina y extractos de plantas sobre la mortalidad de trips de las flores y la calidad de flores de corte

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ABSTRACT

Flower thrips represent a complex of significant quarantine species affecting the cut flower market in Colombia. The aim of this research was to evaluate postharvest treatments using phosphine in conjunction with a plant extract for thrips control and quality control of five cut flower species. Eight treatments were used: six employed a commercial dose of magnesium phosphide as a source of phosphine, one used a double dose and a control group without phosphine application. The first six treatments followed a bi-factorial structure, incorporating three exposure times and the addition of a chili-garlic extract. Thrips control efficacy was evaluated using the Schneider-Orelli index based on field-collected samples. Postharvest quality assessments were conducted on roses, carnations, alstroemerias, chrysanthemums, and hydrangeas over an 18-d period following treatment application. Differences in efficacy were observed between the two locations (the blocking factor). Discrepancies in phosphine efficacy may be related to the variations in populations collected from different crops and locations, both in the departments of Cundinamarca and Antioquia. Variations in magnesium phosphide concentration, both at the commercial dose of 3.4 g m^{-3} and double this amount (2X) did not produce significant differences in treatment efficacy or flower quality. The use of chili pepper and garlic extract applied by nebulization at 3°C combined with phosphine application also did not significantly affect thrips mortality efficacy. The factor most influencing efficacy improvement was exposure time, as longer time periods led to better thrips control. Furthermore, we found that longer exposure times did not affect visual quality or vase life, assessed through changes in color, physiopathies, and chlorophyll content.

Key words: postharvest phytosanitary treatment, chili garlic extract, quarantine pest, rose, chrysanthemum, hydrangea, carnation, alstroemeria.

RESUMEN

Los "trips de las flores" representan un complejo de importantes especies cuarentenarias que afectan al mercado de la flor cortada en Colombia. El objetivo de este estudio fue evaluar tratamientos poscosecha con fosfina y un extracto vegetal para el control de los trips y la calidad de cinco especies de flor cortada. Se aplicaron ocho tratamientos: seis de ellos empleando una dosis comercial de fosfuro de magnesio como fuente de fosfina, uno utilizando una dosis doble, y un grupo de control sin aplicación de fosfina. Los seis primeros tratamientos siguieron una estructura bifactorial, incorporando tres tiempos de exposición y la adición de un extracto de ajo-ají. La eficacia del control de trips se evaluó mediante el índice de Schneider-Orelli basado en individuos colectados en campo. Se realizaron evaluaciones de la calidad poscosecha en rosas, claveles, astromelias, crisantemos y hortensias durante un periodo de 18 d tras la aplicación del tratamiento. Se encontraron diferencias en la eficacia entre las dos ubicaciones (el factor de bloqueo). Las discrepancias en la eficacia de la fosfina podrían estar relacionadas con las variaciones en las poblaciones recolectadas de diferentes cultivos y ubicaciones, tanto en el departamento de Cundinamarca como en el departamento de Antioquia. Las variaciones en la concentración de fosfuro de magnesio, tanto a la dosis comercial de 3.4 g m^{-3} como al doble de esta (2X), no mostraron diferencias significativas en la eficacia del tratamiento ni en la calidad de las flores. El uso del extracto a base de ajo y ají, aplicado por nebulización a 3°C combinado con la aplicación de fosfina, tampoco afectó significativamente la eficacia del control de los trips. El factor que más influyó en la mejora de la eficacia fue el tiempo de exposición, ya que períodos más largos condujeron a un mejor control de los trips. Además, se encontró que los tiempos de exposición más prolongados no afectaron la calidad visual ni la vida útil en el jarrón, evaluada mediante la variabilidad en los cambios en el color, el contenido de clorofila y la presencia de fisiopatias.

Palabras clave: tratamiento fitosanitario poscosecha, extracto de ajo ají, plaga cuarentenaria, rosa, crisantemo, hortensia, clavel, astromelia.

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Introduction

Quarantine pests are organisms that are absent or under active control that pose an economic or environmental risk; and, therefore, their entry into Colombia as pests is controlled (IPPC, 2021). For cut flower exports in Colombia, shipment interceptions because of the presence of quarantine pests are one of the main limitations (ICA, 2018), where thrips, lepidoptera, aphids, and mites stand out as the most significant pests (Brownbridge & Buitenhuis, 2019). For the cut flower market, the term "flower thrips" represents a group of quarantined insect species such as *Frankliniella panamensis* and *Thrips palmi* (Teulon *et al*., 2014). Thrips are an important pest because of their polyphagous character and high reproductive rate (Taylor, 1994) and because they generate direct damage mainly on flower buds and young leaves, tissues, and consumption of cellular content (Herrick *et al*., 2021). In addition, they cause indirect damage because they are potential virus vectors and because of their quarantined nature (Jones, 2005). MacLeod *et al*. (2004) estimate economic losses of £16.9 to £19.6 million over a decade due to *T. palmi* in chrysanthemums in southern England. These losses include reduced yield and quality, extra research, plant health certification expenses, and export losses. They also note a substantial sixfold rise in pest control spending on a 4.8 ha greenhouse site (MacLeod *et al*., 2004).

Aspects such as pest tolerance, decrease in the number of available insecticides, and product market requirements favor the development or innovation of integrated pest management alternatives. Export crops require pest management in the field, complemented with post-harvest strategies. Post-harvest phytosanitary treatments (PPT) include physical methods such as ultrasound, temperature, controlled atmospheres, irradiation (Nicholas & Follett, 2018) or chemicals such as essential oils (Kostyukovsky & Shaaya, 2001), ethyl formate, and fumigants such as methyl bromide (CH_3Br) and phosphine (PH_3) (Sirohi *et al*., 2021). PPTs are an additional phase in the production chain and their improper use can affect quality, especially those that involve high doses (Liu, 2011). For this reason, it is important to ensure that PPTs do not interfere with the cold chain or generate negative physiological effects such as maturation in living tissues (Fields & White, 2002; Su-Kim *et al*., 2016).

Methyl bromide has been used as an effective postharvest pest treatment because of its broad spectrum and versatility. However, since 1987, methyl bromide was listed as harmful to the ozone layer in the Montreal protocol, where the United Nations Environment Program (UNEP) proposed a gradual reduction of its use from 1997 to critical levels in 2005 (UNEP, 2020). Phosphine is a versatile, economical, and effective alternative to methyl bromide (Arora *et al*., 2021) since it acts at the respiratory level, inhibiting the transport of electrons in the mitochondrial complex IV, limiting energy production; this is why it is highly toxic to aerobic organisms such as insects (Karunaratne *et al*., 1997; Zhang *et al*., 2013). Phosphine gas is characterized by rapid diffusion that allows it to reach all spaces in the treated space, has low permeability and low absorption in plant material that makes it relatively harmless, and requires prolonged exposure times to be highly effective (Zhang *et al*., 2013). Because the sensitivity of agricultural products to fumigants varies with the species, it is important to determine aspects such as exposure times, doses and forms of application that control pests without affecting the quality of the agricultural product (Liu & Liu, 2014).

Phosphine gas (PH_3) is generated by hydrolysis produced by contact of ambient humidity with the solid state of phosphides (Carvajal Oviedo *et al*., 2014). The market has two forms of phosphides used for the generation of phosphine: the first is aluminum phosphide (AlP) used mainly in stored products and the second is magnesium phosphide (Mg_3P_2) used in fresh products and grains (Anasac Colombia, 2018; Restrepo-Giraldo, 2019). Commercial presentations of aluminum phosphide contain ammonium carbamate ($NH_2CO_3NH_4$) in its formulation; this reacts exothermically to generate ammonia (NH_3) and carbon dioxide (CO_2). The former is phytotoxic in fresh vegetables, and the latter accelerates ripening by stimulating the production of ethylene; its use is limited in fresh products (Anasac Colombia, 2018; Restrepo-Giraldo, 2019). Magnesium phosphide may or may not contain ammonium carbamate; however, the flower market mostly uses the formulation without ammonium carbamate that only generates phosphine gas and an innocuous solid residue (Huber-Valiño, 2019; Nath *et al*., 2011). Phosphine treatments under conditions of refrigeration require longer exposure times than those carried out at room temperature because the volatilization rate decreases with temperature (Hole *et al*., 1976; Liu, 2011; Zhang *et al*., 2013). However, the exportation of fresh products such as flowers are time-limited in terms of dispatch times and refrigerated storage spaces; so, it is necessary to identify the species to be controlled and the treatment times for control to minimize time.

Species such as garlic (*Allium sativum*) and chili (*Capsicum* sp.) are a source of plant extracts used as shock insecticides at low incidences or as pest repellents (Lema-Jami, 2011). Garlic hydrolate has antimicrobial and antifungal properties because of its ability to limit oxygen absorption by pathogens, affecting their growth and stress responses that cause damage to plants (Juárez-Segovia *et al*., 2019). The chili pepper is characterized by its high content of the alkaloid capsaicin that affects the functioning of chemoreceptors and nociceptors in arthropods, thus, generating signals and stimuli of potentially harmful irritation in the tissue that affect behavior and defense mechanisms and induce stress and evasion reactions (Li *et al*., 2020). Capsaicin is not phytotoxic to ornamental plants and has proven effective in controlling pests such *as Plutella xylostella* in vegetables, reducing their population by up to 55.94% without compromising cabbage production quality (Baidoo & Mochiah, 2016). Given the alterations that some plant extracts produce in insects, their use is proposed as an innocuous alternative that favors the efficacy of phosphine treatments. This research evaluated the effect of treatments with phosphine and its complement with a chili-garlic extract as a postharvest management strategy for controlling flower thrips and quality in the main species of cut flowers for export from Colombia.

Materials and methods

Location

Two trials were established in principle locations for the production and export of cut flowers in Colombia in 2021: The first is eastern Antioquia in the municipality of Rionegro (Antioquia) (6°8'19.42" N, 75°24'53.02" W, altitude 2100 m a.s.l.), and the second in the Bogotá savannah in the municipality of Mosquera (Cundinamarca) (4°41'47.72" N, 74°12'42.54" W, altitude 2550 m a.s.l.). For the Antioquia experiment, daytime temperature conditions were 18.99°C ± 2.16°C and nighttime temperature was 15.96°C ± 1.01°C, while for Cundinamarca, daytime temperature was 15.96°C ± 1.01°C and night time temperature was 15.3°C ± 0.68°C. Relative humidity in both experiments was similar, approximately an average of 79%.

Plant species and insect collect

The five most important flower species in the Colombian export market were included in the experiment. In Antioquia, chrysanthemums (*Chrysanthemum* sp. var. Maisy) and hydrangeas (*Hydrangea macrophylla*. var. White) were used, while, in Cundinamarca, roses (*Rosa* sp. var. Snow Bliss), carnations (*Dianthus caryophyllus* var. Grand Slam), and alstroemerias (*Alstroemeria* sp. var. Himalaya) were used. The flowers were harvested 24 h before the application of the treatments and were subjected to a traditional post-harvest process for export based on Asocolflores (2010) and Fischer and Flórez (1998). The number of stems per bouquet was adjusted according to the species, with 10 stems for chrysanthemum, 3 stems for hydrangea, and 12 stems for rose, carnation, and alstroemeria.

Based on the duration of the developmental stages of *Frankliniella* at a temperature of 25°C-30°C, thrips were directly collected from plants in the field. This suggests that nymphal, preoviposition, and adult longevity stages of both females and males were found on leaves and flowers, establishing them as pests capable of causing damage to the crop (Solís, 2016). Thus, thrips were collected from infested flowers the day before the experiment was begun; they were counted and confined in plastic cups (16 oz or 473 ml) with a lid and a cotton plug to ensure gas exchange. A flower or inflorescence of the species was collected as a source of food and shelter (Kim *et al*., 2016).

Experiment design and treatments

Eight treatments were established based on the dose of magnesium phosphide, the use of the plant extract, and the time of exposure to the treatments (Tab. 1). The first six treatments had a bifactorial structure where the factors were the use or lack of the plant extract and the second factor was exposure time. Additionally, according to the Andean standard for pesticide registration (ICA & ANDI, 2016), two control treatments were included: the first without phosphine (control 0X), and the second with a double dose (control 2X), both using a treatment time (exposure time) of 24 h. Magnesium phosphide (Fumicel Placa®, Anasac, Colombia) was used as a phosphine source with a commercial dose of 3.4 g m^{-3}. The plant extract (with a concentration of 54.2% garlic + 43.4% chili pepper extract, Capsialil SL®, Ecoflora Agro, Medellin, Colombia; a commercial dose of 1.0 ml L^{-1} H_2O) was applied with thermal fogging with a portable device powered with butane gas (KB100, Hyundai, Seoul, South Korea) at a rate of 20 ml m^{-3}.

TABLE 1. Postharvest phytosanitary treatments evaluated, consisting of different doses of magnesium phosphide, their combination with a chili garlic extract, and the exposure time.

Treatment	Magnesium phosphide dose (g m^{-3})	Plant extract (20 ml m^{-3})	Exposure time (h)
1	3.4	No	12
2	3.4	No	18
3	3.4	No	24
4	3.4	Yes	12
5	3.4	Yes	18
6	3.4	Yes	24
7	0.0 (control 0X)	No	24
8	6.8 (control 2X)	No	24

Treatment applications

The applications were carried out under refrigeration conditions in cold rooms (3°C ± 1°C), in which 200-L metal barrels with lids were used as treatment chambers. We used twenty-four barrels, corresponding to the eight treatments with three replicates each. Each barrel contained a glass with 14 thrips to ensure efficacy evaluation, along with two bouquets of flowers per species used for quality assessment. We applied the magnesium phosphide from a cup attached to the wall of each drum. In treatments involving the use of plant extracts, we applied the magnesium phosphide using a thermo-fogger. After the application of the extract and/or phosphide, we immediately sealed each drum. During the treatment period, we monitored for phosphine leaks from the barrels using a portable meter (PAC-8000, Dräguer, Germany). We quantified the concentration of phosphine using colorimetric tubes based on silver salts (Detia-Degesch®, Laudenbach, Germany). We carried out two evaluations for each barrel: the first 7 h after starting the treatments (initial measurement) and the second at the end of the exposure times (final measurement) that were used to estimate the average concentration. The barrels corresponding to each treatment were uncovered and ventilated for 15 min according to their exposure time, *i.e.*, zero day after treatment (DAT). We removed the flowers from the barrels and stored them in boxes for the simulated trip period, where the same refrigeration conditions were maintained.

Environmental variables

During the treatment period, the simulated trip, and the vase quality tests, we monitored the environmental temperature and relative humidity with dataloggers (ELMA DT-171, Elma Instruments, Ryttermarken, Denmark). The average temperature inside the cold rooms was set at 3°C, but it varied between locations, being 2.49°C ±1.40°C for Antioquia and 3.08°C ± 0.50°C for Cundinamarca.

Treatment's efficacy

We evaluated the mortality of thrips at 2 DAT using the count of dead individuals within each vessel. Those individuals that did not show movement after stimulation via repeated contact with a brush under incandescent light at 30 cm were deemed to be dead (Liu, 2011; Zhang *et al*., 2015). We, further, inspected the thrips using a digital microscope at 500x (WiFi Digital Microscope, STPCTOU, China). We used the count of living and dead individuals to estimate the percentage of mortality as the quotient between the number of dead individuals with respect to the total number for each vessel. With these mortality values, we determined the percentage of efficacy with the Schneider-Orelli formula (Ciba-Geigy, 1981; ICA & ANDI, 2016) and Equation 1.

$$\text{Efficacy (\%)} \quad \frac{(\text{b - k})}{(100 \text{ - k})} \times 100 \qquad (1)$$

Where b represented the average mortality percentage for the three replicates of the 0x control (Tab. 1), and k represented the percentage of mortality in each of the replicate of the treatment.

Flower vase quality

We carried out the vase quality evaluations after the simulated travel period occurred under refrigeration conditions in cold rooms (3°C ± 1°C) for 7 d. For each species, there was a total of 9 branches, corresponding to the experimental units. The evaluations were carried out in rooms within the farms, under ambient conditions and under shade. We placed each bouquet in a glass vase to which we added 1.5 L of water from the aqueduct with a pH of 6.3 and an electrical conductivity of 1.8 $\mu S\ cm^{-1}$ and this was replaced every 2 d until the end of the trial to prevent the accumulation of microorganisms and avoid the use of disinfectants in the solution. The quality variables we evaluated were chlorophyll, color, and the presence of physiopathies that were assessed at 12 d, 14 d, 16 d, and 18 d after treatment (DAT) (Caldua-Pohl, 2015; Figueroa *et al*., 2005; López *et al*., 2008; Mosqueda-Lazcares *et al*., 2011).

We assessed the chlorophyll content in the upper third of the foliage using a portable chlorophyllometer (SPAD-502, Konica Minolta, Osaka, Japan) on three randomly selected leaves in duplicate. We measured petal color (CieLab) on three random points of the petal surface in duplicate using a colorimeter (CR-400, Minolta Camera Co., Osaka, Japan). Additionally, at 18 DAT, we recorded the incidence and average severity of physiopathies and diseases described in Table 2, typically assessed in similar experiments.

TABLE 2. Description of the assessed physiopathies in postharvest cut flower species.

Common name of a physiopathy	Description
Bluing	Discoloration in the petals due to loss of pigments or copigmentation with flavonoids or related compounds (Arévalo-Hernández, 2011; Chaudhry, 1997; Cho *et al*., 2020; Halevy & Mayak, 2011).
Dehydration	Loss of generalized turgor of the tissues (Arévalo-Hernández, 2011).
No opening	Absence of the natural opening of the flower, also known as rest or dormancy (Arévalo-Hernández, 2011).
Petal fall	Premature loss of petals (Chaudhry, 1997; Mosqueda-Lazcares *et al*., 2011).
Neck bent or nodding	Loss of turgor and firmness in the portion of the stem immediately below the flower head that causes its curvature (Mosqueda-Lazcares *et al*., 2011).
Oxidation or necrosis in petal and foliage	Presence of oxidation or tissue necrosis in petals or foliage (Chaudhry, 1997; Cho *et al*., 2020).
End of vase life	When more than 50% of the flower petals in each bouquet fell, they began to turn gray (discoloration) or showed necrosis or wilting (Chaudhry, 1997; Mosqueda-Lazcares *et al*., 2011).

Statistical analysis

Analyses were performed with the statistical software R (R Core team, Ver. 1.2.1235, 2020). The average phosphine concentration variable was analyzed with analysis of variance (ANOVA) for all treatments that included phosphine (excluding treatment 7) and taking the locality as a blocking factor. For efficacy, two analyses were performed: the first was an analysis of covariance (ANCOVA) using the "agricolae" library (De Mendiburu, 2021), taking the concentration of phosphine as a covariable, the locality as the first factor and the treatments as the second factor. The second type of analysis for efficacy was limited to treatments 1 to 6 and was performed using ANOVA due to its bifactorial structure, taking the location as the blocking factor, the use of the plant extract as the first factor, and the exposure time as the second factor. The differences between groups were estimated using the Tukey test using the "agricolae" library (De Mendiburu, 2021).

For the evaluation of chlorophyll and color, given its longitudinal nature, we used a longitudinal analysis of variance with the "nparLD" library (Noguchi *et al*., 2012), taking the applied treatment as the first factor and the time in days after treatment as the second factor. In the different analyses of variance, in the absence of interactions, we analyzed the simple factors independently (Montgomery, 2017). Differences between levels were interpreted from error bars (Cumming *et al*., 2007). We evaluated the presence of patho-physiologies descriptively using the count of affected branches and the number of stems with the pathophysiology for each one. We produced graphics using the "ggplot2" library (Gómez-Rubio, 2017).

Results and discussion

There were differences in the average temperature inside the cold rooms between localities even though they were programmed the same (3°C), where Antioquia was lower than Cundinamarca by approximately half a degree although the latter had less variation. These variations responded to aspects of design and the operation of the cold rooms; but, in both cases, they described a traditional scenario of refrigeration in the post-harvest processes of cut flowers. Temperature plays a fundamental role in the conservation of cut flowers since it influences the production of ethylene and maturation (Gómez Rubio *et al*., 2017), and postharvest treatments with phosphine can affect performance (Hole *et al*., 1976; Liu, 2011). During the evaluation of quality in a vase, there were differences in the average temperature between evaluation localities resulting from altitude, with lower values in Cundinamarca than in Antioquia. However, this did not influence the tests since different flower species were evaluated between localities.

The difference between day and night temperatures was similar between the test locations and the difference was not greater than half a degree (Antioquia: 0.33°C, Cundinamarca: 0.44°C). Relative air humidity was higher inside the cold rooms than outside during the vase tests. Differences in relative humidity between environments were identified, and the Cundinamarca trial was higher than in Antioquia although the latter had greater variability. At the environmental level, the relative humidity was similar between environments and between day and night.

We found statistically significant differences for the average concentration of phosphine (*P value*: 0.085), given by treatment 8 (2X control) (287.50 mg L^{-1}) that differed from treatments 1 (195.83 mg L^{-1}), 4 (179.16 mg L^{-1}) and 6 (170.83 mg L^{-1}) (Tab. 3). Between treatments 1 to 6, the difference in phosphine concentration was not statistically different. For the locality factor, there were significant differences (*P* value: <0.001): the concentration used in Antioquia (176.19 mg L^{-1}) was lower than in Cundinamarca (241.66 mg L^{-1}).

TABLE 3. Average phosphine concentration in parts per million (mg L^{-1}) for the eight treatments, the two locations and general average.

Phosphine concentration per treatment			
Group	**At 7 h**	**Final**	**Average**
Treatment 1	183.33 ± 47.14	208.33 ± 34.35	195.83 ± 33.59 b
Treatment 2	183.33 ± 68.71	241.66 ± 53.35	212.50 ± 59.07 ab
Treatment 3	183.33 ± 23.57	233.33 ± 143.37	208.33 ± 75.92 ab
Treatment 4	183.33 ± 23.57	175.00 ± 25.00	179.16 ± 22.43 b
Treatment 5	166.66 ± 37.26	250.00 ± 81.64	208.33 ± 55.27 ab
Treatment 6	150.00 ± 28.86	191.66 ± 67.18	170.83 ± 44.29 b
Treatment 8 (2X)	266.66 ± 106.71	308.33 ± 97.53	287.50 ± 89.84 a
Phosphine concentration per location			
Group	At 7 h	Final	Average
Antioquia	169.04 ± 66.32	183.33 ± 35.63	176.19 ± 43.96 B
Cundinamarca	207.14 ± 58.32	276.19 ± 105.35	241.66 ± 72.10 A
General	188.09 ± 65.29	229.76 ± 91.32	208.92 ± 68.09

Values represent the mean plus/minus the standard deviation for the group. The letter at the end denotes significant differences according to Tukey's test with a 95% confidence estimated for the mean concentration, where lowercase letters describe differences between treatments and uppercase between test sites. The concentration inside the treatment 7 barrel (control 0X) was measured using a portable PAC-8000 meter, without detecting the presence of phosphine in it. Treatment 1: 3.4 g m^{-3} of Mg_3P_2, 0 ml m^{-3} of chili garlic extract (CGE) and 12 h of exposure time (ET). Treatment 2: 3.4 g m^{-3} of Mg_3P_2, 0 ml m^{-3} of CGE and 18 h of ET. Treatment 3: 3.4 g m^{-3} of Mg_3P_2, 0 ml m^{-3} of CGE and 24 h of ET. Treatment 4: 3.4 g m^{-3} of Mg_3P_2, 20 ml m^{-3} of CGE and 12 h of ET. Treatment 5: 3.4 g m^{-3} of Mg_3P_2, 20 ml m^{-3} of CGE and 18 h of ET. Treatment 6: 3.4 g m^{-3} of Mg_3P_2, 20 ml m^{-3} of CGE and 24 h of ET. Treatment 7 (control 0X): 0 g m^{-3} of Mg_3P_2, 0 ml m^{-3} of CGE and 0 h of ET. Treatment 8 (control 2X): 6.8 g m^{-3} of Mg_3P_2, 0 ml m^{-3} of CGE and 24 h of ET.

The differences between treatments with the same dose of magnesium phosphide may respond to variations in the dosage that is carried out manually just before sealing the barrels or to the low volume of these or microleakage of phosphine gas, typical of fumigation.

For average phosphine concentration, no statistically significant effect was found on the response variables (Tab. 4). The locality factor showed significant differences with respect to the thrips mortality variable, where Cundinamarca had a higher mean mortality than Antioquia; however, the efficacy did not show differences between localities. Finally, the treatment factor did affect the response in mortality and efficacy (Tab. 4).

TABLE 4. Results of the analysis of covariance (ANCOVA) for the efficacy variable by the Schneider-Orelli index.

Source of variation	F Value	P Value
Average concentration (covariable)	0.578	0.453
Location (factor 1)	10.663	0.003
Treatment (factor 2)	383.197	<0.001
Location x treatment (interaction)	1.024	0.435

The 0X control (treatment 7) showed the lowest efficacy (2.5%) and described the expected mortality of the population resulting from factors external to the trial (Fig. 1). The efficacy results obtained for the 0X control showed that the insects were adequate for use in the study. The use of insects collected in the field was accepted because it reflected the natural genetic diversity that is desirable if there are enough individuals; however, this can lead to broad experimental limits and mask small differences (Mouratidis *et al*., 2022). The breeding of individuals is the most used method for phytosanitary research (Heather & Hallman, 2008) since it allows control over the stages, uniformity in the species, and a high number of individuals that results in greater strength for statistical tests. In some species, the use of individuals raised in the laboratory for phytosanitary tests is not recommended unless their susceptibility does not differ from wild individuals (Badenes-Pérez & López-Pérez, 2018).

For the treatments with phosphine, the 2X control (treatment 8) was characterized by an efficacy of 97.5%, similar to treatments 3 and 6 (98.6%), all characterized by a long exposure time. A trend of increased efficacy was identified when the exposure time was greater, while the treatments with the lowest exposure times, such as treatment 1 and 4, showed lower efficacy values (92.5% and 94.0%) (Fig. 1). The average concentration of phosphine did not significantly affect the efficacy of the treatments even though concentrations between 170 mg L^{-1} (treatment 6) and 285 mg L^{-1} (treatment 8) were obtained, equivalent to 61% more phosphine (Tab. 5). The commercial dose of 3.4 g m^{-3} of magnesium phosphide (treatments 1 to 6) was adequate to control thrips but it was higher than that used in other thrips studies: *F. occidentalis*, where the maximum

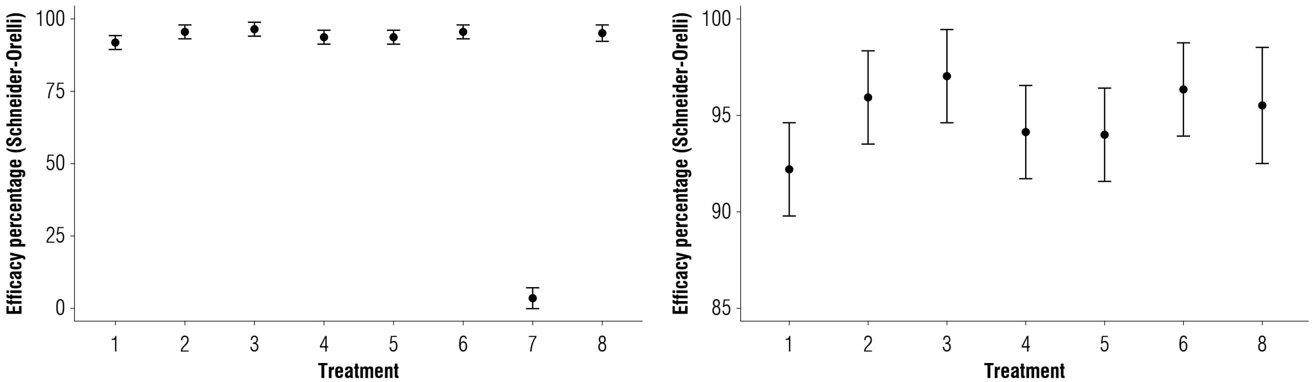

FIGURE 1. Efficacy response of postharvest phytosanitary treatments applied to flower thrips. On the left, the effect for all treatments is shown, while on the right, the treatment was ignored, and the visualization scale was enlarged to show differences between the other groups. The vertical bars represent the 95% confidence intervals for the model. Total data number: 48. Treatment 1: 3.4 g m^{-3} of Mg_3P_2, 0 ml m^{-3} of chili garlic extract (CGE) and 12 h of exposure time (ET). Treatment 2: 3.4 g m^{-3} of Mg_3P_2, 0 ml m^{-3} of CGE and 18 h of ET. Treatment 3: 3.4 g m^{-3} of Mg_3P_2, 0 ml m^{-3} of CGE and 24 h of ET. Treatment 4: 3.4 g m^{-3} of Mg_3P_2, 20 ml m^{-3} of CGE and 12 h of ET. Treatment 5: 3.4 g m^{-3} of Mg_3P_2, 20 ml m^{-3} of CGE and 18 h of ET. Treatment 6: 3.4 g m^{-3} of Mg_3P_2, 20 ml m^{-3} of CGE and 24 h of ET. Treatment 7 (control 0X): 0 g m^{-3} of Mg_3P_2, 0 ml m^{-3} of CGE and 0 h of ET. Treatment 8 (control 2X): 6.8 g m^{-3} of Mg_3P_2, 0 ml m^{-3} of CGE and 24 h of ET.

mortality is reached with 1.52 g m^{-3}, 24 h of exposure and 2°C storage (Liu, 2008) or that of Zhang *et al.* (2015), who reports 100% mortality with a dose of 1.66 g m^{-3} and 16 h of exposure.

For the factorial analysis of efficacy (Tab. 5), we saw no interaction effects in any of the variables, so we analyzed the simple factors separately. For a blocking factor, described as the trial location, there was a statistically significant effect on efficacy. The exposure time factor showed differences for both mortality and efficacy (Tab. 6). Finally, the plant extract use factor did not affect the response in thrips mortality and efficacy (Tab. 5).

TABLE 5. Results of the analysis of variance (ANOVA) for the efficacy variable with the Schneider-Orelli index.

Source of variation	*F Value*	*P Value*
Trial location (block)	17.979	<0.001
Exposure time (factor 1)	5.145	0.012
Plant extract (factor 2)	0.489	0.489
Exposure time x Extract (interaction)	1.141	0.253

The efficacy presented statistically significant differences for the locality factor (Fig. 2) that was lower in Antioquia (93.22%) than Cundinamarca (97.10%). The events that occurred may be attributed to the variation in the population of thrips in the different plant species, a phenomenon influenced by environmental conditions like temperature, rainfall, and the phenological stage of the crops. A high level of genetic diversity is known for the species *F. occidentalis* through ribosomal analysis, indicating the presence of a population structure associated with both geographic region and host plants (Turcios Palomo, 2013). Therefore, we suggest that fluctuations in the effectiveness of phosphine may be attributable to differences in the populations collected from different crops and locations, both in Cundinamarca and Antioquia. This highlights the importance of considering regional and host-specific factors in the management of thrips populations in agricultural settings.

For the exposure time factor (Fig. 2), there were significant differences, with 12 h being the exposure time with the least efficacy (93.22%), followed by 18h (95.47%) and finally 24 h with the greatest efficacy (96.78%). However, only the 12 h and 24 h treatments differed significantly. These results supported the fact that under refrigeration conditions, exposure times should be increased to ensure the absorption of phosphine by insects; Liu (2008) and Liu (2011) find a maximum control effect with 18 h of exposure (250 mg L^{-1} of phosphine). Also, Karunaratne *et al.* (1997) report that higher treatment temperatures can decrease exposure times in adult *Heliothrips haemorrhoidalis*. However, in postharvest cut flowers, temperature is a difficult factor to alter since thrips are highly sensitive to temperature (Gómez *et al.*, 2017) so it would be necessary to understand previous studies and the limits of maximum temperatures that do not affect the quality or durability of the product.

For the factor related to the use of extract at its commercial dose, we found no statistically significant differences between the two groups (Fig. 2). We anticipated that the extract would enhance insect metabolism due to its well-known repellent properties, but this effect was not

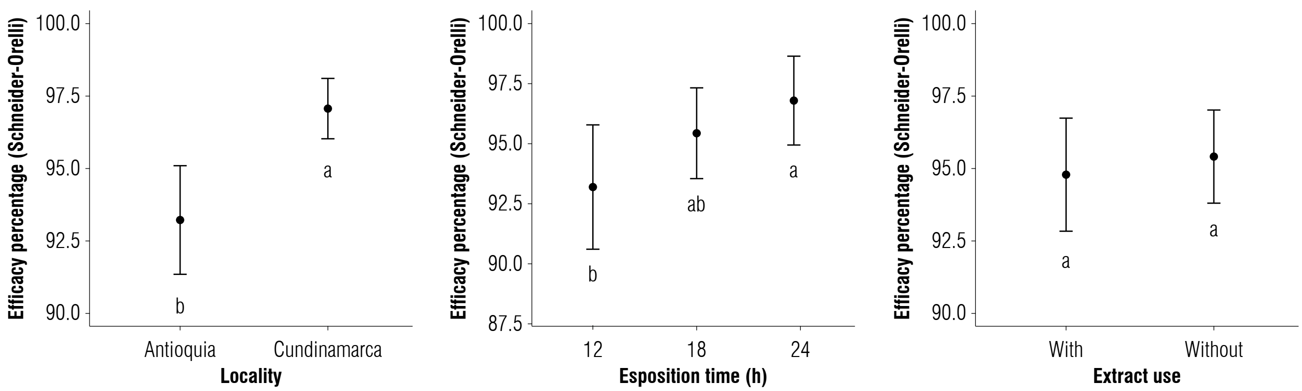

FIGURE 2. Efficacy response of simple factors to postharvest phytosanitary treatments applied to flower thrips. The left figure represents the effect from the blocking factor given by the test location. The central figure represents the effect of the exposure time factor. The figure on the right represents the effect of the factor associated with the use of the plant extract. The vertical bars represent the 95% confidence intervals for the model. Different letters denote statistically significant differences according to Tukey's test (confidence 95.0%). Total data number:48.

significant. This suggests that the presence of the plant extract did not induce sufficient stress in the thrips to elevate their metabolic rate and facilitate the absorption of phosphine. On the other hand, the low influence of the extract on efficacy could be due to the loss of effectiveness of the garlic extract, possibly due to the volatility of the compound called allicin after 2 h (Trabuco *et al*., 2015). Furthermore, the low temperature (3°C) may constrain thrips activity, potentially diminishing extract absorption and, consequently, its impact (Baidoo & Mochiah, 2016; Kazem & El-Shereif, 2010).

The results of quality variables at environmental temperature mostly showed an effect due to sampling time, attributed to changes from maturation to the flower's senescence. However, further research on changes during vase life due to fumigation may be necessary to fully understand the senescence effect of the implemented varieties.

We observed significant differences in chrysanthemums for the a* component due to treatment and time interaction. Hydrangeas exhibited changes in the b* component as a result of treatment effects. Roses showed effects from the interaction for the SPAD variable and treatment factors for both a* and b* components. Carnations displayed changes in the SPAD variable due to interactions. We found no significant differences related to the applied treatments for alstroemerias (Tab. 6).

The senescence of cut flowers depends on different factors, so there is no standard treatment for all species that favors floral longevity (Gómez *et al*., 2017). Senescence is genetically regulated and expressed through the signaling of the hormone ethylene that is the main cause of maturation and senescence in fresh agricultural products. In flowers, the time it takes for the marked symptoms of wilting to appear defines floral longevity (Ciba-Geigy, 1981; Gómez *et al*., 2017; Juárez-Hernández *et al*., 2008; Van-Altvorst & Bovy, 1995). The decrease in chlorophyll content is associated with maturation that derives from an increase in the synthesis of ethylene and chlorophyllase enzymes (Balaguera-López *et al*., 2014). The relative content of chlorophyll (in SPAD units) in roses and carnations showed significant effects from the interaction of the treatments with the quality evaluation time, where treatments 7 and 8 (controls 0X and 2X) showed lower values than the other treatments. A clear trend was not observed, and the variation between the values was low; this means that the treatments do not show negative effects on the quality of the color in the leaves determined by the chlorophylls (Zhang *et al*., 2013).

The color of the petals is one of the most important quality attributes in cut flowers; they manifest by pigments such as carotenoids and flavonoids, the main pigments found in flowers (Tanaka *et al*., 2009). In cut flowers, color changes result from senescence of the flower, which increases with time after removal from the plant, when the flower deteriorates and loses its commercial value (Castellanos *et al*., 2016; Gómez *et al*., 2017). Although white flowers were used for most of the species evaluated in this research, changes in color components over time were identified in almost all of them in the postharvest quality evaluation.

In this trial, minimal responses were observed in color components from the effect of the treatments. The L* component, which describes the changes in luminosity, did not show differences, indicating that the darkening of the petals from browning and tissue oxidation are not related to the treatments evaluated. For component a*, describing the color change between red (positive) and green (negative),

TABLE 6. Results of the non-parametric longitudinal analysis (ANOVA type), for relative chlorophyll content in SPAD units of leaves and for components of the CieLab color space in petals in the cut flower species.

Species	Variable	TTO	DAT	TTO x DAT	CV
Chrysanthemum	SPAD	0.100	0.000	0.118	4.31
Chrysanthemum	L	0.086	0.000	0.454	2.38
Chrysanthemum	a*	0.000	0.000	0.000	34.60
Chrysanthemum	b*	0.059	0.000	0.060	18.80
Hydrangea	SPAD	0.142	0.000	0.103	7.92
Hydrangea	L	0.097	0.000	0.905	2.75
Hydrangea	a*	0.138	0.000	0.715	28.22
Hydrangea	b*	0.067	0.000	0.140	16.58
Rose	SPAD	0.026	0.754	0.007	6.41
Rose	L	0.836	0.004	0.089	1.40
Rose	a*	0.072	0.000	0.195	15.08
Rose	b*	0.000	0.000	0.177	6.71
Carnation	SPAD	0.135	0.000	0.001	3.19
Carnation	L	0.891	0.000	0.911	17.18
Carnation	a*	0.890	0.001	0.873	14.96
Carnation	b*	0.973	0.175	0.509	25.46
Alstroemeria	SPAD	0.691	0.166	0.565	6.57
Alstroemeria	L	0.419	0.000	0.934	8.14
Alstroemeria	a*	0.924	0.226	0.895	55.71
Alstroemeria	b*	0.681	0.000	0.112	71.50

TTO: treatment; DAT: days after treatment; TTO x DAT: interaction of treatments and days after treatment; CV: coefficient of variation in percentage.

the treatments only generated effects in chrysanthemums, probably associated with enzymatic degradation of chlorophyll in chrysanthemums in the initial stages of the evaluation; in the later evaluation days (16 and 18 DAT) this difference is not evident (Pathare *et al*., 2013). For this reason, we inferred that the variations observed respond to experimental error at the time of evaluating the color or variations in the flowers and not to the senescence of the flowers. The b* component, which describes changes between yellow (positive) and blue (negative), showed differences in the rose treatments because of lower values in treatments 6 and 7. This variation did not imply a significant effect in color since changes were imperceptible to the eye as long as they remained close to light colors of the yellow region (b* close to 15), characteristic of white varieties such as Snow Bliss roses (Pathare *et al*., 2013).

Some of the physiopathies were identified in roses and hydrangeas, since both species are characterized by their postharvest susceptibility. In roses, we detected signs of necrosis and reddish discoloration in the petals, possibly due to the oxidation of floral tissues, influenced by environmental conditions such as low temperatures, high light radiation, and deficiencies in calcium and boron (Cabrera *et al*., 2007). Lu *et al*. (2009) found that 52% of the variation in anthocyanin content in petals of different cultivars of *Ipomoea purpurea* is related to temperature and UV radiation. Similarly, previous studies suggest that the genetic predisposition of the cultivar and low temperatures are determinants in the blackening of petals in red roses (Zieslin, 1968). In hydrangeas, necrosis was observed at the stem edge, possibly associated with soil pH. Brouillard (1988) indicates that soil pH can affect flower color through the physical interaction of electrons in pigments. Additionally, hydrangeas grown in soils with a pH of 5.5 exhibit blue flowers, whereas at pH 6.0 they are pink. The aluminum available at low pH accumulates in the petals, forming complexes with anthocyanins that produce the bluish color, and together with low temperatures, can induce tissue necrosis (Quintana *et al.,* 2007).

Regarding the use of the 2X dose, we observed no changes or effects due to the presence of physiopathies. Kim *et al.* (2016) finds no statistically significant effects of phytotoxicity in roses, lilies, and chrysanthemums treated with doses of magnesium phosphide at 2.0 and 4.0 g m^{-3} for 24 h and refrigeration at 8°C. However, it is important to consider for future evaluations that there are reports of phytotoxicity in cut flowers following treatments with phosphine (Zhang *et al*., 2013). Those authors observe effects up to 8 d after the application of treatments in 14 cultivars of cut flowers, with an increase in damage indices in some cultivars as exposure time and plant senescence increases.

Conclusion

The treatments evaluated in this study proved to be effective in controlling flower thrips, with exposure time being the predominant factor over variables such as fluctuations in phosphine concentration and the use of double the commercial dose as well as the inclusion of plant extracts. The simultaneous use of garlic and chili pepper plant extract showed no significant influence on efficacy when applied in conjunction with phosphine. However, given that the storage temperature during treatments and the simulated travel period was 3°C for 7 d, it is plausible that the molecule mechanism of action may be affected by alterations and volatilities after a couple of hours and under low temperatures. To obtain a more precise understanding of the plant extract effects, it would be pertinent to conduct evaluations at different application times, encompassing each of the different varieties implemented in the trial.

Since phosphine treatments had no significant impact on the quality of flower species and their efficacy was over 97% at exposure times of 24 h, the use of phosphine as a tool for postharvest phytosanitary management in cut flowers is proposed. However, further evaluation across multiple cultivars and other species is recommended to corroborate and establish guidelines on its efficacy in flower thrips mortality.

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Conflict of interest statement

The authors declare that there are no conflicts of interests regarding the publication of this article.

Author´s contributions

PLP: conceptualization, investigation, data collect, statistical analysis, writing of original draft, visualization, and writing, review & editing. SBO: investigation, data collect, writing of original draft, and writing, review & editing. AH: supervision, project administration, writing, review & editing. All authors reviewed the final version of the manuscript.

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Evaluation of 1-methylcyclopropene (1-MCP) and temperature in postharvest of banana passion fruit (*Passiflora tripartita* var. *mollissima*)

Evaluación de 1-metilciclopropeno (1-MCP) y temperatura en la poscosecha de curuba (*Passiflora tripartita* var. *mollissima*)

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ABSTRACT

Banana passion fruit or curuba (*Passiflora tripartita* var. *mollissima*) is an exotic fruit liana with fruits of high antioxidant capacity. Its fruits have medicinal, nutritional and industrial properties that make it a product with high nutraceutical potential. This fruit is perishable during postharvest, which is a limiting factor for its conservation. An alternative method for extending the shelf life of fruit postharvest is the use of 1-methylcyclopropene (1-MCP), which inhibits the action of ethylene, delaying the ripening process in fruits and, together with low temperatures, preserves curuba for a longer period without altering its nutritional composition. The effect of the application of 1-MCP and temperature on the quality of curuba fruits was determined. A completely randomized design with four treatments was used: control (14°C), temperature 4°C, 3 mg L^{-1} 1-MCP+14°C, and 3 mg L^{-1} 1-MCP+4°C. The variables evaluated in fruits were color, weight loss, respiration, firmness, total soluble solids, and total titratable acidity. The fruits exposed to the 4°C or 3 mg L^{-1} 1-MCP+4°C treatments had higher firmness and total soluble solids, lower weight loss, and lower color index and titratable acidity with a storage duration of 29 d. The fruits subjected to the 3 mg L^{-1} 1-MCP+4°C treatment showed lower weight loss than in the other treatments. The use of 1-MCP together with low temperatures prolongs the postharvest life of curuba.

Key words: antioxidant, ethylene, ripening, respiration, tropical fruits.

RESUMEN

La curuba (*Passiflora tripartita* var. *mollissima*) es una liana frutal exótica caracterizada por su alta capacidad antioxidante. Sus frutos poseen propiedades medicinales, nutricionales e industriales que hacen de ella un producto con alto potencial nutraceútico. Es un frutal perecedero durante la poscosecha, siendo esto una limitante para su conservación. Un método alternativo para prolongar la vida útil de la fruta después de la cosecha es el uso de 1-metilciclopropeno (1-MCP) que inhibe la acción del etileno, al retardar el proceso de madurez en los frutos, y junto con las bajas temperaturas, los conserva por más tiempo sin alterar su composición nutricional. Se determinó el efecto de la aplicación de 1-MCP y la temperatura sobre la calidad en frutos de curuba. Se utilizó un diseño completamente al azar con cuatro tratamientos: control (14°C), temperatura 4°C, 3 mg L^{-1} 1-MCP+14°C y 3 mg L^{-1} 1-MCP+4°C. Se evaluaron las variables de color, pérdida de peso, respiración, firmeza, sólidos solubles totales y acidez total titulable. Los frutos tratados con la temperatura 4°C o 3 mg L^{-1} 1-MCP+4°C presentaron mayor firmeza y sólidos solubles totales, menor pérdida de peso, índice de color y acidez titulable con una duración de 29 d de almacenamiento. Los frutos sometidos al tratamiento 3 mg L^{-1} 1-MCP+4°C presentaron menor pérdida de peso que los otros tratamientos. El uso de 1-MCP junto con temperaturas bajas prolonga la vida poscosecha de la curuba.

Palabras clave: antioxidante, etileno, maduración, respiración, frutos tropicales.

Introduction

Banana passion fruit or curuba (*Passiflora tripartita*) is a liana native to South America (García-Ruiz *et al*., 2017) known in international markets as an exotic fruit (Salazar & Ramírez, 2017) due to its antioxidant potential and sedative effects (Mayorga *et al*., 2020). It is cultivated in several departments of Colombia, including Antioquia, Boyacá, Huila, Norte de Santander, and Cundinamarca (Parra-Peñalosa & Cancino-Escalante, 2019). This fruit is a source of vitamins A, C, and riboflavin. Its content of phenols, carotenoids, and flavonoids gives it the ability to eliminate free radicals that cause oxidative stress (Chaparro-Rojas *et al*., 2015). It has a higher hypoglycemic and antioxidant capacity than many other fruits (Giambanelli *et al*., 2020). It is utilized as a preservative in lipid peroxidation (Botero *et al*., 2007). Additionally, it is used in the production of fermented beverages (Amorocho-Cruz *et al*., 2022), juices, and desserts. Its seeds are a source of vegetable oil that is processed in the pharmaceutical, food, and cosmetic industries (Hernández-Rivera *et al*., 2018).

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Its importance lies in its health-promoting compounds (Ballesteros-Vivas *et al*., 2019). In Colombia, *arepa* (a traditional flatbread made with corn flour) with curuba pectin could reduce blood glucose levels (Ortiz & Anzola, 2018). Fonseca-Benítez *et al.* (2022) found that curuba seed extract can inhibit the proliferation and growth of oral tumor cells by positively regulating cell death genes, suggesting that it could be a potential treatment for oral cancer.

However, there is a lack of studies on Passifloraceae like the curuba regarding the improvement of their postharvest quality (Fischer *et al*., 2018). Fruits have high water content, which makes them perishable (Zapata *et al*., 2015) and susceptible to damage after harvest (Márquez *et al*., 2017). Therefore, it is important to use alternatives that help maintain fruit quality during the postharvest. One such alternative is 1-methylcyclopropene (1-MCP), which irreversibly binds to ethylene (C_2H_4) receptors (Brasil & Siddiqui, 2018). Some transcriptional regulators of 1-MCP can repress the ethylene response (Gwanpua *et al*., 2017). This blocks fruit maturation; however, the expression of new ethylene receptors would continue with the maturation process. Therefore, it is necessary to develop strategies for each crop based on its ethylene gene expression (Diaz *et al*., 2021). Several studies have been conducted on passion fruit plants, including one on common passion fruit that evaluated the effect of 1-MCP on prolonging shelf life (Dussán-Sarria *et al*., 2011). Another study applied 1-MCP and chitosan at various concentrations to passion fruits stored at 4°C (You *et al*., 2022). Curuba's postharvest life can be extended by storing it at 7°C or 4°C, as reported by Botía-Niño *et al.* (2008). Additionally, Díaz *et al.* (2021) suggest that combining 1-MCP with cold storage is a commonly used method to prolong the shelf life of fruits. The aim of this study was to assess the effect of the application of 1-MCP and the temperature on the postharvest quality of the curuba (*P. tripartita*) fruits.

Materials and methods

Plant material and experimental conditions

Eighty homogeneous curuba (*Passiflora tripartita*) fruits, with approximately 25% yellow color and 75% green color, were collected from a commercial farm in the municipality of Umbita, Boyacá (Colombia). The fruits were free from physical and mechanical damage and had good sanitary conditions. The farm is located at an altitude of 2500 m a.s.l., with an average air temperature of 14°C and monthly precipitation of 100-150 mm (IDEAM, 2022). In October 2022, the selected fruits were transported to the Plant Physiology Laboratory at the University Juan de Castellanos in Soracá, Boyacá.

Sample preparation

The fruits were disinfected with a solution of 0.1 N NaOH in 5 L of water for 5 min. In addition, a commercially available EthylBlocTM (Oasis Grower Solutions, OH, USA) sachet containing 1-MCP 0.014% was applied in two treatments. A dose of 1-MCP was weighed using a precision digital balance (analytical model USS-DB58, Cleveland, OH, USA), then added to 5 L of water. The fruits were immersed in the solution for 10 min and left to dry in the open air. The fruits were subsequently stored at two temperatures: 4°C in a climatic chamber ref C240 (ISAK.S.AS) and 14°C room temperature, monitored with an ambient thermometer (Tab. 1).

TABLE 1. Postharvest treatments of curuba (*Passiflora tripartita*) fruits.

Treatments	Description	1-MCP	Temperature
T1	Control	0	14°C
T2	4°C	0	4°C
T3	1-MCP+14°C	3 mg L^{-1}	14°C
T4	1-MCP+4°C	3 mg L^{-1}	4°C

Variable evaluation

The following variables were evaluated in curuba fruits every 7 d in 4 fruits for each treatment. The epidermal color was measured with a digital colorimeter brand FRU WR-18 (Shenzhen MileSeey Technology Co., Ltd., Shanghai, China), and the parameters of the CIELab system were measured. This color spacing, widely used by researchers to evaluate color attributes, is expressed in numerical values. Colors are classified in terms of luminosity (brightness), hue (color), and saturation (vividity). Three measurements were taken in the equatorial area of the fruits during postharvest storage. The values of "a" <0 indicate the direction towards green and values >0 towards the red, while the values of "b" <0 indicate the direction towards blue and values >0 towards yellow. "L" indicates the luminosity, where zero (0) is black and one hundred (100) is white.

The color index (CI) was established according to Thompson (1998) (Eq. 1).

$$CI = \frac{(1000 \times a)}{(L \times b)} \quad \text{(Eq. 1)}$$

Total soluble solids (TSS), expressed in °Brix, were measured every 7 d using a Hanna HI 9681 digital refractometer (Hanna Instruments®, Woonsocket, RI, USA) with an accuracy of 0.01%, covering a range from 0 to 85%.

The total titratable acidity (TTA) was calculated using Equation 2 with the volume of NaOH until pH 8.2 was reached. To measure, 1 g of curuba juice, 3 drops of phenolphthalein were added to a beaker (AOAC, 2023).

$$\text{TTA\%} = \frac{(A \times B \times C)}{D} \times 100 \qquad \text{(Eq. 2)}$$

where A represents the volume of NaOH used, B represents the normality of NaOH (0.1 N), C represents the equivalent weight in grams of acid in the fruit (citric acid 0.064 g meq^{-1}) (Chaparro-Rojas *et al*., 2015), and D represents the fresh weight in grams of the sample. The maturity ratio was determined as the TSS/TTA ratio.

The respiration rate (mg CO_2 kg^{-1} h^{-1}) was measured according to Garavito *et al*. (2021) using a CO_2 analyzer, AtmoCheck® Double (Hi Tech Systems, Inc., Knoxville, TN, USA). To determine the respiration rate, 100 g of fruit were placed in airtight chambers for 30 min. The weights of the fruit were taken into account and the data was then converted to mg CO_2 kg^{-1} h^{-1}. The firmness of the fruit was measured using a GY-4 digital penetrometer with a 0.05 N approximation (Zhejiang Top Cloud-agri Technology Co., Ltd., Hangzhou, China).

Weight loss % (WL) was calculated according to Equation 3. The fresh weight of the fruits was measured using the analytical balance model USS-DB58, Cleveland, OH, USA, with a precision of 0.0001 g.

$$\%\ WL = \frac{(wi\text{-}wf)}{wi} \times 100 \qquad \text{(Eq. 3)}$$

where wi represents the initial weight of a fruit, and wf represents the weight obtained on the sampling date (measurements were taken weekly starting on day 0 of storage, with four fruits being evaluated per treatment).

Experimental design and statistical analysis

A completely randomized design with four treatments and four replicates was used, with five measurements over time for a total of 80 experimental units (4 fruits per experimental unit). The data was analyzed using R statistical software (R Studio interface, version 1.4). The assumptions of homogeneity (Barlett's test) and the normality of variance (Shapiro-Wilk test) were validated, followed by a one-way ANOVA analysis and Tukey's multiple comparison test (P≤0.05).

Results and discussion

Color

According to the three dimensions of color (Tab. 2), at 8 d of storage the L parameter increased for the control (59.64) and for 1-MCP (61.66). This shows that an increase in luminosity is correlated with a reduction in fruit chromaticity during the ripening process. This was also significantly different from the 4°C (37.66) treatment and 1-MCP+4°C (38.99). The two treatments with the longest

TABLE 2. Color of curuba fruits (*Passiflora tripartita*) in the three dimensions *L*, *a*, *b* during postharvest storage.

Color	Storage days	Treatments							
		Control		Temperature 4°C		1-MCP		1-MCP + Temp (4°C)	
		mean	SE	Mean	SE	mean	SE	mean	SE
L	0	44.98^{a}	1.59	38.24^{a}	1.44	41.74^{a}	1.78	39.15^{a}	0.77
	4	53.22^{a}	3.50	36.90^{b}	2.51	51.40^{a}	2.31	40.92^{ab}	0.65
	8	59.64^{a}	1.72	37.67^{b}	1.39	61.67^{a}	0.85	38.99^{b}	0.62
	15	61.39^{a}	2.08	37.67^{b}	1.81	56.99^{a}	0.48	39.37^{b}	1.37
	22			35.67^{b}	1.69			42.44^{a}	1.31
	29			29.91^{b}	1.60			40.34^{a}	1.24
a	0	-6.76^{a}	0.42	-7.73^{a}	0.46	-7.57^{a}	0.18	-8.38^{a}	0.36
	4	-4.42^{a}	0.64	$-7.98b^{c}$	0.24	-4.78^{ab}	0.84	-8.66^{c}	0.13
	8	2.35^{a}	0.29	-6.44^{b}	0.60	3.35^{a}	0.28	-8.51^{b}	0.16
	15	7.56^{b}	0.21	-4.54^{c}	0.54	12.61^{a}	0.43	-6.54^{c}	0.34
	22			-1.28^{a}	0.91			-5.56^{a}	0.25
	29			2.82^{a}	0.75			-3.06^{b}	0.50
b	0	28.35^{a}	0.75	28.57^{a}	1.39	32.74^{a}	2.25	31.16^{a}	0.54
	4	33.03^{a}	0.77	34.36^{a}	2.02	39.84^{a}	1.27	33.28^{a}	0.25
	8	34.97^{b}	1.38	43.86^{ab}	1.26	48.41^{ab}	1.08	40.04^{ab}	0.65
	15	47.68^{ab}	0.22	42.92^{ab}	1.65	56.18^{a}	1.75	41.71^{b}	1.01
	22			54.20^{a}	2.49			45.87^{a}	2.24
	29			44.85^{a}	2.39			44.44^{a}	0.70

The color parameters are of the CIELab system using the coordinates: L*(luminosity), a*(red/green), b* (yellow/blue). SE corresponds to the standard error of the data. Different letters in the row indicate significant differences according to the Tukey test (P≤0.05).

shelf life have a storage life of 29 d. This is related to weight loss and loss of fruit quality. Significant differences were observed in coordinate *a* between the control (2.35) and 1-MCP (3.34) treatment compared to the 4°C (-6.44) and 1-MCP+4°C (-8.50) treatments at 8 d; at 15 d significant differences were found for the control (7.56) and 1-MCP (12.61) compared to 1-MCP+4°C (-6.54) and 4°C (-4.54). The 4°C treatment (29.91) also showed significant differences from the 1-MCP+4°C treatment (40.33) at 29 d. A gradual increase in red color of fruit skin was observed in both control (7.56) and 1-MCP (12.61) treatments after 15 d of storage, while the fruits in treatments 4°C (2.82) and 1-MCP+4°C (-3.0) remained green until 29 d of storage. This indicates better color preservation at the temperature 4°C and 1-MCP+4°C treatments.

After 8 and 15 d of storage, significant differences were observed in parameter *b* between control (34.96) and 1-MCP+4°C treatment (40.03). The highest values were observed in control (54.14) and 1-MCP (58.92), approaching the yellow color, while the lowest value was observed in 1-MCP+4°C treatment (45.87). According to Téllez *et al.* (1999), a change from green to yellow color in a shorter time at a temperature of 20°C causes fruit wrinkling due to dehydration and the presence of fungi. According to Franco *et al.* (2013), carotenoid content increases as the fruit ripens, which can be related to a decrease in luminosity, with higher a-coordinates and lower b-coordinates, which was evidenced in the temperature 4°C and 1-MCP+4°C treatments.

Color index

At 8 d of storage, both control (1400) and 1-MCP (2631) had a rapid increase in color changes, significantly different from the treatments of 4°C (-7754) and 1-MCP+4°C (-8733). The same trend was observed at 15 and 22 d of storage, with significant differences in the same treatments. In addition, the fruits of the control and 1-MCP treatments lost their postharvest quality after 15 d of storage (Fig. 1). As observed by Fischer *et al.* (2018), the tonality in Passiflora fruit epidermis increases the maturity index. At 29 d of storage, the treatment of temperature 4°C (5092) showed significant differences with the treatment 1-MCP+4°C (-3362), highlighting the color stability with the 1-MCP+4°C treatment. This is consistent with Cheng *et al.* (2012), who found that pear fruits treated with 1-MCP had high chlorophyll content, intact chloroplasts with organized grana thylakoids, and low ethylene production for up to 30 d and 15 d of storage, respectively, compared to untreated fruits.

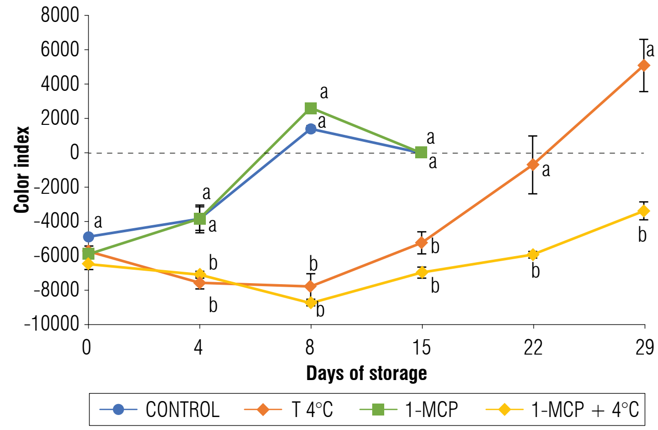

FIGURE 1. Color index of curuba fruits (*Passiflora tripartita*) subjected to preservation treatments during postharvest storage. 1-MCP – 1-methylcyclopropene. Different letters indicate significant differences according to Tukey's test ($P\leq0.05$); vertical bars indicate standard error (n=4). No data are presented for the control and 1-MCP treatments because they lost their postharvest quality after 15 d of storage.

Total titratable solid contents (TSS)

The TSS in fruits at 15 d of storage presents significant differences between the control (9.2 °Brix) and 1-MCP (9.5 °Brix) compared to the treatment of temperature 4°C (11.2 °Brix) and 1-MCP+4°C (9.8 °Brix). Thereafter, the TSS began to decrease in the fruits subjected to the temperature 4°C and 1-MCP+4°C treatments without significant differences between them until 29 d of storage (Fig. 2A). The decrease occurs because, as respiration increases during storage, some sugars are used as substrate in metabolic processes such as glycolysis and conversion to sucrose (Saltveit, 2019). This statement agrees with Téllez *et al.* (1999) regarding curuba fruits, where a decrease in TSS was found after 19 and 22 d of storage. Additionally, Botía-Niño *et al.* (2008) found no significant differences in temperature treatments for curuba fruits at week six of postharvest storage, indicating that continuous refrigeration can preserve the fruits for a longer period.

Total titratable acidity (TTA)

The results indicate that there were significant statistical differences in the TTA of the fruits after 8 d of storage. The fruits treated with 1-MCP+4°C had an increase in TTA (2.92%), while the TTA decreased in the control (1.65%), 1-MCP (1.46%) and temperature 4°C (1.81%) treatments. This decrease may be associated with increased respiratory metabolism in fruits, consuming acids in the tricarboxylic acid cycle (Vallarino & Osorio, 2018). These findings are consistent with a previous study conducted by Mayorga *et al.* (2020), who found higher total titratable acidity in

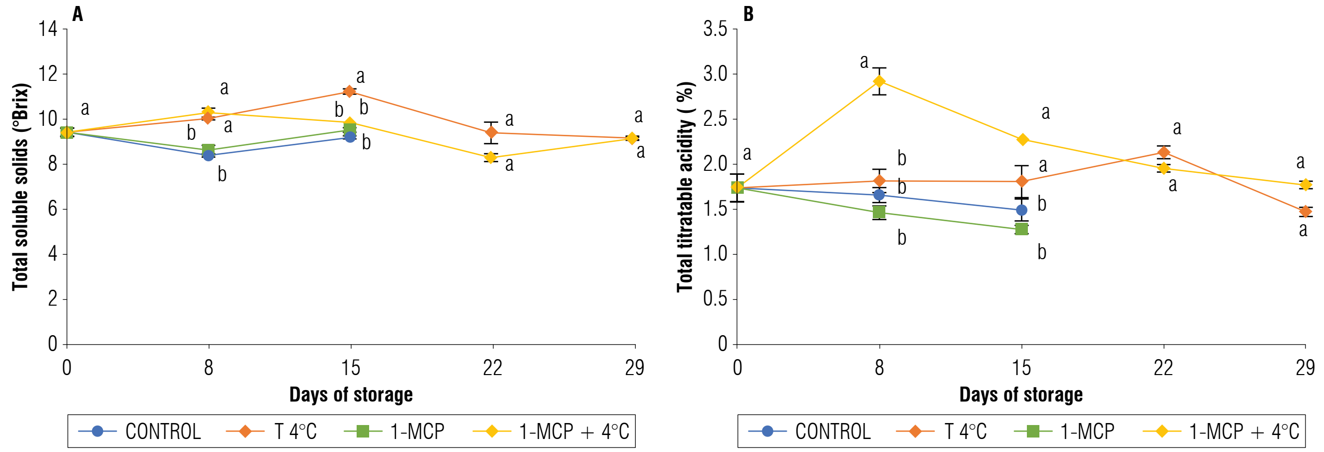

FIGURE 2. A) Total soluble solids and B) Total titratable acidity in fruits of curuba (*Passiflora tripartita*) subjected to preservation treatments during of postharvest storage. 1-MCP – 1-methylcyclopropene. Different letters indicate significant differences according to Tukey's test ($P\leq0.05$); vertical bars indicate standard error (n=4).

curuba fruits. After 22 d of storage, the treatments of temperature 4°C and 1-MCP+4°C showed significant differences in TTA compared with the control and 1-MCP, with a decrease in TTA observed (Fig. 2B). Similarly, Téllez *et al.* (1999) observed a gradual reduction in the TTA of curuba fruits during postharvest. Botía-Niño *et al.* (2008) reported that as fruits ripen, TTA decreases and new components such as organic acids and dehydrogenases are formed.

Maturity ratio

The maturity ratio calculated as TSS/TTA presented significant differences at 29 d of storage for the 4°C (6.56) treatment compared to the 1-MCP+4°C (5.09) (Fig. 3).

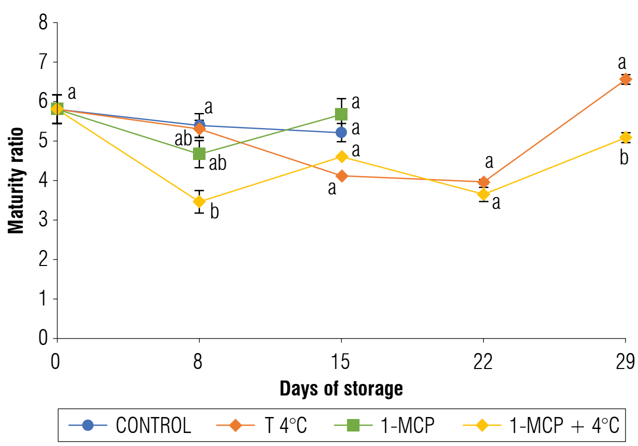

FIGURE 3. Maturity ratio in curuba fruits (*Passiflora tripartita*) subjected to preservation treatments during postharvest storage. 1-MCP – 1-methylcyclopropene. Different letters indicate significant differences according to Tukey's test ($P\leq0.05$); vertical bars indicate standard error (n=4).

According to Fischer *et al.* (2018), in fruits, as the maturity index increases, the TTA decreases and TSS increases. Similarly, Téllez *et al.* (2011) found that curuba fruits presented a higher maturity ratio during storage at room temperature. Likewise, Téllez *et al.* (1999) observed low values of maturity ratio in curuba fruits stored at 8°C. In addition, Botía-Niño *et al.* (2008) found lower maturity ratio values in curuba fruits in treatments with continuous refrigeration, which preserved them for a longer period.

Respiration rate

The respiration rate in fruits presented significant differences at 15 d of postharvest storage, with the control (329.80 mg CO_2 kg^{-1} h^{-1}) and 1-MCP (469.76 mg CO_2 kg^{-1} h^{-1}) differing from the 4°C (267.83 mg CO_2 kg^{-1} h^{-1}) and 1-MCP+4°C (230.41 mg CO_2 kg^{-1} h^{-1}) treatments. At 29 d of storage the 4°C (116.99 mg CO_2 kg^{-1} h^{-1}) and 1-MCP+4°C (146.92 mg CO_2 kg^{-1} h^{-1}) treatments did not present significant differences (Fig. 4). Similar to Téllez *et al.* (1999), curuba fruits presented a climacteric behavior in the respiration rate. On the other hand, Téllez *et al.* (2011) observed a respiration peak in curuba fruits without wax application at 20°C after 11 and 12 d storage and at 8°C after 12 d and 14 d storage. In this study, the respiration peak occurred at 22 d in the 1-MCP+4°C treatment. However, the 4°C and 1-MCP+4°C treatments presented lower respiration rates during the first 15 d compared to the control. This is consistent with Baraza *et al.* (2013), who reported that purple passion fruit (P*assiflora edulis* Sims) fruits treated with 1-MCP had a decrease in respiration rate.

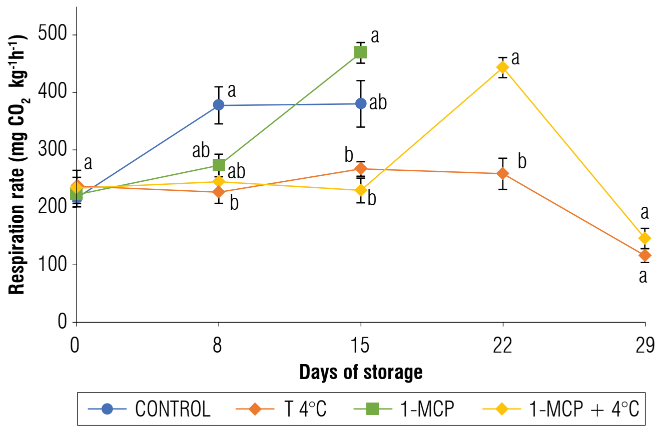

FIGURE 4. Respiration rate in curuba fruits (*Passiflora tripartita*) subjected to preservation treatments during postharvest storage. 1-MCP – 1-methylcyclopropene. Different letters indicate significant differences according to Tukey's test ($P \leq 0.05$); vertical bars indicate standard error (n=4).

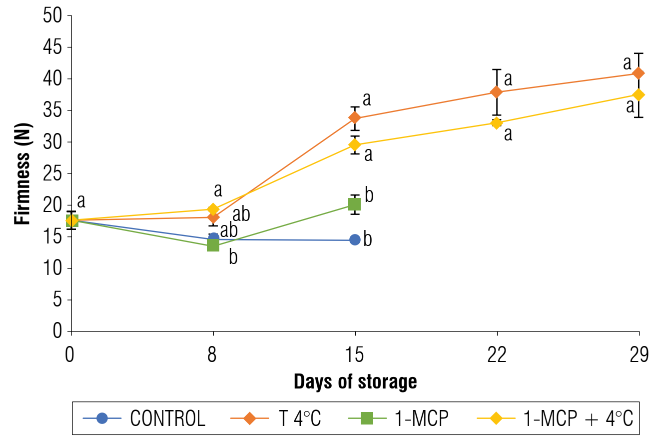

FIGURE 5. Firmness of curuba fruits (*Passiflora tripartita*) subjected to preservation treatments during postharvest storage. 1-MCP – 1-methylcyclopropene. Different letters indicate significant differences according to Tukey's test ($P \leq 0.05$); vertical bars indicate standard error (n=4).

Firmness

After 15 d of storage, fruit firmness differed significantly among the treatments. The 4°C (33.69 N) and 1-MCP+4°C (29.53 N) treatments had higher firmness compared to the control (14.42 N) and 1-MCP (20.08 N), which had lower firmness (Fig. 5). This is consistent with Téllez *et al.* (1999), who found that curuba fruits lose firmness as temperature increases. After 29 d of storage, firmness increased in 4°C (40.8 N) and 1-MCP+4°C (37.5 N) treatments, without significant differences among them. According to Maftoonazad *et al.* (2008), hydrolyzing enzymes of the cell wall ensure the firmness of the fruits during postharvest.

Li *et al.* (2020) found that treatment with 1-MCP in purple passion fruit (*Passiflora edulis* Sims) can regulate cell wall metabolism pathways, increase enzymatic activities, accelerate lignin accumulation, and reduce cell wall degradation during fruit ripening by inhibiting ethylene synthesis. The application of 1-MCP in *Passiflora* fruits helps to improve postharvest life and prevent diseases, as evidenced by a lower incidence of anthracnose, according to Dutra *et al.* (2018). In this sense, Botía-Niño *et al.* (2008) found that continuous refrigeration increased the firmness of curuba fruits, in contrast to the control fruits which experienced reduced firmness.

Weight loss

At 8 and 15 d of storage, weight loss in fruits significantly differed between the treatments 1-MCP (29.6%) and 1-MCP+4°C (18.8%) (Fig. 6). This is consistent with Téllez *et al.* (2011), who found that curuba fruits waxed and stored at low temperatures had lower weight loss and longer preservation. Similarly, Téllez *et al.* (1999) observed less weight loss in curuba fruits stored for 12 d and 14 d at 8°C compared to those stored at 20°C, which accelerated transpiration and oxidative metabolism of the fruits.

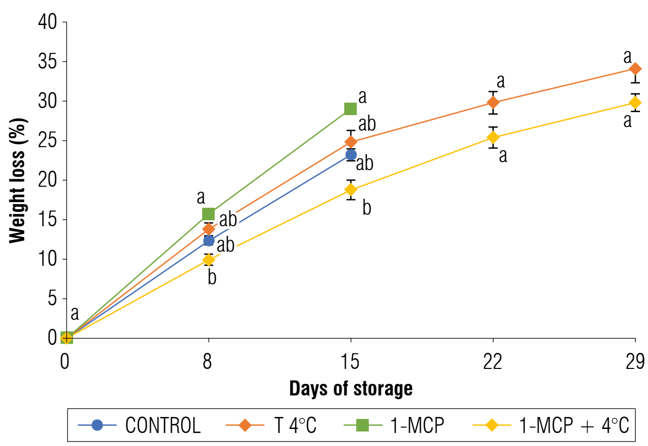

FIGURE 6. Weight loss in curuba fruits (*Passiflora tripartita*) subjected to preservation treatments during postharvest storage. 1-MCP – 1-methylcyclopropene. Different letters indicate significant differences according to Tukey's test ($P \leq 0.05$); vertical bars indicate standard error (n=4).

After 29 d of storage, the fruit weight loss was lower in the 1-MCP+4°C treatment (29.8%) compared to the 4°C treatment (34.1%). This is consistent with Franco-Mora *et al.* (2022), who reported decreased weight loss in *Passiflora biflora* fruits stored at temperatures below 4°C. Additionally, Botía-Niño *et al.* (2008) found that curuba fruits can be stored for a longer time at temperatures between 4°C and 7°C, which reduces weight losses due to respiration and transpiration, making them more suitable for consumption.

Conclusions

Curuba fruits in the 4°C and 1-MCP+4°C treatments exhibited lower values in color index, TSS (8 and 15 d of storage), maturity ratio, respiration rate, and weight loss, and higher values in TTA and firmness. This resulted in a postharvest storage life of 29 d compared to the control and 1-MCP treatments, which had a storage life of 15 d.

The 1-MCP+4°C treatment resulted in lower fruit weight loss, higher TTA and a progressive increase in TSS. It also provided high fruit firmness, lower values in color index and maturity fruit ratio compared to the other three treatments. Thus, this treatment can be considered as an alternative to improve the postharvest life of curuba fruits.

The use of 1-MCP delays the ripening process, and when accompanied by a temperature of 4ºC, is the most effective treatment, with a lower maturity index and weight loss. This slows oxidative metabolism, making it an alternative to improve the postharvest quality of the fruits.

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Conflict of interest statement

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

Author's contributions

DNSS and YADO designed the experiments, developed the methodology, and carried out the laboratory experiments; YADO contributed to the data analysis; and DNSS and YADO wrote the article. All authors reviewed the final version of the manuscript.

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Effect of calcium on fruit quality: A review

Efecto del calcio en la calidad de los frutos: una revisión

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ABSTRACT

Calcium (Ca) plays a crucial role as a nutrient influencing the ripening, post-harvest duration, and quality of fruits. Its impact on the cell wall and its function as a secondary messenger at the cellular level underscore its significance. While recently there has been an increase in studies examining the effects of Ca on fruit quality, there remains a need to consolidate and expand the literature on pre-harvest and post-harvest applications of Ca concerning the physical-chemical properties of fruits. This review aims to compile information on the mechanisms of Ca absorption by plants, as well as the interaction of Ca with the cell wall in fruit development and growth; the review also aims to synthesize literature on the effects of calcium on the physical and chemical properties of fruits that ultimately influence their quality. The review considers a comprehensive analysis of studies published in reputable scientific publishers (Elsevier, Springer, Frontiers, Wiley, MDPI, Hindawi, SciELO) over the last ten years, encompassing various relevant topics. Calcium proves effective in retarding the loss of firmness in fruits, increasing their mass, mitigating mass loss during storage, and extending postharvest life, thereby enhancing marketability. Furthermore, Ca demonstrates a role in decreasing the activity of enzymes responsible for cell wall degradation. Additionally, it reduces ethylene production in fruits, delaying the climacteric peak and reducing its intensity. Its application results in delayed color changes in fruits. For soluble solids, Ca diminishes sugar values and postpones their peak during the post-harvest period while maintaining high total acidity values. Notably, Ca applications contribute to a decreased incidence of certain physiological disorders.

Key words: fertilization, preharvest, postharvest, ripening, fruit development, physiopathy.

RESUMEN

El calcio (Ca) es un nutriente muy importante en la maduración, la duración poscosecha y la calidad de los frutos, debido a su acción en la pared celular y su papel como mensajero secundario a nivel celular. En los últimos años se han incrementado los estudios sobre el efecto del Ca en la calidad de los frutos; no obstante, la literatura sobre sobre las aplicaciones precosecha y poscosecha del Ca en las propiedades físico-químicas de los frutos debe ser consolidada y ampliada. Por lo anterior, esta revisión tiene como finalidad consolidar información sobre cómo es el mecanismo de absorción del Ca por la planta y su interacción con la pared celular en el desarrollo y crecimiento del fruto, así como recopilar literatura sobre el efecto del calcio en las propiedades físicas y químicas de los frutos y su calidad. La revisión considera un análisis exhaustivo de estudios publicados en prestigiosas editoriales científicas (Elsevier, Springer, Frontiers, Wiley, MDPI, Hindawi, SciELO) en los últimos diez años, abarcando diversos temas relevantes. El calcio resulta eficaz para retardar la pérdida de firmeza de los frutos, aumentar su masa, mitigar la pérdida de masa durante el almacenamiento y prolongar la vida poscosecha, mejorando así su comercialización. Además, el Ca desempeña un papel en la disminución de la actividad de las enzimas responsables de la degradación de la pared celular. Así mismo, reduce la producción de etileno en los frutos, retrasando el pico climatérico y reduciendo su intensidad. Su aplicación también produce un retraso en los cambios de color en los frutos. En cuanto a los sólidos solubles, el Ca disminuye los valores de azúcar y retrasa su pico durante el periodo poscosecha, manteniendo altos valores de acidez total. En particular, las aplicaciones de Ca contribuyen a disminuir la incidencia de ciertos desórdenes fisiológicos.

Palabras clave: fertilización, precosecha, poscosecha, maduración, desarrollo de fruto, fisiopatía.

Introduction

In recent years, Colombia has emerged as a significant producer and exporter of fruits and exotic food products. In 2021, fresh fruit production reached a volume of 12.4 million t, exhibiting an average annual growth of 3.7% since 2017 (Procolombia, 2021). This surge in production within the Colombian fruit sector is attributed to its tropical location, enabling year-round harvesting of fruits. Consequently, Colombia has solidified its position as one of the primary exporting nations of exotic fruits in Latin America (Analdex, 2023). In 2022, approximately 246 million kg of fresh fruits were consumed globally, marking a nearly 7 million kg increases from the previous year. This

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trend will persist in the upcoming years, which would allow reaching over 303.5 million kg in 2028 (Statista, 2023).

According to Aune *et al.* (2017), a high consumption of fresh fruits as part of a healthy diet can mitigate the risk of heart disease, cancer, obesity, and diabetes. The authors suggest that a minimum daily intake of 800 g is required to reduce mortality from various causes, offering health and environmental benefits. Moreover, most fruits are low in calories, rich in fiber, contain a high concentration of micronutrients, and are enjoyable for the palate (Guyenet, 2019).

In this context, the term "nutritional density" has been introduced to describe the proportion of nutrients in foods, including vitamins, proteins, fibers, antioxidants, and carbohydrates. This depends on the concentration of nutrients accumulated per unit of mass produced by a crop, and it is directly influenced both by the fertilization practices applied to plants (Barker & Stratton, 2020) as well as by the nutrient availability in the soil, the absorption capacity of the plant, irrigation, and environmental factors (Davis, 2009).

Fertilization in fruit trees has become an indispensable tool for intensive crop production, as the soil lacks the capacity to meet the optimal nutritional requirements demanded by such commercial agricultural operations (Srivastava & Malhotra, 2017). The absence of adequate fertilization leads to nutritional imbalances in plants, resulting in physiological disorders that can impact fruit yield and quality (Kumar & Kumar, 2016).

In this regard, fertilization with calcium (Ca^{2+}) positively influences the growth of organs and tissues and stimulates root growth by facilitating the incorporation of materials into cell walls (Prado, 2021). Initially, the Ca^{2+} ion is absorbed by the plant, transported through the xylem with the transpiration flow, and is considered a slightly mobile element within the plant when transported via phloem (Thor, 2019).

Calcium is more abundant in older leaves, and a deficiency of calcium is common in fruits, young leaves, and storage organs (Prado, 2021). In fruits, a deficiency of calcium is attributed to the reduced number of stomata, impeding the transport of calcium through the xylem (Bonomelli *et al.*, 2022). Plant absorption of Ca^{2+} is faster during the initial stages of fruit growth (Casero *et al.*, 2017), as it serves as an enzyme activator and an essential element for cell division, cell elongation, and growth (Prado, 2021).

Applications of calcium (Ca^{2+}) in fruits contribute to the maintenance of firmness and cell turgor, reducing the incidence of diseases, and preventing physiological disorders (Jain *et al.*, 2019). Additionally, calcium plays a crucial role in the expression of genes influencing fruit production and quality (Wu *et al.*, 2023), as it stabilizes cell walls and ensures their permeability, protecting them from enzymatic degradation (Zhi *et al.*, 2017). This regulation affects ripening in some fruits and influences respiration rates and ethylene production, thereby extending the quality of fruits during storage (Michailidis *et al.*, 2020).

Among the physiological disorders resulting from an insufficient Ca^{2+} supply or alterations in the balance of Ca with other nutrients (Freitas & Mitcham, 2012), various conditions have been identified, including 'blossom end rot' in tomatoes (Karlsons *et al.*, 2023), 'bitter pit' spots in apples (Griffith & Einhorn, 2023), cork spots in pears (Zhang & Cui, 2023), internal browning in pineapples (Zhang *et al.*, 2022), 'tip burn' in strawberries, 'cracking' in cape gooseberries (Fischer *et al.*, 2021), nectarines (Zhu *et al.*, 2023), grapevines (Yu *et al.*, 2020), and melons (López-Zaplana *et al.*, 2020), as well as the recently identified 'crease' skin wrinkling in citrus (Huai *et al.*, 2022), and these conditions result in significant economic losses (Yu *et al.*, 2020).

Among the treatments widely employed for fruit conservation, foliar applications and dips of $Ca(NO_3)_2$ and $CaCl_2$ have been extensively reported. These applications occur during fruit development, at specific intervals, and during the harvest period. The uniform distribution of this solution over the fruits enhances the concentration of Ca in internal tissues, contributing to prolonged shelf life (Dorostkar *et al.*, 2022). However, in some fruits, Ca applications have no effect since sometimes their results are reflected in leaves but not in fruits due to high leaf transpiration (Winkler & Knoche, 2019). While the application of Ca has demonstrated the potential to enhance fruit quality across various crops (Lobos, Retamales, Luengo Escobar *et al.*, 2021), the specific impact of Ca on fruit quality has not been thoroughly studied and summarized. Therefore, the objective of this work was to conduct a comprehensive review that compiles and presents information on the effects of Ca^{2+} on fruit production and quality.

Absorption of Ca by the plants

The movement of calcium (Ca) in the soil towards the roots occurs through mass flow and subsequently it enters the apoplastic space within the apical zone of the root (Doyle *et al.*, 2021). Ca is absorbed as a divalent cation (Ca^{2+}) by the cells of the root endodermis (Gulbagca *et al.*, 2020), specifically through channels located in the plasma membrane that can be voltage-dependent or voltage-independent. The first are activated by depolarization or hyperpolarization and are probably encoded by annexins, while the latter are activated by cyclic nucleotides (Lemtiri-Chliel *et al.*, 2020).

After absorption, Ca is transported with water apoplastically to avoid interference with its role as a secondary messenger. This is because the concentrations of Ca^{2+} in the cytosol must remain in submicromolar ranges (< 0.1 µM) to ensure the ability of cells to generate Ca^{2+} signals (Prado, 2021). Thor (2019) suggests that Ca^{2+} moves mostly via the apoplast from the epidermis of the cortex until it reaches the Caspary band that restricts the apoplastic movement of solutes. Even so, Ca^{2+} can reach the xylem via the apoplast only in regions where the Casparian bands are absent or discontinuous (White & Broadley, 2003). In the selective symplastic pathway, Ca^{2+} enters the cytosol through channel proteins and is pumped to the apoplast of the stele through Ca^{2+}-ATPases or the Ca^{2+}/H^+ antiport pathway, ultimately contributing to its upload in the xylem. From there, Ca^{2+} is transported to the shoots where it is unloaded and distributed to the leaf cells through channels activated by cyclic nucleotides (Wang *et al.*, 2017). In this phase, absorption is quite rapid; and Ca^{2+} supplies from the soil are of fundamental importance. Likewise, the Ca^{2+} flow rate is strongly influenced by transpiration (White & Broadley, 2003).

Calcium absorption by the fruits

In the realm of nutrition and calcium physiology, fruits pose challenges in water and nutrient supply due to their low transpiration rates and limited xylem transport networks compared to other plant organs. This limitation hinders the delivery of calcium to fruits (Hocking *et al.*, 2016). Key factors influencing the supply and distribution of calcium in aerial tissues include the mass flow rate of xylem sap (as Ca^{2+} is not highly mobile in the phloem), competition among ions for binding sites in xylem vessel walls, the presence of ionic transporters (including H^+, influencing pH), membrane channels, and the formation of poorly soluble or insoluble complexes, such as calcium oxalate (Sotiropoulos *et al.*, 2021). Calcium concentration in various cellular compartments can impact water transport through membrane-controlled pathways. An increase in cytosolic calcium concentration may decrease water transport through aquaporins, affecting the relative proportions of apoplastic and symplastic water flow and the magnitude of calcium supply. Symplastic pathways exhibit a lower capacity for long-distance Ca^{2+} movement (Doyle *et al.*, 2021).

In the early stages of fruit development and before the formation of Casparian bands in the root and the suberization of the tissues when the fruit is small, over 50% of the total calcium is contained within it. Therefore, the quantity of calcium fertilization applied to the soil before this stage is of principal importance, as well as the moisture content in the soil, and the presence of many idioblast cells in the root that serve as a Ca sink. Once the Casparian bands are formed, the idioblast cells provide Ca for cell elongation and differentiation (Storey *et al.*, 2003). However, as the fruits expand in later stages, most Ca^{2+} is transported to the leaves, where it cannot be redistributed to the fruits; this results in the dilution of Ca amounts in fruits (Doyle *et al.*, 2021). During this phase, foliar applications of calcium must be implemented through spraying to meet additional nutrient needs and prevent potential physiopathy related to calcium deficiency.

Casero *et al.* (2017) report that in apple (*Malus domestica* Borkh.) cv. 'Golden Smoothee' calcium is absorbed by fruits during the initial stages of development, accumulating until 80 d after full bloom (DAFB). The absorption peaks at 38 DAFB and may be antagonistic with nutrients such as potassium (K^+), ammonium (NH_4^+), and magnesium (Mg^{2+}) at the early stage of fruit development. Similarly, Bonomelli *et al.* (2022) find significant differences in calcium distribution in orange (*Citrus sinensis*) fruits cv. 'Fukumoto' with applications at various stages of development (30 DAFB, 44 DAFB, 66 DAFB, and 99 DAFB). The application at 30 DAFB resulted in the accumulation of 61% of calcium in the peel and 39% of calcium in the fruit pulp. This suggests that calcium mobility to the fruit pulp varies with phenological stages and fruit morphology and is more substantial at the early stages of development.

Doyle *et al.* (2021) note an inverse correlation between fruit growth rate and calcium content in fruits. In blueberry fruits, calcium content increases during early fruit development, with higher levels observed in peel and seed tissues compared to a fruit pulp (Doyle *et al.*, 2021). Calcium deficiency symptoms may manifest a few weeks after anthesis, primarily in the distal zone of the fruits.

Functions of calcium in the fruits

Adequate supply and transport of calcium are crucial for fruit development, as only a small portion of the absorbed calcium is transported to the fruits. Its accumulation relies on xylem flow due to the low mobility of calcium in the phloem (Gao *et al.*, 2019). Calcium accumulation in the fruits primarily occurs during the initial stages of fruit development, where calcium absorption is linked to cell division and metabolism, particularly during the initial fruit expansion. In the later stages of fruit growth, calcium absorption predominantly influences cell-to-cell adhesion (Hocking *et al.*, 2016).

Interactions between Ca and the cell wall during fruit development

Calcium combines with pectic acid in the cell wall to form calcium pectate, constituting the structural skeleton of the cell wall. This structure prevents the disintegration of the gel layer in the cell wall, enhancing the structural strength of the cell wall (Zhang & Wang, 2019). A sufficient calcium supply inhibits the entry of hydrolases and reduces changes in the pectic composition of the cell wall, thereby maintaining fruit firmness. Additionally, being a structural component of the cell membrane, calcium forms bridges connecting phospholipids and proteins within the plasma membrane. This interaction affects phase transition and fluidity, preserving membrane integrity (Xu *et al.*, 2022).

The cell wall comprises various polysaccharides forming a network of cellulose microfibrils linked to a matrix of pectins through α-1,4-glycosidic bonds and hemicelluloses (Hocking *et al.*, 2016). Additionally, it contains minor structural components such as proteins, lignins, and phenolic compounds (Polko & Kieber, 2019). Pectins stand out as the primary polysaccharide of the cell wall in most fruits, and calcium-pectin bonds significantly influence their physical, structural, and resistance properties (Cui *et al.*, 2021).

The prevalence of either ionic or ester bonds among pectins plays a crucial role in the physical properties of fruit cell walls, impacting the solubility of pectins (Hocking *et al.*, 2016). Homogalacturonan, the primary pectic polysaccharide, constitutes 60% of all pectins in the cell wall (Gawkowska *et al.*, 2018), interconnected with Ca^{2+} ions to form pectates, collectively known as the egg-box (Huang *et al.*, 2023).

During cell growth and expansion, acidification of the cell wall takes place, displacing calcium bound to pectin through the protonation of carboxyl groups (Hocking *et al.*, 2016). This phenomenon is triggered by changes in pectin concentration, leading to the release of apoplastic Ca^{2+} [Ca^{2+}]. These changes induce secretion and modification of pectins, crucial for fruit growth (Gao *et al.*, 2019). According to Hocking *et al.* (2016), apoplast pH is influenced by xylem sap pH under conditions of ample water supply, potentially resulting in significant alterations in cell wall dynamics and composition.

During fruit growth, pectins undergo de-esterification, catalyzed by pectin methylesterases (PMEs), releasing carboxylic residues linked to calcium (Hocking *et al.*, 2016). The level of PME activity and the availability of Ca^{2+} within the apoplast significantly impact the strength of the cell wall, its expansion, as well as the disassembly and remodeling of pectin (Khan *et al.*, 2019). In the ripening phase, calcium inhibits polygalacturonase (PG) activity, and a reduction in calcium concentration favors increased PG activity (Hocking *et al.*, 2016). PG exhibits its maximum expression at 100 d after anthesis (DAA) in grapes, indicating a programmed role in cell wall disassembly at maturity (Khan *et al.*, 2019). At the initial stages of fruit growth, low activity of PG and pectate lyases (PL) is regulated by specific isoforms of these enzymes (Hocking *et al.*, 2016). However, as the fruit matures, extensive depolymerization of pectins occurs, resulting in shorter subunits that are more soluble, indicating a decrease in the xyloglucan and cellulose networks in the cell wall. This is attributed to the action of PG and PL in tomato fruits. In this context, Nie *et al.* (2022) tested targeted mutations of the SlPG gene and found that it was possible to delay the loss of firmness and water in tomato fruits.

The strength of the bonds forming Ca pectates is dependent on pH, with apoplastic values between 6 and 7 enhancing these bonds. Furthermore, the formation and dissolution of pectate gels by Ca bonds depend on the level of de-esterification, specifically the concentration of free carboxyl groups and free calcium ions (Frempong *et al.*, 2022). As the dissolution and hydration of pectic gels occur, the breaking of calcium bonds with pectins increases, resulting in a reduction in cell wall rigidity evident in small deformations. This process occurs at a pH of 3, where the resistance of the links is minimal (Lara-Espinoza *et al.*, 2018).

According to Gao *et al.* (2019), an adequate calcium concentration in the fruits delays and decreases the climacteric peak and ethylene production. This is largely dependent on the phosphorylation of calcium-dependent proteins, inhibiting the activity of 1-aminocyclopropane-1-carboxylic acid oxidase (ACO) and ethylene-forming enzyme (EFE), subsequently reducing the contents of 1-aminocyclopropane-1-carboxylic acid (ACC).

Moreover, calcium treatments also diminish microbial growth and susceptibility to pathogens, delaying fruit ripening and extending postharvest life (Gao *et al*., 2019). However, an excessive calcium supply can elevate cytosolic calcium concentration, potentially causing membrane damage and increase respiratory intensity.

Calcium exerts an inhibitory effect on enzymes and co-enzymes during fruit ripening and softening (Huai *et al*., 2022). It significantly suppresses the expression of genes that encode cell wall-degrading enzymes such as cellulase, pectinesterase, polygalacturonase, β-galactosidase, and pectate lyase. However, when pectin degradation occurs, there is an increase in free Ca^{2+}, resulting in the loss of cell-to-cell polymerization and fruit softening (Gao *et al*., 2019). Additionally, in the cell membrane, calcium inhibits lipid peroxidation initiated by the activation of the enzyme lipoxygenase. Calcium also enhances the activity of enzymes neutralizing reactive oxygen species, including superoxide dismutase, peroxidase, catalase, and the enzymes of ascorbate-glutathione cycle (Xu *et al*., 2022).

According to Saure (2005), the concentration of calcium varies according to the different growth rates of each part of the fruit. Calcium in the fruit can be found as water-soluble Ca or exchangeable Ca; the latter increases during fruit development until reaching 84% of the total Ca that indicates that as the fruit matures, Ca is found in more available forms. Likewise, Ca can be redistributed, since, in ripe fruits, the concentration of Ca is higher in the peel and lower in the pulp section just below the peel.

As a secondary messenger calcium elevates the level of phosphorylation in fruits, inducing the activation of MAKP (mitogen-activated protein kinases) cascades. These cascades control protein stability and regulate the expression of genes that inhibit ethylene synthesis, ultimately prolonging the post-harvest life of the fruits (Yu *et al*., 2018).

Effect of calcium on the physical properties of the fruits

The physical transformations within fruits play a pivotal role in determining their postharvest handling. These changes primarily manifest in the cell walls of the fruits and are associated with the modification and continuous solubilization of pectins. Generally, controlled depolymerization of the cell wall structure occurs, leading to the loss of cell adhesion, which results in the associated reduction of fruit firmness and the leakage of internal juices during the ripening process. Consequently, this leads to mass loss and a decline in overall quality (Fig. 1).

Mass loss

Calcium mitigates mass loss in fruits by reducing the phosphorylation of phosphatidylinositol bisphosphate. This reduction diminishes the activity of aquaporins, thereby likely decreasing the movement of water from the cytoplasm to the apoplast. This, in turn, limits evaporation and helps maintain fruit weight for an extended period (Xu *et al*., 2022).

In support of this, Sinha *et al.* (2019) observed that a pre-harvest application of 2% $Ca(NO_3)_2$ by spraying on plum trees resulted in approximately 1% less mass loss in fruits compared to the control during all post-harvest measurements. Similarly, Ali *et al.* (2021) sprayed pre-harvest peach fruits with 1% $CaCl_2$ and found lower mass losses (ranging from 21.6% to 26.9%) compared to other treatments and the control (ranging between 34.66% and 35.16%). Additionally, Thakur *et al.* (2019) applied $CaCl_2$ at the time of harvest in melon (*Cucumis melo*) and papaya, reducing the loss of fruit mass after 20 d after harvest (DAH). In lemon, at 42 DAH, mass loss was 28.7% for control fruits and 23.2% for those treated with 0.18 M $CaCl_2$ (Frempong *et al*., 2022). Xu *et al.* (2022) immersed apple fruits at harvest in doses of 2% $CaCl_2$ for 30 min resulting in mass losses ranging between 4.7% and 5.6% for treated and control fruits. Similarly, calcium application is known to decrease mass loss in grapes (Shi *et al*., 2023) and blueberries (Lobos, Retamales, Luengo Escobar *et al*., 2021).

Firmness

Firmness is a critical quality parameter in fruit marketing (Huang *et al*., 2023). Calcium enhances fruit firmness by strengthening the cell wall structure, reinforcing cell adhesion, and forming Ca pectate when combined with pectic acid (Zhang & Wang, 2019). Additionally, exogenous calcium application causes the accumulation of homogalacturonans and an increased number of pectin networks (Huang *et al*., 2023).

Calcium applications are typically done pre-harvest alongside fertilization, while in other instances, they are directly administered to fruits during the final stages of development or post-harvest. These applications have demonstrated positive outcomes in enhancing or sustaining fruit firmness over an extended period. However, according to

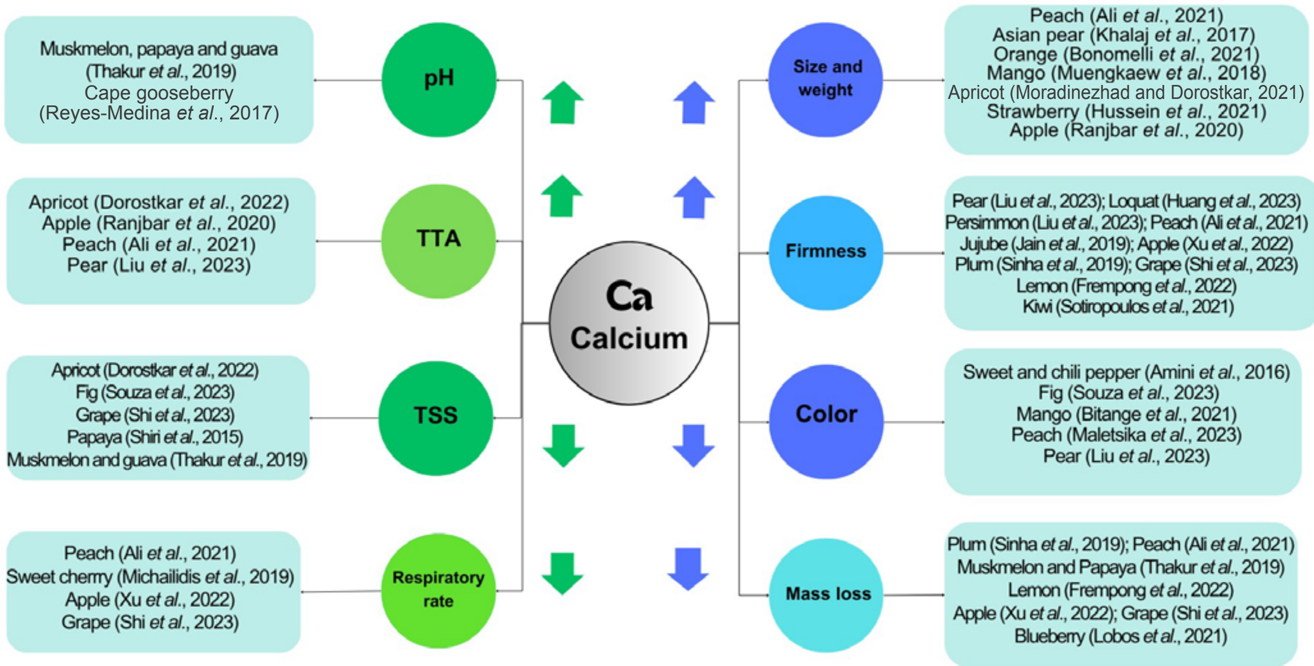

FIGURE 1. Effect of calcium on the physical and chemical properties of fruits. TTA – total titratable acidity, TSS – total soluble solids.

Sena *et al.* (2024), foliar Ca fertilizations prove to be more effective than soil applications in augmenting Ca levels within the fruits. In this context, Khakpour *et al.* (2022) observe that the soil application of 150 g of $Ca(NO_3)_2$ per apple tree results in fruits exhibiting increased firmness compared to the control group. Conversely, Álvarez-Herrera *et al.* (2022) found no significant disparities in fruit firmness when employing fertilizations ranging from 0 to 100 kg ha^{-1} of Ca.

Liu *et al.* (2023) further notes that $CaCl_2$ application reduces the amount of water-soluble pectins, indicating prolonged firmness in persimmon (*Diospyros kaki*) fruits. Ali *et al.* (2021) applied $CaCl_2$ through pre-harvest sprays (1%, 2%, and 3%) and significantly increased peach fruit firmness by 28%, 13%, and 24%, compared to the control. Overall, calcium applications have successfully maintained fruit firmness for longer durations in various fruits such as jujube (*Ziziphus mauritiana* Lamk.) (Jain *et al.*, 2019), papaya, melon, guava (Thakur *et al.*, 2019), apple (Xu *et al.*, 2022), plum (Sinha *et al.*, 2019), grapevine (Shi *et al.*, 2023), blueberry (Lobos, Retamales, Luengo Escobar *et al.*, 2021), lemon (Frempong *et al.*, 2022), and kiwi (Sotiropoulos *et al.*, 2021). Also, Melo *et al.* (2022) find that two weekly applications of 0.3 g L^{-1} of $CaCl_2$ increase the calcium content and firmness in tomato fruits. During postharvest, Liu *et al.* (2023) immersed freshly harvested pear fruits in 5 g L^{-1} of Ca lactate for 10 min, resulting in fruits with greater firmness at 8 DAH (28.8 N) compared to the control (27.3 N). A similar effect was observed in loquat (*Eriobotrya japonica* Lindl), where Ca lactate increased fruit firmness from 4.1 to 4.6 N at 30 DAH.

Color

Color significantly influences the physical appearance and market acceptability of fruits, making it a crucial quality parameter (Sinha *et al.*, 2019). Calcium is known to delay changes in fruit color by retaining chlorophyll content. The application of Ca reduces respiratory intensity and ethylene production, thereby slowing down the ripening phase (Souza *et al.*, 2023). This retention of green pigments is attributed to the inhibition of enzymes, such as chlorophyllase, by Ca, which is the initial enzyme involved in chlorophyll degradation (Nassarawa *et al.*, 2024). In this context, Bitange *et al.* (2021) notes that the application of Ca slows chlorophyll degradation and pigment synthesis, aligning with Amini *et al.* (2016), who observed a reduction in carotenoid content in sweet pepper fruits treated with nano-calcium.

Souza *et al.* (2023) reported that spraying the plants with 1% $CaCl_2$ maintained high chromaticity and hue values in fig fruits. Similarly, Bitange *et al.* (2021) observed higher hue values in mango fruits treated with $CaCl_2$ compared to the control throughout the postharvest period. Additionally, in peaches, the epidermis of the fruits exhibited a greener color after treatment with $CaCl_2$ (Maletsika *et al.*, 2023). Liu *et al.* (2023) found that the values of L*, a*, and b* in pear fruits increased to a lesser extent with the application of Ca lactate compared to the control, indicating that postharvest fruits subjected to Ca treatments experience a delay in color development, making them less perishable.

Fruit size and mass

Calcium plays a pivotal role in the growth and development of fruits, particularly during the cell division stage; it impacts the size and mass of the fruits, as observed in peaches (Ali *et al.*, 2021). Foliar application of 1% $CaCl_2$ in peach trees improved the fresh mass, size, and pulp-to-stone ratio, resulting in larger-diameter fruits and a higher economic return. Khalaj *et al.* (2017) demonstrated that foliar application of Ca increased pectic substances in the cell walls, leading to an increase in pericarp tissue thickness and overall fresh mass of the fruits. Additionally, the influence of Ca on the contents of endogenous growth substances, especially cytokinins, has been reported (Ali *et al.*, 2021). In this context, Bonomelli *et al.* (2022) found that early applications of Ca were more effective in increasing the dry mass of orange fruits.

Similar results have been reported for the improvement of physical traits in various horticultural products through the use of $CaCl_2$. In mango fruits, applications of $CaCl_2$ increase length, width, thickness, and average fresh mass (Muengkaew *et al.*, 2018). In apricot, these increase the diameter and fresh mass of the fruits (Moradinezhad & Dorostkar, 2021). In strawberries, the diameter increases (Hussein & Al-Doori, 2021), and in apples, the fresh mass and diameter increases following by applications of $CaCl_2$ (Ranjbar *et al.*, 2020). In contrast, Hirzel (2023) found that, for Ca applications ranging between 0 and 4 kg ha^{-1}, the size and mass of blueberry fruits are not affected; similarly, Yu *et al.* (2020) show that the application of Ca did not significantly affect the diameter of grapefruits.

Effect of Ca on the chemical properties of the fruits

Among the chemical changes during fruit development are depolymerization and modifications in the side chains of pectin, degradation of xyloglucan, increasing activity of non-catalytic proteins (arabinogalactan proteins, AGP), and processes related to ripening such as the accumulation of solutes and acid degradation, some of which are detailed below.

Total soluble solids (TSS)

One of the most impactful characteristics of fruits on the market is flavor, primarily determined by the amount of TSS. During ripening and storage, the starch in fruits slowly converts into sugars. However, calcium slows down respiration and metabolism, thereby delaying processes like the hydrolysis of polysaccharides to monosaccharides, which, in turn, delays ripening and reduces the TSS of fruits during postharvest (Dorostkar *et al.*, 2022). Similarly, Souza *et al.* (2023) mention that the application of $CaCl_2$ decreases the concentration of TSS and reduces the content of reducing sugars in figs, attributed to the greater stabilization of pectin structures provided by calcium. Shi *et al.* (2023) find that the application of Ca in grapevine fruits delayed the maximum TSS peak from 14 DAH in the control to 28 DAH for the treated fruits. Shiri *et al.* (2015) report that TSS decreases in papaya fruits with increasing calcium concentration. Furthermore, Thakur *et al.* (2019) mention that the application of $CaCl_2$ decreases the amount of TSS by 20%, 12.6%, and 9.34% compared to the control in papaya, melon, and guava fruits, respectively, after 20 d of storage. However, in some fruits, the application of calcium does not affect the TSS concentration, such as apples (Fallahi & Mahdavi, 2020), blueberries (Lobos, Retamales *et al.*, 2021), and cape gooseberry (Álvarez-Herrera *et al.*, 2022).

Total titratable acidity (TTA)

The effects of Ca application on TTA in fruits vary depending on the application period, storage time, concentration, and species evaluated (Souza *et al.*, 2023). Ali *et al.* (2021) mention that the application of calcium decreases the activity of oxidative enzymes, such as ascorbate oxidase, peroxidase, oxidase, catalase, and polyphenol oxidase, which, during storage, decrease the amount of organic acids (Shi *et al.*, 2023). This indicates that the treatment of fruits with Ca maintains high TTA values (Dorostkar *et al.*, 2022).

Ranjbar *et al.* (2020) applied $CaCl_2$ and nano-calcium to pre-harvest apple fruits and found that TTA increased at the time of harvest, attributed to the decrease in respiratory activity, leading to a decrease in the hydrolysis of organic acids. Ali *et al.* (2021) mention that fruits treated with 1% $CaCl_2$ maintain high TTA values during storage due to a decrease in respiratory intensity, preventing the decrease in acids used as a respiratory substrate (Saltveit *et al.*, 2019). Similarly, Liu *et al.* (2023) achieve higher TTA values throughout the postharvest in pear fruits with the application of Ca, consistent with that reported in apricot (Dorostkar *et al.*, 2022). In contrast, Maletsika *et al.* (2023) find that the application of calcium in peach fruits decreases TTA as well as the concentration of citric and quinic acid. However, these authors mention that the lower values of organic acids found are not related to the ripening of the fruits. Nevertheless, they obtain higher contents of succinic acid in fruits. Ribeiro *et al.* (2020) report that the preharvest application of 4% $CaCl_2$ decreases TTA in guava fruits. In other studies, the application of Ca through fertilization does not affect TTA values in gooseberry fruits (Álvarez-Herrera *et al.*, 2022) and blueberries (Lobos, Retamales *et al.*, 2021).

pH

During ripening, fruit filling occurs mainly by symport, in which H^+ plays an important role, as they are part of substrates such as glucose and sucrose. Consequently, the concentration of H^+ at the vacuolar level decreases, and the pH increases slightly. Therefore, it is likely that the application of Ca slows down the decrease in pH by decreasing respiratory and fruit metabolism, minimizing the oxidation processes of organic acids (Álvarez-Herrera *et al.*, 2022). Thakur *et al.* (2019) mention that the application of 2% $CaCl_2$ in papaya, melon, and guava maintain higher pH values in fruits (4.15, 4.48, and 3.85, respectively) at the end of storage (20 DAH) compared to the control (3.80, 4.05, and 2.30, respectively). Similarly, Reyes-Medina *et al.* (2017) obtained higher pH values in cape gooseberry fruits at 35 DAH with the application of 0.5% $CaCl_2$.

Respiration

The respiratory intensity of the fruits is affected by the concentration of Ca (Michailidis *et al.*, 2019). According to Khlopkov *et al.* (2021), sudden changes in respiration are associated with a drastic change in the concentration of some ions. Ca has a significant effect on the activity of a series of enzymes and membrane complexes involved in respiration, including alternative NADPH-dehydrogenases or complex IV. Once the concentration of Ca increases at the cytoplasmic level during the generation of electrical signals, it leads to an increase in Ca in the mitochondrial matrix, where Ca is likely involved in the formation of the respiratory response (Sweetman *et al.*, 2020). Similarly, Gao *et al.* (2019) mention that the application of Ca can delay and reduce the intensity of the climacteric peak. Ali *et al.* (2021) applied Ca at preharvest and managed to reduce the climacteric values in peach fruits by 1.4 times on average. Michailidis *et al.* (2019) find a reduction in respiratory activity with calcium applications in cherry fruits (*Prunus avium* L., cv. 'Tsolakeika'), while Xu *et al.* (2022) demonstrate that the application of 2% $CaCl_2$ in apples managed to reduce respiration by 17.5% during all measurements over 20 d of storage. Furthermore, as the preharvest application of Ca increases, ethylene production decreases in peach fruits (Ali *et al.*, 2021) and grapevines (Shi *et al.*, 2023).

Ca in physiological fruit disorders

Calcium deficiencies usually appear in fruits at the initial stages, and although sometimes they are not noticeable to the naked eye, they can cause disorders that affect the development and quality of fruits and, consequently, the postharvest behavior. In this regard, low levels of Ca^{2+} in fruits cause bitter spots, blossom end rot, cracking, and watery fruits, among other physiological disorders (Gao *et al.*, 2019). On the other hand, if high concentrations of calcium occur, it can cause cellular toxicity, cell walls that are too rigid, and abnormalities in the growth and development of the fruits (Hocking *et al.*, 2016).

Bitter pit

Bitter pit manifests as concentrated depressions at the end of the calyx of apple fruit caused by the physiological degradation of cells under the epidermis. This phenomenon is associated with localized calcium deficiency, occurring between the fourth and sixth week after anthesis during the stage of cell division and elongation (Griffith & Einhorn, 2023). The decrease in Ca levels leads to an increase in the concentration of potassium and magnesium, affecting membrane permeability and potentially resulting in cell death (Yahia *et al.*, 2019). Similarly, Ranjbar *et al.* (2020) note that apple fruits treated with nano-calcium exhibit reduced sensitivity to bitter spots, as measured by the K/Ca ratio. Additionally, Griffith and Einhorn (2023) suggest that abscisic acid, as a gibberellic acid antagonist, mitigates bitter pit stains by reducing transpiration, improving xylem function, and facilitating calcium transport to the fruits.

Blossom end rot (BER)

Blossom end rot occurs at the apical end, specifically in the section of the fruit opposite the stem. It is associated with low absorption and distribution of calcium during stages of it's high demand, leading to a lack of union between the cell wall and pectins, resulting in reduced cellular resistance. The lesion begins as a dark-colored depression that oxidizes as it grows. Gao *et al.* (2019) mention that increased PME activity raises the Ca^{2+} contents bound to the cell wall, decreasing the availability of Ca^{2+} for other functions and generating susceptibility to blossom-end rot.

Moreover, Watanabe *et al.* (2021) find that the lowest incidences of BER in tomatoes occur with less fruit growth and high concentrations of Ca during the initial stages of growth, particularly when Ca distribution is preferentially directed towards the fruits instead of the leaves. BER prevention involves pre-harvest Ca application. In this regard, BER is lower in plants treated with calcium (Coulibaly *et al.*, 2023). Similarly, Reitz *et al.* (2021) applied $CaCl_2$ to tomato fruits, reducing BER severity with a 10 mg L^{-1} dose.

Cracking

Cracking involves the fissuring of the fruit peel and outer pulp during cell expansion (Yahia *et al.*, 2019), occurring in the final stage of fruit growth where the layer of newly enlarged cells exhibits weak elasticity, in addition to the biochemical changes that the exocarp undergoes, leading to cracking (Santos *et al.*, 2023). This phenomenon occurs in fruits of the crops such as cape gooseberry (Fischer *et al.*, 2021), nectarines (Zhu *et al.*, 2023), grapevines (Yu *et al.*, 2020), pears (Seo *et al.*, 2022), melons (López-Zaplana *et al.*, 2020), and others. Choi *et al.* (2020) suggest that cracking arises from rapid cell division, non-uniform arrangement of epidermal cells, and intense rain periods (Seo *et al.*, 2022). In that sense, excessive water contents in soil increase turgor pressure at the cellular level, rapidly increasing the volume of the fruits, which exceeds the extensibility of the peel and causes cracking (Santos *et al.*, 2023).

Numerous studies link fruit cracking to cell wall metabolism, affirming that Ca^{2+} can inhibit cell wall softening, thereby increasing the structural strength of fruit peel (Fan *et al.*, 2023). Zhu *et al.* (2023) report that nano-calcium application decreases cracking in nectarines by up to 20%. Ca applications have also decrease fruit cracking incidence in cherries (Matteo *et al.*, 2022), grapevines (Shi *et al.*, 2022), and cape gooseberry (Álvarez-Herrera *et al.*, 2012). Additionally, Cooman *et al.* (2005) found, in cape gooseberry, that fruit cracking is influenced by the presence of Ca or B in the fertilizers applied, with this physiopathy increasing between 5.5% to 13.0% when either of these two elements was absent from the nutrient solution.

Cork spot

Cork spot, primarily occurring in pears and apples, are attributed to calcium deficiencies. The main symptoms involve the appearance of round, sunken spots on the fruit peel, followed by subsequent browning and lignification of the pulp beneath the damaged area (Zhang & Cui, 2023). In apples, the spots acquire a bitter taste and impact the fruit size, with their occurrence being more frequent in larger fruits (Yahia *et al.*, 2019). Cui *et al.* (2021), inducing Ca deficiency with nitrendipine in pear fruits, find a 12% higher incidence of cork spots than in the control. However, they mention that Ca^{2+} concentration is not the sole biochemical indicator of cork spot.

Spongy tissue

Spongy tissue, a physiological disorder associated with calcium deficiency, results in poor quality and an unpleasant flavor in mango fruits (Ma *et al.*, 2023). External symptoms are not evident, and the physiopathy becomes apparent only upon cutting the fruits (Yahia *et al.*, 2019). Alterations develop in the pulp closest to the seed, acquiring a spreading brown color over time, leading to a decrease in nutritional properties and affecting marketing (Yahia *et al.*, 2019). Positive regulation of specific genes increases Ca accumulation in the cell wall and vacuoles of the pulp, reducing available Ca for other metabolic functions, thus, altering cellular Ca homeostasis and causing localized Ca deficiency, resulting in the development of spongy tissue (Ma *et al.*, 2023).

Watery fruits

This physiopathy, reported by Gao *et al.* (2019), primarily occurs in Passifloraceae, such as passion fruit, banana passion fruit, and purple passion fruit. It involves the alteration of textural and ultrastructure properties of the peel, causing softening and giving the impression of a watery fruit with low quality (Xu *et al.*, 2023). Applying $CaCl_2$, as mentioned by Xu *et al.* (2023), maintains the structural integrity of the passion fruit peel, reduces enzymatic activity, and preserves postharvest quality, delaying peel wrinkling and fruit senescence.

Sunburn

Sunburn is damage occurring in the epidermis of fruits caused by a combination of events such as water deficit, salinity, high temperature, and intense solar radiation, expected to be more common due to climate change (Park *et al.*, 2022). This disorder is primarily observed in high tropic areas, and a strategy to mitigate its effects is the application of calcium carbonate nanoparticles (Teixeira *et al.*, 2022), which act as a suppressor and reduce sunburn in pineapple (Fischer *et al.*, 2022).

Conclusions

Foliar applications of calcium have a more significant impact on fruits when carried out in the initial stages of development. Calcium positively influences fruit mass and reduces fresh mass loss during storage. The softening of fruits is delayed due to calcium inhibitory action on enzymes degrading the cell wall, maintaining firmness for an extended period, and favoring fruit storage. Calcium applications maintain high total acidity values while generating low total soluble solids (TSS) values, delaying TSS peaks during postharvest. Calcium decreases ethylene production, delays the climacteric peak, and reduces its intensity, along with delaying color changes in fruits. Calcium also reduces the appearance of physiological disorders related to deficiencies, playing a crucial role in preventing disorders that affect fruit quality. It's noteworthy that the deficiency of this nutrient causes specific symptoms in each species.

Conflict of interest statement

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

Author's contributions

MJG wrote the initial draft and carried out the final revision of the manuscript. JAH wrote, carried out the revision of the manuscript, and translated the initial draft. GF revised and complemented the manuscript. All authors reviewed the final version of the manuscript.

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Effect of planting density and fertilization on yield and tuber characteristics of potato of Group Andigenum

Efecto de la densidad de siembra y la fertilización en el rendimiento y las características de tubérculos de papa del grupo Andigenum

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ABSTRACT

Fertilization and planting density are important practices in potato-producing regions, particularly in regions where yields are not optimal. In addition, appropriate characteristics are required for industrial processing of potato, prompting farmers to improve their productivity and produce varieties demanded by the agroindustrial market. The aim of this study was to determine the effect of two planting densities: 0.20 m x 1.00 m (50,000 plants ha^{-1}) and 0.40 m x 1.00 m (25,000 plants ha^{-1}) and three NPK fertilization doses (60-60-60, 120-120-120, 180-180-180) on the yield and tuber characteristics of potato (*Solanum tuberosum* Group Andigenum) varieties Serranita, Luyanita, and Capiro. A randomized block design was used with subdivided plots and three replicates. The results indicate higher total yield and commercial yield (42.87 and 38.74 t ha^{-1}) with the 180-180-180 kg ha^{-1} dose of NPK. The Luyanita variety stood out with yields of 36.03 and 30.97 t ha^{-1} of total and commercial tuber yield, respectively. The highest total and commercial tuber yields were obtained with the density 0.20 m x 1.00 m. The Luyanita variety showed acceptable physicochemical characteristics for agribusiness with 24.73% dry matter, low content of reducing sugars (0.21%), acceptable color of fries (3.11), specific gravity (1.11 g cm^{-3}) and adequate oil content (20.44%). The planting density factor showed no statistically significant differences.

Key words: varieties, *Solanum tuberosum*, soil, fertility.

RESUMEN

La fertilización y densidad de siembra son las prácticas más importantes en zonas productoras de papa, en las cuales los rendimientos no son óptimos; además se requiere de características apropiadas de los tubérculos para procesos industriales. Por lo tanto, los agricultores quieren mejorar su productividad y producir variedades que exige el mercado agroindustrial. El objetivo de este estudio fue determinar el efecto de dos densidades de siembra: 0.20 m x 1.00 m (50 000 plantas ha^{-1}) y 0.40 m x 1.00 m (25 000 plantas ha^{-1}) y tres dosis de fertilización NPK (60-60-60, 120-120-120, 180-180-180), en el rendimiento y características de los tubérculos de papa (*Solanum tuberosum* grupo Andigenum) de las variedades Serranita, Luyanita y Capiro. Se utilizó un diseño de bloques al azar con parcelas subdivididas y tres repeticiones. Los resultados indican mayor rendimiento total y rendimiento comercial (42.87 y 38.74 t ha^{-1}) con la dosis de NPK de 180-180-180 kg ha^{-1}. Se destacó la variedad Luyanita con rendimientos de 36.03 y 30.97 t ha^{-1} de rendimiento total y comercial de tubérculos. El mayor rendimiento total y comercial de tubérculos se obtuvo con la densidad 0.20 m x 1.00 m. La variedad Luyanita presentó características de tubérculos aceptables para la agroindustria debido a 24.73% de materia seca, bajo contenido de azúcares reductores (0.21%), color de fritura aceptable (3.11), gravedad específica (1.11 g cm^{-3}) y contenido de aceite (20.44%) adecuados. Para el factor densidad de siembra, no hubo diferencias estadísticamente significativas.

Palabras clave: variedades, *Solanum tuberosum*, suelo, fertilidad.

Introduction

In 2022, potato production in Peru reached 6.0 million t across nineteen departments of Peru, with 340.9 thousand ha of harvested area and an average yield of 17.6 t ha^{-1} (MIDAGRI, 2023). The average consumption of potatoes per person was 63.5 kg per year (INEI, 2023). In the highlands, approximately 68% of agricultural soils are affected by erosion processes of medium to extreme severity due to the lack of management techniques and the destruction of vegetation cover on the slopes. Most soils are predominantly stony, with rock outcrops on the slopes, that is, lithosols (Brack & Mendiola, 2000; Paulet Iturri & Amat León, 1999). Potato crop yield is often limited due to

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low and unbalanced rates of inorganic fertilizers and inadequate row and plant spacing (Tadesse & Mulugeta, 2023).

Fertilization and planting density are important practices in potato-producing areas, as they impact the yield and quality of the tubers (Flores-López *et al*., 2020; Pinedo-Taco *et al*., 2020). A positive correlation has been found between yield and different levels of fertilization in the potato, indicating that as yield per hectare grows, nutrient extraction by the plants also increases linearly (Oliver Cortez, 2017). Cheng *et al.* (2023) demonstrated that a high fertilization rate improved nutrient absorption, plant growth, and yield of potato tubers. In relation to planting density, Flores-López *et al.* (2016) obtained higher potato tuber yields when a higher density was applied.

In Peru, the average yield of potato production per unit area (17.6 t ha^{-1}) is not optimal (MIDAGRI, 2023). The industrialization of potatoes and the availability of varieties Serranita, Luyanita, and Capiro, which have potential industrial use, is a technological production opportunity for growers. It is necessary to increase productive yields while ensuring the following characteristics of tuber quality: dry matter content (≥ 20%), low content of reducing sugars (glucose and fructose) -preferably less than 0.25%, high specific weight (≥ 1.080 g cm^{-3}) and optimal size, shape, and presence of sprouts. These parameters are influenced by genotype, mineral nutrition, production environment, and storage conditions of the tubers (Casagrande *et al*., 2014; Tirado-Lara *et al*., 2020; Vásquez *et al*., 2019; Vázquez-Carrillo *et al*., 2016). Therefore, the aim of this study was to determine the effect of two planting densities and three doses of NPK fertilization on the yield and physicochemical characteristics of three potato varieties of Group Andigenum under the conditions of the northern highlands of Peru.

Materials and methods

The potato varieties evaluated were Serranita (V1), Luyanita (V2), and Capiro (V3) from the Instituto Nacional de Innovación Agraria (INIA), Estación Experimental Baños del Inca, Cajamarca (Peru).

The experiment was conducted in the town of Santa Rosa de Chaquil, located in the District of La Encañada, province of Cajamarca, Peru, at 2980 m a.s.l., with coordinates 07°07'78" S and 78°19'59.3" W, average temperature of 16.6°C, and annual rainfall of 674.4 mm (SENAMHI, 2022). The soil is characterized by a sandy loam texture. Planting took place on November 20, 2020 and tubers were harvested during the months of April and May 2021 after growing periods of 140, 150, and 165 d for Luyanita, Serranita, and Capiro, respectively.

The soil characteristics were: pH 4.8, organic matter contents 2.10% (medium), phosphorus contents 30.52 mg kg^{-1} (high) and potassium 220.0 mg kg^{-1} (high) (INIA, EAA Baños del Inca, Cajamarca, 2022).

Eighteen treatments were carried out with three doses of N-P-K fertilizers (low (F1) =60-60-60, medium (F2) =120-120-120 and high (F3) =180-180-180)), with two planting densities: d1= 1.0 m x 0.20 m (50,000 plants ha^{-1}); d2= 1.0 m x 0.40 m (25,000 plants ha^{-1}), and three potato varieties (V1= Serranita, V2= Luyanita, V3 = Capiro. The percentage of dry matter in tubers was determined by the method recommended by Bonierbale *et al.* (2010). This consisted of taking approximately 500 g of small tuber fractions of 1 to 2 cm, mixing them thoroughly and taking sub-samples of 200 g each. The exact weight of each subsample was recorded and each subsample was then placed in a paper bag or open container in the oven at 80°C for 72 h, checking the weight of the samples at regular intervals until they reached a constant weight. Finally, the percentage of dry matter content of each subsample was calculated using the following equation:

$$Dry\ matter = \frac{dry\ weight}{fresh\ weight} \times 100\% \qquad \text{(Eq. 1)}$$

The amount of reducing sugars in the 200 g samples was evaluated using the 3,5-dinitrosalicylic acid (DNS) method. The content of reducing sugars was calculated using the following equation:

$$\%\ RS = \frac{(Abs\text{-}B)\text{-}a}{b \times M} \times 100\% \qquad \text{(Eq. 2)}$$

where % RS: percentage of reducing sugars; Abs: sample absorbance; B: blank sample absorbance; M: sample fresh weight (g); a is an intercept of the calibration curve; b is a slope of the calibration curve.

The color of the fries was evaluated using the color chart recommended by the International Potato Center (Bonierbale *et al*., 2010), with a scale from 1 to 5, where 1 is an acceptable yellow color and 5 is a very dark yellow or brown color.

Determination of specific gravity (SG) was done using the weight-in-air/weight-in-water method recommended by Bonierbale *et al.* (2010). The specific gravity was obtained using the equation:

$$Specific\ gravity = \frac{weight\ in\ air}{weight\ in\ air\text{-}weight\ in\ water} \quad \text{(Eq. 3)}$$

To determine the oil content in tubers of potato varieties for frying and production and the degree of oil absorption, the pressure method was used according to Bonierbale *et al*. (2010).

The experimental design was in randomized complete blocks, with subdivided plots and three replicates, where the large plot based on was two densities (d1, d2), the intermediate plot on three levels of fertilization (F1, F2, and F3), and the small plot on the potato variety factor (V1, V2, and V3). The distance between rows was 1.00 m and the distance between plants was 0.20 and 0.40 m. The furrow length was 3.0 m. Fertilization was carried out in a continuous jet with 50% N and 100% P and K at planting. Nitrogen source was urea 46%; phosphorus source was simple calcium superphosphate 20%; and potassium source was potassium chloride 60%. Application doses were 50% N plus 100% P and K at sowing, and the remaining 50% of N during the potato tuber filling.

During the harvest phase, total yield, commercial yield, and non-commercial yield of tubers were evaluated. The commercial yield of tubers was evaluated according to the Colombian classification by diameter (Arias *et al*., 1996; Bautista *et al*., 2012; Escallón *et al*., 2005; Rodríguez *et al*., 2009) as cited by Seminario *et al*. (2018). The classification considered the following three size categories: first class > 4 cm, second class 2-4 cm, and third class <2 cm. Commercial yield consisted of first-class and second-class tubers. Commercial yield met quality conditions for sale, while non-commercial yield did not meet these conditions.

For the evaluation of other variables, 20 tubers were taken per treatment and sent to the International Potato Center (Lima, Peru) for analysis of frying quality, dry matter content, oil extraction, and specific gravity.

Statistical analysis

The data was analyzed using Statistical Analysis Systems (SAS Institute, 2004). The assumptions of homogeneity (Barlett's test) and the normality of variance (Shapiro-Wilk test) were validated, followed by a one-way ANOVA. The treatment comparisons were carried out using the least significant difference (LSD) test (P<0.01).

Results and discussion

According to the analysis of variance for total yield, commercial yield, and non-commercial yield of tubers of the three varieties, highly significant differences (P≤0.01) were found for the factors of fertilization (F) and varieties (V) in all yield categories. This indicates that the different levels of these factors affected the yield, except in the case of planting density, with no statistically significant differences detected (P>0.01) (Tab. 1).

TABLE 1. Analysis of variance in the study of planting densities and fertilization levels in three potato (*Solanum tuberosum* Group Andigenum) varieties Serranita, Luyanita, and Capiro.

Source of variation	Degrees of freedom	Yield		
		Total	Commercial	Non-commercial
Replicates	3	ns	ns	ns
Density (D)	1	ns	ns	ns
Error (a)	3			
Fertilization (F)	2	**	**	**
DxF	2	ns	ns	ns
Error (b)	12			
Varieties (V)	2	**	**	**
DxV	2	ns	ns	ns
FxV	4	ns	ns	ns
DxFxV	4	ns	ns	ns
Error (c)				
Total	71			
Variation coefficient		16.10%	16.96%	35.06%

**P≤0.01; ns: not significant.

These results show that there is genetic variability between the varieties evaluated and that each level of NPK fertilization elicits a different response in yields. The coefficient of variability (CV) used as a measure of accuracy in conducting experiments (Vásquez-Arce, 2014) for total tuber yield and commercial yield was 16.1% and 16.96%, respectively, indicating the accuracy of the experiment. The coefficient of variation for non-commercial yield was 35.06%, considered high (Gordón-Mendoza & Camargo-Buitrago, 2015). When comparing the means of the fertilization factor, a significant difference was identified in the three levels (Fig. 1), with the highest level of fertilization, 180-180-180 kg NPK ha^{-1}, achieving total and commercial yields of 42.87 t ha^{-1} and 38.74 t ha^{-1}, respectively, and a non-commercial yield of 4.74 t ha^{-1}.

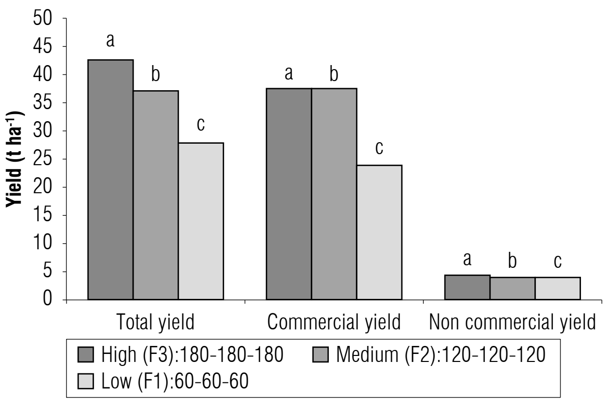

FIGURE 1. Averages of total, commercial and non-commercial yields in three varieties of potato (*Solanum tuberosum* Group Andigenum) Serranita, Luyanita, and Capiro at two planting densities and three levels of fertilization. Different letters indicate significant differences according to the LSD test ($P \leq 0.01$).

The average yields for increasing fertilizer doses – low (60 kg ha^{-1} of NPK), average (120 kg ha^{-1} NPK) and high (180 kg ha^{-1} NPK) – were 28.31, 37.53, and 42.87 t ha^{-1}, respectively; for commercial yields the averages were 24.10, 32.79, and 38.74 t ha^{-1}, which indicates an upward trend, that is to say, the higher the fertilizer dose, the higher the productivity (Fig. 1). The results are consistent with those of Flores-López *et al.* (2020), Ríos Quinchoa *et al.* (2010), and Vega Cobos (2018), who found high tuber yields with the NPK fertilization levels studied (high and medium). In this regard, Jiang *et al.* (2021) found that increasing nitrogen application rates in two potato varieties improved both tuber yield and processing quality, with the optimal dose of N about 98-200 kg N ha^{-1} for yield and high quality of potato chips. However, Valverde Samekash and Bobadilla Rivera (2017) achieved lower yields (24.95 t ha^{-1}) with an NPK fertilization of 180-160-120. This low yield was probably due to the low potassium (K_2O) dose of 120 kg ha^{-1} since the potato is a species with a high response to the application of fertilizers due to its low root density (Campos, 2014). Furthermore, the potato crop extracts a high amount of potassium; for example, Westermann (2005) reported that a yield of 56 t ha^{-1} of potato corresponds to an extraction of 400 kg ha^{-1} of K_2O by the plants.

Figure 2 shows the yields of the three varieties, indicating that the Luyanita (V2) and Serranita (V1) had total yields of 36.03 and 34.05 t ha^{-1}, respectively, and 30.97 and 30.80 t ha^{-1} of commercial yield, respectively, but did not show statistical differences between them. Both varieties statistically outperformed the Capiro variety (V3), whose total and commercial yields were 22.48 t ha^{-1} and 19.05 t ha^{-1}.

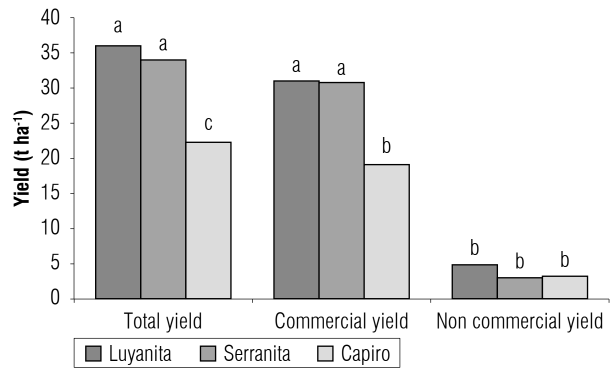

FIGURE 2. Averages of total, commercial and non-commercial yields in three varieties of potato (*Solanum tuberosum* Group Andigenum) Serranita, Luyanita, and Capiro at two planting densities and three levels of fertilization. Different letters indicate significant differences according to LSD ($P \leq 0.01$).

Yields of the Serranita variety (34.05 t ha^{-1}) are lower than those reported by Cárdenas Huamán (2018), who in a comparative study of five varieties from sexual seed obtained a yield of 35.0 t ha$^{-1.}$ The difference may be due to greater uniformity in plant growth, which leads to a better distribution of nutrients, water and light, resulting in a higher yield. The Capiro variety reached average yields of 22.48, 19.05, and 3.43 t ha^{-1} (Fig. 2). Commercial yields of the Luyanita and Serranita varieties (30.87 and 30.80 t ha^{-1}) were lower than those reported by Tirado-Lara *et al.* (2020), who achieved commercial yields of 31.8, 32.0, and 33.0 t ha^{-1} in advanced potato genotypes with pigmented pulp. The high yield obtained by the varieties under study is consistent with the reports of Morales *et al.* (2013), who obtained a high response with doses of 150-250-70 kg of N-P-K.

For the planting density of 0.20 m x 1.00 m, the highest total yield of 31.25 t ha^{-1} was achieved, with a commercial yield of 27.14 t ha^{-1} and a non-commercial yield of tubers of

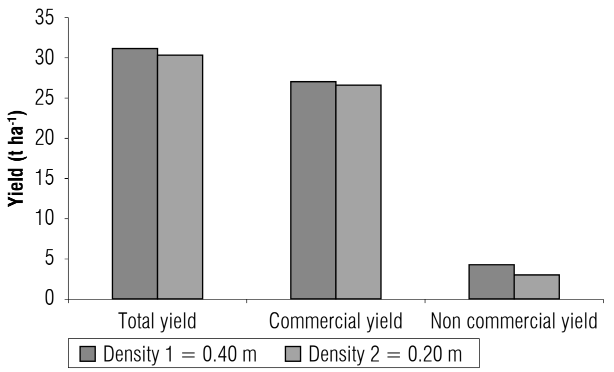

FIGURE 3. Averages of total, commercial and non-commercial yields in three potato varieties (*Solanum tuberosum* Group Andigenum) Serranita, Luyanita, and Capiro at two planting densities and three levels of fertilization. For each yield category, no significant differences were found between the planting densities (LSD, $P>0.05$).

4.50 t ha^{-1} (Fig. 3). These results are consistent with Vargas *et al.* (2021) who found that the higher planting density favored an increase of approximately 17% in tuber yield in the potato cultivars evaluated. Valverde and Bobadilla (2017) found that a density of 1.0 m x 0.20 m achieved a 24.95 t ha^{-1} yield, while a decreased density (1.0 m x 0.40 m) reduced yield to 13.2 t ha^{-1}. Vega (2018) stated that the highest commercial yield was obtained with a density of 0.40 m x 0.90 m, and Almeida *et al.* (2016.) achieved a yield of 16.34 t ha^{-1} with spacing of 0.90 m x 0.30 m. These reports confirm that potato plantations with relatively high densities give higher yields, as observed in the present research. Variations in yield can be attributed to the edaphoclimatic conditions (Alemeida *et al.*, 2016). Our results surpassed those achieved by Rojas Mercado and Seminario Cunya (2014), who obtained yields of 5 to 11.0 t ha^{-1} with a planting density of 0.40 m x 0.90 m. However, our results were not in agreement with those obtained by Rodríguez *et al.* (2004), who had high yields with greater planting density. Likewise, the results obtained in the present experiment are not consistent with those found by Arismendi (2002), who obtained a higher tuber yield with a planting density of 0.20 m between plants. These different results are attributed to the phenotypic plasticity of the varieties studied in each case (Rodríguez *et al.*, 2004). However, our results agree with the notion that higher fertilization leads to greater yield of potato tubers.

The results of the present experiment in terms of planting density differ from those of López Crus and Guevara Hoyos (2022), who obtained greater yield with a density of 0.30 m x 0.90 m. This confirms that high planting density in potato crop influences tuber yields due to its impact on the competition between plants (competitive ability), which impacts accumulation of dry matter and the use of soil and air space (Dogliotti *et al.*, 2011; López Crus & Guevara Hoyos, 2022).

The variance analysis (Tab. 2) of the five physicochemical variables studied of tuber quality is interpreted considering them as a fixed effects model, as they are important for the processing industry.

In the source of variation for fertilization (F) and variety (V), there are highly significant differences ($P\leq0.01$) for dry matter, reducing sugars, color (C), and oil content, indicating statistical differences between fertilization levels and varieties (Tab. 2). There was no statistical difference

TABLE 2. Analysis of variance and statistical significance for the variables of tuber quality of three potato varieties grown at two planting densities and three doses of NPK fertilization.

Source of variation	Degrees of freedom	Dry matter	Reducing sugars	Color of the fries (C)	Specific gravity	Oil content
Repetitions	3	ns	ns	ns	ns	ns
Density (D)	1	ns	ns	ns	ns	ns
Error (a)	3					
Fertilization (F)	2	**	**	ns	ns	**
DxF	2	ns	ns	ns	Ns	ns
Error (b)	12					
Varieties	2	**	**	*	ns	**
DxV	2	ns	ns	ns	ns	ns
FxV	4	ns	ns	ns	ns	ns
DxFxV	4	ns	ns	ns	ns	ns
Error (c)	36					
Total	71					
Variation coefficient		9.5%	6.6%	18.8%	1.2%	7.5%

** Significant ($P<0.01$); ns: not significant.

for the source of variation of density (D). These results are not consistent with those obtained by Almeida *et al.* (2016), who found statistical significance for the different distances between potato plants. This result was due to the fact that they tested extreme distances between plants (15, 20, 25, or 30 cm) and 90 cm between rows. The coefficients of variation for the evaluated variables ranged from 1.2% to 18.8%, revealing the good experimental accuracy of this evaluation methodology. Table 3 presents the comparison of LSD averages for dry matter (DM), reducing sugars (RS), color (C), specific gravity (SG) and oil content (OC) evaluated in tubers of three potato varieties under the effect of two planting densities and three levels of fertilization.

The variation between varieties (Tab. 3), for dry matter (DM), reducing sugars (RS), color (C) and oil content (OC), especially in the behavior of the three varieties, Serranita (V1), Luyanita (V2) and Capiro (V3), suggests that they can be selected by their values obtained for each variable studied.

For dry matter (DM), the multiple comparison ranges of LSD at 5% probability (Tab. 3) showed that the average dry matter content of the Serranita variety (22.53%) was statistically similar to that obtained for the Capiro variety (22.24%). The Luyanita variety stood out (24.73%), statistically surpassing Serranita and Capiro. This variation is due to several factors, such as sowing date, soil moisture content, harvest time, and physiological age of the tubers, among others, all of which influence the different potato genotypes. These factors contribute to increasing the variability of dry matter content from one season to another due to their high heritability (Vásquez-Carrillo *et al.*, 2016). The 24.73% dry matter percentage of Luyanita exceeds the results of Silveira *et al.* (2020), who characterized 25 potato clones and obtained values between 17.19 and 23.5% dry matter; as well as those of Aliaga *et al.* (2016) who obtained 22.5% dry matter with the Capiro variety. Therefore, the Luyanita variety would be considered the most suitable for the processing industry since, according to Ticsihua Huamán *et al.* (2021), the tubers used for the production of fried flakes must have a dry matter content higher than 20%.

For reducing sugars (RS), the comparison of the means of the three varieties (Tab. 3) indicates that the Serranita variety had an average of 0.28%, surpassing the Luyanita and Capiro varieties with values of 0.21% and 0.22%, respectively. These results are lower than those reported by Aliaga *et al.* (2016), who found values of 0.288% to 0.466% with the Capiro variety cultivated at soil pH of 4.8 to 7.1, respectively. However, they are not consistent with those of Vázquez-Carrillo *et al.* (2016), who evaluated six genotypes at altitudes of 2,600 m a.s.l. and 3,500 m a.s.l. and found values of 0.04% to 0.58%.

Considering that the three varieties evaluated in this trial had the same growing conditions, these differences are likely due to a varietal (genetic) factors. It is possible that these varieties have different development cycles (150 d for Serranita, 140 d for Luyanita and 165 d for Capiro), which could affect the level of reducing sugars during the time of harvest as a result of the interaction with the environment (Morales-Fernández *et al.*, 2018; Moreta Villacrés, 2021; Tirado-Lara *et al.*, 2020). This indicates that for good frying quality, reducing sugars of 0.20% and 0.30% are recommended. Our results are consistent with these reports, since the variation shown in sugar content between

TABLE 3. Comparison of means (LSD) of variables evaluated in tubers of three potato varieties with three levels of fertilization and two planting densities.

Fertilization	Variables				
	Dry matter (%)	Reducing sugars (%)	Color of the fries	Specific gravity	Oil content (%)
High (F3): 180-180-180	24.59 a	0.36 a	2.92 a	1.080	24.8 b
Medium (F2): 120-120-120	22.42 b	0.35 a	2.77 b	1.100	27.0 a
Low (F1): 60-60-60	20.50 b	0.25 b	2.61 b	1.110	27.2 a
Variety					
Serranita (V1)	22.53 b	0.28 a	2.84 a	1.090	27.30 a
Luyanita (V2)	24.73 a	0.21 b	3.11 a	1.110	20.44 b
Capiro (V3)	20.24 b	0.22 b	2.35 b	1.080	27.30 a
Density					
D1 (1.0 m x 0.20 m)	33.57	0.92	2.01	1.47	39.80
D2 (1.0 m x 0.40 m)	33.94	0.42	2.29	1.10	39.20

Treatments with the same letter do not differ significantly according to the 5% LSD test.

varieties reflects their ability to accumulate carbohydrates in tubers during crop development.

The multiple comparison test of LSD showed that the average percentage of reducing sugars corresponding to the Luyanita variety (0.21%) was statistically lower than those obtained by the Serranita (0.28%) and Capiro (0.22%) varieties, making Luyanita and Capiro suitable for frying due to the low content of reducing sugars. This characteristic is directly responsible for the adequate or inadequate coloration of the product after frying (Tab. 3). Palermo *et al.* (2016) indicated that varieties with a lower content of reducing sugars are associated with a lower production of acrylamide during frying. They recommend using varieties with a content of reducing sugars less than 0.30% of fresh weight, since values greater than 0.33% are unacceptable for frying.

For frying quality, no statistical differences were obtained between the Serranita and Luyanita varieties, whose averages for color of the fries were 2.84 and 3.11, respectively, which according to the CIP scale correspond to the creamy yellow color with low presence of dark spots, surpassing the average obtained by the Capiro variety, which had a value of 2.35 (white or creamy yellow) (Bonierbale *et al.*, 2010) (Tab. 3). The color of the fries is a very important variable in tuber quality and largely depends on the reducing sugar content, since a lower content of sugars produces a lighter frying color, making it acceptable for the industrialization and commercialization of French fries (Ticsihua Huamán *et al.*, 2021).

The cultivars Serranita and Capiro had the most acceptable color of fries of 2.35 to 2.84, due to their low values on the scale of the Bonierbale *et al.* (2010), as a result of their lower content of reducing sugars (Tab. 3). This suggests that the three varieties have the best frying quality, as they presented the best processing characteristics in this study. These results are not consistent with those obtained by Tirado-Lara *et al.* (2020), who achieved values of 1.7 to 3.5 on the CIP scale when evaluating frying quality of improved pigmented pulp genotypes.

The specific gravity (SG) values were 1.090, 1.110 and 1.080 for Serranita, Luyanita, and Capiro, respectively (Tab. 3). No statistical differences were found between the specific gravity values. These results are higher than those reported by Alcon Callejas and Bonifacio (2020), who, in trials with bitter potato varieties, found values between 1.002 and 1.040. However, they are similar to those obtained by Silveira *et al.* (2020). When evaluating 25 potato clones, they found values ranging from 1.050 to 1.110, indicating that they are suitable for the potato chip industry.

According to Lizarazo *et al.* (2022), specific gravity is influenced by variety and environmental conditions such as soil type, water availability, and soil fertility. The three varieties were grown in the same place and under the same conditions, so the differences between them in this variable are due to the variety interaction with the environment (Vásquez *et al.*, 2019).

It can be inferred that the varieties evaluated are suitable for agribusiness, as the Bonierbale *et al.* (2010) recommends specific gravity values between 1.09 and 1.11. Likewise, the variables of specific gravity and dry matter evaluated (Tab. 3) show a high and positive correlation (r = 0.979). This is consistent with the reports of Alcon Callejas and Bonifacio (2020) and Silveira *et al.* (2020).

For oil content (OC), there were no statistical differences between the Serranita and Capiro varieties, whose average was 27.30%, surpassing the Luyanita variety with an average of 20.44%. This indicates that the Luyanita variety had lower oil absorption (Tab. 3). These results are lower than those reported by Mora (2016), who obtained 28.8% to 31.5% and those of Valencia-Flórez *et al.* (2017), who reported averages of 27.8 to 29.5%, respectively. For the Serranita variety, Espitia Sotelo and Soto Sáenz (2015) obtained an average oil content of 17.62%, well below the varieties studied in this experiment. These reports indicate that these percentages are obtained if they are not subjected to any pre-frying treatment.

The higher dry matter content (≥24.73%), which corresponds to a higher specific gravity (≥1.110) and low level of reducing sugars (≤0.21), results in lower oil absorption in the frying process (20.44%) (Tab. 3). These results are consistent with Silveira *et al.* (2020), who stated that potatoes with higher dry matter content (≥20%) and higher specific gravity (≥1.080) with low levels of reducing sugars result in fried foods with lower oil content. These data imply that the Luyanita variety (Fig. 2 and Tab. 3) is ideal for industrial processing.

The fertilizer doses affected dry matter content, reducing sugars, color, and oil content (Tab. 3). Dry matter values were higher (24.59%) with the high fertilization rate (180-180-180), statistically surpassing the medium and low doses, whose averages were 22.42% and 20.50% respectively. With low NPK applications (60-60-60 kg ha^{-1}), low values of reducing sugars (0.25%), color (2.61) and specific gravity

(1.110) were obtained, which are suitable for processing (Tab. 3), indicating that this low level of NPK favors the improvement in product quality.

These results are consistent with Palacios *et al.* (2008), who found an improvement of the terminal product, such as chips and canes, with low levels of fertilization. However, these results are not consistent with those reported by Giletto *et al.* (2013), who found lower dry matter content (19%) when N-P-K fertilization levels increased. It was observed that the content of dry matter, reducing sugars, specific gravity and color decreased with low NPK doses (Tab. 3).

Regarding specific gravity, there were no statistical differences for the three fertilization doses (Tab. 3), which indicates that fertilization did not influence this variable, with averages ranging 1.080 to 1.110. The lowest specific gravity, 1.080 was obtained with the formula 180-180-180 NPK kg ha^{-1}, indicating an inverse relationship. These results are consistent with those of Ozturk *et al.* (2010), who stated that the specific gravity of the potato decreases with increasing levels of NPK. They also conform to Rivera *et al.* (2006) who established that for potatoes to be suitable for chips, the specific gravity must be greater than 1.080. An upward trend was found between NPK fertilization levels and reducing sugar content (Tab. 3).

No significant differences were found for oil content between the 120-120-120 and 60-60-60 doses, with averages of 27.0 and 27.2%, respectively. However, at the 180-180-180 dose, the oil content was 24.8% (Tab. 3). This is consistent with Öztürk *et al.* (2010), who stated that the oil content decreases with increasing phosphorus levels. Based on the results obtained in this experiment, we recommend low applications of NPK (60-60-60 kg ha^{-1}) for potato producers whose production is destined for industrial processing, as this formulation helps to improve the quality of the final product (chips, sticks). Regarding the sowing density, there were no statistical differences for the variables of dry matter contents, reducing sugars, color, specific gravity, and oil content in tubers.

Conclusions

The results obtained suggest that high planting density and high doses of NPK fertilization in potato crop influenced the yield and physicochemical characteristics of the three varieties studied, with potential use for the industry. The superiority of the Luyanita potato variety over other varieties, in the conditions of the Cajamarca highlands (Peru), is marked by its higher yield and acceptable physicochemical properties for industrial processing. This achievement contributes to agricultural development, benefitting growers and the agro-industrial sector. It will be important to continue research on this topic, focusing on planting density with a wider spacing between rows and potato plants cultivated in different agroecological areas.

Conflict of interest statement

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

Author's contributions

VV and HC designed the experiments; VV, LJ and RP carried out the field and laboratory experiments; PH, VC and CC contributed to data analysis; PH and BA wrote the article. All authors reviewed the final version of the manuscript.

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Spatial variability of soil penetration resistance in cotton growing areas

Variabilidad espacial de la resistencia del suelo a la penetración en las zonas algodoneras

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ABSTRACT

Resistance to soil penetration is a problem in cotton growing areas; this harms plant development and crop productivity. An analysis of spatial variability allows studying the variability of soil physical properties, especially those related to compaction and resistance to penetration. The hypothesis of this study was based on the possibility of evaluating soil compaction in areas cultivated with cotton crops through an analysis of spatial variability. Thus, the aim of the study was to evaluate the spatial variability of soil resistance to penetration and its effects on cotton productivity. The research was carried out in Paraíso das Águas, Mato Grosso do Sul, Brazil, in an agricultural area belonging to Fazenda Indaiá II. The experimental area consisted of a plot of 60 ha, where cotton seeds of the FM 978 6LTP cultivar were planted. The *x* and *y* directions of the Cartesian coordinate system were defined and the experimental mesh was staked, spaced 9.9 m apart. Cotton productivity was evaluated (t ha^{-1}) as well as gravimetric soil moisture at a depth of 0 to 0.20 m as well as mechanical resistance to penetration in the following layers: 0 to 0.10 m (RP1), 0.10 to 0.20 m (RP2), 0.20 to 0.30 m (RP3), 0.30 to 0.40 m (RP4), 0.40 to 0.50 m (RP5), 0.50 to 0.60 m (RP6), including average penetration resistance (RPM). For each evaluated variable, classical descriptive analysis and analysis of spatial variability were carried out, with the construction of semi-variogram and later kriging and cokriging maps. To analyze the dependence and spatial interdependence between the variables, the soil resistance to penetration showed spatial variability in the cotton crops; and there was an inversely proportional relationship between cotton productivity and soil resistance to penetration, where the lower the penetration resistance, the higher the cotton productivity.

Key words: soil compaction, geostatistics, *Gossypium hirsutum*, kriging.

RESUMEN

La resistencia a la penetración del suelo es un problema en las zonas de cultivo de algodón, ya que perjudica el desarrollo de las plantas y la productividad de los cultivos. El análisis de variabilidad espacial permite estudiar la variabilidad de las propiedades físicas del suelo, especialmente aquellas relacionadas con la compactación, como la resistencia a la penetración. La hipótesis de este trabajo se basó en la posibilidad de evaluar la compactación del suelo en áreas cultivadas con cultivos de algodón a través del análisis de variabilidad espacial. Así, el objetivo del trabajo fue evaluar la variabilidad espacial de la resistencia a la penetración y sus efectos sobre la productividad del algodón. El trabajo se realizó en Paraíso das Águas, Mato Grosso do Sul, Brasil, en una zona agrícola perteneciente a la Fazenda Indaiá II. El área experimental estuvo compuesta por una parcela con una superficie de 60 ha, donde se sembraron semillas de algodón del cultivar FM 978 6LTP. Se definieron las direcciones *x* y *y* del sistema de coordenadas cartesianas y se plantó la malla experimental, espaciada 9,9 m. Se evaluó la productividad del algodón (t ha^{-1}), la humedad gravimétrica del suelo a una profundidad de 0 a 0,20 m y la resistencia mecánica a la penetración en las capas: de 0 a 0,10 m (RP1), de 0,10 a 0,20 m (RP2), 0,20 a 0,30 m (RP3), 0,30 a 0,40 m (RP4), 0,40 a 0,50 m (RP5), 0,50 a 0,60 m (RP6) y resistencia media a la penetración (RPM). Para cada variable estudiada se realizó un análisis descriptivo clásico y un análisis de variabilidad espacial, con la construcción de semivariograma y posteriormente mapas de kriging y cokriging, para analizar la dependencia e interdependencia espacial entre las variables. La resistencia a la penetración del suelo presentó variabilidad espacial en áreas algodoneras y existió una relación inversamente proporcional entre la productividad del algodón y la resistencia a la penetración del suelo, donde a menor resistencia a la penetración, mayor es la productividad del algodón.

Palabras clave: compactación de suelo, geoestadística, *Gossypium hirsutum*, kriging.

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Introduction

Cotton (*Gossypium hirsutum* L.) has economic and social importance for Brazilian agriculture. It is cultivated to obtain seeds and fiber and is widely used in the production of feed and the textile industry (INDEA, 2023). In the 2022/23 harvest, production exceeded 3,150.1 million t, with a forecast increase in the planted area in the 2023/24 harvest (CONAB, 2023).

An expansion of cotton cultivation in Brazil occurred in the early 1990s, extending across the biome with intense application of technology (Sari *et al*., 2023). Knowledge of the factors that affect cotton production is important for defining regions with potential for the production of seeds and fiber of high quality; differences in production are associated with cultivars and physical quality of the soil (Ramos *et al*., 2022).

Soil management implies knowing its physical, chemical, and biological characteristics, aiming at practices for agricultural production by understanding its potential and limitations (Silva *et al*., 2020). Soil compaction is a limiting factor in agricultural production. It impacts the reorganization of particles and aggregates, limiting the adsorption and absorption of nutrients, infiltration, and redistribution of water and gas exchange; and these factors limit agricultural productivity (Moraes *et al*., 2020).

Resistance to soil penetration is an indication of the physical quality of soils; it is a property related to compaction, and it mechanically quantifies the impediment of root growth (Jamali *et al*., 2021). Penetration resistance is a practical and complete parameter related to texture, soil density and water content that is used to monitor the state of soil compaction. Critical values varying between 1.5 and 4.0 MPa (Cortez *et al*., 2018) define a compacted soil with necessary assessment depending on the crop to be planted. Responding to resistance to penetration, plants reduce leaf area so that photoassimilates are redirected to the roots, to mitigate the effects of resistance. This harms the productivity of the cotton plants (Nikkel & Lima, 2020).

Soil resistance to penetration is evaluated with a penetrometer, equipment that measures the resistance of soil layers. It is characterized as one of the main tools for diagnosing and evaluating soil compaction (Vogel *et al*., 2017). Oliveira *et al*. (2023) emphasize the importance of knowing gravimetric soil moisture, as it is closely related to soil resistance to penetration.

The analysis of the spatial variability of soil resistance to penetration through geostatistics allows detection of the variation and spatial distribution of soil properties, especially those related to compaction (Cortez *et al*., 2018). The use of spatial variability promotes recurrent monitoring of the state of soil compaction in a practical way, characterizing the compaction caused by soil use and management in agricultural cultivation areas (Machado *et al*., 2015). Thus, it is classified as a precision agriculture technique.

Based on the above, the hypothesis of this study was based on the possibility of evaluating soil resistance to penetration in areas cultivated with cotton crops using the analysis of spatial variability. Thus, the objective of the work was to evaluate the spatial variability of gravimetric water contents in soil, soil resistance to penetration, and their effects on cotton productivity.

Materials and methods

The study was carried out in Paraíso das Águas, Mato Grosso do Sul, Brazil, in an agricultural area belonging to Fazenda Indaiá II, close to the geographic coordinates 19º1'33" S, 53º0'37" W and an altitude of 608 m a.s.l. According to Köppen and Geiger, the climate is humid tropical (Aw), characterized by a mean temperature varying between 14°C and 31°C and mean annual rainfall of 1303 mm.

The soil in which the experimental meshes were measured was classified as Entisols (USDA, 1987) with a sandy texture, containing 12% clay. The physical characteristics of soil were determined in the 0-0.20 m depth layer as follows: particle size distribution (%) of clay, silt, sand, 12, 5, 83; soil density of 1.3 g cm^{-3}; water content (g g^{-1}) of 0.088 and 0.053 for soil field capacity and permanent wilting point, respectively, determined by the Richards method. All soil physical analysis followed the methods described in Teixeira *et al*. (2017).

The following soil chemical characteristics were determined in the Soil Laboratory of the Brazilian Institute of Analysis (IBRA), where all the analysis followed the methods described in Teixeira *et al*. (2017): pH: 5.020; soil organic matter OM (g kg^{-1}): 57.100; phosphorus P (mg dm^{-3}): 10.190; potassium K (mg dm^{-3}): 0.060; calcium Ca (mg dm^{-3}): 1.540; magnesium Mg (mg dm^{-3}): 0.710; aluminum Al (mg dm^{-3}): 0.000; potential acidity H+Al: (cmol$_c$ dm^{-3}): 0.011; sum of bases SB (cmol$_c$ kg^{-1}): 0.041; effective cation exchange capacity ECEC (cmol$_c$ kg^{-1}): 0.045; cation exchange capacity CEC (meq 100 g^{-1}): 0.020; base saturation BS (%): 43.020.

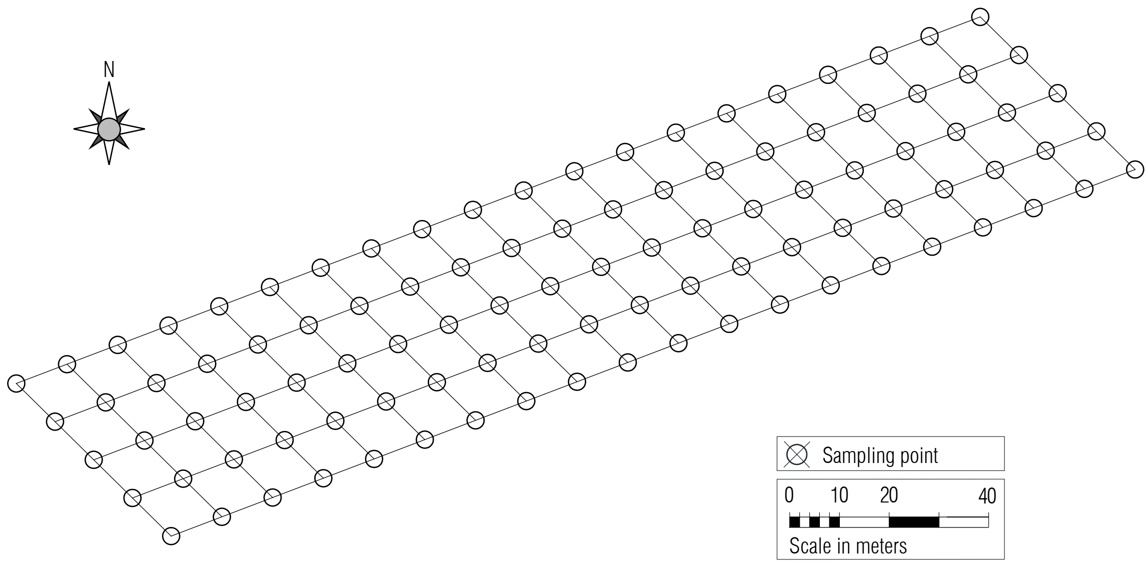

FIGURE 1. Sampling mesh with 100 points, from the experimental grid.

In the experimental area of 60 ha, we planted cotton seeds of the cultivar FM 978 6LTP. Planting was carried out on November 20, 2022, with a spacing of 90.0 cm x 11.7 cm, totaling a population of 95,000 plants ha^{-1}.

The x and y directions of the Cartesian coordinate system were defined and grid, spaced 9.9 m apart (Fig. 1). Each experimental mesh consisted of three transects measuring 49.5 m x 198.0 m. Transects were spaced 9.9 m apart, with sample points squared at 9.9 m x 9.9 m, containing 100 of them. The area corresponding to each experimental mesh was 0.98 ha.

The mechanical resistances of soil to penetration were: RP1, RP2, RP3, RP4, RP5, RP6, and RPM, where the number that accompanies the soil property referred to the soil depth as follows: 1 – depth from 0 to 0.10 m; 2 – depth of 0.10 to 0.20 m; 3 – depth of 0.20 to 0.30 m; 4 – depth of 0.30 to 0.40 m; 5 – depth of 0.40 to 0.50 m; 6 – depth of 0.50 to 0.60 m; RPM referred to the resistance to soil penetration in the layer from 0 to 0.60 m calculated by the average of all readings taken. The determination of soil resistance to penetration was measured up to 60 cm deep as this is an effective depth of the root system of the cotton crop.

To determine soil resistance to penetration, a Falker digital penetrometer model PenetroLOG–PLG 1020 was used. It was configured to record readings at constant penetration speed with the unit in kilopascal (kPa). The measurements were taken one week before the cotton harvest, during the dry season.

To determine gravimetric soil moisture content (W) at each sampling point, soil samples were collected at a depth of 20 cm and taken to the laboratory. The samples were placed in capsules and weighed to obtain the moist soil weight (MSW). They were then placed in an oven at 105°C for 24 h to obtain the dry soil weight (DSW). Finally, the gravimetric moisture content W was determined using Equation 1.

$$W = \left[\frac{(MSW - DSW)}{MSW} \right] \times 100 \tag{1}$$

Cotton productivity (CP) was determined by harvesting all bolls (fiber and seeds) from three randomly harvested plants at each sampling point. The material was weighed on a precision scale to determine the average per plant in grams. The value was transformed from g per plant to t. The results were multiplied by the number of plants per ha and the cotton productivity (yield) was presented in t ha^{-1}. Khamidov *et al.* (2023), Pereira *et al.* (2023), and Ferreira and Resende (2023), among other authors, carried out experiments with cotton and measured its productivity in t ha^{-1}.

Statistical analysis

For each soil property and for cotton productivity, a classic descriptive analysis was carried out, with the statistical program Rbio (biometrics in R) version 17, in which the mean, median, minimum and maximum values, standard deviation, coefficient of variation, and kurtosis were calculated. Skewness and analysis of the frequency distributiwere carried out. To test the hypothesis of normality or lognormality of the productive components (x), the Shapiro and Wilk test (1965) at 5% was used. In it, the null hypothesis as tested, which is judged to be the sample coming from a population with normal distribution.

Spatial correlation analysis was performed using free and open source software using the Gamma Design Software package (GS+, 2004). For each variable (soil property or plant productivity), its spatial dependence was analyzed by calculating the simple semivariogram which presents the spatial dependency evaluator (SDE). However, for those that showed spatial interdependence, their crossed semivariograms were also calculated, based on the stationarity assumptions of the intrinsic hypothesis. For each variable, the nugget effect (Co), range (Ao) and threshold (Co + C) were related (GS+, 2004).

Working to obtain the ideal number of neighbors, kriging and cokriging maps were obtained, through interpolation, to analyze the dependence and spatial interdependence between the variables.

Results and discussion

Table 1 presents the descriptive analysis of soil properties. From this analysis, it is possible to observe a variation in relation to the evaluated data. Cotton productivity showed a high standard deviation due to the data dispersion, as it is a sampling grid with a large number of points (100). A greater number of sample points provides greater solidity in the resul

Soil resistance to penetration with high values, is the principal symptom resulting from soil compaction creating an impediment from root growth, water and nutrient availability for cotton cultivation (Anghinoni *et al*., 2019). According to Aime *et al.* (2019), in situations of soil compacted in the 0.0-0.25 m layer, the volume of cotton roots is greater on the soil surface, regardless of the cultivar used, due to the limitation of root growth in deeper layers.

Table 2 presents the correlation matrix between productivity and gravimetric moisture and soil resistance to penetration.

Results show the presence of a high correlation between the soil resistance to penetration RP1xRP2, RP1xRPM, RP2xRP3, RP2xRPM, RP3xRP4, RP3xRPM, RP4xRPM, and RP5xRP6. Positive correlations were observed between all penetration resistance depths studied. This result indicates that, at different depths, penetration resistances are correlated with each other in this soil. According to Oliveira *et al.* (2020), who studied resistance to penetration in Oxisols, the same behavior is known.

The correlation matrix also shows a negative correlation between cotton productivity and RP1, RP2, and RP3. This indicates that the greater the soil compaction, that is, the greater soil resistance to penetration, the lower the cotton productivity. The productivity of agricultural crops is affected by soil compaction (Cortez *et al*., 2018). Cotton is a crop susceptible to soil compaction (Nouri *et al*., 2019).

Geostatistical analysis showed the occurrence of a negative spatial correlation between productivity and RP1 (Tab. 3). This result demonstrates an inversely proportional relationship between penetration resistance and cotton productivity, where the lower the resistance to soil penetration, the higher the crop productivity.

TABLE 1. Descriptive analysis of cotton productivity, gravimetric soil moisture content, and soil resistance to penetration in cotton growing area.

Property	Mean	Minimum	Maximum	Standard deviation	Kurtosis	Skewness
CP	3.755	1.311	8.924	93.940	1.154	0.889
RP1	0.644	0.069	1.756	0.380	0.145	0.656
RP2	2.250	0.486	4.302	0.762	-0.367	0.090
RP3	2.665	0.542	4.244	0.845	-0.475	-0.490
RP4	3.035	1.268	4.335	0.637	0.179	-0.394
RP5	3.061	1.906	4.023	0.451	-0.462	-0.246
RP6	2.622	1.646	3.896	0.477	0.197	0.586
RPM	2.343	1.469	3.031	0.346	-0.392	-0.268
W	2.338	1.014	3.779	0.597	-0.778	0.021

CP: cotton productivity, t ha^{-1}; RP1: soil resistance to penetration in 0.0-0.10 m, kPa; RP2: soil resistance to penetration in 0.10-0.20 m, kPa; RP3: soil resistance to penetration in 0.20-0.30 m; RP4: soil resistance to penetration in 0.30-0.40 m, kPa; RP5: soil resistance to penetration in 0.40-0.50 m; RP6: soil resistance to penetration in 0.50-0.60, kPa m; RPM: medium soil resistance to penetration, kPa; W: gravimetric soil moisture content.

TABLE 2. Correlation matrix between cotton productivity, gravimetric soil moisture content, and soil resistance to penetration in cotton growing areas.

	CP	RP1	RP2	RP3
CP	1	-	-	-
RP1	-0.095	1	-	-
RP2	-0.108	0.505**	1	-
RP3	-0.041	0.122	0.556**	1
RP4	0.041	0.155	0.335*	0.690**
RP5	0.151	0.018	-0.200	-0.098
RP6	0.181	-0.068	-0.209	-0.067
RPM	0.012	0.450**	0.686**	0.795**
W	0.041	-0.143	0.024	0.021
	RP4	**RP5**	**RP6**	**RPM**
RP1	-	-	-	-
RP2	-	-	-	-
RP3	-	-	-	-
RP4	1	-	-	-
RP5	0.196	1	-	-
RP6	0.069	0.499**	1	-
RPM	0.785**	0.277*	0.239*	1
W	-0.070	-0.147	-0.032	-0.068

CP: cotton productivity, t ha^{-1}; RP1: soil resistance to penetration in 0.0-0.10 m, kPa; RP2: soil resistance to penetration in 0.10-0.20 m, kPa; RP3: soil resistance to penetration in 0.20-0.30 m; RP4: soil resistance to penetration in 0.30-0.40 m, kPa; RP5: soil resistance to penetration in 0.40-0.50 m; RP6: soil resistance to penetration in 0.50-0.60 m, kPa; RPM: medium soil resistance to penetration, kPa; W: gravimetric soil moisture content. * significant at the 5% level and ** significant at the 1% level according to the Tukey´s test.

TABLE 3. Estimated parameters for the simple and crossed semi-variogram of the components in relation to cotton productivity, gravimetric soil moisture content, and soil resistance to penetration in cotton growing areas.

Property	Adjust -ment	Nugget effect	Level	Range (m)	SSR	SDE (%)	Class
CP	Exp	0.336	2.1	38	0.1400	0.840	Strong
RP1	Gau	0.035	0.150	15	8.74E-04	0.767	Strong
RP2	PNE	x	x	x	x	x	x
RP3	PNE	x	x	x	x	x	x
RP4	Exp	0.160	0.425	25	0.0158	0.624	Moderate
RP5	Exp	0.085	0.205	27	2.25E-03	0.585	Moderate
RP6	Sph	0.140	0.228	34	5.70E-03	0.386	Moderate
RPM	Gau	0.063	0.125	17	7.60E-04	0.496	Moderate
W	PNE	x	x	x	x	x	x
CP x RP1	Gau	-0.003	-0.080	35	1.19E+01	0.934	Strong

CP: cotton productivity, t ha^{-1}; RP1: soil resistance to penetration in 0.0-0.10 m, kPa; RP2: soil resistance to penetration in 0.10-0.20 m, kPa; RP3: soil resistance to penetration in 0.20-0.30 m, kPa; RP4: soil resistance to penetration in 0.30-0.40 m, kPa; RP5: soil resistance to penetration in 0.40-0.50 m; RP6: soil resistance to penetration in 0.50-0.60 m, kPa; RPM: medium soil resistance to penetration, kPa; W: gravimetric soil moisture content. CPxRP1 – correlation between cotton productivity and penetration resistance in 0-0.10 m; SSR – the sum of squared residuals; SDE – spatial dependency evaluator; Exp = exponential; Gau = gaussian; Sph = spherical; PNE = pure nugget effect.

Soil in ideal conditions for root development is explored homogeneously by plant roots and its volume is relatively greater than in soils with compaction problems since compacted soils have increased density, reduced porosity, and increasing resistance to root penetration (Cortez *et al*., 2018). Therefore, the formation of compacted surface layers, especially in relation to the continuous traffic of agricultural machinery, affects root formation, harming plant development and cotton productivity (Ramos *et al*., 2022).

Figure 2 presents the simple semi-variograms and kriging maps of penetration resistance at different depths and cotton productivity.

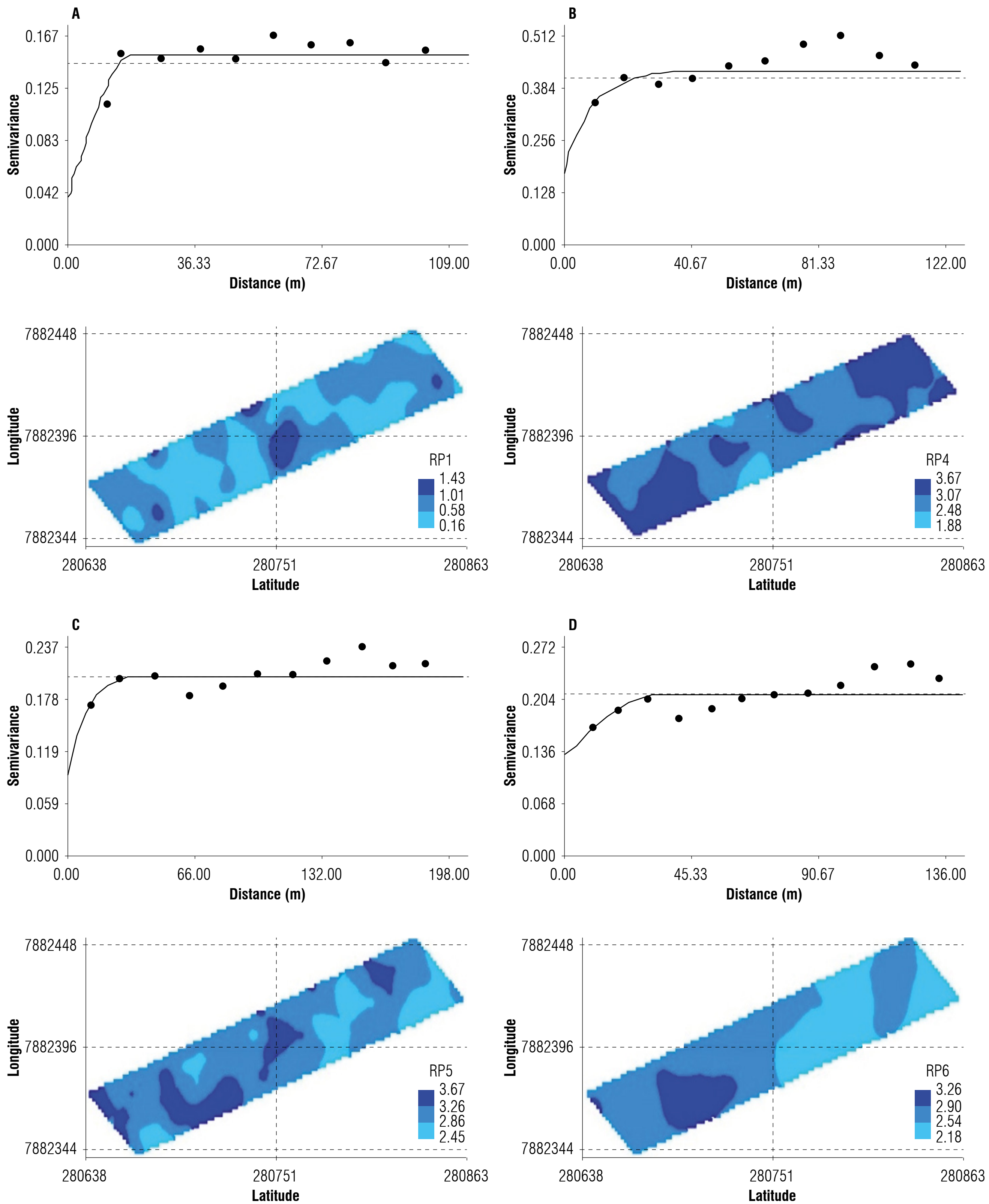

FIGURE 2. Semivariograms and kriging maps: (A) RP1: resistance to soil penetration in 0.0-0.10 m; (B) RP4: resistance to soil penetration in 0.30-0.40 m; (C) RP5: resistance to soil penetration in 0.40-0.50 m; (D) RP6: resistance to soil penetration in 0.50-0.60 m. Continue in the next page

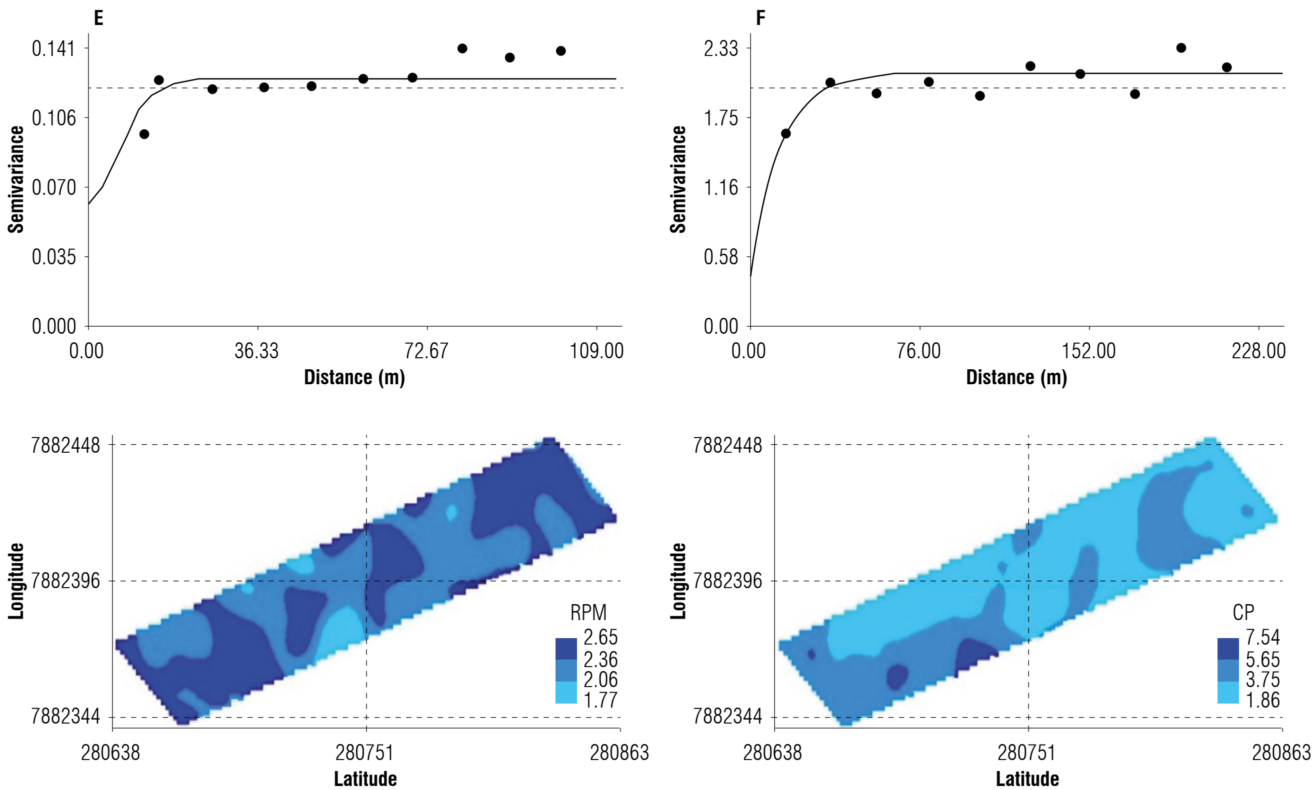

FIGURE 2 continuation. Semivariograms and kriging maps: (E) RPM: medium soil resistance to penetration; (F) CP: cotton productivity.

Analyzing the map (RP1), it is possible to observe that the central part presents higher soil resistance to penetration values in the 0-0.10 m layer, while the southwest region presented the lowest values (Fig. 2A).

On the map that represents the RP4 property, we noted that in the northeast region there are higher penetration resistance values, while in the southwest the values are lower (Fig. 2B). In RP5, the northeast region had the lowest values for soil resistance to penetration in the 0-0.50 m layer, while the central and southwest regions had the highest values (Fig. 2C).

The study area has been cultivated for many years. In addition to cotton cultivation, corn and beans, among other crops, have been planted; and the area has also been used as pasture for cattle. There is a possibility that the traffic of heavy machinery or of animals may have had a strong influence on increasing the soil resistance to penetration. These are reasons why some regions on the maps present higher values of soil compaction.

The RP6 property had the lowest soil resistance to penetration values in the northeast and southeast regions, while the southwest region had the highest values (Fig. 2D). The kriging map that had the average resistance to soil penetration in the center and right and left margins had the highest values (Fig. 2E).

In relation to crop productivity, the lowest values were observed in the central, north and northeast, while the southwest region had the highest values (Fig. 2F).

Figure 3 presents the crossed semi-variograms and kriging and cokriging maps of cotton productivity.

The regions on the map where the highest values of cotton productivity were presented are precisely those in which there was better interaction between the crop and the physical aspects of the soil. That is, regions where the soil presented the lowest mechanical resistance to soil penetration had greater productivity of cotton. This fact was verified in the crossed semi-variogram (CP x RP1) of cotton productivity as a function of the soil's mechanical resistance to penetration at a depth of 0-0.10 m where the semivariogram curve is turned downwards, indicating a negative correlation. This implies that the higher the CP, the lower the RP1 (Fig. 3).

Soil resistance to penetration with high values is a negative and unwanted characteristic that concerns compaction, negatively influencing plant root growth (Silva *et al.*, 2021).

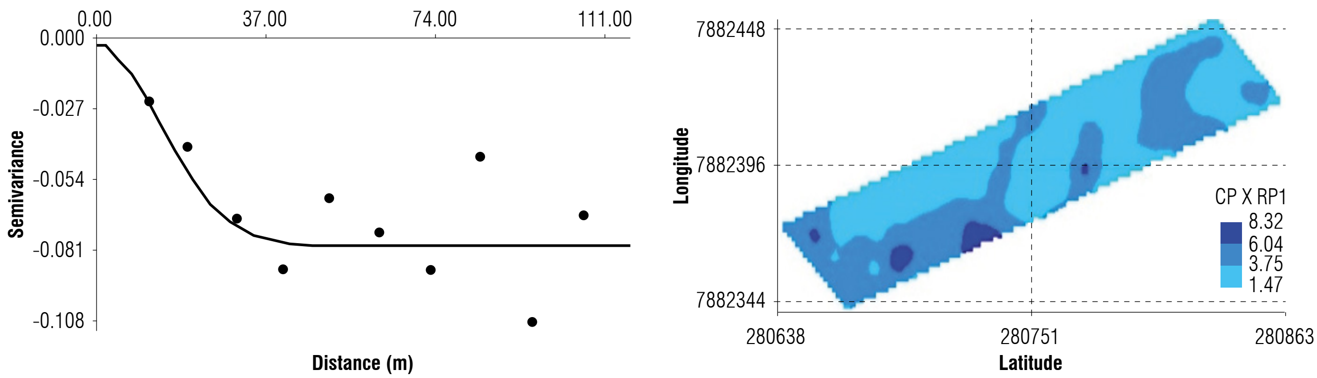

FIGURE 3. Cross semi-variogram and cokriging map. CPxRP1 represents a correlation between cotton productivity and soil resistance to penetration in 0-0.10 m.

The main problem with high resistance of soil to penetration consisted in that it caused negative effects on root growth; and, thus, it reduces the number of leaves, plant height and dry matter (Gubiani *et al*., 2017).

Soils that are resistant to penetration are characterized by roots found in the superficial layers, where plants aim to maximize the absorption of water and nutrients due to the difficulty of exploring deeper layers (Gabriel *et al*., 2021). However, the surface layers of the soil easily lose water through evaporation, subjecting plants to water deficit (Nouri *et al*., 2019). Additionally, compacted soils have lower infiltration rates, leading to waterlogging, which is harmful to crops such as cotton that have low tolerance to waterlogging (Aime *et al*., 2021).

In this study, the variability of soil resistance to penetration in areas cultivated with cotton and its influence on productivity was observed; the greater the resistance to soil penetration, lower the cotton productivity is found. Future perspectives are based on the possibility of continuing the study in the same areas, observing the increase or decrease in soil resistance to penetration using the methods to minimize soil compaction.

Conclusions

Cotton productivity (CP), soil resistance to penetration (RP1), and correlation between cotton productivity and soil resistance to penetration (CPxRP1) showed spatial variability in cotton growing areas presenting a strong degree of spatial dependence.

The penetration resistances RP4, RP5, RP6, and RPM showed spatial variability in cotton growing areas varying in degrees of spatial dependence from higher to moderate.

There is an inversely proportional relationship between cotton productivity and soil resistance to penetration, where the lower the soil resistance to penetration, the higher the cotton productivity, indicating that adequate soil management, prioritizing the maintenance of straw and consequently organic matter and avoiding the movement of heavy machinery, are paths to less compacted and more productive soil.

Conflict of interest statement

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

Author's contributions

JPOR: conceptualization, methodology, validation, formal analysis, research, writing - original draft; JTO designed the experiment, analyzed the data, wrote, and edited the manuscript; TRC: resources, writing, review and editing; CGR: validation, resources, writing – review and editing; FFC: validation, writing - review and editing. All authors approved the final version of the manuscript.

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Technological and socioeconomic characterization of *Cucurbita argyrosperma* Huber production systems in Champotón, Campeche, Mexico

Caracterización tecnológica y socioeconómica de los sistemas de producción de *Cucurbita argyrosperma* Huber en Champotón, Campeche, México

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ABSTRACT

The state of Campeche, Mexico, occupies first place in the production of *Cucurbita argyrosperma* Huber, with the municipality of Champotón contributing 7% of state production. Chihua squash production systems were characterized based on their technological and socioeconomic level by applying a semi-structured questionnaire to those responsible for the Chihua squash production systems. The data were analyzed using principal components, clusters, and comparison of means with the Tukey test ($P\leq0.05$). Three groups of producers were identified: G1, G2, and G3, grouping 28, 100, and 8 people, respectively. G1 has intermediate land availability, intermediate salaried labor, producer organizations, seed processing, average purchase-sale contract, medium use of technologies, higher income per unit of area, and represents 20.58% of producers; G2 has lower land availability, lower number of salaried labor, low organization of producers, seed processing, no purchase-sale agreement, low use of technologies, lower income per unit of area and utility, and concentrates 73.53% of producers; G3 has greater availability of land, greater number of salaried labor and producer organizations, does not process seeds, has greater purchase-sale agreement, high use of technologies, average income values, and represents 5.88% of the producers. The group with the highest technological index was not the one with the highest profitability, which was determined by producer organizations and the availability of land.

Key words: agricultural producers, Chihua squash, agricultural profitability.

RESUMEN

El estado de Campeche, México, ocupa el primer lugar en producción de *Cucurbita argyrosperma* Huber, donde el municipio de Champotón aporta el 7% de la producción estatal. Se caracterizaron los sistemas de producción de calabaza chihua, en función de su nivel tecnológico y socioeconómico, aplicando un cuestionario semiestructurado a los responsables de los sistemas de producción de calabaza chihua. Los datos se analizaron mediante componentes principales, conglomerados y comparación de medias con la prueba de Tukey ($P\leq0.05$). Se identificaron tres grupos de productores, G1, G2 y G3, agrupando a 28, 100 y 8 personas cada uno. G1 tiene disponibilidad de tierra intermedia, mano de obra asalariada intermedia, organizaciones de productores, procesamiento de semillas, contrato de compra-venta media, uso de tecnologías medio, mayor ingreso por unidad de superficie y concentra al 20.58% de los productores; G2 presenta menor disponibilidad de tierra, menor número de mano de obra asalariada, baja organización de productores, procesamiento de semillas, sin acuerdo de compra-venta, bajo uso de tecnologías, obtiene menores ingresos por unidad de área y utilidad, y concentra el 73.53% de los productores; G3 presenta mayor disponibilidad de tierra, mayor número de mano de obra asalariada y organizaciones de productores, no procesa semillas, mayor acuerdo de compra-venta, alto uso de tecnologías, tiene valores de ingresos promedio y representa el 5.88% de los productores. El grupo de mayor índice tecnológico no fue el más rentable, debido a que estuvo determinado por las organizaciones de productores y la disponibilidad de tierras.

Palabras clave: productores agrícolas, calabaza chihua, rentabilidad agrícola.

Introduction

In Mexico, agriculture is an important economic activity as it is the main source of income for 13.21% of the rural population (INEGI, 2016), in which women and youth actively participate (FAO, 2015). In the state of Campeche, the high availability of natural resources destined to promote agriculture has been documented; however, there is

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a low production rate, and the development outlook is not very encouraging, mainly due to low investment in infrastructure and technology (Uzcanga Pérez *et al*., 2012). The agricultural systems in the state of Campeche reported a total production of USD 137,848,113.8 for the 2022 agricultural cycle; the main crops, both irrigated and seasonal, were grain corn (*Zea mays*), soybean (*Glycine max*), Chihua squash (*Cucurbita argyrosperma*), grain sorghum (*Sorghum bicolor*) and rice (*Oryza sativa*) with production values of USD 51,810,860.2; 15,208,653.4; 5,297,773.6; 3,443,426.6; and 5,280,917.4, respectively. The state leads the country in Chihua squash cultivation, with 15,426 ha of planted area as of 2022 and an average yield of 0.53 t ha^{-1} of seeds, equivalent to 31.69% of the total national production (SIAP, 2024).

Within the Chihua squash producing municipalities, over the past five years, the Champotón municipality has gone from third to fifth place in planted area (2,217 to 2,257 ha) and from fourth to fifth in seeds production (309.30 to 825.16 t) per agricultural cycle, reporting the lowest seed yield (0.37 t ha^{-1}) compared to other municipalities with yields ranging 0.40 to 0.86 t ha^{-1} (SIAP, 2024).

Cucurbita argyrosperma cultivation is important due to the high demand for its seed, mainly by agribusiness to produce paste for traditional meals and snacks (INIFAP, 2014). This product reaches competitive prices in regional markets (Ireta-Paredes *et al*., 2018) and represents an economic opportunity for peasant families (Camino & Müller, 1993). In Campeche, Chihua squash cultivation is an important component for agrobiodiversity maintenance (Salazar-Barrientos *et al*., 2016); it is also the second most important economic element after maize for producers in rural areas (Dorantes-Jiménez *et al*., 2016). The crop is sown in temporary conditions in the spring-summer cycle (May-June) and under irrigation systems in the autumn-winter cycle (October-December) (INEGI, 2015). The most common technologies are soil preparation (plow and harrow), application of fertilizers, herbicides, and chemical insecticides (SAGARPA, 2015).

In Champotón, Campeche, the production of *C. argyrosperma* is traditionally an important economic activity for families due to the adaptation of the crop to the soil and climate conditions (Eguiarte *et al*., 2018). However, in recent cycles, a decrease in the area dedicated to its cultivation was observed as well as a decrease in productivity, negatively affecting the profitability of the production systems and the welfare of the producing families. To positively impact the profitability of the production system, the interaction of social, technological, and economic elements (Apollin & Eberhart, 1999) should be established. Given that references or consultation documents for the management of this crop are minimal, the objective of the study was to determine the technological and socioeconomic factors that influence the profitability of Chihua squash production systems in Champotón, Campeche, Mexico. This contribution aims to support future studies and development plans and to increase profitability through the use of appropriate technologies for the region in *C. argyrosperma* crop production systems.

Materials and methods

Study area location and sample size selection

The research took place in the municipality of Champotón, Campeche, Mexico (19°21'20" N, 90°43'24" W). The region is dominated by a warm subhumid climate with rains in summer (García, 2004), annual precipitation between 900 and 1200 mm, and an average temperature of 26.4°C.

To select the *C. argyrosperma* producing localities, the directory of producers at the office for attention to agricultural and fishing sectors of the Champotón municipality was used. Ten percent of the localities were selected, prioritizing those with the largest cultivation area: Santo Domingo Kesté, Mayatecun Module 1, Mayatecun Module 2, and Felipe Carrillo Puerto, with 683, 686, 233, and 190 families, respectively (INEGI, 2015). To determine the sample size, the finite population formula was used (Sierra Bravo, 2001):

$$n = Z^2pq \, \frac{N}{NE^2} \, Z^2 \, pq \qquad (1)$$

where n = sample size, Z = confidence level, p = positive variability, q = negative variability, N = population size, E = error precision.

In this research, a mixed quantitative-qualitative approach was used to obtain numerical results and information on the qualities of the systems through closed and open questions included in the surveys. The participatory actions method was used, where the object of research was the producer as a participant. The producer decides to participate, expresses their knowledge and experience on the phenomenon addressed and helps define the basis of improvement actions (Bernal, 2002). This approach made it possible to define and analyze the main study variables that characterize Chihua squash producers.

Technological and socioeconomic data compilation

A survey was applied to 136 producers responsible for the production units; the questionnaire had two sections: a) socioeconomic level, with 18 items: Age, schooling, main economic activity, total area of the plot, area destined for Chihua, years in activity, type of land tenure, type of soil, number of family members, reception of credit for agricultural production, access to domestic services, membership in a constituted organization, income from activity, family workforces, wage workforce, total workforce, purchase-sale agreement, profitability (production costs and labor costs), and b) technological level, with 38 items: more than five years of experience in cultivation, access to training courses, access to technical assistance, determination of wage needs, depth control of planting, density of 3,000 to 6,000 plants ha^{-1}, biological pest control, physical pest control, seed selection, pest and disease occurrence registry, crop relay, crop association, access to exclusive area for seed drying, seed separation, pre-harvest production estimate, production record, production cost record, harvest loss record, identification of potential buyers and transformation, soil nutrient analysis, tracking, burning of agricultural residues in the plot, plowing, furrowing, and use of agricultural calendar, use of synthetic herbicides, use of synthetic nematicides, fertilizer in sowing, in the development phase and in the flowering phase, use of organic fertilizers and application of insecticides, mechanical seed extraction and use of special dryer for seed, use of irrigation, irrigation water analysis, and calculation of irrigation sheet.

A consultation was conducted with experts in Cucurbitaceae cultivation and the characterization of agricultural production systems to define the variables of each section. The experts were researchers from the National Institute of Forestry, Agricultural and Livestock Research, College of Postgraduates, National Technological Institute of Mexico, and entrepreneurs specializing in the cultivation of Chihua squash. As a management reference, the technological package for the cultivation of Chihua squash developed by INIFAP (2015) was used. To produce a technological level index, the technologies applied by each producer were quantified from a total of 38 possible options. According to the sum of the used technologies, three technological levels were defined: low (<16), medium (16 to 30) and high (>30).

The total production costs (including the cost of family labor) and the total income from commercialization of the dehydrated Chihua squash seeds were calculated; these variables were calculated following the algebraic expressions based on economic theory (Samuelson *et al.,* 2009) as follows:

$$TC = P_x XTC \qquad (2)$$

where TC=total cost, Px=price of input or activity x and X=activity or input,

$$TI = P_y Y \qquad (3)$$

where TI=total income (US\$ ha^{-1}), Py=market price of crop Y (US\$ t^{-1}); and Y=crop yield (t ha^{-1}),

$$R = TI - T \qquad (4)$$

where R=profitability, TI=total income and TC=total cost.

Data analysis

The information gathered in the survey was captured in an Excel program and the data were analyzed with descriptive statistics. Subsequently, a principal component analysis (PCA) was applied to reduce the size of the data set and to allow the identification of a small number of variables representing most of the original variability of observations, corresponding to the variables with the highest variances (0.5) in factor 1 and 2 (Fig. 1).

With the most relevant variables of the PCA, multivariate statistical analyses were carried out (cluster analysis with complete distance chaining and Euclidean distances) to determine the groups in the systems (Peña, 2002), considering each group resulting from the analysis as a variation factor. Finally, to determine and contrast the variables of the technological and socioeconomic factors that affect the profitability of each group, a Spearman correlation, an analysis of variance and Tukey comparison of means (P<0.05) were performed using the statistical software STATISTICA v7.

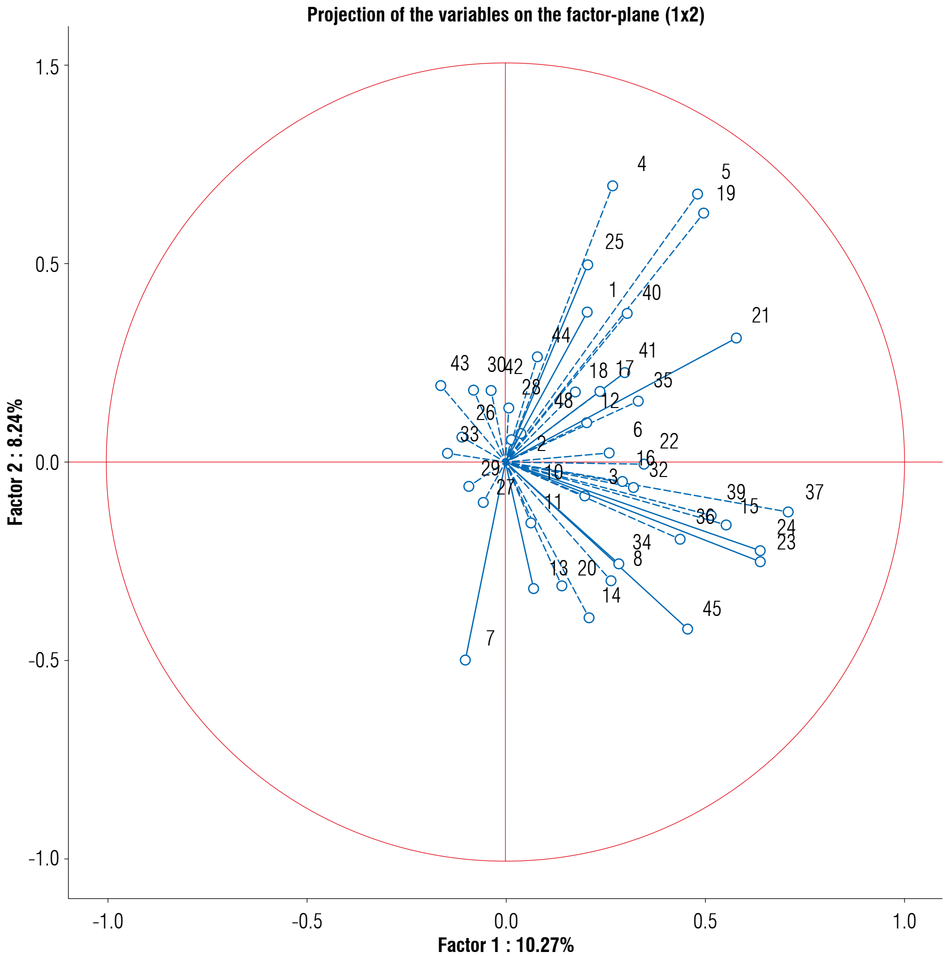

FIGURE 1. Principal components analysis with variables that affect the groups of the production systems of *Cucurbita argyrosperma*.

Results and discussion

Out of the total interviewed producers, 90.4% were men and 9.6% women, therefore, the cultivation is primarily supported by males. The participation of women is mainly oriented to postharvest management, such as the extraction and drying of the seeds, and to a lesser extent to the cultural activities around cultivation and decision-making. Fifty four percent of the producers mentioned that the decision and reason to grow Chihua squash is due to the high demand for dry seed in the regional and national market.

The low participation of women in the management of the crop was due to the fact that the activities of soil preparation, sowing, cleaning of weeds, pest control, fertilization and harvesting require high physical effort, while the activities of post-harvest management require greater manual dexterity. This situation is also observed in bean (*Vicia faba*) production systems at the state of Puebla and in grape cultivation in Aguascalientes (Mexico), where 4 and 2% of the producers were women (Borja-Bravo *et al*., 2016; Rojas-Tiempo *et al*., 2012). However, in milpa systems in Yucatán, Mexico, it was found that women are essential for the conservation of plant species (Salazar-Barrientos *et al*., 2016). In banana production systems in Colombia, women have participated in the decision-making, indicating that women in the rural sector are moving from a secondary role (labor) to a primary one (management) (León-Agatón *et al*., 2015).

Description of the grouped *C. argyrosperma* production system

Three groups of Chihua squash production systems were identified: G1 presents intermediate values of land availability, intermediate wage workforce, medium producer organization, seed processing, medium purchase-sale agreement, medium use of technologies. They obtain the highest income per unit of area and profitability; G2 reports average values of land availability, lower number of wage workforce, low producer organization, seed processing, no purchase-sale agreement, low use of technologies, low income and profitability; and G3 has the highest land availability, the highest number of wage workforce and producer organization, no seed processing, the highest purchase-sale agreement, high use of technologies, medium income and profitability (Fig. 2, Tab.1).

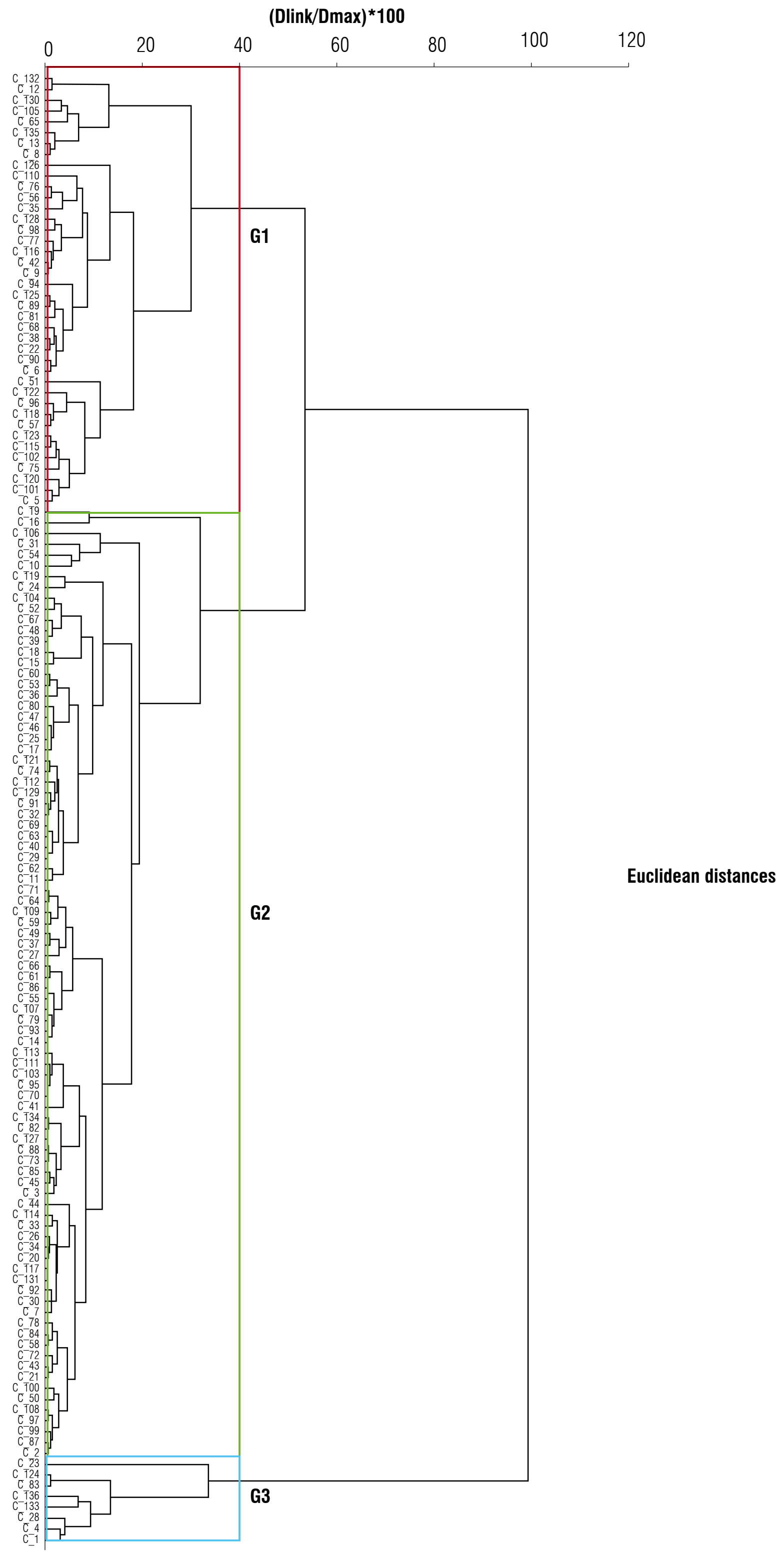

FIGURE 2. Groups of Chihua squash (*Cucurbita argyrosperma*) production systems in Champotón, Campeche, Mexico.

TABLE 1. Socioeconomic characteristics of the producers of *Cucurbita argyrosperma* in Champotón, Campeche, Mexico.

Variables	G1	G2	G3
Number of producers	28	100	8
Number of endemic dialects	7	6	2
Participation of women (%)	50	35	25
Number of public services	5	5	5
*Seed transformation (%)	10.7	12.0	0
Age (years)	43.39 ± 13.84^{a}	44.94 ± 14.46^{a}	55.62 ± 16.36^{a}
Schooling (years)	6.46 ± 3.38^{a}	5.04 ± 4.07^{a}	5.12 ± 3.94^{a}
Total area of the plot (ha)	14.50 ± 26.67^{a}	8.80 ± 18.31^{a}	46.94 ± 51.78^{b}
*Area destined for Chihua crop (ha)	3.30 ± 2.83^{a}	2.25 ± 1.53^{a}	10.31 ± 4.43^{b}
Years in activity (years)	16.86 ± 9.40^{a}	15.50 ± 10.58^{a}	15.5 ± 8.48^{a}
Family members (number)	4.53 ± 1.89^{a}	4.32 ± 2.21^{a}	4.25 ± 2.19^{a}
*Belongs to a constituted organization	0.21 ± 0.42^{ab}	0.11 ± 0.32^{a}	0.50 ± 0.53^{b}
Receive credit for agricultural production	0.071 ± 0.26^{a}	0.11 ± 0.31^{a}	0.38 ± 0.52^{a}
Family workforce	12.32 ± 9.68^{a}	15.44 ± 9.12^{a}	16.25 ± 13.74^{a}
*Wage workforce	24.64 ± 13.63^{ab}	13.10 ± 11.60^{a}	26.38 ± 18.83^{b}
Total workforce	36.96 ± 11.82^{a}	28.54 ± 14.53^{a}	42.63 ± 11.76^{a}
*Purchase-sale agreement	0.04 ± 0.19^{a}	0^{a}	0.25 ± 0.46^{b}
*Income (USD ha^{-1})	946.42 ± 457.92^{b}	629.25 ± 404.23^{a}	780.86 ± 370.82^{ab}
*Profitability	507.31 ± 528.27^{b}	272.16 ± 417.71^{a}	362.94 ± 347.55^{b}
*Technological index	18.11 ± 3.79^{ab}	17.34 ± 3.78^{a}	21 ± 3.62^{b}

[a, b, c] Different letters in the same row indicate significant differences according to the Tukey's test ($P<0.05$). *Variables with an asterisk are those that served to form the groups.

Socioeconomic characteristics of *C. argyrosperma* production systems

The age and education of the producers were similar in the three groups, with an average age of 47.99 and 5.54 years of education (Tab. 1). These values were similar to those reported in agricultural production systems in Colombia, where those responsible have an average age of 50 years (Rocha-Rodríguez *et al*., 2016). In this sense, the FAO mentions that the rural population is tending to grow old, and young people are migrating to urban environments (FAO, 2014). Agriculture represented the main economic activity for 89.3, 96.0, and 87.5% of the G1, G2, and G3 producers, respectively.

Of the three groups, G3 presented the largest total land area (P=0.00007) with an average of 46.94 ha, of which 22% was allocated to planting Chihua squash. Despite the large planting area, G3 had the lowest yields (rs=-0.879582; P<0.05), negatively affecting profits (rs=-0.904762; P<0.05), and utility (rs=-0.952381; P<0.05) and profitability of this crop (rs=-0.785714; P<0.05).

The highest seed yield was recorded in G1 with 635.24 kg ha^{-1} (P=0.00280) (Tab. 3), generating higher income (rs=0.948435, P<0.05), profitability (rs=0.879417, P<0.05), and economic utility of production (rs=0.918839, P<0.05).

The experience of the producers in the management of the Chihua squash crop ranged between 1 to 50 years, with an average of 15.95 in the three groups. The longer they have been in activity, the more access they have to governmental support (rs=0.495393, P<0.05). In the last five years, 82% of producers received government support for agricultural production, 59% of which came from the Secretary of Agriculture and Rural Development.

Land tenure in G1 was private, ejido, loaned, rented, and a combination of these, with 64.29, 3.57, 3.57, 3.57, and 17.85% each, respectively. In G2, it was privately owned, ejido, rented, loaned, communal property, and a combination of these, with 41.41, 10.94, 10.94, 3.13, 0.78, and 16.40%, respectively. In G3, it was ejido, private property, and loaned-private property, with 50.0, 37.5, and 12.5%, respectively. The type of soil according to the Maya (Bautista *et al.,* 2012) and INEGI (2017) classification was as follows: in G1, 58.62% of the plots were k'an kab or Luvisol type soil; in G2, the predominant soils were yaxx kom-ak' al che or Gleyic Vertisol and k'an kab or Luvisol in 53 and

39% of the cases, respectively; in G3, the predominant soils were yaxx kom-ak' al che or Gleyic Vertisol and k'an kab or Luvisol with 50 and 25%, respectively.

The average family in the three groups consisted of 4.37 individuals. In G2, 50% of adult women (mostly the producer wives) participate in the different processes of the Chihua squash cultivation. In G2, 35% participated. In G3, 25% contributed as labor, 12.5% supported other activities such as the seeds marketing, and 12.5% contributed financial resources for cultivation.

The use of different endemic dialects from southern Mexico and Guatemala was recorded among the producers of the three groups. In G1, Kanjobal, Maya, Mam, Chuj, Q'eqchi, Cachiquen, and Quiché were registered (28.57, 14.28, 10.71, 7.14, 3.57, 3.57, and 7.14%). In G2, the main dialects were Kanjobal, Maya, Mam, Quiché, Q'eqchi, and Chuj (31, 14, 10, 6, 6, and 4%), while, in G3, only 12.5% spoke Chuj combined with Kanjobal.

The families in the three groups (G1, G2, and G3) have access to 71.4% public services, including piped water, electricity, garbage recollection, road access, telephony (telecommunications and networks), education and health services. The use of private loans is increasingly common among producers without sufficient capital: these were used by 7.1, 11.0, and 37.5% of the producers of G1, G2, and G3, respectively.

The presence of organized producers was higher in G3 (P=0.00770) followed by G1 and G2, with an average of 50, 21, and 11%. Affiliation to constituted organizations showed a high correlation with the years dedicated to Chihua squash production (rs=0.888889, P<0.05).

The origin of the employed labor in the three groups was similar. In the G1 systems, the labor distribution was family workforce-wage workforce, family workforce, and wage workforce (78.57, 14.29, and 7.14%). In G2, it was family workforce-wage workforce, family workforce, and wage workforce (79, 20, and 1%). In G3, it was family workforce-wage workforce, family workforce, and wage workforce (75, 12.5, and 12.5%). G3 presented the highest use of labor per each cultivated hectare with 42.6 wages (P=0.00135) followed by G1 and G2 with 36.9 and 28.5 wages.

Additionally, of the total organized producers, 25% in G1 and 3.57% in G3 (P=0.00001) carried out purchase-sale contracts before the Chihua squash planting for the 2016 agricultural cycle. These sale-purchase contracts were mainly conducted by producers with fewer academic degrees (rs=-0.765092, P<0.05).

The production cost per ha was similar among groups, with an average value of USD 404.70 during the 2016 cycle, mainly due to wages, payments and purchase of inputs. The highest incomes were reported in G1 (P=0.00206) with USD 946.5.00 ha^{-1}, compared to G3 and G2 (USD 780.85 and 629.25 ha^{-1}). The calculated profitability varied among the three groups; iG1 had the highest (P=0.04726) with USD 507.30, followed by G3 (USD 363.00) and G2 (USD 272.10). The higher profitability observed in G1 was due to productivity, product quality, and production organization amongst producers. When there are deficiencies in these elements, the systems cannot negotiate with intermediaries and become vulnerable (Muñoz *et al*., 2014).

Seed transformation for an added value gain is an uncommon activity. Only 10.71 and 12% of the producers in G1 and G2 registered such activity. It mainly consists of roasting the seeds on a "comal" in an artisanal way for its commercialization in the main cities of the state (Fig. 3), at an average price of USD 3.50 kg^{-1}. The three groups presented a medium technological index. However, G3 stands out for its greater use of technologies, with 21 of the possible 38 (P=0.02819) compared to 18.11 and 17.34 in G1 and G2. The generation of added value in agricultural products is a strategy that promotes the development of agrarian systems by increasing their income. In this study, only G1 and G2 producers (10.71 and 12%) carry out some process to include an added value into a part of their production. These values coincide with those reported when analyzing the marketing schemes of agricultural producers in the market of Costa Rica (Rodríguez-Sáenz & Riveros, 2016), who mention that low added value, poor product

FIGURE 3. Seed transformation of *Cucurbita argyrosperma* in Champotón, Campeche, Mexico.

differentiation and little diversity in the supply are the most relevant characteristics and challenges faced by small and medium-scale producers, as well as rural micro, small, and medium-sized enterprises.

Technological characteristics of *C. argyrosperma* production systems

In Table 2, the technologies used in the Chihua squash production systems are recorded, covering stages from sowing to postharvest handling, including use of machinery, equipment, infrastructure, application of agrochemicals and the techniques to carry out each of the activities. Among the groups, only G1 and G2 carry out seed processing, while G3 invests more in irrigation systems.

Over 90% of the producers considered that the seed selection activity most influences crop yield; 91.91% manually conducted this activity considering the following criteria: 1) squash size, 2) seed size, and 3) presence of complete seeds (Fig. 4). The soil preparation for planting mainly includes tracing, plowing, furrowing, and chemical analysis of available mineral nutrients (Tab. 3). Seed selection is a practice that is traditionally carried out by Chihua squash producers, consisting of choosing large and robust pumpkins, extracting their seeds by cutting the fruits in half, and then drying the seeds under the sun without removing

TABLE 2. Main uses of technology in *Cucurbita argyrosperma* production systems in Champotón, Campeche, Mexico.

	Producers (%)		
Technologies	**G1**	**G2**	**G3**
Agrochemicals (pesticides)	100	100	100
Machinery and equipment	100	100	100
Seed selection	90.3	91	89.7
Crop relay	92.9	88	87.5
Seed separation	35.7	50	50
Cultivation record	28.9	18	38.9
Crop association	17.8	16	15.2
Technical assistance	17.9	8	25
Training courses	14.3	12	25
Transformation	10.7	12	0
Chromotropic traps	9.3	8	11.5
Irrigation	3.6	8	14.9

G1, G2, and G3 – three groups of the plant production systems.

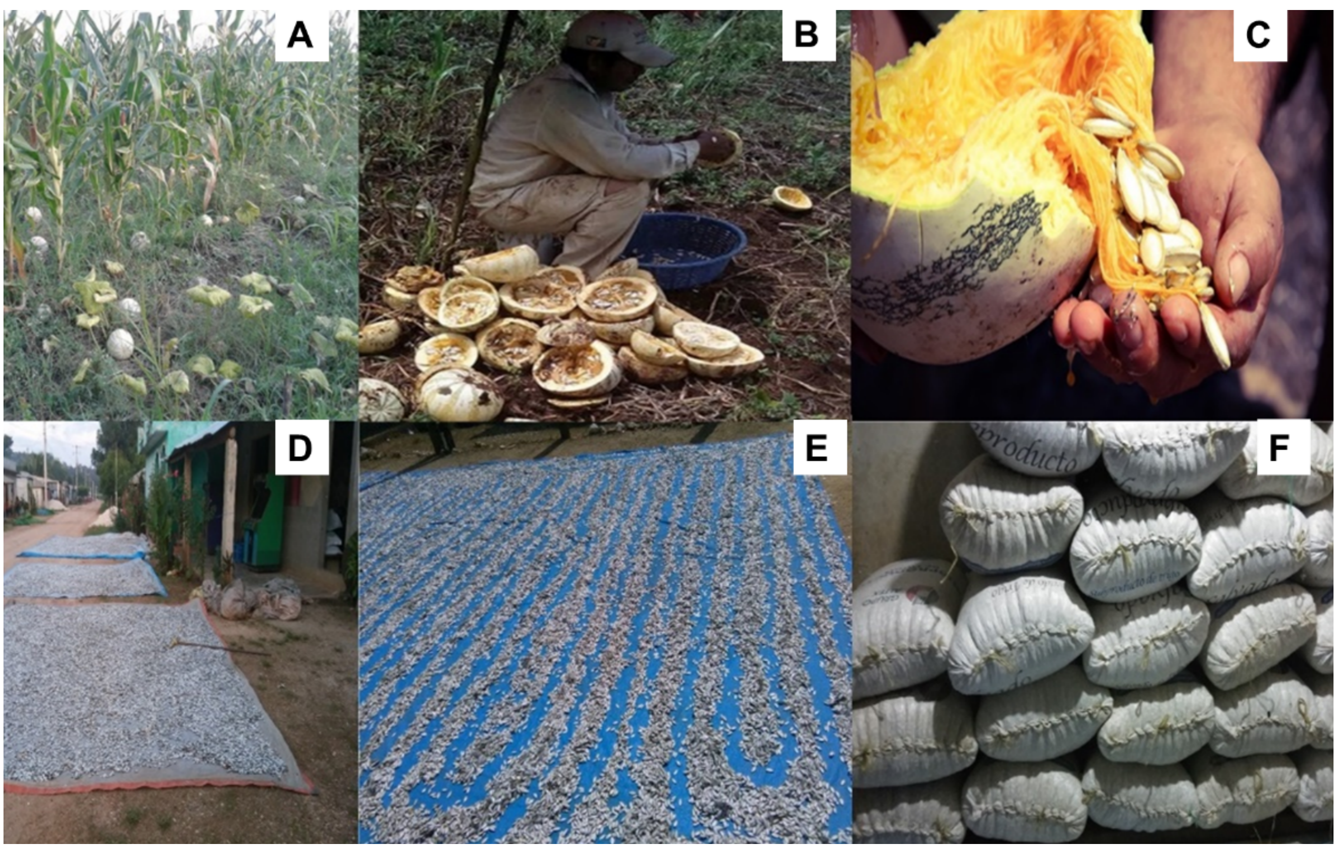

FIGURE 4. Seed selection and packaging process of *Cucurbita argyrosperma*: A) fruit harvest, B) manual seed extraction, C) manual seed selection, D) solar drying of seeds, E) final drying phase, and F) seed packing.

the remaining pulp or separate vain seeds. This activity is carried out in the same way by producers in Jesús Carranza, Veracruz (Ortiz-Timoteo *et al*., 2014). In contrast, in bean crops with squash in Yaxcabá, Yucatán, only 38% of producers select squash seed before sowing, while 62% do so at the time of harvest (Latournerie - Moreno *et al*., 2005). However, Rönicke *et al*. (2023) mention that the seeds used for planting are not improved varieties, so the crop has serious problems such as low germination percentage, low vigor, high heterogeneity in the size and shape of fruits, varying number and weight of seeds per fruit, and incidence of pests and diseases.

The cultivation of Chihua squash is mainly associated with the cultivation of corn. This association was recorded in 16.2% of the evaluated cases and demonstrates an efficient horizontal use of the plot. The incorporation of Chihua squash in the corn crops preserves the "milpa" traditional system (Ebel *et al*., 2017; Lira *et al*., 2016), a polyculture practice by agricultural peasants since pre-Columbian times to preserve the diversification in the systems (Gliessman, 1998; Morales Tapia & Guzmán Gómez, 1998).

TABLE 3. Main practices in land preparation for the sowing of *Cucurbita argyrosperma* in Champotón, Campeche, Mexico.

	Percentage of producers		
Practices	**G1**	**G2**	**G3**
Tracking	75	69	75
Plowing	67.8	68	75
Furrows	10.7	15	25
Use of agricultural calendar	11	11	14
Soil chemical analysis	14.3	10	12.5

G1, G2, and G3 – three groups of the plant production systems.

Other relay crops were also recorded, mainly corn (80%) and other species such as *Hibiscus sabdariffa, Arachis hypogaea*, and *Phaseolus vulgaris* (11%). In all the evaluated systems, agrochemicals are applied for pest control, disease management, weeds management, and fertilization. Only 9.56% of the interviewed producers mentioned carrying out preventive management to control pests and diseases through chromotropic traps.

The application of agrochemicals in the cultivation of Chihua squash is the most widely used technology in the region. It is generally carried out without any control, prevention, and adequate equipment and represents a high economic cost to the systems (Schiesari *et al*., 2013). In contrast, the use of agricultural irrigation in Chihua squash production systems is minimal, due to the high cost of installation and operation. This situation also occurs in the peasant agricultural systems in the Río Segundo basin, Argentina, where irrigation represents a costly investment and a financial risk that farmers are not willing to assume (Riera & Pereira, 2013).

About 9% of the interviewed producers had access to irrigation, while 91% stated that it is an inaccessible option due to the high cost of installation and limited access to wells. In all cases, the harvest is done manually, starting with the harvest, dividing the fruits, and extracting the seeds; due to their manual agility, mainly women and youth participate in this activity. About 29% of the producers mentioned keeping production records, a practice learned through training courses and technical assistance (13.23 and 11.03%, respectively). The technological index is the sum of the technologies adopted and adapted to the production systems (Borja-Bravo *et al*., 2016). The production systems in G3 recorded the highest technological index, due to use of technology irrigation systems. This trend coincides with that reported in small production agricultural systems (Ayala-Garay *et al*., 2013; Vilaboa-Arroniz *et al*., 2009).

Regarding the seed commercialization, a variation in sale price was recorded, ranging from USD 2.25 per seed kg at the beginning and USD 1.55 at the end of the season, which represents a 31% difference. These prices were set by buyers, including local, regional, and national collectors (Tab. 4). In the production systems evaluated, profitability did not improve with a greater sowing or a higher technological index. Additionally, it seems that the use of irrigation systems affects the profitability of the system, making irrigation an unsuitable technology for the cultivation of *C. argyrosperma* under the conditions of Champotón, Campeche.

TABLE 4. Main forms of commercialization of the *Cucurbita argyrosperma* production systems in Champotón, Campeche, Mexico.

Variable	Number of producers/%
Not identified	24/17.65
[1]In the community	4/2.94
[2]Internal collector	52/38.23
[3]External collector	45/33.09
[4]Intermediary	11/8.09
Total	136/100

[1]Sold to a neighbor who in turn takes it to the Campeche or Ciudad del Carmen market, [2]Sold to a neighbor who acts as an intermediary collector, [3]A person from another municipality or state (Champotón, Escárcega and Hopelchén belonging to Campeche or Yucatán, Veracruz, and Puebla), [4]A neighbor of another community of Champotón, Campeche, who resells it. Source: Authors, based on the data obtained in the interviews, 2016.

Conclusions

In the Champotón municipality, Campeche, three types of Chihua squash production systems are clearly differentiated by area of the plot, constituted organization, wages workforce, seed processing, purchase-sale agreement, income (USD ha^{-1}), profitability, and use of technologies. These differences determine three technological levels that in turn, affect the economic profitability of the systems. In this sense, the group with the highest technological index ranked second in profitability, so this variable does not improve by increasing the number of technologies used. Therefore, to increase the profitability of Chihua squash production systems, it is necessary to organize producers to receive technical advice that reduces production cost, manage financing, industrialize the seeds to increase their value and explore innovative and fair marketing channels.

Likewise, the results of the study showed that in rural systems some technologies suitable for industrialized systems, such as irrigation, do not have the same effect and can negatively impact the system. Similarly, within the family labor force, the participation of women is evident, contributing 25 to 50% of the labor, focused on the management of seeds after harvest. Therefore, future studies should encourage the participation of women in the different cultivation activities and promote fair compensation for their work.

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Conflict of interest statement

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

Author's contributions

Conceptualization: HCKC, BCM, and CFB; methodology: JCI; software and validation: SFC, VRM; writing-review and editing: JCI, BCM, CFB. All authors have read and approved the final version of the manuscript.

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Rooting for pasta: Unleashing the rheological potential of tannia (*Xanthosoma sagittifolium*)

Enraizamiento de la pasta: liberando el potencial reológico de la malanga (*Xanthosoma sagittifolium*)

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ABSTRACT

The quest for technological advancements in food products has led to the exploration of unconventional raw materials and innovative formulations. This study investigated the feasibility of incorporating tannia (*Xanthosoma sagittifolium*) starch as a partial substitute for wheat flour in pasta formulations. Tannia tubers were sourced, and native starch was extracted following a wet method. Four pasta formulations were prepared with varying percentages of tannia starch substitution (5%, 10%, 15%, and 20%), alongside a control sample. Physicochemical analyses applied for moisture content, ash content, acidity, and pH revealed 11.97% moisture, 0.4% ash, 0.007% acidity, and 4.6 pH in tannia starch. The rheological analysis denoted as the parameters in the Mixolab showed alterations in hydration, moisture, and stability with increasing tannia starch substitution. Cooking tests demonstrated a reduction in optimal cooking time with higher levels of tannia starch substitution, attributed to lower gelatinization temperatures of the tannia starch. Weight loss increased with greater substitution of tannia starch, while water absorption varied, showing a non-linear trend. Quality indices reflected changes in dough characteristics and gluten strength with tannia starch substitution. Further optimization of formulations is recommended to balance technological enhancement with pasta quality attributes, paving the way for the development of novel pasta products.

Key words: starch, starchy raw material, sustainable agriculture, physicochemical properties, wheat, new cocoyam.

RESUMEN

La búsqueda de mejoras tecnológicas en productos alimenticios ha llevado a la exploración de materias primas no convencionales y formulaciones innovadoras. Este estudio investigó la viabilidad de incorporar almidón de malanga (*Xanthosoma sagittifolium*) como un sustituto parcial de la harina de trigo en formulaciones de pasta. Se extrajo el almidón nativo de tubérculos de malanga siguiendo el método húmedo. Se prepararon 4 formulaciones de pasta con diferentes porcentajes de sustitución de almidón de malanga (5%, 10%, 15% y 20%), junto con una muestra de control. Los análisis fisicoquímicos ejecutados, humedad, cenizas, acidez y pH, revelaron 11,97% de humedad, 0,4% de cenizas, 0,007% de acidez y 4,6 de pH en el almidón de malanga. El análisis reológico denotado como los parámetros de Mixolab mostró alteraciones en la hidratación, humedad y estabilidad con el aumento de la sustitución de almidón de malanga. Las pruebas de cocción demostraron una reducción en el tiempo de cocción óptimo con niveles más altos de sustitución de almidón de malanga, atribuido a las temperaturas de gelatinización más bajas del almidón de malanga. La pérdida de peso aumentó con una mayor sustitución de almidón de malanga, mientras que la capacidad de hinchamiento varió, mostrando una tendencia no lineal. Los índices de calidad reflejaron cambios en las características de la masa y la fuerza del gluten con la sustitución de almidón de malanga. Se recomienda una mayor optimización de las formulaciones para equilibrar la mejora tecnológica con atributos de calidad de la pasta, allanando el camino para el desarrollo de productos de pasta novedosos.

Palabras clave: almidón, materia prima amilácea, agricultura sostenible, propiedades fisicoquímicas, trigo, quequesque.

Introduction

The escalating demand for food products with enhanced technological properties has spurred a continuous quest for unconventional raw materials and innovative formulations (De & Goswami, 2021). Due to its versatility, wheat has become an indispensable component of the human diet (Mefleh *et al*., 2019). Globally, it stands as a mainstay crop, serving as the primary livelihood for countless farmers across the planet. In terms of contribution of nutrients,

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wheat outshines many agricultural commodities, offering the global populace a rich source of both calories and proteins (Boland & Hill, 2020). One of the remarkable attributes of wheat lies in its unique viscoelastic properties, which are primarily manifested in its dough. Unlike many other grains, wheat dough exhibits a distinctive combination of viscosity and elasticity, allowing it to stretch and hold its shape during the kneading and shaping processes (Van Rooyen *et al*., 2023). This inherent characteristic of wheat dough is essential to produce a wide array of wheat-based products, including pasta (Uthayakumaran & Wrigley, 2010). In the context of pasta production, the viscoelastic nature of wheat dough plays a salient role as it enables the dough to be molded into various shapes, such as *spaghetti*, *penne*, or *lasagna*, while retaining its structural integrity during cooking. The elasticity of the dough ensures that the pasta maintains its shape and texture, even when subjected to boiling water (Gasparre & Rosell, 2022). Therefore, the viscoelastic properties of wheat dough are significant in the manufacturing process of pasta, facilitating the creation of diverse pasta shapes and ensuring the desired texture and consistency of the final product (Shang *et al*., 2023). In this context, pasta, conventionally crafted from durum wheat has undergone a notable evolution through the integration of novel constituents (Sarkar & Fu, 2022). These include rice, corn, pseudocereals, legumes, and fiber-enriched variants, contributing to the diverse landscape of dry pasta offerings in the market (Schmidt & Raczyk, 2023). The ubiquity of pasta across retail shelves owes to its economic accessibility, prolonged shelf life, and user-friendly preparation protocols (Shirhatti *et al*., 2023). Traditionally, pasta formulations predominantly feature starch-rich grains, complemented by proteins, bioactive compounds, vitamins, and minerals (Gull *et al*., 2018). In 1964, the Food and Agriculture Organization (FAO) introduced the concept of composite flours as a solution for nations devoid of wheat cultivation (Noorfarahzilah *et al*., 2014). These composite flours, incorporating wheat alongside up to 40% of alternative cereals or supplementary ingredients, have been extensively studied for their implications in pasta production (Baiano *et al*., 2011; Chillo *et al*., 2007). The adoption of composite flours has played a role in yielding pasta endowed with commendable attributes, catering to the dietary exigencies of demographics constrained by limited access to nutrient-rich sustenance (Nilusha *et al*., 2019). With the global agro-industrial panorama witnessing a surge in demand, there arises an imperative for economically viable products endowed with superior technological attributes (Podio *et al*., 2019). Pasta, as a dietary staple embraced worldwide, offers myriad benefits but also exerts a significant calorific load attributable to its elevated carbohydrate content *vis-à-vis* modest nutrient density (Yadav & Gupta, 2015). To redress this asymmetry, the food industry has endeavored to fortify pasta formulations with nutrient-rich adjuncts, envisaging a diminution in dependence on wheat imports (Nkwonta *et al*., 2023). The partial replacement of wheat with starch derived from alternative botanical sources has engendered the inception of technological-modified food commodities, wielding substantial influence over consumer dietary patterns (Sharif *et al*., 2022). This emphasis underscores the importance of carefully selecting ingredients, refining cooking techniques, and optimizing processing methods (Podio *et al*., 2019). Starches derived from tubers and roots offer a promising alternative, promoting the use and cultivation of local resources (Pacheco de Delahaye & Techeira, 2009). This shift goes beyond traditional sources of starch such as wheat, potatoes, and rice, reflecting a landscape with starches of different structural characteristics that can impact product manufacturing (Bertoft, 2017). The potential benefits of using non-conventional roots such as yam, cassava, and sweet potato in pasta production have been already reported. The integration of these roots can improve cooking qualities and introduce new functional properties. Moreover, these alternative raw materials can contribute to food diversity, sustainability, and meet the demands of health-conscious consumers (Alvis *et al*., 2008; Djeukeu *et al*., 2017; Meaño Correa *et al*., 2014; Odey & Lee, 2020; Singh *et al*., 2004). The aim of this research was to investigate the impact of substituting wheat flour with tannia starch (derived from *Xanthosoma sagittifolium*) and varying cooking and cooling temperatures on the rheological properties and overall quality attributes of pasta.

Materials and methods

Starch source

Tannia (*Xanthosoma sagittifolium*) tubers were sourced from Pucará (Ecuador) during the rainy season. *Triticum durum* semolina and other ingredients for pasta production were procured from local stores.

Extraction of native tannia *(Xanthosoma sagittifolium)* starch

The extraction of native tannia starch followed the wet method (Quezada-Correa *et al*., 2021) at pilot scale. Fifty kg of tannia tubers were disinfected, peeled, sliced into 1-cm-thick slices, and immediately immersed in a 2% (w/v) citric acid solution. Wet blending was conducted using a semi-industrial blender for 3 min at low speed. Subsequently, the blend was filtered through a sieve cloth, and the residue obtained was washed until no apparent traces of starch remained. The filtrate was allowed to settle for 24 h to facilitate solid precipitation. The sediment obtained, corresponding to starch, was dried in trays at 55°C for 24 h in a laboratory oven (INB 500, Memmert GmbH + Co. KG, Schwabach, Germany), then crushed and sieved through a 0.25 mm (ASTM No. 60) mesh sieve.

Physicochemical analysis of *Xanthosoma sagittifolium* starch

Moisture analysis

Moisture content was determined using a MB90/120 halogen analyzer (Ohaus Corporation, Parsippany, USA) at a temperature range of 40-160°C. A 0.5 g sample of tannia starch was placed in the analyzer pan, initiating the drying process (Calero-Jiménez *et al*., 2021). The equipment registered consecutive weight loss, and drying was considered complete once the weight stabilized. The moisture content present in the starch was automatically obtained by the difference in weight. Each analysis was performed in triplicate.

Ash determination

Ash content was determined following the procedure outlined by Mitchell (1990). Five grams of the sample were weighed into crucibles, then placed in a muffle furnace and calcined at temperatures of 550 ± 3°C for 3 h until reaching a constant weight. The sample was subsequently allowed to cool in a desiccator to 25°C. The ash percentage was calculated using Equation 1:

$$\text{Ash content (\%)} = \frac{A - B}{C} \times 100 \qquad \text{Eq. (1)}$$

where

A = weight of crucible with sample (g);

B = weight of crucible with ashes (g);

C = weight of the sample (g).

Titratable acidity

To determine the acidity in starch samples, the AOAC method (AOAC, 2005) standardized protocol was applied. Ten grams of starch were weighed and added to 100 ml of distilled water in a beaker. The mixture was stirred for 30 min to ensure the formation of a homogeneous slurry. Fifteen ml of the starch slurry were pipetted into another beaker. A few drops of phenolphthalein were added to the sample slurry. Titration was then initiated by adding a standardized solution of NaOH, 0.1 N, from a burette while stirring the mixture. The endpoint of the titration was determined by monitoring the color change of the indicator solution up to a permanent color change. The volume of NaOH 0.1 N required to reach the endpoint was recorded and Equation 2 was applied:

$$\%\ \text{Acidity} = \frac{V_{NaOH} \times N_{NaOH} \times P_{\text{meq acetic acid}}}{P_{\text{sample}}} \times 100 \qquad \text{Eq. (2)}$$

where

V_{NaOH} = volume of NaOH used (ml);

N_{NaOH} = NaOH normality (0.1 N);

$P_{\text{meq acetic acid}}$ = milliequivalent weight of acetic acid (0.06);

P_{sample} = sample weight (g).

Determination of pH

To determine the pH value in starch, the methodology indicated by Medina and Catarí (2022) was used with adaptations. Five grams of starch were weighed and dispersed in 100 ml of deionized water in a flask. The mixture was heated in a water bath at 50°C for 15 min. Subsequently, the mixture was cooled to room temperature to stabilize the gelatinized starch slurry. A Bante900P pH meter (Bante Instruments, Shanghai, China) was used to read the values in the samples. The electrode of the pH meter was immersed in the cooled starch slurry. The pH value of the starch sample was recorded.

Blends formulation

Four formulations were prepared, each incorporating varying percentages of starch (5%, 10%, 15%, and 20%) into wheat flour. These were compared against a standard sample consisting of 100% wheat flour. The total mixture weight for each formulation was standardized to 250 g.

Rheological analysis of the blends

The rheological analyses were conducted using the Mixolab Chopin instrument (KPM Analytics, Westborough, USA) in accordance with ICC-173 standards and the methodologies outlined by Contreras Dioses *et al*. (2017). These tests were carried out in the laboratories facilities of the Ambato University of Technology (UTA). Each analysis utilized 75 g of the respective blend and evaluated 5 key parameters (Švec & Hrušková, 2015) detailed in Table 1.

Additionally, the analysis included the determination of water absorption necessary for dough development (%) and dough stability (min), as per the methodology described by Acurio *et al*. (2018). Quality indices such as water absorption index, kneading index, gluten strength, viscosity, amylase resistance, and retrogradation index were also evaluated.

TABLE 1: Description of parameters assessed in the rheological analysis.

Parameter	Description	Usage
C1	Maximum torque during mixing.	To assess the initial mixing phase for information regarding water absorption capacity and development during kneading of the dough.
C2	Protein quality, represents the weakening of the protein based on the mechanical work and the increasing temperature.	To measure the stability of dough proteins during mixing. It is indicative of the quality of the gluten network, which affects its elasticity and extensibility, essential for bread-making quality.
C3	Starch gelatinization, expresses the rate of starch gelatinization.	To evaluate the temperature at which starch granules begin to gelatinize and swell; relevant for the viscosity and gas-holding capacity of doughs during baking.
C4	Amylase activity, indicates the stability of the hot-formed gel.	To measure the activity of enzymes that break down starches into sugars, influencing the fermentation process of the dough and the crumb structure and sweetness in final products.
C5	Starch retrogradation, represents starch retrogradation during the cooling period.	To assess the recrystallization of gelatinized starch during cooling, which affects the shelf life and staling rate in bread.

Source: Švec and Hrušková (2015).

Manufacturing of pasta

Pasta formulations were prepared following the procedures indicated by Alamprese *et al.* (2007) with adaptations using an HR2375/06 extruder (Koninklijke Philips N. V., Amsterdam, The Netherlands). The percentages of water, egg, and salt remained unchanged for all formulations. The established formulations are presented in Table 2.

TABLE 2. Formulations for pasta manufacturing.

Ingredients	Formulations				
	F0	F1	F2	F3	F4
Wheat flour (%)	69.3	64.3	59.3	54.3	49.3
Tannia starch (%)	0	5	10	15	20
Egg (%)	4	4	4	4	4
Water (%)	26	26	26	26	26
Salt (%)	0.7	0.7	0.7	0.7	0.7

The ingredients of the pasta formulations were weighed in accordance with predetermined formulations. Subsequently, a preliminary blending of flour and starch was conducted before combining all ingredients. The resultant mixture was then cold-extruded in the HR2375/06 extruder with all the ingredients to form the pasta shapes, which were subsequently dried in an oven set to 65-70°C for 4 h to achieve a moisture content of 13%. The pasta was then allowed to cool at room temperature for 60 min before packaging.

Cooking trials in pasta

Determination of optimal cooking time

Fifty grams of pasta from the control sample and those prepared with varying substitution percentages were individually immersed in 400 ml of boiling water. After 9 min, a piece of pasta was extracted, placed between two glass slides, and subjected to compression. The ideal cooking time was established as the point at which the pasta exhibited no whitening upon compression (Granito *et al.*, 2014).

Assessment of pasta weight loss

To assess the weight loss of the pasta, the water used for boiling was collected and evaporated at 100°C until a constant weight was achieved. The percentage of solids remaining in the cooking water for each pasta formulation was subsequently determined (Granito *et al.*, 2014).

Determination of water absorption

An adapted methodology, based on the approach outlined by Granito *et al.* (2014), was employed for the determination of water absorption. Fifty grams of dry pasta from each formulation were cooked to their respective optimal times, drained, and cooled to 25°C. The cooked and dry pasta weights were then measured using an analytical balance. The water absorption in the pasta was calculated using Equation 3:

$$\text{Water absorption (\%)} = \frac{W_{cooked} - W_{dry}}{W_{dry}} \times 100 \qquad \text{Eq. (3)}$$

where

W_{cooked} = weight of cooked pasta;

W_{dry} = weight of dry pasta.

Statistical analysis

Statistical analyses were conducted to investigate the effect of varying levels of tannia starch substitution (5%, 10%, 15%, and 20%) for wheat flour in pasta manufacturing using a completely randomized design (CRD). The evaluated variables included cooking tests (cooking time, weight loss, and water absorption percentage) and rheological analyses.

Each parameter was assessed in triplicate, and the results were reported as mean ± standard deviation. Analysis of variance (ANOVA) and Tukey's multiple comparison test were employed to assess statistical significance, with a significance level set at $P<0.05$. Data analysis was performed using IBM SPSS Statistics version 20 (International Business Machines Corporation, Armonk, USA).

Results and discussion

Moisture content

The moisture content found in this study for tannia starch was 11.97%. Palomino *et al.* (2010) reported lower moisture content in tannia starch (10.82%). Madrigal-Ambriz *et al.* (2018) found lower values for *X. sagittifolium* flour (6.87%). However, Torres Rapelo *et al.* (2013) recorded values higher than those obtained in this study (14.49--14.29%).

Ash content

The ash content found in tannia was 0.4%, higher than that reported by Palomino *et al.* (2010) for Criollo tannia starch (0.09%). Torres Rapelo *et al.* (2013) reported lower values for taro (*Colocasia esculenta* L. Schott) starch of around 0.27%.

Acidity and pH values

The acidity and pH values obtained were 0.007% and 4.6, respectively, both lower than those reported by Madrigal-Ambriz *et al.* (2018), who reported acidity values of 0.47% and pH of 5.91. Rodríguez- Miranda *et al.* (2011) also reported a pH of 6.78, both values derived from taro starch analysis. Palomino *et al.* (2010), however, reported higher acidity (0.04%) and pH (6.0) values for *Xanthosoma sagittifolium* starch. Differences in acidity and pH values can be attributed to the methods of extraction and processing, the inherent properties of the starch source, and storage conditions (Ashogbon & Akintayo, 2014). Environmental factors such as soil composition, climate, and cultivation practices can also influence the chemical composition of the starch (Patindol *et al.*, 2015). Lower acidity and pH values can affect the functional properties of the starch, including its gelatinization temperature, pasting properties, and shelf life (Awuchi *et al.*, 2019). A lower pH can enhance the solubility of the starch and affect its interaction with other ingredients in food formulations (Zhu, 2017). Table 3 details the results for physicochemical parameters found for tannia starch in the present study.

TABLE 3. Physicochemical parameters found for tannia starch.

Parameters	Percentage (%)
Ash	0.4 ± 0.001
Acidity	0.007 ± 0.003
pH	4.6 ± 0.002
Moisture	11.97 ± 0.002

Values reported are the means of 3 replicates ± standard deviation.

Cooking test of pasta

The results obtained from this analysis, as presented in Figure 1, confirmed that as the percentage of tannia starch increases, the optimal cooking time for pasta decreases.

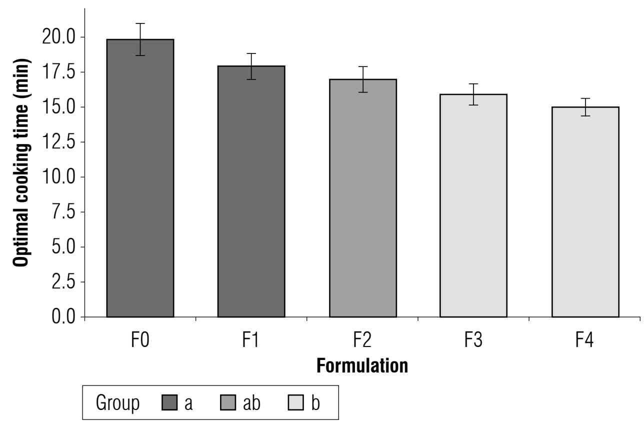

FIGURE 1. Optimal cooking time (min) by formulation. Error bars in the columns represent the standard errors (n= 3) of the mean. F0, F1, F2, F3, and F4 correspond to the formulations described in Table 2, where the percentages of wheat flour, tannia starch, egg, water, and salt are specified. The groups (a, ab, b) represent statistical differences in optimal cooking times among the formulations. Group "a" indicates significantly longer cooking times, group "b" represents shorter cooking times, and group "ab" includes formulations with intermediate cooking times that are not significantly different from either "a" or "b." Tukey's multiple comparison tests were employed ($P<0.05$).

The reduction in cooking time (Fig. 1) is attributed to the lower gelatinization temperatures exhibited by tannia starch. Consequently, as the substitution of tannia starch increases, less energy is required for complete cooking (Torres Rapelo *et al.*, 2013). Supporting this, Littardi *et al.* (2020) suggest that the optimal cooking time for pasta increases with gluten content. Li *et al.* (2017) affirm that the addition of non-wheat starches prolongs the time required for noodles to reach readiness. This finding is consistent with observations by Criollo Feijoo *et al.* (2017), who noted an increase in cooking times with increased banana starch content, attributable to its higher gelatinization temperatures.

Weight loss

The recorded weight losses ranged from 5.39% (F0) to 11.12% (F4), as depicted in Table 4. Notably, with each incremental substitution of tannia starch, weight loss increased accordingly as shown in Figure 2. This phenomenon shows the impact of partially replacing durum wheat with an alternative starch source on pasta properties (García-Valle *et al*., 2021). The incorporation of starch from a different nature into pasta formulations can significantly impact the weight loss observed during cooking (Giuberti *et al*., 2015). Tannia starch may have higher hydration capacity compared to durum wheat (Suparthana & Putu Timur Ina, 2020), meaning it can absorb more water during cooking, leading to increased weight loss when the pasta is drained after cooking. On the other hand, durum wheat is high in gluten, which helps form a strong protein network that traps starch granules and retains water during cooking (Sissons, 2008). Replacing durum wheat with tannia starch reduces the gluten content, weakening the pasta's structural integrity. This weaker network allows more water to escape, contributing to higher weight loss. Additionally, tannia starch might interact differently with gluten compared to wheat starch, potentially leading to a less cohesive matrix that releases more water during cooking. Tannia starch could also have a higher swelling power than wheat starch (Krisbianto & Minantyo, 2024), causing it to expand more during cooking. This higher expansion may lead the pasta produced to break apart more easily, leading to greater weight loss.

This pattern mirrors findings reported by Gianibelli *et al.* (2005), who noted that reducing the percentage of wheat starch and substituting it with other starch sources leads to increased cooking losses. Similarly, Granito *et al.* (2014) observed results consistent with those described in this study in formulations consisting in 100% wheat (3.00±0.15), 90% wheat:10% *Vigna sinensis* (5.35±0.15), 88% wheat:12% *Phaseolus vulgaris* (15.13±0.12), and 88% wheat:12% *Cajanus cajan* (19.7±0.08).

Water absorption in pasta

Water absorption in pasta relates to the amount of water the starch retains during cooking, causing an enlargement in its volume. The cooking test analysis is linked to the size of the pasta, as boiling water hydrates the gluten network, leading to an increase in volume. Consequently, as less

TABLE 4. Weight loss in pasta formulations with varied levels of tannia starch substitution.

Formulation	Weight loss
F0	5.39 ± 0.12[a]
F1	5.75 ± 0.14[a]
F2	6.16 ± 0.35[a]
F3	9.68 ± 0.27[b]
F4	11.12 ± 0.52[b]

Values reported are the mean of 3 replicates ± standard deviation. Values containing a, b differ significantly according to the Tukey's test ($P<0.05$).

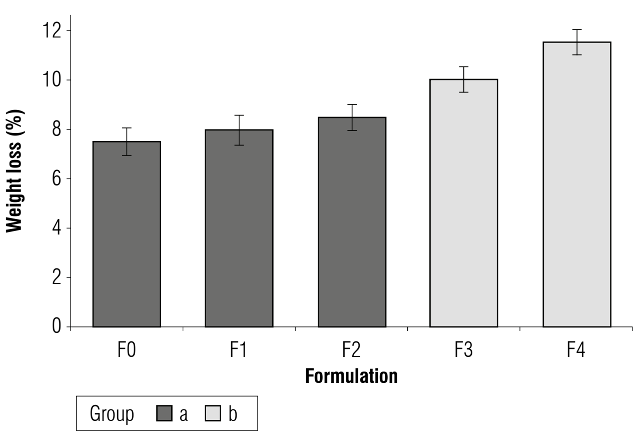

FIGURE 2. Weight loss in pasta formulations with varying levels of starch substitution. Error bars in the columns represent the standard errors (n= 3) of the mean. F0, F1, F2, F3, and F4 correspond to the formulations described in Table 2, where the percentages of wheat flour, tannia starch, egg, water, and salt are specified. The groups (a, b) represent statistical differences in weight loss among the formulations. Group "a" indicates significantly lower weight loss, and group "b" represents higher weight loss. Tukey's multiple comparison tests were employed ($P<0.05$).

TABLE 5. Water absorption in the pasta formulations proposed.

Formulation	Water absorption (%)
F0	152.20 ± 1.42[a]
F1	149.07 ± 2.87[a]
F2	181.71 ± 2.28[b]
F3	185.43 ± 4.13[b]
F4	252.57 ± 5.61[c]

Values reported are the mean of 3 replicates ± standard deviation. Values containing a and b differ significantly according to the Tukey's test ($P<0.05$).

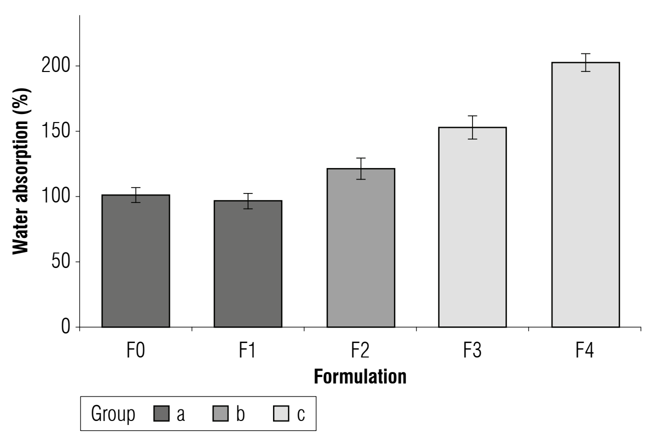

FIGURE 3. Water absorption in pasta formulations with varying levels of starch substitution. Error bars in the columns represent the standard errors (n = 3) of the mean. F0, F1, F2, F3, and F4 correspond to the formulations described in Table 2, where the percentages of wheat flour, tannia starch, egg, water, and salt are specified. The groups (a, b, c) represent statistical differences in water absorption among the formulations. Group "a" indicates significantly lower water absorption, group "b" indicates intermediate levels, and group "c" represents the highest water absorption. Tukey's multiple comparison tests were employed ($P<0.05$).

wheat flour is used, the water absorption capacity increases, as observed in Figure 3; this behavior has been observed by Akanbi *et al.* (2011). Interestingly, in this study, a different trend is observed; substituting 5% of tannia starch decreases the water absorption, whereas increasing quantities of 10%, 15%, and 20% result in an increment in the water absorption. Table 5 depicts the water absorption in the pasta formulations evaluated. The described phenomenon is due to the high values of water absorption in the starch (Akanbi *et al.*, 2009). Similar findings were reported by Adepeju *et al.* (2011) when increasing the percentage of breadfruit starch by 20% and 30% resulted in an increase in the water absorption capacity.

Rheological characterization of the blends

Hydration refers to the percentage of water needed to be added to the flour to form a dough with suitable properties for manipulation. As mentioned by Witczak *et al* (2016), absorption is linked to the endosperm of the grain and the gluten present therein. As observed in the results, mixtures with different substitution percentages, as well as the standard mixture, exhibited inversely proportional outcomes; as the amount of tannia starch increased, the moisture percentage decreased. This behavior aligns with findings reported by Sacón-Vera *et al.* (2016), primarily due to the varying quantity of proteins present in the different formulations. The stability percentage obtained for F1, F2, and F3 treatments were similar to that in the control treatment (F0), corresponding to a strong dough. The stability percentage for F4 was significantly different (P<0.05) from the others, showing lower stability as the substitution percentage increased. Table 6 indicates the values found for the parameters related to the rheological characterization in the formulations. The results of this analysis ranged between 8.1 and 10.6 min. This is associated with the formation of a weak dough, which would not withstand thorough kneading or fermentation without breaking apart.

TABLE 6. Parameters for the rheological characterization in the formulations evaluated.

Formulation	Hydration (%)	Moisture (%)	Stability (min)
F0	62.5 ± 0.04^a	14.1 ± 0.2^a	10.2 ± 0.6^a
F1	62.2 ± 0.02^a	13.6 ± 0.6^a	10.6 ± 0.4^a
F2	62.1 ± 0.01^a	13.7 ± 0.5^a	9.9 ± 0.1^a
F3	62.4 ± 0.07^a	13.5 ± 0.7^a	9.4 ± 0.5^a
F4	60.2 ± 0.03^b	13.2 ± 0.2^a	8.1 ± 0.3^b

Values reported are the mean of 3 replicates ± standard deviation. Values containing a and b differ significantly according to the Tukey's test (P<0.05).

Rheological analysis of doughs

When doughs are placed in the equipment to determine rheological parameters and reach the highest point, which is 1.1 N×m at 30°C, they are considered stable in their transformations; therefore, at that moment and classified as a solid mass (Sandoval *et al.*, 2012). The values of the analyzed properties ranged from C1, with values between 1.05 to 1.12 N×m, Typical C1 values for wheat dough range from 1.1 to 1.5 N×m (Sandoval *et al.*, 2012). The study values are slightly lower but still within an acceptable range, indicating that the dough develops well, albeit slightly weaker when more tannia starch is added. For the C2 parameter, which reflects the water content retained by the starch granules, inversely proportional values were obtained in relation to the addition of tannia starch, ranging from 0.48 to 0.33 N×m; the higher the C2 value, the higher the water content retained by the starch granules. C2 values often range from 0.3 to 0.6 N×m depending on the type of starch and protein content (Dubat, 2013). The values in the study fall within this range, but a decrease in C2 with increased tannia starch indicates less swelling power, likely due to reduced gluten content. The values obtained in parameter C3, which correspond to starch gelatinization, ranged from 1.46 to 1.25 N×m, with the first value belonging to the control sample. These results indicate that higher values correspond to masses composed of high-quality starches, with a tendency to achieve good volume during the production. C3 values for wheat dough typically range from 1.3 to 1.8 N×m (Koksel *et al.*, 2009). The study values are slightly lower but still indicate good gelatinization, with higher values for formulations with less tannia starch, signifying better starch quality and volume during product production; the higher the value, the higher the increase in the viscosity of the dough under heat. F1 showed starch gelatinization similar to F0, whereas the rest were significantly different (P<0.05). The results obtained for C4, representing amylase activity, ranged from 0.89 to 0.70. F0 and F1 showed similar amylase activity, while the remaining treatments were significantly different (P<0.05). Values in the range of 0.7 to 1.0 N×m are common for doughs with moderate to low amylase activity (Arcangelis *et al.*, 2020). The study results aligned well, showing lower amylase activity, which increases water absorption and makes the dough easier to handle. Lastly, C5, corresponding to retrogradation, is directly related to the values of C4. The results should not vary much between them, as this will determine the shelf life for the product (Coțovanu *et al.*, 2020). The values observed in C5 ranged from 1.19 to 0.98 N×m. Typical C5 values range from 1.0 to 1.3 N×m (Coțovanu *et al.,* 2020). The values in the study are consistent with these ranges, with higher tannia starch content indicating better retrogradation characteristics and potentially longer shelf life. Comparing the results for C5 with the parameters of C4 indicate that the more tannia starch is added in pasta production, the longer the shelf life of the product. Table 7 presents the results obtained from Mixolab analysis.

Quality indices of the blends

The water absorption index (WAI) remained unchanged across formulations with substitution percentages of 5%, 10%, and 15%, as well as the control sample. However, when substituting 20% of tannia starch with wheat starch, WAI decreased. This decrease is attributed to the reduction in protein content in the mixture with higher substitution levels. Wheat doughs typically have WAIs between 8.0 and 9.0 (Ma *et al.*, 2021). The study shows a slight decrease with higher tannia starch, indicating that lower protein content affects water absorption. The kneading index (KI) values show differences starting from a 10% substitution compared to the control sample, which consists of 100% wheat starch. These differences arise due to the gluten percentage present in the various mixtures; higher substitution leads to lower protein content, resulting in a dough that lacks proper stretching during fermentation (Sandoval *et al.*, 2012). The KI is an indicator of the resistance of dough to this stretching process. As the water and flour combination undergoes this process, its texture changes, becoming soft, viscoelastic, and easily extendable. KI values vary based on gluten content, typically between 3.0 to 6.0 for doughs (Marchenkov *et al.*, 2021). The study values decrease significantly with higher tannia starch, reflecting reduced dough resistance and stretching ability due to lower gluten. The gluten strength index (GSI) results were inversely proportional to the percentages of tannia starch substitution. This parameter relates to the quality of proteins in the dough rather than their quantity. Glutenins and gliadins, found in wheat, contribute to forming a good dough by providing elasticity, strength, extensibility, and viscosity (Quezada Correa *et al.*, 2019). GSI for good quality wheat doughs are usually around 4.0 to 8.0 (Marchenkov *et al.*, 2021). The decrease in the study indicates a loss in protein quality and gluten strength with higher tannia starch content. The starch viscosity index (SVI) and amylase resistance index (ARI) analyses yielded consistent results across all formulations, indicating no significant differences. This highlights the importance of considering the amylase percentages in the mixtures to maintain food properties; higher amylase content results in a very soft paste, making it difficult to handle (Dubat & Rosell, 2013). The consistent values indicate that amylase activity is balanced across formulations, maintaining similar starch viscosity and resistance to enzyme activity. The retrogradation index (RI) is associated with the shelf life of the product. Results for this parameter were consistent across all mixtures, including the control sample. Lower retrogradation values correspond to longer product shelf life, and the consistent values suggest that despite variations in starch content, the retrogradation properties remain stable, indicating good shelf life potential (Bárcenas & Rosell, 2007). During this stage, starch molecules that have been gelatinized reassociate to form a crystalline double-helix structure, transferring some of the water and reducing elasticity due to instability (De Arcangelis *et al.*, 2020). Table 8 features the quality indices in the doughs analyzed.

TABLE 7. Results of Mixolab analysis.

Formulations	C1 (N×m)	C2 (N×m)	C3 (N×m)	C4 (N×m)	C5 (N×m)
F0	1.12 ± 0.03^{a}	0.48 ± 0.02^{a}	1.42 ± 0.005^{a}	0.89 ± 0.006^{a}	1.19 ± 0.04^{a}
F1	1.10 ± 0.05^{b}	0.45 ± 0.04^{a}	1.43 ± 0.003^{a}	0.86 ± 0.002^{a}	1.12 ± 0.06^{b}
F2	1.08 ± 0.01^{a}	0.42 ± 0.01^{a}	1.37 ± 0.02^{b}	0.81 ± 0.004^{b}	1.10 ± 0.01^{b}
F3	1.06 ± 0.03^{b}	0.37 ± 0.06^{b}	1.27 ± 0.04^{c}	0.71 ± 0.001^{c}	1.02 ± 0.02^{c}
F4	1.05 ± 0.07^{ab}	0.33 ± 0.04^{b}	1.25 ± 0.06^{c}	0.70 ± 0.007^{c}	0.98 ± 0.02^{a}

Development of dough (C1), protein quality (C2), starch gelatinization (C3), amylase activity (C4), and retrogradation (C5). Values reported are the mean of 3 replicates ± standard deviation. Values containing a, b, c differ significantly according to the Tukey's test (P<0.05).

TABLE 8. Quality indices in doughs analyzed.

Parameter	F0	F1	F2	F3	F4
WAI	8.0 ± 0.03^{a}	8.0 ± 0.01^{a}	8.0 ± 0.06^{a}	8.0 ± 0.05^{a}	7.0 ± 0.38^{a}
KI	7.0 ± 1.02^{a}	6.0 ± 0.92^{a}	4.5 ± 0.43^{b}	3.0 ± 0.67^{b}	2.0 ± 0.26^{c}
GSI	4.0 ± 1.13^{a}	3.0 ± 1.02^{a}	1.5 ± 0.97^{b}	2.0 ± 0.42^{b}	0.5 ± 0.89^{c}
SVI	2.0 ± 0.04^{a}	2.0 ± 0.83^{a}	2.0 ± 0.27^{a}	2.0 ± 0.09^{a}	2.0 ± 0.05^{a}
ARI	1.0 ± 1.08^{a}	2.0 ± 0.76^{a}	2.0 ± 0.93^{a}	2.0 ± 1.24^{a}	2.0 ± 1.14^{a}
RI	2.0 ± 0.87^{a}	2.0 ± 0.36^{a}	2.0 ± 0.68^{a}	2.0 ± 0.08^{a}	2.0 ± 0.53^{a}

WAI: water absorption index; KI: kneading index; GSI: gluten strength index; SVI: starch viscosity index; ARI: amylase resistance index; RI: retrogradation index. Values reported are the mean of 3 replicates ± standard deviation. Values containing a, b, and c differ significantly according to the Tukey's test (P<0.05).

Conclusions

This study explored the feasibility of using tannia starch as a partial substitute for wheat flour in pasta formulations. The results demonstrated that substituting up to 20% of the wheat flour with tannia starch yielded pasta with acceptable cooking properties and quality indices. Rheological analysis revealed significant alterations in hydration, moisture, and stability as the proportion of tannia starch increased, highlighting the need for careful formulation adjustments. Despite these challenges, the potential of tannia starch to enhance the technological advancements of pasta warrants further exploration and optimization. Based on these findings, pasta manufacturers could consider incorporating tannia starch into their formulations to improve technological properties of their products. Furthermore, optimizing the formulation to maintain desirable cooking properties and pasta quality could open new market opportunities for innovative pasta products.

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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

HMGV and FPCM set research goals. SECM and FPCM annotated and maintained scrub data. HMGV, MLPE, SECM, and FPCM analyzed data. HMGV, MLPE, and SECM conducted experiments and developed methodology. FPCM managed research planning. MLPE, SECM, and FPCM provided resources. MLPE and FPCM led planning and execution. SECM and FPCM ensured reproducibility. All authors prepared and presented work. HMGV and FPCM drafted, revised, and translated the manuscript. All authors revised the final version of the manuscript.

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SCIENTIFIC NOTE

Use of dynamic simulation and Forrester diagrams to describe the growth of lettuce (*Lactuca sativa* L.) under field conditions

Uso de simulación dinámica y diagramas de Forrester para describir el crecimiento de lechuga (*Lactuca sativa* L.) en condiciones de campo

Alexis Valery[1], Rossana Timaure[1], Aquiles Enrique Darghan Contreras[2*], and Nair José González Sotomayor[2]

ABSTRACT

The use of computational tools to describe some processes of crop growth has evolved in recent decades and remains an area of active research, where more and more applications are incorporated with the integration of a greater number of mathematical tools, statistics, and computational calculation efficiency, simplifying the tasks of modeling and visualizing the components of the system used. The present research proposes a dynamic growth model for lettuce cultivation using Forrester diagrams to evaluate different scenarios involving five growth functions and five lettuce cultivars in field conditions of the Bailadores region (Venezuelan Andes, 2550 m a.s.l.). The lettuce variety Coastal Star achieved the greatest accumulation of dry matter used as a response in each model. The logistics of growth function was properly adjusted to the experimental data compared to the other models. The proposed diagram model can be used as a basis for the construction of more complex models that incorporate other physiological variables of the crop and the growth environment.

Key words: state variables, maximum system capacity, Vensim.

RESUMEN

El uso de herramientas computacionales para describir algunos procesos del crecimiento de los cultivos ha evolucionado en las últimas décadas y sigue siendo un área de investigación activa, donde se incorporan cada vez más aplicaciones con la integración de una mayor cantidad de herramientas matemáticas, estadísticas y de eficiencia de cálculo computacional, simplificando las tareas de modelado y las de visualización de los componentes del sistema utilizado. La presente investigación propone para el cultivo de lechuga un modelo de crecimiento dinámico utilizando diagramas de Forrester, para evaluar diferentes escenarios involucrando cinco funciones de crecimiento y cinco variedades de lechuga en condiciones de campo de la región de Bailadores (Andes venezolanos, 2550 m s.n.m). La variedad de lechuga Coastal Star fue la que logró la mayor acumulación de materia seca (usada como respuesta en cada modelo). Se encontró que la función de crecimiento logístico ajustó adecuadamente los datos experimentales en comparación con los otros modelos. El modelo diagramado propuesto puede ser usado como base para la construcción de modelos más complejos que incorporen otras variables fisiológicas y el ambiente del cultivo.

Palabras clave: variables de estado, máxima capacidad del sistema, Vensim.

Introduction

Agricultural systems are complex and exhibit a multitude of input and output variables that may exhibit complex relationships with patterns not easily described (Namirembe *et al*., 2020). The advancement of computer systems is a tool for the management of information derived from crop fields. When this information is processed using mathematical or statistical models together with computational tools, the results of the adjustment of suitable models can guide decision-making in the management of crops in favor of optimal resource use and increased food production. At present, there are countless models available in the scientific literature that address specific aspects of agricultural production, highlighting water management, nutrients, pests, and diseases (Negus *et al*., 2024). Crop development models can be divided into two types: mechanistic and empirical (El Jarroudi *et al*., 2014). Mechanistic models mathematically describe our knowledge and hypotheses about the physiological processes of the crop and their interactions with their development in the environment (Landsberg & Sands, 2010). Empirical models apply statistical models of crop development, describing the cumulative processes based on the involved parameters in the functional form that is used. These models are generally adapted to agricultural field data (Junk *et*

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al., 2016) and their primary application is the prediction of the development of growth under certain regional and climatic conditions for some plant varieties. The suitability of these models for a particular farmer will depend on his individual needs (Kundathil *et al*., 2023). The dynamic nature of crop development related to their phenological stages has allowed the use of dynamic simulation models for more than a decade to describe and evaluate plant growth (Hernández *et al.,* 2009) and to explain their ecophysiological relationships and their changes due to adverse factors (Sommer, 2023). However, defining growth is complex in nature, as it is the result of a multitude of interrelated processes, among which division, elongation, and specialization of cells stand out as well as several external factors, such as plant species, soil, moisture, light, nutrient availability, agricultural management, etc. (Hilty *et al*., 2021; Logachev & Goncharov, 2024; Rauff & Bello, 2015; Van Keulen, 2013).

There are several methods for analyzing plant growth; the functional approach is one of the most important. This may generate some confusion due to the existence of a specific field of mathematics that deals with functional analysis and can generate confusion from a semantic viewpoint, since the growth approach does not use the tools of functional analysis at all. Therefore, the phrase "fit functional forms" is recommended (Kantorovich & Akilov, 2016). In this approach, measurements are taken at discrete time intervals and include mathematical functions that are used to describe the growth of plants or their structures (Hunt, 2016), using all the data collected during the sampling process that covers morphometric, physiological, soil and climatic variables to identify the functional form that could properly fit the data (Alvar-Beltrán *et al*., 2023).

It is appropriate to use Forrester diagrams to represent the dynamic process of crop modeling (Haefner, 2005; Logachev & Goncharov, 2024) since these are an invaluable tool for understanding and analyzing intricate systems, particularly those exhibiting nonlinear dynamics. Forrester diagrams represent a system in a simple way using direct symbology that allows the connection of a series of processes of the systemin an interactive and visual way that facilitates an understanding of the interactions of the different system compartments.

In this type of diagram, eight types of symbols are employed. The first of these corresponds to the state variable (compartments) where the matter or energy of the system is stored, and it is represented by a rectangle. The second symbol represents the flow variables that determine the movements of matter and/or energy between the state variables or another external system. This is represented by a continuous double-line arrow with a key symbol in the middle that allows for the flow velocity to be controlled. The matter and/or energy can be transferred to another level of study or to an external system that is referred to as a source or sink. This is represented by a cloud figure that is the third symbol. The fourth symbol represents the auxiliary and exogenous variables that are constant and provide information that facilitate the interpretation of the equations used in the flow variables, and this is represented by a circle. The fifth symbol represents the information channel between exogenous or auxiliary variables, and it is represented by a continuous arrow (Logachev & Goncharov, 2024).

The importance of model development lies primarily in its ability to describe experimental or observational data over time in order to facilitate prediction or its use as a sub-model in more complex models. The selection of models depends on their adjustment; and, in the case of dynamic models, it is necessary to specify the appropriate differential equation. This allows a consideration whether the parameters of the equation are of biological importance (not always directly interpretable) and finally evaluate the metrics associated with differences between observed and estimated values of the model (Hamner *et al*., 2018). In the case of simple models, the differential equation is typically described in terms of the first derivative of the growth function that by means of numerical integration allows an estimate of the parameters of the model of the indicators of growth of plant organs as well as of the whole plant. Among the functional forms of differential equations there is the logistic model of Blumberg, Von Bertalanffy, Richards and Gompertz; however, other models are known to characterize the development of the crops that could be involved (Fernández-Chuairey *et al*., 2019; Reyes-Medina *et al*., 2019; Rodríguez-Perez, 2013).

Models of crop growth have been proposed worldwide and include the cultivation of tomato (*Solanum lycopersicum*) (Luo *et al*., 2020), corn (*Zea mays*) (Attia *et al*., 2021), potato (*Solanum tuberosum*) (Divya *et al*., 2021), lettuce (*Lactuca sativa*), etc. The models are applicable to both field and greenhouse conditions for many crops; however, in the case of lettuce, a leafy crop that has food and medicinal uses leading to an increase in its consumption worldwide, little has been developed in dynamic modeling of field data in tropical conditions (Das & Bhattacharjee, 2020; Díaz-Pérez *et al*., 2024; Lipton & Ryder, 2021). Most simulation models in this field are designed to simulate cultivation under controlled conditions in greenhouses. One such model is the NICOLET model (Nitrate Control in Lettuce) that is well-known and widely used (Chang *et al*., 2021; Juárez-Maldonado *et al*., 2010; Jung *et al*., 2018; Li *et al*., 2022). Other models of horticultural crops include the DSSAT and SUBSTOR models (Jones *et al*., 2003; Rodríguez-González *et al*., 2020; Sarmiento & Bowen, 2002) that have been used in South America with high frequency in the dynamic modeling of potato cultivation.

Because of the aspects described above, this research proposes a model of lettuce (*Lactuca sativa*) cultivation under field conditions using Forrester diagrams for its construction with the separate incorporation of different growth functions for the aerial and root parts of the plant. This represents a preliminary stage in the creation of a more comprehensive model that will be applicable to tropical conditions.

Materials and methods

Location

The research was carried out in the Agricultural and Environmental Biotechnology Research Group (GIBAA) at the National Experimental University of Táchira, Venezuela. The site is located at an elevation of 2,550 m in the region of Bailadores in the Venezuelan Andes, with air temperatures ranging from 9°C to 16°C and an annual rainfall between 1500 and 2000 mm year^{-1}.

Plant material

Five varieties of lettuce (*Lactuca sativa*): V1 - Green Towers, V2 – Thoreau, V3 - Coastal Star, V4 – Arroyo, and V5 – Altura were used in this study. The total biomass (shoots and roots) of 18 plants per variety was measured weekly for 8 weeks. The collected plant material was placed in prelabeled paper bags and then dried at 65°C in a forced-air oven until a constant mass was achieved. The dry matter (g m^{-2}) was determined and expressed as a result of having a field density of 10 plants per m^2.

Implemented model

Simulation and calibration were performed using Vensim software (Professional license version 5.11a). The model was configured with a single compartment or state variable to represent the dry weight of the response variable for each part of the plant (shoot Hs or roots Rs) (Fig. 1). Each compartment has two flows; in the case of leaves (Fig. 1A), the input flow represents gross primary production, and the output flow represents translocation of matter from the aerial parts to the roots. For the roots (Fig. 1B), the input flow is the material coming from the aerial part, while the output flow corresponds to the root exudates.

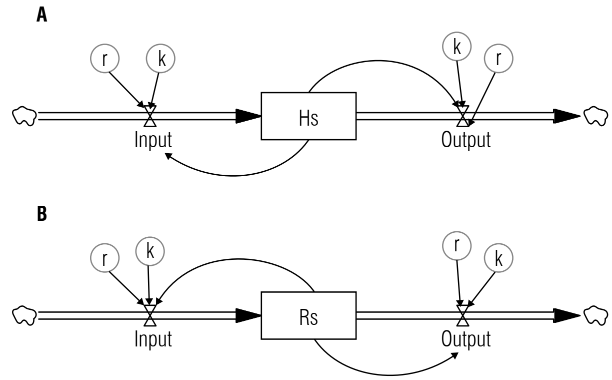

FIGURE 1. Forrester diagram of the proposed model for vegetative growth of the shoot system (A) and the root system (B) of lettuce.

After identifying the state variables and flows affecting each compartment (Fig. 1), five growth functions (Tab. 1) were selected to estimate dry matter accumulation in each compartment over time. For this, the first derivative was used, depending on the flow rates of each compartment. These functions have similarities in that they present the growth rate (r) and a maximum system capacity (k) as auxiliary variables, while the functions of Blumberg and Richards present other parameters related to the turning point where the growth rate reaches its maximum expression (Tsoularis & Wallace, 2002). All functions require as input information the size of the compartment (N) over time that is provided by the information channel (continuous arrows) as well as the auxiliary variables (Figs. 1 and 2).

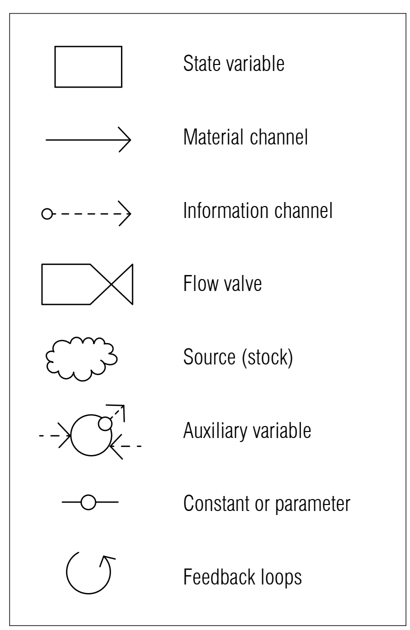

FIGURE 2. Symbols of Forrester diagrams and their interpretations.

TABLE 1. Growth functions and their first applied derivatives (Rodriguez, 2013; Szabelska *et al*., 2010).

Model	Functional form (N(t))	First derivative (*dN/dt*)
Logistic	$\frac{k}{1+\left[\left(\frac{k}{N_0}-1\right)e^{-rt}\right]}$	$rN\left(1-\frac{N}{k}\right)$
Blumberg	$\int_{N_0/k}^{N(t)/k} x^{-\alpha}(1-x)^{-\gamma}dx = rk^{\alpha-1}t$	$rN^{\alpha}\left(1-\frac{N}{k}\right)^{\gamma}$
Von Bertalanffy	$k\left[1+\left[1-\left(\frac{N_0}{k}\right)^{1/3}\right]e^{-\frac{1}{3}rk^{1/3}t}\right]^3$	$rN^{2/3}\left[1-\left(\frac{N}{k}\right)^{1/3}\right]$
Richards	$k\left[1-e^{-\beta rt}\left[1-\left(\frac{N_0}{k}\right)^{-\beta}\right]\right]^{\frac{1}{\beta}}$	$rN\left[1-\left(\frac{N}{k}\right)^{\beta}\right]$
Gompertz	$kexp\left\{\left[ln\left(\frac{N_0}{k}\right)\right]^{1-\gamma}+r^{\prime}(-1)^{\gamma}(1-\gamma)t\right\}^{\frac{1}{1-\gamma}}$	$rN\left(ln\left(\frac{N}{k}\right)\right)$

Model fitting

Calibration of the estimated parameters for each growth function used was performed using the calibration module of the Vensim software that provided the simulated values. Given the bivariate nature of the response (dry weight of shoots and root parts), the adjustment tools applied consisted of statistics based on the criterion of approximation of the distance between a set of data points. For this purpose, Euclidean, Manhattan, and Mahalanobis distances were determined. These distances are assessed based on the principle that a lower value indicates a better fit (Haefner, 2005; Hamner *et al*., 2018). The distances were calculated using R (R Core Team, 2023).

Results and discussion

The experimental data showed that varieties V2, V3, and V4 were the ones that achieved the highest weight at the end of the trial, with 460 g m^{-2}, 459 g m^{-2}, and 445 g m^{-2} compared to varieties V1 (362 g m^{-2}) and V5 (411 g m^{-2}). When adding the root dry weights to the shoot dry weights (V1: 107 g m^{-2}; V2: 81 g m^{-2}; V3: 150 g m^{-2}; V4: 121 g m^{-2}; V5: 151 g m^{-2}), V3 obtained the highest total dry weight compared to the others. It should be noted that, in both leaves and roots, an increase in dry matter accumulation was observed from day 40. The results were considerably higher than those reported by Ortiz Mackinson *et al*. (2022), who obtained 150 g m^{-2} dry weight in their optimal treatment for the aerial part, influenced by a multitude of factors, including variety and management, etc.

Model

Plant growth is usually described in three stages (Hilty *et al.*, 2021), starting with germination, followed by a phase of rapid growth known as the logarithmic stage, and finally, a senescence stage. In this research the evaluated stage corresponded to the logarithmic phase, making the proposed model a good descriptor of the process when using the logistic function (Tab. 2). The logistic model was properly adjusted to the experimental data compared to the other functions tested for both variables (Figs. 3 and 4). These results differed from Mathieu *et al*. (2006), who obtains a better description of the experimental data using a second order exponential polynomial.

Computational tools in the modeling processes allowed for the evaluation of different options to describe a process and select equations that provided a good fit during model development (Antle *et al*., 2016). These tools are leading researchers to formulate various hypotheses about growth-related processes (Berger *et al*., 2019; Muller & Martre, 2019). Constantly, strategies are being sought for the selection of materials with specific good performance, using models that describe their development (Zohary, 1991). In this regard, the proposed model can lead to the development of management plans with various aspects such as fertilization timing and planting times (Chiesa *et al*., 2001; Rauff & Bello, 2015), aiming to provide the crop with the necessary resources for its growth. In this case study, growth is most pronounced in both the aerial and root parts after 40 d (Figs. 3 and 4). This approach would allow the determination of crop growth stages that could be used to facilitate a more comprehensive understanding of lettuce growth behaviour (Li *et al*., 2022). This, in turn, would allow the identification of the optimal timing of fertilization that, in turn, would improve growth and yield.

Another case where the logistic model is appropriate was that of Carini *et al*. (2020) who evaluate four different lettuce cultivars. Similarly, Tan *et al*. (2022) demonstrate that

TABLE 2. Distances obtained for each growth function in lettuce.

Distance	Logistic	Richards	Von Bertalanffy	Gompertz	Blumberg
Manhattan	4.72	6.87	11.11	25.28	18.56
Euclidean	1.01	1.54	2.62	6.14	3.38
Mahalanobis	0.09	0.44	0.51	0.91	0.92

the NICOLET B3 model is capable of accurately predicting the fresh and dry matter production of lettuce. Conversely, Li *et al*. (2022) utilise three models to characterise the growth of lettuce crops. Their findings indicate that the Verhulst growth function accurately reflects the observed growth characteristics. However, the logistic function demonstrates a superior fit to the sigmoidal curve derived from the height measurements, suggesting the potential for evaluating different functions according to the specific characteristics of the crop under evaluation. It is important to note that the evaluations presented above correspond to the growth of the crop under controlled greenhouse conditions.

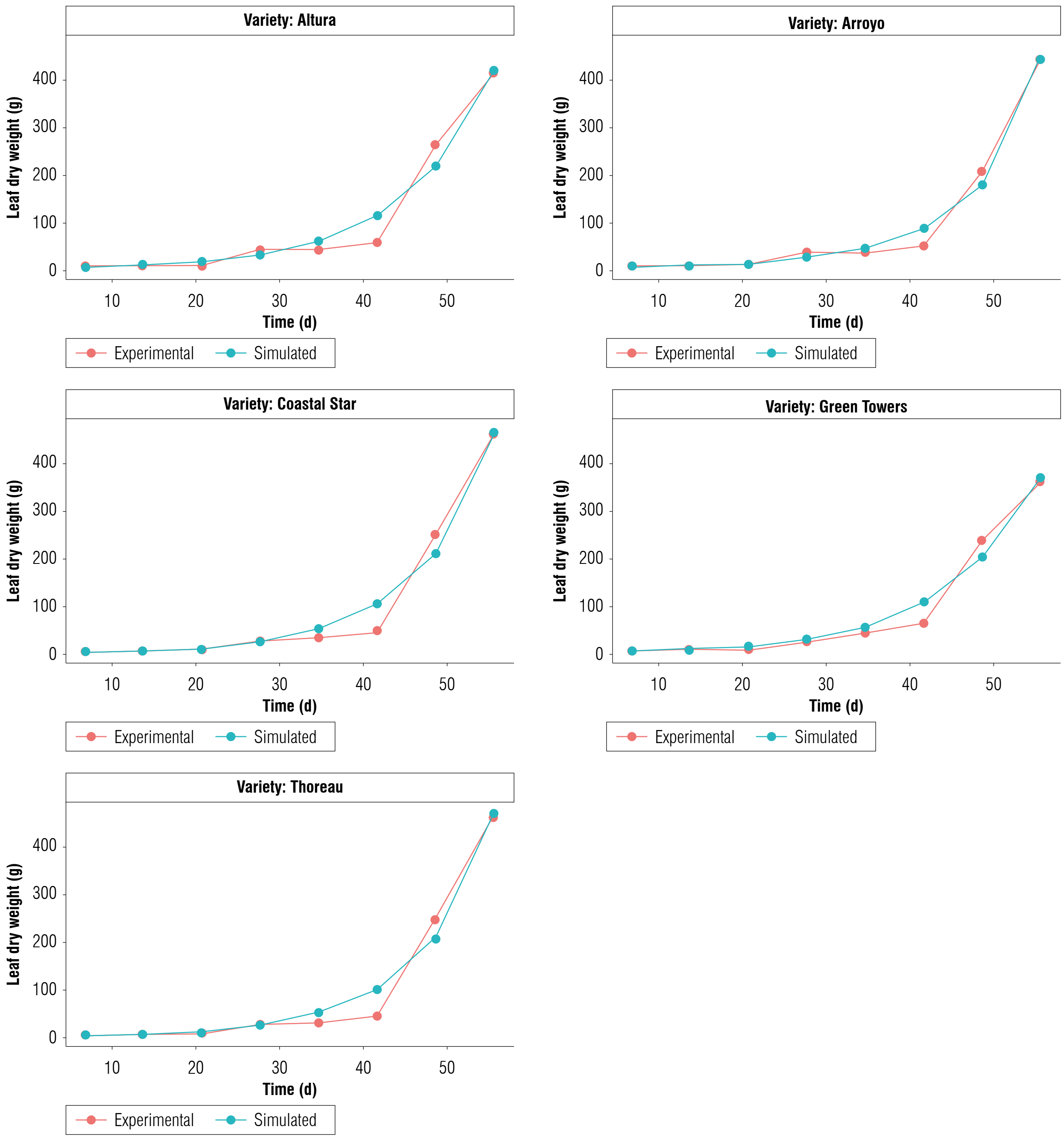

FIGURE 3. Experimental and simulated values for leaf dry weight of lettuce using the logistic growth function.

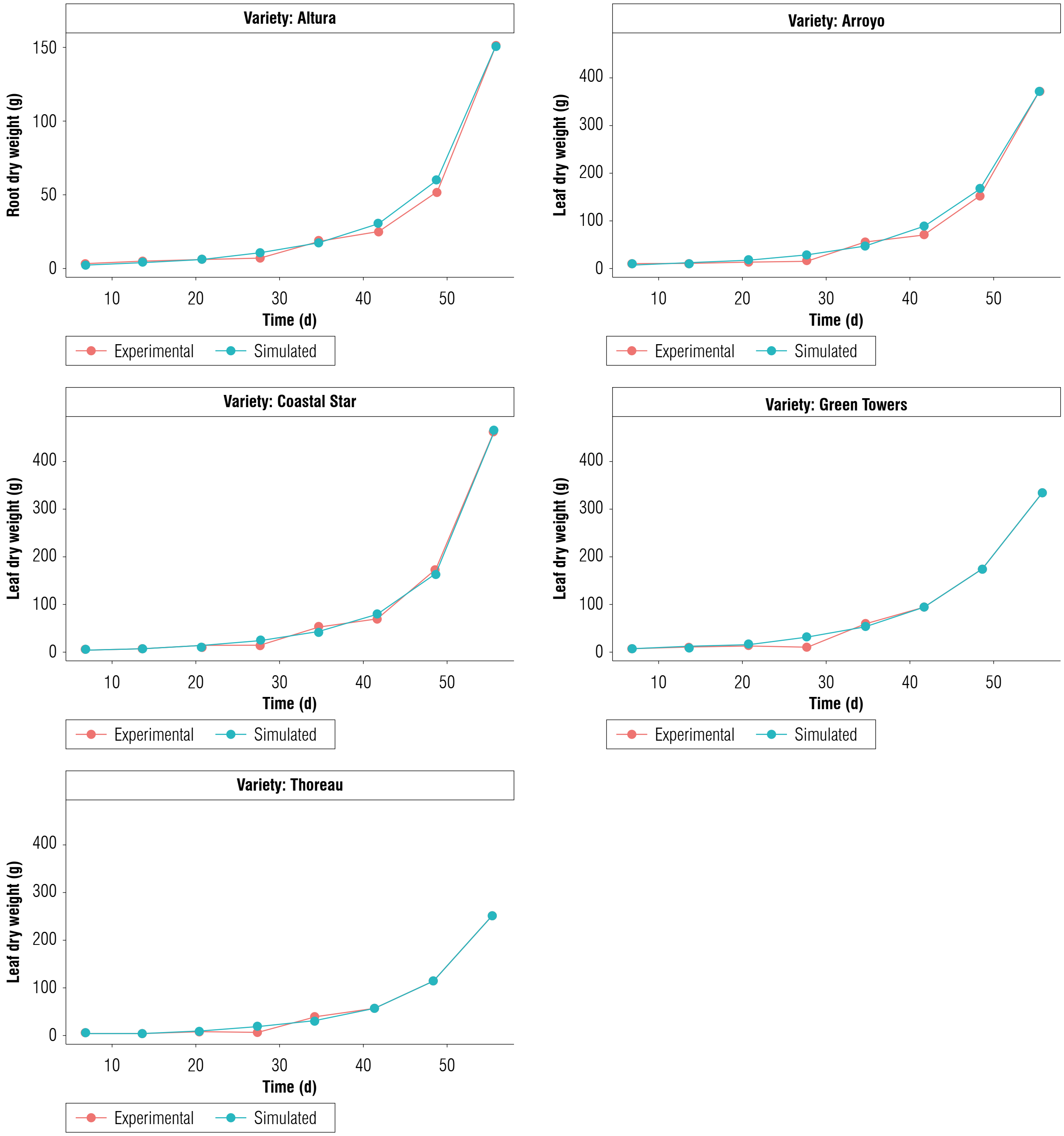

FIGURE 4. Experimental and simulated values for root dry weight of lettuce using the logistic growth function.

The proposed model allowed the comparison of different growth functions; and, although it may be preliminary, it offers scalability, providing the opportunity to evaluate various options and incorporate different conditions as well as other functions, allowing increasingly complex models such as the MOMOS model to evolve, beginning with the description of soil carbon (Pansu *et al*., 2004; Pansu *et al*., 2010), involving both soil carbon and soil nitrogen (Pansu *et al*., 2014).

Conclusions

Dynamic simulation models are a tool for studying the different stages of plant growth by selecting equations and representing biological processes in a more realistic way. In this research, discriminant analysis showed that the logistic growth function had the best goodness of fit for simulating the case study that would allow the identification of growth stages and improve the use of resources and management.

Constructing these types of models with data from high-altitude tropical climates enables the evaluation of agricultural production systems and research into the incorporation of new tools to better utilize environmental resources for food production.

In the teaching of dynamic crop modeling, Forrester diagrams certainly facilitate the interpretation of the components considered in the modeling process, whether this is preliminary in nature or how a sub-model has been used in a more complex model.

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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

AV and RT designed the experiments and conducted the field and laboratory experiments. AEDC carried out the statistical analysis of the study. NG and AEDC interpreted the results. AV and RT wrote and translated the initial draft. All authors revised the final version of the manuscript.

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SCIENTIFIC NOTE

Seed germination and seedling emergence of *Gundelia tournefortii* L. in response to some environmental factors

Germinación de semillas y emergencia de plántulas de *Gundelia tournefortii* L. en respuesta a algunos factores ambientales

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ABSTRACT

Gundelia tournefortii has long been considered a valuable medicinal and edible plant in the Mediterranean areas. Seeds are the main means through which *G. tournefortii* can propagate in space and in time. The aim of this experiment was to evaluate the impact of environmental factors on seed germination of this wild medical and food plant. In this study, we investigated the germination and emergence responses of seeds of *G. tournefortii* collected from Kamyaran and Sonqor in western Iran to the environmental factors of light, temperature, salt stress, water potential, and sowing depth. The results showed that the optimal temperature for germination was 20°C, with a maximum germination percentage of about 70% for both populations. In general, the two populations responded to the tested factor(s) similarly. Light was not required for germination of *G. tournefortii.* The species was tolerant to water stress (germinating more than 50% under water potential up to -1.0 MPa), while sensitive to salt stress. More than 50% of the seedlings of *G. tournefortii* were able to emerge from depths more than 12 cm. According to the results, rain-fed fields located in the western parts of Iran are suitable for the planting of *G. tournefortii*.

Key words: water potential, salt stress, sowing depth, seedling emergence.

RESUMEN

Gundelia tournefortii es considerada como una valiosa planta medicinal y de alimento en las zonas mediterráneas. Las semillas son el principal medio por el cual *G. tournefortii* puede garantizar su permanencia en el espacio y en el tiempo. El objetivo de este estudio fue evaluar el impacto de los factores ambientales en la germinación de las semillas de esta planta medicinal silvestre. En este estudio, investigamos las respuestas de germinación y emergencia de semillas de *G. tournefortii* recolectadas en Kamyaran y Sonqor, ubicadas en el oeste de Irán, a los factores ambientales de temperatura, luz, estrés salino, potencial osmótico y profundidad de siembra. Los resultados mostraron una temperatura óptima de 20°C con un porcentaje máximo de germinación de aproximadamente 70% para ambas poblaciones. En general, ambas poblaciones respondieron de manera similar a los factores evaluados. La luz no se requirió para la germinación de *G. tournefortii*. Las plantas fueron tolerantes al estrés hídrico (germinando más del 50% bajo potencial hídrico hasta -1,0 MPa), mientras que fueron sensibles al estrés salino. Más del 50% de las plántulas de *G. tournefortii* pudieron emerger de profundidades de enterramiento de más de 12 cm. Acorde a los resultados, los campos de secano ubicados en la parte occidental de Irán son favorables para la plantación de *G. tournefortii*.

Palabras clave: potencial hídrico, estrés salino, profundidad de siembra, emergencia de plántulas.

Introduction

Gundelia tournefortii L., native to western Asia (Lev-Yadun & Abbo, 1999), is a well-known edible and important medicinal wild plant from the Asteraceae family (Vitek *et al*., 2017). It is a native plant that grows naturally in some regions of Iran, particularly in the rangelands of the western part (Karimzadeh *et al*., 2023).

This wild species has been used to treat a wide range of diseases (Abu-Lafi *et al*., 2019; Han *et al*., 2022; Keskin *et al*., 2022). The compounds found in *G tournefortii* have several pharmacological effects (Dastan & Yousefzadi, 2016; Hani *et al*., 2024). Furthermore, the flowers, leaves, seeds and stems of *G. tournefortii* are used as food sources (Konak *et al*., 2017; Saraç *et al*., 2019).

Nevertheless, multiplication of this medicinal plant *in vitro* and *in vivo* is challenging as its seeds are dormant and need specific conditions to break dormancy and to germinate (Mattana *et al*., 2022). Seed germination is one of the most important steps in a plant's life cycle, and the transition from seed to seedling is a crucial event (Rajjou *et al*., 2012). Temperature, light, moisture, salinity, and soil

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burial depth are the main environmental factors affecting seed germination and seedling emergence (Mattana *et al*., 2009; Nosratti *et al*., 2017).

Globally, seed germination and early growth of seedlings are negatively affected by environmental stress factors, especially drought and salinity (Llanes *et al*., 2016). These stress factors inhibit plant growth and reproductive development, leading to substantial reductions in crop production (Nosratti *et al*., 2023). Among environmental factors, temperature has a major role in determining the periodicity of seed germination and the distribution of the species (Guan *et al*., 2009).

Seed culture is a cost-effective substitute for commercial propagation methods and is now extensively used for the commercial propagation of a wide range of plant species, *e.g.*, medicinal plants (Chen *et al*., 2016). Nevertheless, the propagation of medicinal plants by seeds could be affected by numerous abiotic factors such as temperature, light, salt stress, and pH (Elhindi *et al*., 2016; Hesami *et al*., 2018; Murch *et al*.,2004).

There is little research on the effect of environmental factors on *G. tournefortii* seed germination. A better understanding of the effect of these factors on seed germination of *G. tournefortii* is required to create a better environmental situation for its cultivation as well as its propagation under controlled conditions. Hence, the aim of this research was to study the impact of temperature, light, burial depth, moisture, and salinity on the germination and seedling emergence of *G. tournefortii*.

Materials and methods

Site and seed description

Mature secondary capitula, hereafter "capitula", of two populations of *G. tournefortii* were harvested from nearly 600 plants growing in pastures in Sonqor County (34°46'58" N, 47°35'54" E), Kermanshah province, Iran, and Kamyaran County (34°47'43" N, 46°56'12" E), Kurdistan province, Iran, during June 2022 (Tab. 1). Capitula were considered mature when the plants of *G. tournefortii* had turned yellow and completely senesced. All tests in the present research were conducted using the seeds of both populations of *G. tournefortii* (Sonqor and Kamyaran populations).

Because of the low germination percentage observed in preliminary tests (lower than 20%), the fruit complex (*i.e.*, the secondary capitulum or disseminule) was manually removed, leaving only the seed coat, which is a thin, papery layer surrounding the embryo (Hind, 2013). For both populations of *G. tournefortii*, the 1,000-seed weight and 1,000-capitula weight were about 110 and 240 g, respectively.

TABLE 1. Precipitation and average daily air temperature of the study site during the growth season of *Gundelia tournefortii* in Sonqor and Kamyaran (Iran) in 2022.

	Precipitation (mm)		Temperature (ºC)	
Month	**Sonqor**	**Kamyaran**	**Sonqor**	**Kamyaran**
March	120	93.5	6.0	10.5
April	89.1	61.1	11.2	13.3
May	63.5	93.3	17.5	21.3
June	32.2	41.0	21.5	25.0

Germination test procedure

Five replicates of 20 seeds of *G. tournefortii* were put in 9 cm diameter Petri dishes containing two sheets of filter paper (Whatman No. 1, Maidstone, UK) moistened with 9 ml of distilled water or treatment solution. All Petri dishes were firmly wrapped with clear parafilm to reduce evaporation and then placed in a germination chamber (JAL TEB, model 200L) under a constant temperature of 20°C. The recording of germinated seeds was done when the radicle appeared (2 to 3 mm), and the measurement of this variable continued daily for two weeks.

Effect of light and temperature on germination

Constant temperature treatments during seed germination consisted of 10, 15, 20, 25, 30, and 35°C under light/dark or continuous darkness. To apply the light treatments, Petri dishes with *G. tournefortii* seeds were sealed with transparent polyethylene bags, while the dark treatments were wrapped in aluminum foil. The light intensity in the germination chamber was 150 µmol m^{-1} s^{-1} using fluorescent lamps.

Effect of salinity on germination

Seeds of *G. tournefortii* were exposed to six levels of salt stress using solutions containing 0 (control), 4, 8, 16, 32, 64, 128, and 256 mM of sodium chloride (NaCl) (Payamani *et al*., 2018). Treated seeds in Petri dishes were germinated under conditions described in the procedure for the germination test.

Effect of water stress on germination

The effect of different water potential on seed germination of *G. tournefortii* was assessed by incubating seeds in polyethylene glycol (6000) solutions with osmotic potentials of 0.0 (distilled water), –0.1, –0.2, –0.4, –0.6, –0.8, –1.0, or –1.2

MPa. The solutions were prepared based on the method described by Michel (1983). Seeds of both populations were then incubated in the light/temperature conditions as described in the germination test procedure.

Effect of soil burial depth on seedling emergence

The influence of different burial depths of 0, 2, 4, 6, 8, 10, and 12 cm on the appearance of *G. tournefortii* seedlings was examined. Seeds were placed at the desired depth in pots (25 cm in diameter by 15 cm in height), which were filled with a soil mixture (24% sand, 33% silt, 37% clay, 0.57% of total organic matter, and a pH 7.0). For each depth, 20 seeds were distributed on the surface of the soil and then covered with the same soil mixture to reach the desired depth. All the pots were placed in the greenhouse at 25/18 °C with a 12-h photoperiod. Pots were watered as needed to maintain sufficient soil moisture. Emergence was recorded weekly for a month. Seedlings were considered emerged when two fully developed leaves appeared on the soil surface. Seedlings were carefully separated to avoid soil problems after weekly measurement.

Statistical analyses

All trials, including those for soil burial depth, salinity, water stress, light and temperature were conducted as a completely randomized design with factorial arrangement of treatments with five replicates. The Kolmogorov–Smirnov test using the statistical software SPSS showed that the data are normal. Data were analyzed by the ANOVA procedure in MSTAT-C, and the LSD (Least significant difference) mean comparison test was applied. Regression analysis was done on data achieved from NaCl and water stress sowing depth experiments using the SigmaPlot software (v. 14.0, SyStat Software, Point Richmond, CA, USA). Germination percentages obtained from various concentrations of salt or in water were fitted to the following functional three-parameter exponential model (Nosratti *et al.*, 2019):

$$G_{max}/[1+(X/\ X_{50})^{Grate}] \qquad (1)$$

where G is the total germination (%) at each NaCl or osmotic potential X, G_{max} is the maximum germination (%), X_{50} is the NaCl concentration or osmotic potential required for 50% reduction of the maximum germination, and G_{rate} is the slope.

The seedling emergence percentage obtained at different soil depths was fitted to the following two-parameter exponential decay model (Nosratti *et al.*, 2019):

$$E_{max}e^{(-Erate.x)} \qquad (2)$$

In this model, E is the final seedling emergence percentage at burial depth x, E_{max} indicates the maximum seedling emergence (%), and E_{rate} is the slope.

Results and discussion

Temperature and light

Seed germination of both populations of *G. tournefortii* was not affected by light. Germination percentage increased linearly with temperature up to an optimal temperature (20°C), after which the germination rate declined (Fig. 1). Maximum seed germination percentage was achieved when seeds were incubated at 20°C (69% for both Sonqor and Kamyaran populations) (Fig. 1). In both populations, germination declined further as temperature increased to 25°C. Negligible seed germination (20%) occurred at low temperature (about 20 to 24%).

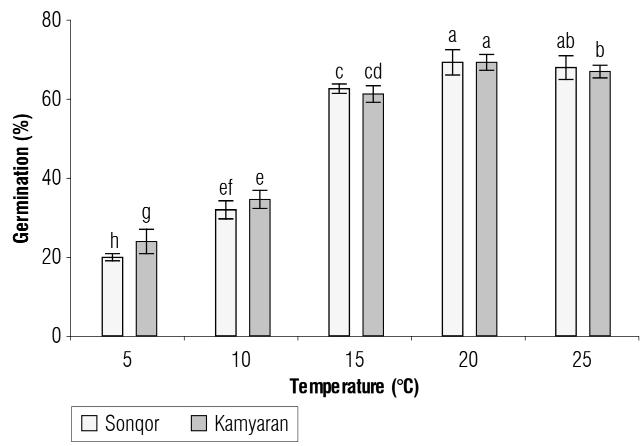

FIGURE 1. Seed germination of two populations (Sonqor and Kamyaran) of *Gundelia tournefortii* in response to temperature. Vertical bars represents the standard error of mean. Means with the same letter are not significantly different according to the LSD test.

The results of this study indicate that the germination of this medicinal species is limited during winter and summer in Iran. Seedlings of *G. tournefortii* typically emerge in the early spring when temperatures range between 15 and 20°C in western parts of Iran. Similar to our results, Mattana *et al.* (2022) concluded that the best temperature for seed germination of *G. tournefortii* collected from various areas of the Mediterranean was 15°C. Hence, it is recommended to incubate seeds of *G. tournefortii* at 15 to 20°C temperature range for successful germination under controlled condition.

Salinity

Seed germination of both populations of *G. tournefortii* collected for this study decreased as salt concentrations increased, described well by a three-parameter logistic model (P<0.001) (Fig. 2). The germination percentage was 43% and 39% at 64 mM of NaCl for the Sonqor and Kamyaran populations, respectively. Less than 5% germination was observed at 256 mM salinity in both populations (Fig. 2). The mM concentration of NaCl necessary for 50% reduction of the highest germination (X_{50}) of *G. tournefortii* seeds was 95 and 92 mM for the Sonqor and Kamyaran populations, respectively.

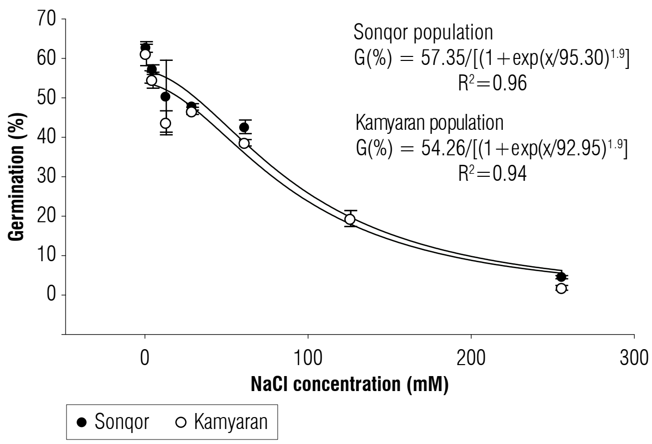

FIGURE 2. Seed germination of two populations (Sonqor and Kamyaran) of *Gundelia tournefortii* in response to sodium chloride (NaCl) in the germination media. Vertical bars represents the standard error of mean (n= 5) and the lines represent a three-parameter logistic model fitted to the data.

Salinity, via increased osmotic pressure in germination media, reduces water absorption by seeds, which in turn affects metabolic and physiological processes that reduce or prevent seed germination (Gupta & Huang, 2014; Tlahig *et al*., 2021; Uçarlı, 2020).

In general, the germination of *G. tournefortii* under different salinity stresses indicates that a higher percentage of seeds can germinate in soils predominantly found in western provinces of Iran, where NaCl concentrations in the soil are less than 250 mM (Emadodin *et al*.,2012). Our results suggest that *G. tournefortii* could not tolerate saline soils like halophytes (salt-tolerant plants). Based on our results, *G. tournefortii* can be considered a salt-sensitive species (glycophyte) when compared with other species (Uçarlı, 2020).

Water potential

In both populations, seed germination decreased as water potential decreased (Fig. 3). A sigmoid model accurately described the germination of both populations in response to water stress. Non-stressed seeds germinated more than 70% for both populations, while germination decreased to about 50% at –1.2 MPa. The water potential required for 50% inhibition of the maximum germination was –1.91 and –1.85 MPa for Sonqor and Kamyaran population, respectively (Fig. 3).

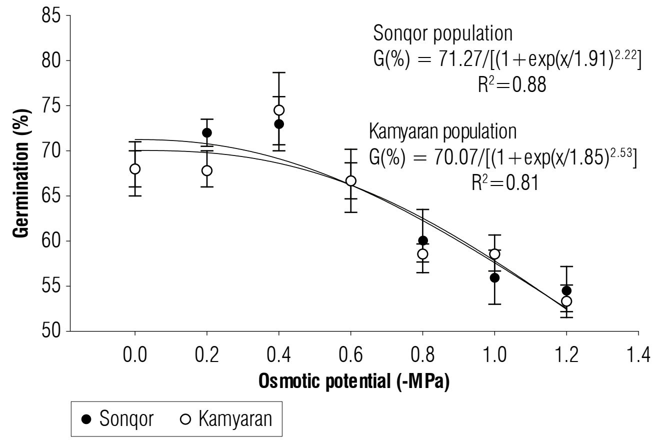

FIGURE 3. Seed germination of two populations (Sonqor and Kamyaran) of *Gundelia tournefortii* in response to a water potential (-MPa) in germination media. Vertical bars represents the standard error of mean (n= 5) and the lines represent a three-parameter logistic model fitted to the data.

As evident from the X_{50} parameter, seed germination in both populations of *G. tournefortii* was more tolerant to water stress compared to other crops and medical plants (Khojasted & Chahouki, 2013; Sheikh-Mohamadi *et al*., 2018; Xiong & Zhu, 2002). These results show that *G. tournefortii* is well adapted to water shortage during germination. Therefore, this medical species can germinate well in rain-fed farmlands. The higher tolerance to water stress by *G. tournefortii* is consistent with its natural distribution, as this medical species frequently populates dryland rangelands (Esbati *et al*., 2021; Khojasted & Chahouki, 2013). Based on these results, it can be concluded that planting *G. tournefortii* under rain-fed conditions is feasible.

Sowing depth

Seedlings of both populations of *G. tournefortii* emerged favorably (higher than 60%) from sowing depths up to 6 cm (Fig. 4). Increasing burial depth reduced the emergence percentage similarly for both Sonqor and Kamyaran populations. The maximum germination (68% for both populations) was observed for seeds located on soil surface, while seeds buried 12 cm deep in the soil managed to emerge seedlings in both populations (Fig. 4).

It is well documented that the seedling emergence of most plant species decreases as seed burial depth increases in the soil (Grundy *et al*., 2003). In general, the burial depth of

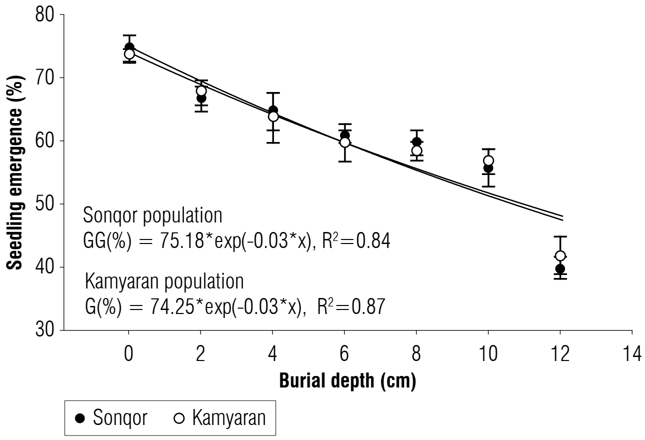

FIGURE 4. Seedling emergence from seeds of two populations (Sonqor and Kamyaran) of *Gundelia tournefortii* in response to burial depth. Vertical bar represents the standard error of mean (n= 5) and the lines represent a two-parameter exponential decay model fitted to the data.

most crop species is less than 15 cm (Kluyver *et al*., 2013). Therefore, *G. tournefortii* is well suited for cultivation in farmlands as a medicinal plant. The hypogeal germination characteristic of *G. tournefortii*, where cotyledons remain below the soil surface during seedling development, is the main reason for its ability to emerge from deeper layers of soil. Therefore, it doesn't require extra force to penetrate soil when compared with epigeal emergence in which the cotyledons extend above the soil surface (Gardarin *et al*.,2016). In addition, seeds of *G. tournefortii* are large enough to support seedling emergence from greater depth.

Conclusions

There was only a slight and non-significant difference in seed germination between the Kamyaran and Sonqor populations in response to various environmental factors evaluated in this study. Seed germination of *G. tournefortii* at the very high and low temperature conditions examined in our study indicates its potential for cultivation in a broad range of temperature conditions. Seeds of *G. tournefortii* still germinated under high levels of osmotic stress, while they were sensitive to salt stress. This suggests that *G. tournefortii* has a capacity to be planted in a broad range of farmlands, especially in rain-fed areas in western provinces of Iran and other parts of the world affected by drought stress and low concentration of NaCl in the soil. The highest soil depth from which seedling of *G. tournefortii* can emerge is higher than the typical depth of regular tillage in Iran. Therefore, such information can be valuable in developing its cultivating methods such as tillage systems and best soil conditions. Furthermore, the data obtained from this study may be helpful for development of species-specific propagation protocols and *ex situ* conservation of *G. tournefortii*.

Acknowledgments

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Conflict of interest statement

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

Author's contributions

IN: conceptualization, research. MEGH: conceptualization, writing, and editing supervision. HF: conceptualization, visualization, writing, and editing. IN: conceptualization, writing, and editing supervision. All authors have read and approved the final version of the manuscript.

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SCIENTIFIC NOTE

Physiological responses and initial growth of eggplant under nutrient exclusion from nutrient solution

Respuestas fisiológicas y de crecimiento inicial de la berenjena bajo la exclusión de nutrientes en la solución nutritiva

Lucas Aparecido Manzani Lisboa[1*], Marcos Antonio dos Santos[1], Marcelo da Cruz Francisco[1], and Marcelo Henrique Ribeiro Pereira[1]

ABSTRACT

In order to grow eggplant, a certain amount of mineral nutrients is needed to meet plant requirements at the initial phase of growth; with the absence of some nutrients, its physiological responses become compromised. This research aimed to study the physiological responses and initial growth of eggplant under nutrient omission in nutrient solution. The experiment was carried out in 2023 at the Fundação Educacional de Andradina located in the municipality of Andradina, state of São Paulo (Brazil). The experimental design was completely randomized, with nutrient exclusion of magnesium (Mg), boron (B), zinc (Zn), manganese (Mn), or copper (Cu) plus a control group with the supply of all mineral nutrients, with four replicates totaling 20 plots. Magnesium exclusion caused greater damage to the initial growth of eggplant in nutrient solution, with a 33.76% reduction in the concentrations of chlorophylls *a* and *b*; the contents of chlorophylls correlated with the concentration of organic nitrogen in the leaves. Boron exclusion caused deformations of leaf blades.

Key words: *Solanum melongena* L., magnesium, boron, zinc, manganese.

RESUMEN

Para cultivar berenjena, se necesita una cantidad de nutrientes minerales para cubrir sus necesidades en la fase inicial de crecimiento y con la exclusión de algunos nutrientes, sus respuestas fisiológicas se ven comprometidas. Este trabajo tuvo como objetivo estudiar las respuestas fisiológicas y el crecimiento inicial de berenjenas cultivadas bajo exclusión de nutrientes de la solución nutritiva. El experimento se realizó en 2023, en la Fundaçión Educacional de Andradina, ubicada en el municipio de Andradina, estado de São Paulo (Brasil). El diseño experimental fue completamente al azar, con omisión de los siguientes nutrientes: magnesio (Mg), boro (B), zinc (Zn), manganeso (Mn) o cobre (Cu) más un grupo control con el aporte de todos los nutrientes, con cuatro repeticiones y en total 20 parcelas. La eliminación de magnesio causó mayor daño al crecimiento inicial de la berenjena en la solución nutritiva, lo que provocó una reducción del 33,76% en las concentraciones de clorofilas *a* y *b*, y los contenidos de clorofilas se correlacionaron con la concentración de nitrógeno orgánico en las hojas. La exclusión de boro provocó deformaciones de las láminas foliares.

Palabras clave: *Solanum melongena* L., magnesio, boro, zinc, manganeso.

Introduction

Eggplant (*Solanum melongena* L.) stands out among vegetables as it is grown all over the world due to its nutritional characteristics; it contains dietary fiber, vitamins, and several essential elements such as zinc and iron. Furthermore, it is rich in flavonoids, phenolics and thiamine, which play an important role in human health, with anti-cancer, anti-asthmatic, antioxidant and anti-diabetic effects (Abubakar *et al*., 2023).

The eggplant requires certain quantities of mineral nutrients for its growth and development. This means the plant demands an exact amount of each essential mineral element to complete its cycle (Ghani *et al*., 2023; Rengel *et al*., 2023).

Magnesium (Mg) is a macronutrient that can be found in the soil solution in the form of Mg^{2+}. When its availability is restricted, plants present interveinal chlorosis in older leaves, and, in fruits, magnesium deficiency results in a reduction in size, altering fruit acidity and vitamin C content. Magnesium has several functions in plant metabolism, such as in the activation of enzymes and as part of pigments, where it is positioned at the center of chlorophyll and coordinated by four nitrogen atoms. In addition, Mg acts in ribosome function in the formation of the RNA polymerase

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enzyme, which is fundamental for the synthesis of proteins (Lisboa *et al*., 2024; Shaul, 2002; Taiz & Zeiger, 2013).

The micronutrient boron (B) can be found in the soil as boric acid (H_3BO_3) or borate anion $[B(OH)_4]^-$. This element participates in the biosynthesis of the cell wall along with other physiological processes, including translocation of organic metabolites and biosynthesis of some proteins (Sharma *et al*., 2022). Symptoms of boron deficiency vary between plant species. The most common are a reduction in growth and deformations of organs in the growth zones, especially in the stem apices, with brittle leaves that may be more intensely green (Kohli *et al*., 2023).

Zinc (Zn) can be found in the soil solution in the form of the Zn^{2+} cation, which moves in the soil by diffusion. The loss of Zn from soil through leaching and basic soil pH create the conditions for Zn deficiency in plants. A pH lower than 7.0 is favorable for the uptake of this element by plants (Fernandes, 2006). Zn deficiency manifests in the youngest plant parts as the shortening of the nodes, chlorosis of leaves, reduction in size and deformations in leaf shape (Lisboa *et al*., 2024). It is estimated that 8% to 10% of all eukaryotic proteins contain at least one Zn atom, particularly in oxidoreductases, transferases, hydrolases, lyases, isomerases or ligases, which implies that this element is an activator in the vast majority of enzymes. Zn is now recognized as the most widely used trace element in nature, along with iron (Fe) (Clemens, 2021).

Manganese (Mn) could be highly available in soils, second to iron (Fe) under suitable pH conditions (6.5). It plays an important role in photosynthesis, respiration, and activation of enzymes involved in oxidative stress, particularly in the elimination of reactive oxygen species (ROS). Manganese deficiency compromises growth, development and productivity of plants due to metabolic disorders (Silva & Berti, 2022).

In addition, to the importance of iron and molybdenum in plant growth at the initial stages, copper (Cu) also acts in the formation of the cell wall and regulates the transport of transcription proteins. Under copper limitation, biological processes such as photosynthesis, respiration, antioxidant protection against stress, and metabolic functions of plants are interrupted, affecting the productivity of crops (Yruela, 2005). This study aimed to understand the physiological responses and initial development of eggplant grown under nutrient exclusion from nutrient solution.

Materials and methods

Conditions of the experiment and experimental design

The experiment was carried out in 2023 in the Plant Morphology and Physiology laboratory, with an average temperature of 26°C and relative humidity of 75%, under artificial light provided by light-emitting diode (LED) at a constant intensity of 1000 μmol m^{-2} s^{-1} of photosynthetically active radiation (PAR). The laboratory is located at the Andradina Educational Foundation in the municipality of Andradina, state of São Paulo (Brazil). The experimental design was completely randomized, with exclusion of nutrients, namely magnesium (Mg), boron (B), zinc (Zn), manganese (Mn), and copper (Cu), with a control group with all nutrients. There were four replicates totaling 20 plots, where each plot consisted of one seedling. The seedlings of the Napoli® Sakata variety were obtained from a commercial nursery located in the same municipality, with an average size of 11.95 cm and 3±1 leaflets per seedling.

The seedlings were grown in a nutrient solution with the following concentrations of each nutrient (Furlani, 1998): 0.75 g L^{-1} $Ca(NO_3)_2$; 0.53 g L^{-1} KCl; 0.15 g L^{-1} P_2O_5; 0.4 g L^{-1} $MgSO_4$; 1.5x10^{-2} g L^{-1} $CuSO_4$; 2.0x10^{-2} g L^{-1} $ZnSO_4$; 1.5x10^{-1} g L^{-1} $MnSO_4$; 1.5x10^{-1} H_3BO_3; 1.5x10^{-2} g L^{-1} Na_2MoO_4; 3.0 g L^{-1} Fe-EDTA (6%). The electrical conductivity was adjusted daily to 2,000±100 μS cm^{-1}, with a pH of 6.4±0.2. Black plastic pots with a capacity of 10 L were used for plant growth, and the nutrient solutions were changed weekly.

Growth variables

After 30 d from the beginning of the experiment, the following variables were measured: plant height (PH), obtained as the difference between the final length minus the initial length expressed in cm using a ruler graduated in mm; number of fully expanded leaves (NL), obtained by the difference between the final and initial number of leaves; leaf area (LA), measured using the Easy Leaf Area application (Easlon & Bloom, 2014); and total dry mass (TDM), weighing together the aerial part and roots, obtained through drying the plants in a circulation and air renewal oven at a constant temperature of 65°C until reaching a constant weight.

Contents of chlorophylls *a* and *b* and organic nitrogen

The first fully expanded leaf was selected from the apex of the plants, where the levels of chlorophyll *a* and *b* (Chlo *a* and Chlo *b*, μmol m^{-2}) were determined through direct reading using a Falker® clorofiLOG device, with values recorded in SPAD units (Parry *et al.*, 2014) and subsequently converted into absolute concentrations of pigments as described by Chang and Troughton (1972). The content of organic nitrogen (N-org) in the leaves, expressed in g kg^{-1} of dry mass, was estimated according to Ferreira (2006).

Statistical analysis

For statistical analysis, the variables were subjected to normality tests using the Shapiro-Wilk test; analysis of variance was performed using the F test (P<0.05) and the means were compared using the Scott & Knott test at 5% probability (Banzatto & Kronka, 2013). A Pearson correlation analysis was performed using the statistical program R (R Core Team, 2015).

Results and discussion

A statistical difference between the treatments was observed in plant height (PH), where the exclusion of magnesium and then boron and manganese presented the lowest averages, reflecting a reduction of approximately 14.81% in relation to plants that received all nutrients (Tab. 1).

The photosynthetic rate may have been reduced due to magnesium restriction, as this element is a key component of the chlorophyll molecule (Taiz & Zeiger, 2013). Its restriction may compromise photosynthetic electron transport, impairing water photolysis and decreasing the availability of H^+ ions necessary for the formation of $NADPH^+$. As a consequence, CO_2 fixation would be compromised, potentially affecting the development of the eggplant aerial parts (Lisboa *et al.*, 2024). A strong positive correlation was observed between LA and NL, as shown in Figure 1.

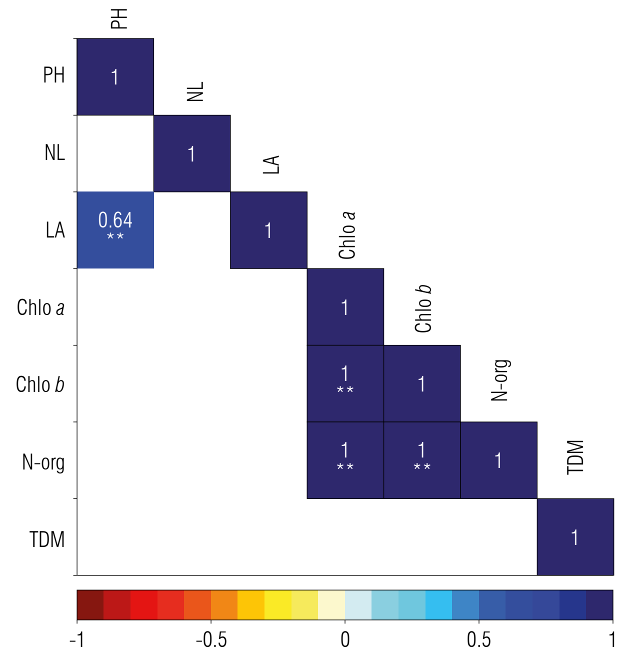

FIGURE 1. Significant Pearson correlation matrix between the variables of plant height (PH), number of leaves (NL), leaf area (LA), chlorophylls *a* and *b* (Chlo *a* and Chlo *b*), organic nitrogen content (N-org) and total dry mass (TDM) of eggplant grown under nutrient exclusion from the nutrient solution.

Therefore, a reduction in the LA of plants may reduce the photosynthetic rate, negatively influencing PH, as shown in Figure 2A.

A statistical difference was not observed for NL; however, a difference was observed in LA where the omission of either boron, magnesium or zinc resulted in the lowest averages of this variable (Tab. 1). Boron withdrawal from the nutrient

TABLE 1. Average plant height (PH), number of leaves (NL), leaf area (LA) and total dry mass (TDM) of eggplant grown under nutrient exclusion from the nutrient solution.

Nutrient solution	PH (cm)	NL	LA (cm^2)	TDM (g)
Complete	31.25^a	8.00	359.31^a	1.21^a
-Mg	26.62^b	7.00	270.68^b	0.49^b
-B	27.25^b	7.25	269.08^b	0.63^b
-Zn	31.00^a	7.50	336.33^a	1.12^a
-Mn	28.12^b	7.50	272.01^b	0.72^b
-Cu	30.37^a	7.75	318.89^a	0.83^b
P-value	0.0056**	0.3485^{ns}	0.0077**	0.0001**
CV%	6.30	8.60	12.12	19.64
OA	29.10	7.50	304.38	0.83

OA: Overall average; CV: Coefficient of variation; ** - significant at 1% probability level (P<0.01); * - significant at the 5% probability level (0.01=<P<0.05). Means followed by the same letter do not differ statistically. The Scott & Knott test was applied at a level of 5% probability of the event occurring.

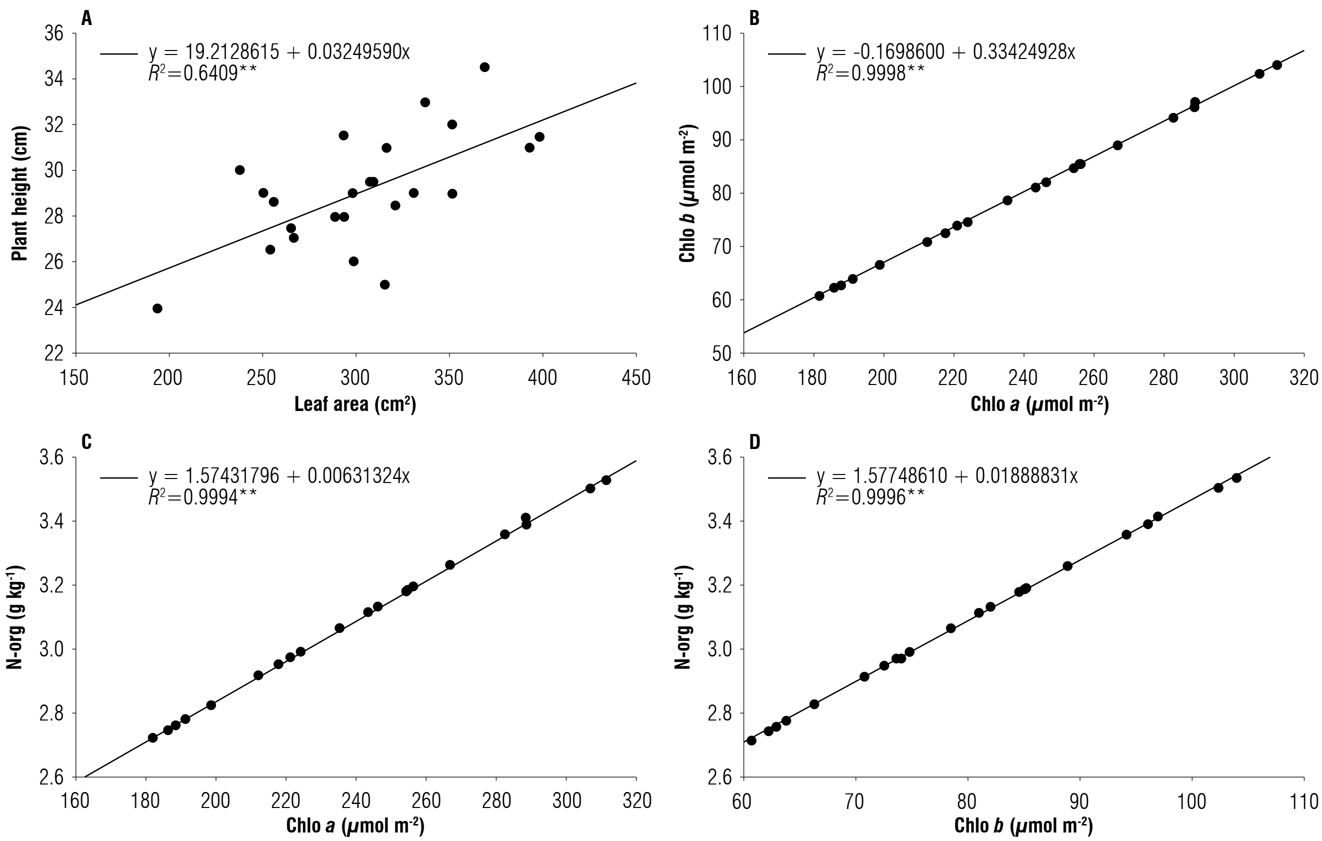

FIGURE 2. Significant linear regressions after Pearson's correlation between the variables plant height (PH), leaf area (LA), contents of chlorophylls *a* and *b* (Chlo *a* and Chlo *b*), organic nitrogen content (N-org) of eggplant grown under nutrient exclusion from the nutrient solution.

solution presented the lowest average, with a difference of approximately 25.11% in relation to the plants that received all nutrients. Notably, the poor formation of the eggplant leaves that were grown under exclusion of boron is seen in Figure 3, where the leaf blade is characterized by ribbed intervein spaces.

It is worth noting that cultivation under the restriction of magnesium, manganese and copper most contributed to the negative effects on the growth of eggplant in a nutrient solution, as shown in Figure 4.

The restriction of these elements, especially magnesium and boron, can compromise the metabolism of biomolecules such as sucrose (primary) and cellulose and lignin (secondary), which can alter morphology of leaf tissues, especially leaf size. These results corroborate with Lisboa *et al*. (2024). Furthermore, significant changes in temperature, light intensity, and water availability can further exacerbate these changes in plant morphology (Ohnishi *et al*., 2023).

Nutrient restrictions also influenced the TDM of eggplant. The crop with all nutrients and those without zinc presented the highest averages. The crop with all nutrients presented a difference of 59.20% higher in relation to the crop with magnesium restriction, which presented the lowest average total dry mass (Tab. 1).

This response to magnesium restriction was expected as similar results were reported by Lisboa *et al*. (2024) and Lisboa, Galindo *et al*. (2024), who highlighted the importance of magnesium in gas exchange and development in tomato and pepper. When the plant undergoes sudden changes in leaf gas exchange, its dry mass is compromised due to impaired carbon fixation, which is dependent on electrons captured by $NADP^+$ in the light-dependent phase of photosynthesis. This restriction affects the metabolic action of the 1,5-bisphosphate carboxylase/oxygenase, commonly known as Rubisco, crucial in the Calvin cycle (Taiz & Zeiger, 2013).

This negative response due to the restriction of nutrition in the formation of chlorophylls *a* and *b* is observed in Table 2. The absence of the Mg, Zn, and Mn resulted in lower averages, with approximately 33.76% and 33.91% lower contents of chlorophylls *a* and *b*, respectively, in relation to plants that were grown with all nutrients. Due to their same metabolic origin, chlorophylls *a* and *b* present a significant correlation, as seen in Figures 1 and 2B.

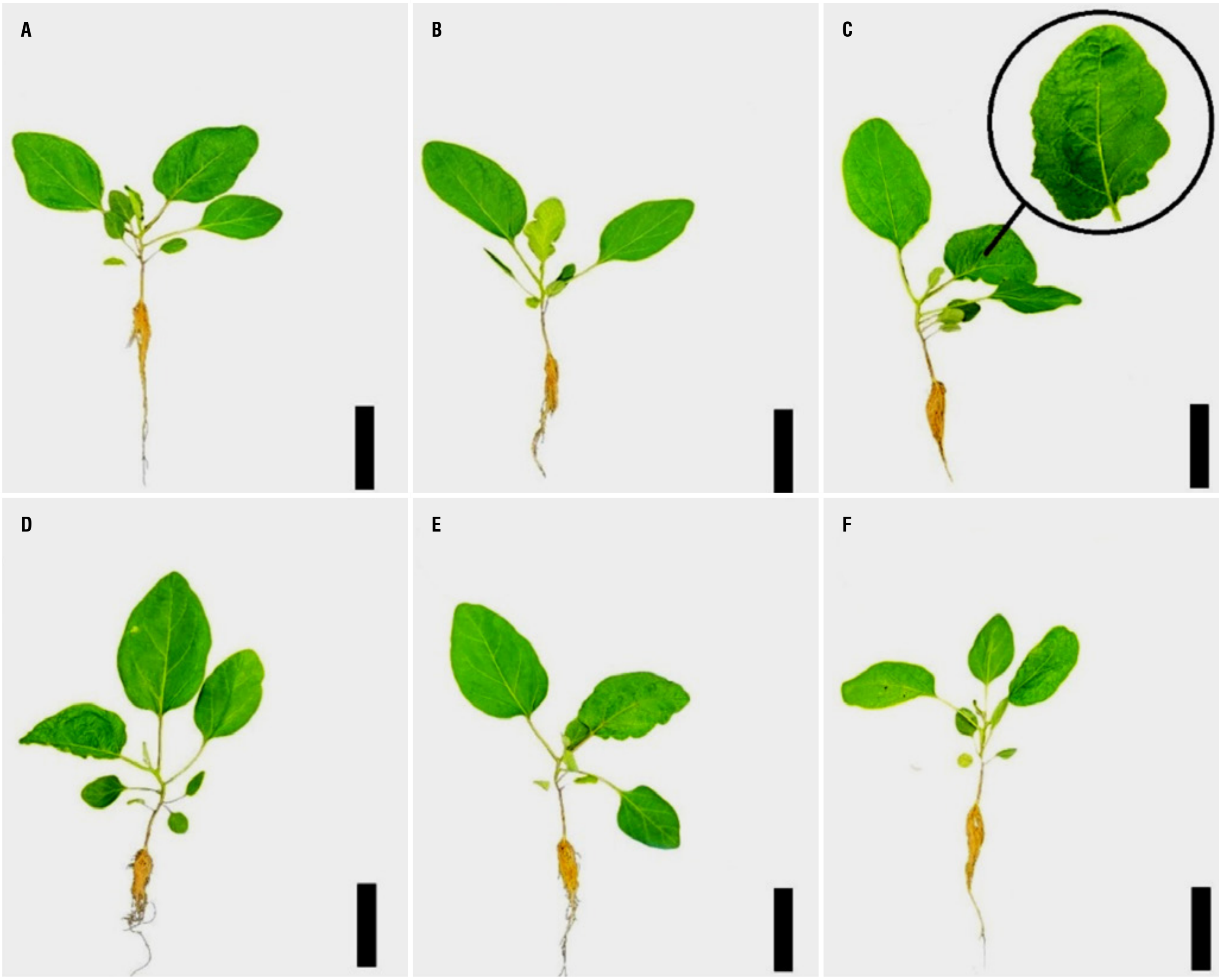

FIGURE 3. Eggplant plants grown under nutrient exclusion from the nutrient solution: A = Complete nutrient solution; B = absence of Mg; C = absence of B; D: absence of Mn; E = absence of Cu, and F = absence of Zn. Bar = 10 cm.

TABLE 2. Average contents of chlorophyll *a* and *b* (Chlo *a* and Chlo *a*, μmol m^{-2}) and organic nitrogen content (N-org, g kg^{-1}) in leaves of eggplant grown under nutrient exclusion from nutrient solution.

Nutrient solution	Chlo *a*	Chlo *b*	N-org
Complete	294.32^{a}	98.32^{a}	3.43^{a}
-Mg	194.93^{c}	64.97^{c}	2.80^{c}
-B	249.78^{b}	83.26^{b}	3.15^{b}
-Zn	206.62^{c}	68.87^{c}	2.87^{c}
-Mn	232.94^{c}	77.64^{c}	3.04^{c}
-Cu	259.85^{b}	86.61^{b}	3.21^{b}
P-value	0.0003**	0.0003**	0.0003**
CV%	10.54	10.51	5.14
AO	239.74	79.96	3.08

OA: Overall average; CV: Coefficient of variation. ** - significant at 1% probability level (P<0.01); * - significant at the 5% probability level (0.01=<P<0.05). Means followed by the same letter do not differ statistically. The Scott & Knott test was applied at a level of 5% probability of the event occurring.

Due to the low concentration of nutrients in the leaves, particularly magnesium, which is a critical component of chlorophyll molecules (Pranckietienė *et al.*, 2020), where it is linked with four nitrogen atoms (N), the biochemical phase of photosynthesis is compromised. This restriction reduces the assimilation of atmospheric carbon dioxide, which is necessary for conversion into glucose. Magnesium is also required for the activation of the Rubisco enzyme, which is present in chloroplasts (Taiz & Zeiger, 2013).

When plants are under stress, they experience the production of reactive oxygen species (ROS), which increases the concentrations of antioxidant enzymes. If the stress is for a long period and/or high intensity, ROS can cause serious damage to the cell structures, mainly DNA, lipids, cell membranes, and proteins (Reis *et al.*, 2018).

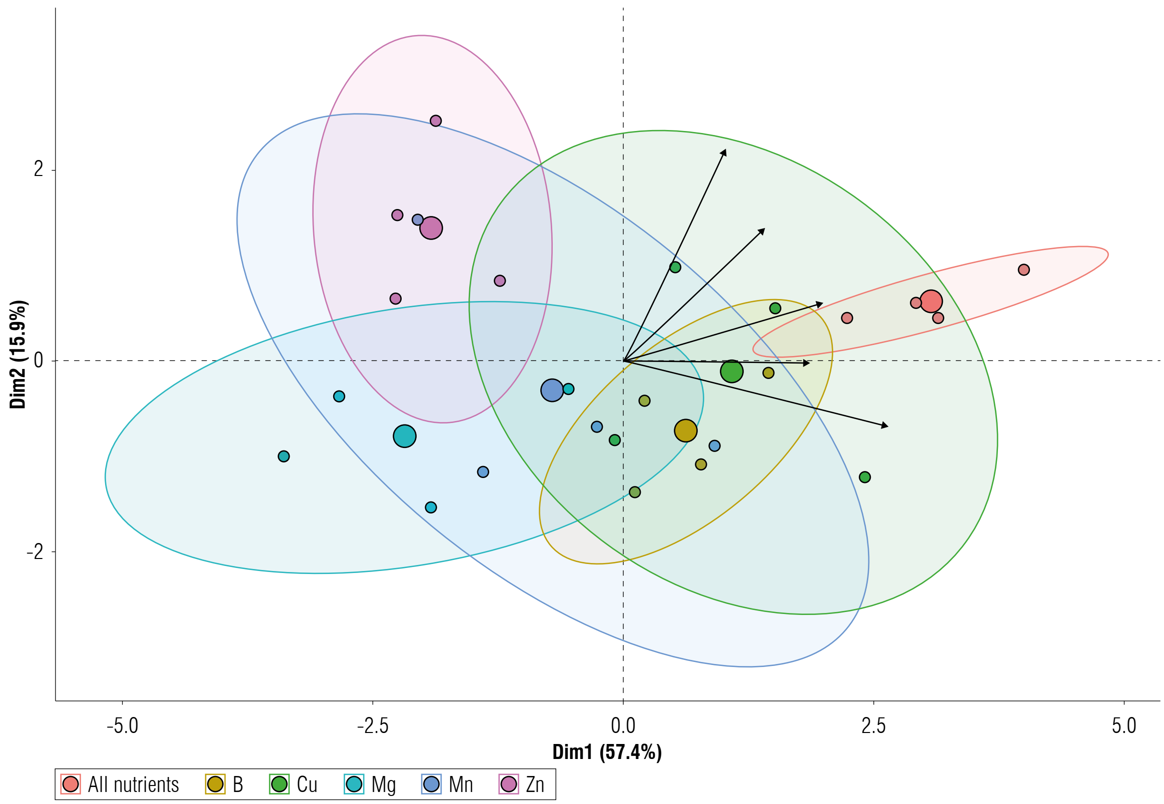

FIGURE 4. Biplot chart of the significant principal component analysis of eggplant grown under nutrient exclusion from the nutrient solution.

With this bond between the magnesium atom and nitrogen (Mg-4N) in the chlorophyll molecule, the magnesium restriction may have caused a lower concentration of N-org in eggplant leaves. This effect is well illustrated in Figures 1 and 2D, demonstrating this correlation between chlorophyll contents and N-org concentration.

Therefore, due to this correlation, nitrogen deficiency in the leaves caused by magnesium restriction (Fig. 1) starts to influence the intensity of the green color in the leaves. Many studies have already reported the correlation between the intensity of the green color and nitrogen concentrations, which can be an important factor when calculating fertilization in crops (Chang & Troughton, 1972; Silva *et al*., 2014).

Conclusions

Magnesium exclusion from the nutrient solution causes significant damage to the initial growth of eggplant grown in nutrient solution, resulting in a 33.76% reduction in the concentrations of chlorophylls *a* and *b*. The contents of these pigments correlated with the concentration of organic nitrogen in the leaves. Boron exclusion from the nutrient solution caused deformations in the leaf blades.

Conflict of interest statement

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

Author's contributions

LAML: conceptualization, research, writing - original draft, visualization, writing, and editing. MHRP: conceptualization, writing, and supervision editing. MAS: conceptualization, visualization, writing, and editing. MCF: conceptualization, writing, and editing supervision. All authors have read and approved the final version of the manuscript.

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