

Foliar application of salicylic acid mitigates water deficit in guava¹

Aplicação foliar de ácido salicílico mitiga o déficit hídrico em goiabeira

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HIGHLIGHTS:

Guava growth, chlorophyll content, and gas exchange decrease if grown with 50% of the reference evapotranspiration (ET_r). 'Crioula' guava plants irrigated with 50% ET_r have less electrolyte leakage under 2.4 mM salicylic acid foliar application. Salicylic acid increases the chlorophyll content in 'Paluma' guava at concentration of 1.2 mM and 50% ET_r.

ABSTRACT: Fruit growing is an activity of great relevance for agribusiness in Brazil, with emphasis on guava. However, water deficiency is one of the limiting factors for plant productivity, so the use of phytohormones such as salicylic acid has been employed as a strategy to mitigate the effects of water deficit. The objective of this study was to evaluate the foliar application of salicylic acid as an attenuator of water deficit in guava genetic materials. The experimental design adopted was randomized blocks, in a 2 × 2 × 5 factorial scheme, considering two water regimes (irrigation depths equivalent to 50 and 100% of the reference evapotranspiration - ET_r), two guava genetic materials ('Crioula' and 'Paluma') and five concentrations of salicylic acid (0, 1.2, 2.4, 3.6, and 4.8 mM). Foliar application of salicylic acid at a concentration of 2.4 mM promoted growth, gas exchange, and chlorophyll a fluorescence of 'Crioula' guava under irrigation with 50% ET_r. Water deficit reduced growth, gas exchange, chlorophyll contents, and chlorophyll a fluorescence of guava. Among the genetic materials, greater growth, gas exchange, chlorophyll contents, and chlorophyll a fluorescence was observed in 'Paluma' compared to 'Crioula' under irrigation with 50% ET_r without foliar application of salicylic acid. Foliar application of salicylic acid at a concentration of 1.2 mM increases leaf area, number of leaves, and total dry mass in the 'Crioula' genetic material under 50% ET_r.

Key words: *Psidium guajava* L., phytohormone, abiotic stress

RESUMO: A fruticultura é uma atividade de grande relevância para o agronegócio no Brasil, com destaque para a goiabeira. Entretanto, a deficiência hídrica é um dos fatores limitantes para a produtividade das plantas, onde a utilização de fitohormônios, como o ácido salicílico, tem sido empregado como uma estratégia para mitigar os efeitos do déficit hídrico. O objetivo deste estudo foi avaliar a aplicação foliar de ácido salicílico como atenuador do déficit hídrico em materiais genéticos de goiabeira. O delineamento experimental adotado foi em blocos ao acaso, em esquema fatorial 2 × 2 × 5, considerando dois regimes hídricos (lâminas de irrigação equivalentes a 50 e 100% da evapotranspiração de referência - ET_r), dois materiais genéticos de goiabeira ('Crioula' e 'Paluma') e cinco concentrações de ácido salicílico (0, 1,2, 2,4, 3,6 e 4,8 mM). A aplicação foliar de ácido salicílico na concentração de 2,4 mM promoveu efeito benéfico no crescimento, nas trocas gasosas e na fluorescência da clorofila a da goiabeira 'Crioula' sob irrigação com 50% da ET_r. O déficit hídrico reduziu o crescimento, as trocas gasosas, o conteúdo de clorofila e a fluorescência da clorofila a da goiabeira. Dentre os materiais genéticos, a 'Paluma' obteve maior crescimento, trocas gasosas, teores de clorofila e fluorescência da clorofila a em comparação com a 'Crioula' sob irrigação com 50% da ET_r sem aplicação foliar de ácido salicílico. A aplicação foliar de ácido salicílico na concentração de 1,2 mM aumentou a área foliar, o número de folhas e a fitomassa seca total no material genético 'Crioula' sob 50% de ET_r.

Palavras-chave: *Psidium guajava* L., fitohormônio, estresse abiótico

INTRODUCTION

Guava (*Psidium guajava* L.) is a fruit crop widely cultivated in large commercial orchards in Brazil, standing out for its socioeconomic relevance. Its leaves and fruits contain secondary metabolites such as terpenes and phenolic compounds (Macedo et al., 2021). In addition to its medicinal use, guava is commonly consumed fresh or used in the food industry to produce pulp, nectars, jams, jellies, and syrups (Angulo-López et al., 2021).

In 2022, Brazil produced 582,832 tons of guava, with a harvested area of 22,533 hectares. The States of Pernambuco, São Paulo, and Rio de Janeiro stood out as the main producers, with 205,960, 182,077, and 18,057 tons, respectively (IBGE, 2023). However, its production is limited in some semi-arid regions, due to the low availability of water, caused by rainfall concentrated in a few months, followed by long periods of drought and high evapotranspiration (Lucena et al., 2018).

Under water deficit conditions, plants reduce the frequency of stomatal opening, a process mediated by abscisic acid, which results in restrictions on CO₂ absorption, transpiration, photosynthesis and allocation of photoassimilates, compromising plant growth (Usman et al., 2022; Soares et al., 2023). This condition also leads to an increase in the production of reactive oxygen species, such as O₂⁻ and H₂O₂, causing changes in plant metabolism that culminate in the oxidation of important cellular components such as membranes, lipids, pigments, and cell genetic material (Usman et al., 2022). In a study with guava crop under irrigation with water deficit, Lacerda et al. (2025) observed that a 50% ETr depth reduced the growth in diameter of the rootstock, graft, scion, and scion volume, as well as gas exchange: stomatal conductance and transpiration, and the maximum and variable fluorescence indices of guava cv. Paluma. In the literature, there are incipient studies evaluating the effects of water deficit on guava crop. Therefore, it is extremely important to determine the level of tolerance or sensitivity of the crop to water deficit and to establish viable production strategies.

Thus, exogenous application of osmoregulators is shown to be a strategy to mitigate the effects of water deficit on plants, especially foliar application of salicylic acid, a phenolic

compound composed of seven carbons and a signaling molecule synthesized naturally and endogenously in plants (Ahanger et al., 2019), contributing to physiological activities, such as nutrient absorption, photosynthesis, flower induction, and stomatal closure (Silva et al., 2022). Salicylic acid acts at the molecular level as one of the plant's defense signaling agents, increasing antioxidant activities and synthesis of osmoregulators, such as proline, glycine-betaine, and total carbohydrates, contributing to the reduction of oxidative stress (Kaur et al., 2022).

Acclimatization of plants under water deficit can occur from enzymatic and non-enzymatic mechanisms for cellular detoxification and osmotic regulation of leaves and roots, maintaining turgidity through the accumulation of low-molecular-weight components, maintaining a high osmotic pressure (Saheri et al., 2020). Plant tolerance to water stress can also vary between cultivars of the same species (Usman et al., 2022). In this context, the objective of this study was to evaluate the foliar application of salicylic acid as an attenuator of water deficit in guava genetic materials.

MATERIAL AND METHODS

The experiment was conducted from September 2022 to March 2023, under greenhouse conditions, at the 'Rolando Enrique Rivas Castellón' Experimental Farm belonging to the Center of Sciences and Agri-Food Technology - CCTA of the Universidade Federal de Campina Grande (UFCG), in the municipality of São Domingos, Paraíba, Brazil, located at 6° 50' 4" South and 37° 53' 9" West, at an altitude of 190 m. Data of maximum and minimum air temperature and relative air humidity, collected during the experimental period, are presented in Figure 1.

The experimental design was randomized blocks in a 2 × 2 × 5 factorial scheme, referring to two guava genetic materials ('Crioula' and 'Paluma'), two water regimes (100 and 50% of the reference evapotranspiration - ETr), and five concentrations of salicylic acid (0, 1.2, 2.4, 3.6, and 4.8 mM), with four replicates and two plants per plot, totaling 160 plants. Salicylic acid concentrations were defined based on a study conducted by Lacerda et al. (2022).

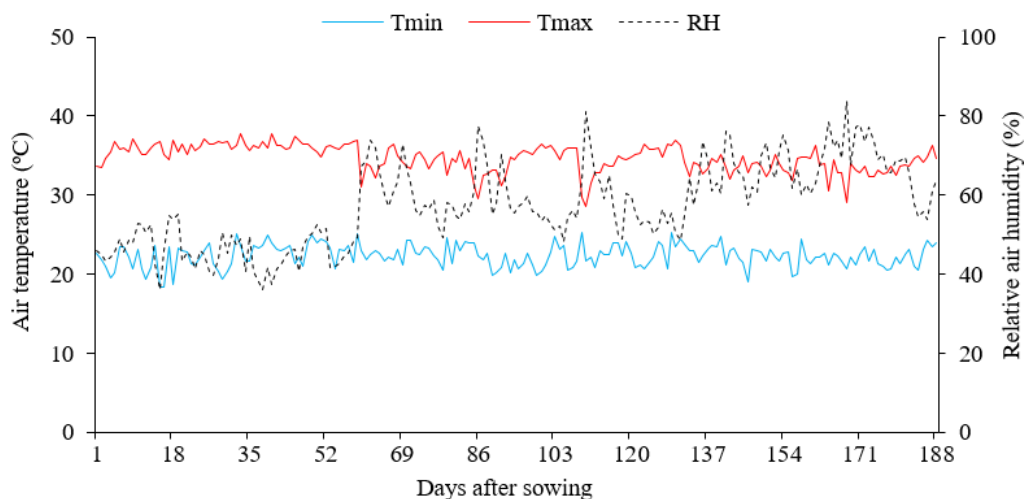


Figure 1. Maximum (Tmax) and minimum (Tmin) air temperature and relative air humidity (RH) during the experimental period from September 06, 2022 to March 04, 2023

Sowing was carried out in plastic bags with a volume of 1.4 dm³ filled with a substrate composed of soil, sand, and well decomposed manure in a volumetric ratio of 2:2:1, using two seeds; after emergence, thinning was performed, leaving one plant per bag. The genetic materials used were 'Crioula', obtained from natural crossing and with vast genetic diversity, that is, it is not defined as a commercial variety, but it is a genetic material adapted to the edaphoclimatic conditions of the semi-arid northeast (Melo et al., 2021), and 'Paluma', a widely marketed cultivar in Brazil due to the quality of its fruits and its high nutritional value, being rich in vitamin C, potassium, calcium and magnesium, and bioactive compounds, in addition to having high yield (Souza et al., 2016). The physical-hydraulic and chemical attributes of the soil were determined according to the methodology of Teixeira et al. (2017).

Fertilization management followed the recommendation of Novais et al. (1991), applying 100 mg of N (urea), 300 mg of P₂O₅ (monoammonium phosphate) and 150 mg of K₂O (potassium chloride) kg⁻¹ of soil, applied as top-dressing, starting at 60 days after sowing (DAS), split into eight applications. Micronutrients were applied every 15 days through foliar fertilization, with the commercial product Dripsol micro[®], which contains in its constitution: 1.2% magnesium, 0.85% boron, 3.4% iron, 4.2% zinc, 3.2% manganese, 0.5% copper, and 0.06% molybdenum.

In all experimental units, soil moisture was maintained at field capacity until the beginning of the application of the treatments (90 days after sowing - DAS). The water used for irrigation came from the local supply system of the city of São Domingos, PB, and the irrigation depth for each treatment was determined based on plants under full irrigation (100% ETr), obtained by weighing lysimetry. To this end, the bags with soil were weighed prior to sowing to determine the dry weight of the soil (g) and its weight at field capacity (g), and after sowing, the actual weight (g) was determined at each irrigation event. With these values, soil moisture at field capacity was calculated according to Eq. 1 (Silva et al., 2023):

$$U_{fc} = \frac{M_{sfc} - M_{ds}}{M_{ds}} \quad (1)$$

where:

U_{fc} - gravimetric moisture content of soil at field capacity (g g⁻¹);

M_{sfc} - mass of soil at field capacity (g); and,

M_{ds} - mass of dry soil (g).

Then, volumetric soil moisture (θ) at the actual field capacity at each irrigation event was estimated, according to Eq. 2:

$$\theta_{fc} = U_{fc} \times BD \quad (2)$$

where:

θ_{fc} - volumetric moisture of soil at field capacity (cm³ cm⁻³);

U_{fc} - gravimetric moisture of soil at field capacity (g g⁻¹);

and,

BD - bulk density (g cm⁻³).

Salicylic acid (SA) solution was prepared by dissolution in 30% ethyl alcohol (95.5% purity) on the days of SA application at 5 p.m. Foliar application began 72 hours prior to the beginning of the differentiation of the irrigation depths and was subsequently performed every 15 days (Sá et al., 2024) by spraying the abaxial and adaxial sides of the leaves, during the entire period of seedling formation, in a total of six applications, the mean value per plant being 21.87 mL.

Gas exchange was analyzed at 188 DAS, using an infrared gas analyzer - IRGA (Infra-Red Gas Analyser, model LCpro - SD, from ADC BioScientific, UK), by measuring stomatal conductance - g_s (mol H₂O m⁻² s⁻¹), transpiration - E (mmol H₂O m⁻² s⁻¹), CO₂ assimilation rate - A (μmol CO₂ m⁻² s⁻¹) and internal CO₂ concentration - C_i (μmol CO₂ m⁻² s⁻¹). These data were then used to estimate the instantaneous water use efficiency - WUE (A/E) [(μmol CO₂ m⁻² s⁻¹) (mmol H₂O m⁻² s⁻¹)⁻¹] and the instantaneous carboxylation efficiency - iCE (A/C_i) [(μmol CO₂ m⁻² s⁻¹) (μmol CO₂ m⁻² s⁻¹)⁻¹]. Readings were taken between 6:30 and 10:00 a.m. under natural conditions of air temperature, CO₂ concentration, and using an artificial radiation source of 1,200 μmol m⁻² s⁻¹.

At 188 days after sowing, photosynthetic pigments were determined from the contents of chlorophyll a, chlorophyll b, total chlorophyll and carotenoids of the leaves, by means of a sample of four discs of the third mature leaf from the apex, previously weighed, according to the methodology proposed by Arnon (1949). At the same time, the relative water content in the leaf blade of guava seedlings was determined according to the methodology of Weatherley (1950). Electrolyte leakage was also measured, in accordance with Scotti-Campos (2013).

Photochemical efficiency was determined at 188 DAS using a pulse-modulated fluorometer (Sciences Inc. - Model OS-30p, Hudson, USA), by placing leaf clips on the selected leaves for readings after 30 minutes of adaptation to the dark, and then determining initial fluorescence - F₀, maximum fluorescence

Table 1. Physical and chemical attributes of the soil used in the experiment

Chemical attributes								
pH (H ₂ O) (1:2.5)	OM (g kg ⁻¹)	P (mg kg ⁻¹)	K ⁺	Na ⁺	Ca ²⁺	Mg ²⁺	Al ³⁺	H ⁺
7.19	1.40	59.5	0.49	0.07	4.70	3.63	0.00	0.00
Chemical attributes				Physical attributes				
EC _{se} (dS m ⁻¹)	CEC (cmol _c kg ⁻¹)	SAR _{se} (mmol L ⁻¹) ^{0.5}	ESP (%)	Particle-size fraction (g kg ⁻¹)			Moisture (dag kg ⁻¹)	
				Sand	Silt	Clay	33.42 kPa ¹	1519.5 kPa ²
0.58	8.82	1.40	33.33	735.10	201.4	63.5	15.78	6.41

pH - Hydrogen potential, OM - Organic matter: Walkley-Black Wet Digestion; Ca²⁺ and Mg²⁺ extracted with 1 M KCl at pH 7.0; Na⁺ and K⁺ extracted with 1 M NH₄OAc at pH 7.0; Al³⁺+H⁺ extracted with 0.5 M CaOAc at pH 7.0; EC_{se} - Electrical conductivity of the saturation extract; CEC - Cation exchange capacity; SAR_{se} - Sodium adsorption ratio of the saturation extract; ESP - Exchangeable sodium percentage; ^{1,2} Referring to the moisture contents in the soil corresponding to field capacity and permanent wilting point, respectively

- Fm, variable fluorescence - Fv and quantum efficiency of photosystem II - Fv/Fm.

The growth of guava seedlings was measured at 188 DAS, based on the number of leaves - NL, obtained by manual counting of the leaves, considering those that were longer than 3 cm; plant height - PH (cm), measured from the plant collar to the insertion of the apical bud, using a graduated ruler; stem diameter - SD (mm), measured with a digital caliper at 2 cm from soil surface; and leaf area - LA (cm²), obtained by measuring the midrib length of each leaf with a graduated ruler, following the methodology of Queiroga et al. (2023), and calculated according to Eq. 3.

$$LA = 0.3205 \times L^{2.0412} \quad (3)$$

where:

LA - leaf area (cm²); and,

L - midrib length of the leaf (cm).

In this same period, after harvesting the plants were dried at 65 °C for 48 hours in a forced air circulation oven and then weighed on a semi-analytical scale, to obtain the total dry mass - TDM (g per plant).

The collected data were subjected to principal component analysis (PCA), synthesizing the amount of relevant information contained in the original data sets in a smaller number of dimensions, resulting from linear combinations of the original variables generated based on the eigenvalues ($\lambda \geq 1.0$) in the correlation matrix, explaining percentages greater than 10% in the total variance (Govaerts et al., 2007). Only variables with a correlation coefficient equal to or greater than 0.5 remained in the PCA, performed with the FactoMineR (Factor Analysis and Data Mining with R) package. From the reduction of dimensions, the original data of the variables of each component were subjected to multivariate analysis of variance (MANOVA) by the Lawley-Hotelling test (Hotelling et al., 1947) at a 0.05 probability level for water regime, salicylic acid concentrations, and genetic material, as well as for the interaction between them. The justification for using MANOVA is to analyze the combined influence of multiple independent variables on multiple dependent variables, providing a comprehensive understanding of their joint effects. Additionally, a Pearson correlation analysis was performed using the Corrplot package. All analyses were executed on the R 4.3.2 platform (R Core Team, 2022).

RESULTS AND DISCUSSION

Principal component analysis (PCA) revealed that the first two components explained 61.40% of the total variance of the data. However, most of the significant associations ($p \leq 0.01$) were concentrated in the first component, which alone explained 48.92% of the variance of the data (Tables 2, 3 and 4).

In the first component, there were correlations between number of leaves ($r = 0.87$), total dry mass ($r = 0.85$), leaf area ($r = 0.85$), plant height ($r = 0.83$), CO₂ assimilation rate ($r = 0.85$), stem diameter ($r = 0.84$), initial fluorescence ($r = 0.77$), transpiration ($r = 0.74$), quantum efficiency of

photosystem II ($r = 0.67$), maximum fluorescence ($r = 0.64$), variable fluorescence ($r = 0.63$), relative water content ($r = 0.61$), stomatal conductance ($r = 0.51$), carotenoids ($r = -0.50$), instantaneous carboxylation efficiency ($r = -0.51$), instantaneous water use efficiency ($r = -0.55$), electrolyte leakage ($r = -0.69$) and internal CO₂ concentration ($r = -0.74$), while in the second component, which explained 12.54% of the original variance, only the pigments were negatively correlated: chlorophyll a ($r = -0.80$), chlorophyll b ($r = -0.65$) and chlorophyll total ($r = -0.77$) (Tables 2, 3 and 4).

The application of salicylic acid (SA) had a remarkable effect on the growth and physiological aspects of the 'Crioula' and 'Paluma' genetic materials, especially in response to different levels of water availability, since plants under 50% ETr for both genetic materials had lower values of growth and gas exchange, as well as high levels of carotenoids, electrolyte leakage, and relative water content (Figure 2). Growth inhibition due to water restriction is often associated with loss of cell turgor, which limits cell division and elongation, resulting in physiological changes such as disturbances in photosynthesis and increased reactive oxygen species (Oliveira et al., 2023).

In the 'Crioula' genetic material, the application of 2.4 mM of SA (M₁SA₃) resulted in considerable increments in the mean values of CO₂ assimilation rate, stomatal conductance and transpiration, recording increases of 146, 820, and 93%, respectively, compared to plants that did not receive SA, under conditions of 100% ETr (Table 2). Similarly, Queiroga et al. (2023) observed that SA, at concentration of 2.9 mM, increased stomatal conductance in guava plants. The authors suggest that this beneficial effect may be related to the ability of SA to regulate the antioxidant defense system, maintain the integrity of the chloroplast, influence the control of stomatal opening and closing, and maintain the stability of the cell membrane.

Plants of the 'Crioula' genetic material under water restriction (50% ETr) showed lower values of CO₂ assimilation rate, stomatal conductance and transpiration, without the application of SA, being 4.16, 62.88, and 83.33% lower compared to those observed in 'Paluma' guava (Table 2). This occurs because, under water deficit conditions, the activities of some essential enzymes, including ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) and RuBisCO activase, are inhibited, leading to a reduction in CO₂ fixation (Kang et al., 2023).

Abrar et al. (2022), when studying the tolerance to water deficit and physiological responses of guava, also observed that under water restriction the stomata were closed to conserve leaf moisture; consequently, stomatal conductance decreased, which led to a decrease in photosynthesis and transpiration. Considering that both genetic materials were propagated via seminiferous plants (seeds), the superiority observed in cv. Paluma over 'Crioula' is possibly due to genetic variability and the variation in the degree of tolerance to water deficit that occurs between genetic materials of the same species.

Despite the reductions observed in the 'Crioula' genetic material under 50% ETr, the application of SA at concentration of 2.4 mM increased CO₂ assimilation rate, stomatal conductance and transpiration compared to plants without SA (Table 2). This increase occurs because SA acts in the

Table 2. Eigenvalues, percentage of total variance explained in the multivariate analysis of variance, and probability of significance by Hotelling's test (p-value) for the factors

			Principal components (PC)			
			PC1		PC2	
Eigenvalues (λ)			10.27		2.63	
Percentage of total variance ($S^2\%$)			48.92		12.54	
			Manova p			
Lawley-Hotelling test for water condition (C)			<0.01		<0.01	
Lawley-Hotelling test for salicylic acid concentrations (SA)			<0.01		<0.01	
Lawley-Hotelling test for genetic material (M)			<0.01		<0.01	
Lawley-Hotelling test for C $\times$ SA			<0.01		<0.01	
Lawley-Hotelling test for C $\times$ M			<0.01		<0.02	
Lawley-Hotelling test for SA $\times$ M			<0.01		<0.01	
Lawley-Hotelling test for C $\times$ SA $\times$ M			<0.01		<0.03	
PCs	Correlation coefficients (r)					
	A	E	gs	Ci	WUE	iCE
PC1	0.85	0.74	0.51	-0.74	-0.55	-0.51
PC2	0.03	0.12	-0.2	0.30	0.07	0.33
Treatment						
C ₁ M ₁ SA ₁	2.60 $\pm$ 0.06	2.47 $\pm$ 0.46	0.03 $\pm$ 0.0003	283.50 $\pm$ 10.26	0.54 $\pm$ 0.15	0.004 $\pm$ 0.0001
C ₁ M ₁ SA ₂	4.35 $\pm$ 0.08	3.68 $\pm$ 0.66	0.05 $\pm$ 0.0008	316.25 $\pm$ 10.03	0.50 $\pm$ 0.13	0.01 $\pm$ 0.0004
C ₁ M ₁ SA ₃	6.40 $\pm$ 0.28	4.78 $\pm$ 0.33	0.18 $\pm$ 0.01	234.00 $\pm$ 11.43	0.41 $\pm$ 0.06	0.007 $\pm$ 0.0004
C ₁ M ₁ SA ₄	3.57 $\pm$ 0.02	2.87 $\pm$ 0.13	0.03 $\pm$ 0.0004	294.25 $\pm$ 7.30	1.30 $\pm$ 0.04	0.01 $\pm$ 0.0005
C ₁ M ₁ SA ₅	4.95 $\pm$ 0.06	1.14 $\pm$ 0.02	0.18 $\pm$ 0.02	258.25 $\pm$ 1.44	1.10 $\pm$ 0.03	0.007 $\pm$ 0.0006
C ₁ M ₂ SA ₁	4.07 $\pm$ 0.03	4.48 $\pm$ 0.23	0.12 $\pm$ 0.04	277.00 $\pm$ 12.06	0.91 $\pm$ 0.07	0.01 $\pm$ 0.0001
C ₁ M ₂ SA ₂	4.50 $\pm$ 0.34	2.07 $\pm$ 0.15	0.06 $\pm$ 0.0002	298.25 $\pm$ 5.71	2.20 $\pm$ 0.12	0.02 $\pm$ 0.0003
C ₁ M ₂ SA ₃	3.70 $\pm$ 0.40	3.35 $\pm$ 0.14	0.10 $\pm$ 0.0002	299.00 $\pm$ 12.02	2.00 $\pm$ 0.09	0.01 $\pm$ 0.0002
C ₁ M ₂ SA ₄	2.45 $\pm$ 0.16	2.37 $\pm$ 0.04	0.05 $\pm$ 0.0004	292.50 $\pm$ 9.67	2.17 $\pm$ 0.25	0.01 $\pm$ 0.0001
C ₁ M ₂ SA ₅	4.50 $\pm$ 0.40	4.18 $\pm$ 0.12	0.04 $\pm$ 0.0001	279.75 $\pm$ 8.37	1.14 $\pm$ 0.20	0.01 $\pm$ 0.0004
C ₂ M ₁ SA ₁	1.15 $\pm$ 0.34	1.11 $\pm$ 0.19	0.01 $\pm$ 0.0001	340.25 $\pm$ 15.42	4.00 $\pm$ 0.19	0.02 $\pm$ 0.0001
C ₂ M ₁ SA ₂	1.50 $\pm$ 0.12	1.38 $\pm$ 0.16	0.14 $\pm$ 0.03	298.00 $\pm$ 5.65	0.95 $\pm$ 0.16	0.02 $\pm$ 0.0002
C ₂ M ₁ SA ₃	1.80 $\pm$ 0.20	1.95 $\pm$ 0.05	0.03 $\pm$ 0.0002	289.75 $\pm$ 5.33	1.62 $\pm$ 0.18	0.01 $\pm$ 0.0001
C ₂ M ₁ SA ₄	1.70 $\pm$ 0.13	1.18 $\pm$ 0.22	0.03 $\pm$ 0.0001	296.25 $\pm$ 2.42	1.45 $\pm$ 0.22	0.01 $\pm$ 0.0002
C ₂ M ₁ SA ₅	1.25 $\pm$ 0.16	1.53 $\pm$ 0.09	0.01 $\pm$ 0.0001	341.25 $\pm$ 7.66	1.29 $\pm$ 0.08	0.02 $\pm$ 0.0003
C ₂ M ₂ SA ₁	1.20 $\pm$ 0.14	2.99 $\pm$ 0.03	0.08 $\pm$ 0.0004	306.50 $\pm$ 4.94	0.89 $\pm$ 0.16	0.01 $\pm$ 0.0001
C ₂ M ₂ SA ₂	1.60 $\pm$ 0.24	1.54 $\pm$ 0.04	0.06 $\pm$ 0.0002	300.50 $\pm$ 9.79	1.84 $\pm$ 0.05	0.01 $\pm$ 0.0004
C ₂ M ₂ SA ₃	1.45 $\pm$ 0.30	1.82 $\pm$ 0.06	0.06 $\pm$ 0.0003	300.00 $\pm$ 2.25	0.80 $\pm$ 0.04	0.01 $\pm$ 0.0001
C ₂ M ₂ SA ₄	2.60 $\pm$ 0.24	1.88 $\pm$ 0.04	0.04 $\pm$ 0.0002	303.25 $\pm$ 17.14	1.39 $\pm$ 0.03	0.01 $\pm$ 0.0004
C ₂ M ₂ SA ₅	1.43 $\pm$ 0.18	1.55 $\pm$ 0.22	0.08 $\pm$ 0.0004	285.00 $\pm$ 7.77	0.95 $\pm$ 0.04	0.01 $\pm$ 0.0001

$\pm$ - Standard error of the mean (n = 4). C₁ - Irrigation with 100% ETr; C₂ - Irrigation with 50% ETr; M₁ - 'Crioula' genetic material; M₂ - 'Paluma' genetic material; SA₁ - Salicylic acid (0 mM); SA₂ - Salicylic acid (1.2 mM); SA₃ - salicylic acid (2.4 mM); SA₄ - Salicylic acid (3.6 mM); SA₅ - Salicylic acid (4.8 mM); A - CO₂ assimilation rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$); E - Transpiration ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$); gs - Stomatal conductance ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$); Ci - Internal carbon concentration ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$); WUE - Instantaneous water use efficiency [$(\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}) / (\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1})$]; iCE - Instantaneous carboxylation efficiency [$(\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}) / (\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1})$]

maintenance of the photosynthetic apparatus, stimulating the activity of antioxidant enzymes that help protect plants against damage caused by oxidative stress, besides influencing stomatal opening, facilitating the entry of CO₂, increasing its assimilation rate (Zafar et al., 2021). Likewise, Fatima et al. (2023) studied the application of SA in yellow passion fruit and observed that foliar application of SA at concentration of 1.30 mM attenuates the effects of water deficit (50% ETr) on gas exchange and growth.

Internal carbon concentration, water use efficiency and instantaneous carboxylation efficiency in plants of the 'Crioula' genetic material without SA application under 50% ETr were respectively 11.10, 349.43 and 61.54% higher than those observed in the 'Paluma' genetic material (Table 2). This result can be explained by the fact that 'Crioula' guava is a rustic plant material, adapted to the edaphoclimatic conditions of the northeastern semi-arid region, being more efficient in the use of water and CO₂ carboxylation (Souza et al., 2016).

It was also observed that 'Crioula' guava under 100% ETr and application of SA at concentration of 2.4 mM showed the lowest values of internal carbon concentration (234 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), instantaneous water use efficiency (0.41 [$(\mu\text{mol CO}_2$

$\text{m}^{-2} \text{ s}^{-1}) / (\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1})$]) and instantaneous carboxylation efficiency (0.003 [$(\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}) / (\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1})$]), while plants under 50% ETr showed increments in the values of these variables, which decreased with the application of SA concentrations (Table 2).

This is consistent with the reduction of stomatal conductance and transpiration under 50% ETr, which increases water use efficiency under water deficit. The stomatal response observed in plants subjected to this deficit can be attributed to the production of abscisic acid, contributing to the maintenance of leaf water potential even under conditions of water scarcity (Soares et al., 2023). However, the application of SA increased enzymatic and photosynthetic functions, reducing the efficiency (Silva et al., 2022).

In the 'Crioula' genetic material, the contents of chlorophyll a, chlorophyll b, and chlorophyll total increased by 29.79, 18.26 and 25.96% with application of SA up to 2.4 mM under 100% ETr and carotenoid levels decreased (14.34%); likewise, for these same plants under water restriction, chlorophyll contents increased up to SA concentration of 3.6 mM and carotenoid contents decreased (Table 3). 'Paluma' guava under water restriction showed higher mean values than those of

Table 3. Eigenvalues, percentage of total variance explained in the multivariate analysis of variance, and probability of significance by Hotelling's test (p-value) for the factors

					Principal components (PC)				
					PC1		PC2		
Eigenvalues (λ)					10.27		2.63		
Percentage of total variance ($S^2\%$)					48.92		12.54		
Manova p									
Lawley-Hotelling test for water condition (C)					<0.01		<0.01		
Lawley-Hotelling test for salicylic acid concentrations (SA)					<0.01		<0.01		
Lawley-Hotelling test for genetic material (M)					<0.01		<0.01		
Lawley-Hotelling test for C $\times$ SA					<0.01		<0.01		
Lawley-Hotelling test for C $\times$ M					<0.01		<0.02		
Lawley-Hotelling test for SA $\times$ M					<0.01		<0.01		
Lawley-Hotelling test for C $\times$ SA $\times$ M					<0.01		<0.03		
PCs		Correlation coefficients (r)							
		Chla	Chlb	Chlt	Carot	F ₀	F _m	F _v	F _v /F _m
PC1		0.52	0.64	0.60	-0.50	0.77	0.64	0.63	0.67
PC2		-0.80	-0.65	-0.77	0.10	-0.12	0.38	0.27	0.32
Treatment									
C ₁ M ₁ SA ₁		2414.30 $\pm$ 159.73	1199.08 $\pm$ 166.40	3613.37 $\pm$ 220.25	619.16 $\pm$ 25.50	85.50 $\pm$ 4.99	407.75 $\pm$ 27.01	404.50 $\pm$ 16.74	0.78 $\pm$ 0.02
C ₁ M ₁ SA ₂		2400.10 $\pm$ 111.34	1005.14 $\pm$ 90.94	3405.23 $\pm$ 148.38	713.73 $\pm$ 30.67	93.50 $\pm$ 2.95	503.50 $\pm$ 17.87	460.00 $\pm$ 14.66	0.80 $\pm$ 0.01
C ₁ M ₁ SA ₃		3133.37 $\pm$ 161.65	1418.09 $\pm$ 80.12	4551.46 $\pm$ 249.39	530.38 $\pm$ 39.00	120.00 $\pm$ 3.48	550.75 $\pm$ 14.90	455.25 $\pm$ 27.41	0.84 $\pm$ 0.01
C ₁ M ₁ SA ₄		2359.05 $\pm$ 200.72	861.76 $\pm$ 70.96	3220.82 $\pm$ 199.72	710.49 $\pm$ 33.22	101.75 $\pm$ 2.75	547.00 $\pm$ 24.94	445.25 $\pm$ 22.63	0.81 $\pm$ 0.02
C ₁ M ₁ SA ₅		2382.38 $\pm$ 129.06	933.21 $\pm$ 116.25	3315.59 $\pm$ 217.13	709.05 $\pm$ 36.21	90.50 $\pm$ 4.87	467.50 $\pm$ 27.36	377.00 $\pm$ 25.36	0.80 $\pm$ 0.01
C ₁ M ₂ SA ₁		2193.59 $\pm$ 220.02	932.52 $\pm$ 60.10	3126.11 $\pm$ 120.61	642.03 $\pm$ 32.73	85.00 $\pm$ 2.35	497.00 $\pm$ 21.95	412.00 $\pm$ 15.19	0.83 $\pm$ 0.00
C ₁ M ₂ SA ₂		2209.71 $\pm$ 183.09	988.02 $\pm$ 95.67	3197.73 $\pm$ 114.30	643.46 $\pm$ 44.09	99.00 $\pm$ 2.79	553.50 $\pm$ 26.79	358.00 $\pm$ 13.97	0.83 $\pm$ 0.01
C ₁ M ₂ SA ₃		2446.37 $\pm$ 158.26	1174.97 $\pm$ 66.97	3621.34 $\pm$ 196.61	645.22 $\pm$ 31.01	95.50 $\pm$ 0.85	457.50 $\pm$ 12.59	337.50 $\pm$ 18.56	0.80 $\pm$ 0.02
C ₁ M ₂ SA ₄		2727.19 $\pm$ 217.73	1123.67 $\pm$ 118.72	3850.86 $\pm$ 217.13	781.51 $\pm$ 37.40	103.2 $\pm$ 3.70	496.50 $\pm$ 32.47	393.25 $\pm$ 13.78	0.83 $\pm$ 0.03
C ₁ M ₂ SA ₅		2186.29 $\pm$ 199.48	915.81 $\pm$ 101.13	3102.09 $\pm$ 111.13	791.06 $\pm$ 41.37	98.25 $\pm$ 2.96	525.75 $\pm$ 15.32	427.50 $\pm$ 23.10	0.81 $\pm$ 0.01
C ₂ M ₁ SA ₁		1993.73 $\pm$ 160.13	764.62 $\pm$ 133.51	2758.35 $\pm$ 221	832.34 $\pm$ 15.82	80.00 $\pm$ 2.82	373.50 $\pm$ 16.45	283.50 $\pm$ 18.56	0.72 $\pm$ 0.02
C ₂ M ₁ SA ₂		2202.82 $\pm$ 78.40	865.69 $\pm$ 110.70	3068.51 $\pm$ 141.12	698.92 $\pm$ 19.33	91.25 $\pm$ 1.47	467.75 $\pm$ 23.41	376.50 $\pm$ 20.90	0.80 $\pm$ 0.01
C ₂ M ₁ SA ₃		2123.45 $\pm$ 120.97	872.32 $\pm$ 131.74	2995.77 $\pm$ 153.21	602.94 $\pm$ 20.82	87.25 $\pm$ 1.46	505.00 $\pm$ 16.87	417.75 $\pm$ 11.24	0.83 $\pm$ 0.03
C ₂ M ₁ SA ₄		2328.79 $\pm$ 143.39	923.39 $\pm$ 144.23	3252.18 $\pm$ 174.15	672.07 $\pm$ 35.94	95.25 $\pm$ 1.02	485.50 $\pm$ 28.56	390.25 $\pm$ 33.96	0.80 $\pm$ 0.01
C ₂ M ₁ SA ₅		2162.00 $\pm$ 154.49	890.87 $\pm$ 142.50	3052.87 $\pm$ 253.12	605.32 $\pm$ 13.50	94.00 $\pm$ 7.39	500.25 $\pm$ 20.82	406.25 $\pm$ 31.16	0.81 $\pm$ 0.02
C ₂ M ₂ SA ₁		2354.16 $\pm$ 185.91	864.33 $\pm$ 102.21	3218.49 $\pm$ 132.45	688.65 $\pm$ 12.77	93.00 $\pm$ 3.52	534.00 $\pm$ 9.48	441.00 $\pm$ 16.77	0.79 $\pm$ 0.01
C ₂ M ₂ SA ₂		2851.99 $\pm$ 148.87	1128.40 $\pm$ 127.68	3980.39 $\pm$ 147.12	662.13 $\pm$ 35.42	89.50 $\pm$ 2.32	453.00 $\pm$ 11.24	363.50 $\pm$ 14.41	0.72 $\pm$ 0.02
C ₂ M ₂ SA ₃		2482.47 $\pm$ 154.49	936.26 $\pm$ 56.12	3418.73 $\pm$ 126.12	743.38 $\pm$ 25.62	86.00 $\pm$ 5.86	446.50 $\pm$ 23.96	360.50 $\pm$ 24.13	0.80 $\pm$ 0.01
C ₂ M ₂ SA ₄		2640.19 $\pm$ 185.91	1087.16 $\pm$ 111.04	3727.36 $\pm$ 147.14	766.32 $\pm$ 40.90	90.00 $\pm$ 2.59	438.00 $\pm$ 21.16	322.25 $\pm$ 26.88	0.81 $\pm$ 0.03
C ₂ M ₂ SA ₅		2274.46 $\pm$ 141.86	891.81 $\pm$ 20.57	3166.28 $\pm$ 128.14	743.35 $\pm$ 12.77	85.00 $\pm$ 4.50	460.25 $\pm$ 16.77	375.25 $\pm$ 11.24	0.81 $\pm$ 0.02

$\pm$ - Standard error of the mean (n = 4). C₁ - Irrigation with 100% ETr; C₂ - Irrigation with 50% ETr; M₁ - 'Crioula' genetic material; M₂ - 'Paluma' genetic material; SA₁ - Salicylic acid (0 mM); SA₂ - Salicylic acid (1.2 mM); SA₃ - Salicylic acid (2.4 mM); SA₄ - Salicylic acid (3.6 mM); SA₅ - Salicylic acid (4.8 mM); Chl a - Chlorophyll a ($\mu\text{g mL}^{-1}$); Chl b - Chlorophyll b ($\mu\text{g mL}^{-1}$); Chl t - Chlorophyll total ($\mu\text{g mL}^{-1}$); Carot - Carotenoid carotenoids ($\mu\text{g mL}^{-1}$); Fo - Initial fluorescence; Fm - Maximum fluorescence; Fv - Variable fluorescence; Fv/Fm - Quantum efficiency of photosystem II

the 'Crioula' genetic material under 50% ETr for chlorophyll a (18.07%), chlorophyll b (13.03%) and chlorophyll total (16.68%) with 0 mM of SA (Table 3). Additionally, in plants of the cv. 'Paluma' chlorophyll content increased and carotenoid content decreased up to 1.2 mM of SA under 50% ETr.

During periods of water deficit, plants reduce chlorophyll synthesis and increase carotenoid synthesis as part of their antioxidant defense mechanism (Zafar et al., 2021). However, with the application of SA, there was a reversal of the process, because the acid reduces chlorophyll degradation under stressful conditions, reducing damage to the photosynthetic apparatus induced by the accumulation of compatible solutes and reducing the level of reactive oxygen species, as well as improving the activity of chlorophyll-synthesizing enzymes (Saheri et al., 2020). Xavier et al. (2022) found that application of SA to guava at concentrations of 1.60 and 1.50 mM increased the concentrations of chlorophyll b and chlorophyll total, respectively.

The initial, maximum and variable fluorescence and quantum efficiency of photosystem II in guava genetic materials were reduced with water restriction, but 'Paluma' guava had 16.25, 42.97, 55.55, and 11.26% higher values, respectively, compared to 'Crioula' under water restriction of 50% ETr and 0 mM of SA (Table 3). As observed for the chlorophyll

content, the initial, maximum and variable fluorescence and the instantaneous carboxylation efficiency of 'Crioula' guava plants were higher with the application of SA at concentration of 2.4 mM under 100% ETr, while under water restriction there were reductions in the values of these variables, but SA application mitigated this decrease, with higher values at the SA concentration of 4.8 mM (Table 3). The reductions in Fm, Fv and Fv/Fm highlight the deleterious effects of water deficit on the photoactivation of photosystem II (Medyouni et al., 2021).

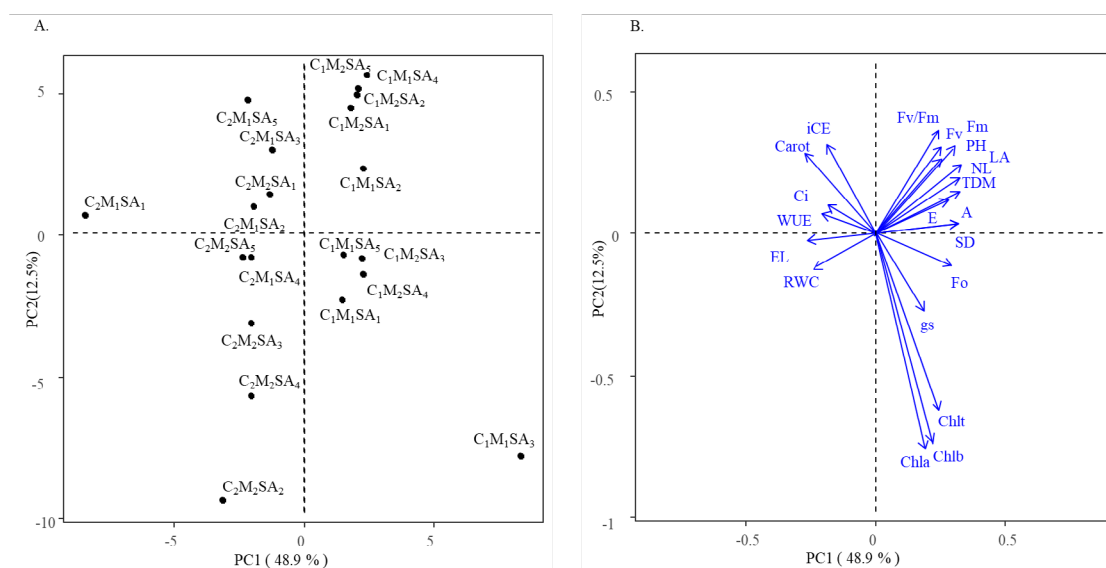
However, the application of SA increased chlorophyll a fluorescence, and this protective effect can be attributed to the ability of SA to increase the electron transport rate and the quantum yield of photosystem II, as well as increasing the relative content of protein D1 in stressed plants (Silva et al., 2023). Xavier et al. (2022) also reported increments in fluorescence with foliar application of SA at concentration of 0.9 mM in guava.

In 'Crioula' guava plants without SA application and subjected to water restriction (50% ETr), the mean values of relative water content and electrolyte leakage increased, being equal to 76.18 and 23.12%, respectively. SA application reduced these variables, and the most notable effects were observed with the concentration of 1.2 mM for the relative water content and 2.4 mM for electrolyte leakage.

Table 4. Eigenvalues, percentage of total variance explained in the multivariate analysis of variance, and probability of significance by Hotelling's test (p-value) for the factors

	Principal components (PC)						
	PC1						PC2
Eigenvalues (λ)	10.27						2.63
Percentage of total variance ($S^2\%$)	48.92						12.54
Manova p							
Lawley-Hotelling test for water condition (C)	<0.01						<0.01
Lawley-Hotelling test for salicylic acid concentrations (SA)	<0.01						<0.01
Lawley-Hotelling test for genetic material (M)	<0.01						<0.01
Lawley-Hotelling test for C $\times$ SA	<0.01						<0.01
Lawley-Hotelling test for C $\times$ M	<0.01						<0.02
Lawley-Hotelling test for SA $\times$ M	<0.01						<0.01
Lawley-Hotelling test for C $\times$ SA $\times$ M	<0.01						<0.03
PCs	Correlation coefficients (r)						
	RWC	EL	PH	SD	NL	LA	TDM
PC1	-0.61	-0.69	0.83	0.84	0.87	0.85	0.85
PC2	-0.10	-0.02	0.36	0.03	0.25	0.21	0.15
Treatment							
C ₁ M ₁ SA ₁	69.10 $\pm$ 2.52	13.78 $\pm$ 0.51	79.06 $\pm$ 3.89	7.16 $\pm$ 0.27	27.75 $\pm$ 1.68	1154.48 $\pm$ 73.53	18.76 $\pm$ 0.77
C ₁ M ₁ SA ₂	59.63 $\pm$ 1.74	13.37 $\pm$ 1.62	77.13 $\pm$ 3.91	7.42 $\pm$ 0.17	24.88 $\pm$ 0.74	1033.29 $\pm$ 57.44	19.65 $\pm$ 0.39
C ₁ M ₁ SA ₃	76.19 $\pm$ 2.43	13.05 $\pm$ 1.57	82.25 $\pm$ 4.74	7.74 $\pm$ 0.34	29.13 $\pm$ 0.51	1257.00 $\pm$ 61.16	19.98 $\pm$ 0.10
C ₁ M ₁ SA ₄	51.24 $\pm$ 1.39	13.47 $\pm$ 0.71	77.50 $\pm$ 2.52	7.21 $\pm$ 0.49	27.88 $\pm$ 1.23	1122.40 $\pm$ 46.29	17.75 $\pm$ 0.88
C ₁ M ₁ SA ₅	60.29 $\pm$ 2.82	18.55 $\pm$ 1.42	73.00 $\pm$ 1.76	6.96 $\pm$ 0.27	27.00 $\pm$ 1.53	1225.90 $\pm$ 32.51	17.49 $\pm$ 0.95
C ₁ M ₂ SA ₁	68.22 $\pm$ 3.80	18.64 $\pm$ 1.37	79.00 $\pm$ 1.16	7.54 $\pm$ 0.59	28.13 $\pm$ 1.42	1094.03 $\pm$ 58.18	17.43 $\pm$ 0.51
C ₁ M ₂ SA ₂	56.95 $\pm$ 2.08	13.04 $\pm$ 1.48	77.38 $\pm$ 3.89	7.41 $\pm$ 0.24	28.88 $\pm$ 0.71	1240.20 $\pm$ 59.34	18.05 $\pm$ 0.70
C ₁ M ₂ SA ₃	53.18 $\pm$ 2.86	13.09 $\pm$ 2.25	81.13 $\pm$ 2.89	6.57 $\pm$ 0.52	28.75 $\pm$ 2.31	1017.07 $\pm$ 64.76	19.88 $\pm$ 0.83
C ₁ M ₂ SA ₄	59.50 $\pm$ 1.44	13.70 $\pm$ 1.16	77.50 $\pm$ 3.59	7.31 $\pm$ 0.25	28.00 $\pm$ 1.95	1200.53 $\pm$ 68.49	19.84 $\pm$ 0.48
C ₁ M ₂ SA ₅	57.17 $\pm$ 1.64	17.83 $\pm$ 1.41	81.75 $\pm$ 2.97	7.13 $\pm$ 0.27	25.88 $\pm$ 0.42	1192.62 $\pm$ 58.45	18.28 $\pm$ 0.50
C ₂ M ₁ SA ₁	48.20 $\pm$ 2.70	23.13 $\pm$ 1.50	47.50 $\pm$ 3.55	4.99 $\pm$ 0.38	14.38 $\pm$ 1.32	497.54 $\pm$ 79.21	8.68 $\pm$ 0.71
C ₂ M ₁ SA ₂	61.46 $\pm$ 2.77	17.89 $\pm$ 1.46	65.38 $\pm$ 3.25	5.19 $\pm$ 0.51	22.13 $\pm$ 1.39	787.22 $\pm$ 48.45	14.58 $\pm$ 0.49
C ₂ M ₁ SA ₃	67.96 $\pm$ 3.57	16.36 $\pm$ 1.95	67.00 $\pm$ 3.20	5.81 $\pm$ 0.38	22.00 $\pm$ 1.95	764.00 $\pm$ 61.70	13.53 $\pm$ 0.74
C ₂ M ₁ SA ₄	69.94 $\pm$ 1.52	16.43 $\pm$ 1.72	61.13 $\pm$ 3.99	5.11 $\pm$ 0.24	20.88 $\pm$ 1.32	704.60 $\pm$ 64.77	11.81 $\pm$ 0.63
C ₂ M ₁ SA ₅	52.01 $\pm$ 3.36	17.73 $\pm$ 1.50	59.88 $\pm$ 0.32	5.34 $\pm$ 0.07	20.83 $\pm$ 1.33	619.01 $\pm$ 22.70	9.11 $\pm$ 0.55
C ₂ M ₂ SA ₁	65.47 $\pm$ 0.57	13.06 $\pm$ 1.46	63.63 $\pm$ 3.17	5.58 $\pm$ 0.23	18.13 $\pm$ 1.29	586.79 $\pm$ 51.02	10.59 $\pm$ 0.37
C ₂ M ₂ SA ₂	72.44 $\pm$ 2.77	19.59 $\pm$ 1.72	50.13 $\pm$ 0.94	6.26 $\pm$ 0.07	16.75 $\pm$ 0.86	592.87 $\pm$ 48.19	10.48 $\pm$ 0.56
C ₂ M ₂ SA ₃	54.57 $\pm$ 3.40	15.64 $\pm$ 0.62	47.75 $\pm$ 3.30	5.70 $\pm$ 0.25	19.75 $\pm$ 1.66	717.37 $\pm$ 62.29	9.73 $\pm$ 0.28
C ₂ M ₂ SA ₄	59.24 $\pm$ 2.91	16.36 $\pm$ 1.84	48.25 $\pm$ 3.87	6.27 $\pm$ 0.10	18.13 $\pm$ 0.61	560.12 $\pm$ 47.67	10.95 $\pm$ 0.42
C ₂ M ₂ SA ₅	61.61 $\pm$ 1.65	17.47 $\pm$ 1.20	54.00 $\pm$ 2.47	5.31 $\pm$ 0.21	17.00 $\pm$ 1.19	552.55 $\pm$ 61.70	11.48 $\pm$ 0.50

$\pm$ - Standard error of the mean (n = 4). C₁ - Irrigation with 100% ETr; C₂ - Irrigation with 50% ETr; M₁ - 'Crioula' genetic material; M₂ - 'Paluma' genetic material; SA₁ - Salicylic acid (0 mM); SA₂ - Salicylic acid (1.2 mM); SA₃ - Salicylic acid (2.4 mM); SA₄ - Salicylic acid (3.6 mM); SA₅ - Salicylic acid (4.8 mM); RWC - Relative water content (%); EL - Electrolyte leakage (%); PH - Plant height (cm); SD - Stem diameter (mm); NL - Number of leaves; LA - Leaf area (cm²); TDM - Total dry mass (g per plant)



C₁ - Irrigation with 100% ETr; C₂ - Irrigation with 50% ETr; M₁ - 'Crioula' genetic material; M₂ - 'Paluma' genetic material; SA₁ - Salicylic acid (0 mM); SA₂ - Salicylic acid (1.2 mM); SA₃ - Salicylic acid (2.4 mM); SA₄ - Salicylic acid (3.6 mM); SA₅ - Salicylic acid (4.8 mM); A - CO₂ assimilation rate; E - Transpiration; gs - Stomatal conductance; Ci - Internal carbon concentration; WUE - Instantaneous water use efficiency; iCE - Instantaneous carboxylation efficiency; Chl a - Chlorophyll a; Chl b - Chlorophyll b; Chl t - Total chlorophyll; Carot - Carotenoids; Fo - Initial fluorescence; Fm - Maximum fluorescence; Fv - Variable fluorescence; Fv/Fm - Quantum efficiency of photosystem II; RWC - Relative water content; EL - Electrolyte leakage; PH - Plant height; SD - Stem diameter; NL - Number of leaves; LA - Leaf area; TDM - Total dry mass

Figure 2. Two-dimensional projection of the principal component scores for the factors irrigation water regimes (C), genetic materials (M) and salicylic acid concentrations (A) and the analyzed variables (B) in the two principal components (PC1 and PC2)

The reduction of electrolyte leakage in SA-treated plants under conditions of water deficit suggests greater cell membrane integrity, which can be attributed to the activation of antioxidant defense mechanisms induced by SA (Kaur et al., 2022). On the other hand, the increase in electrolyte leakage in plants without SA treatment under water restriction indicates greater cell membrane permeability, a common phenomenon in situations of water deficit due to abscisic acid activation (Soares et al., 2023).

Plants of the 'Crioula' genetic material irrigated with 50% ETr had the highest relative water content and electrolyte leakage without application of SA (Table 4). This is justified because the integrity of cell membranes is essential for the maintenance of cell homeostasis and for the proper function of plant cells; thus, when the membranes are not damaged under water stress, the cell does not lose its capacity to regulate ion gradients and water entry, which does not compromise vital processes such as nutrient absorption and the synthesis of organic compounds, maintaining growth (Chavouski et al., 2023).

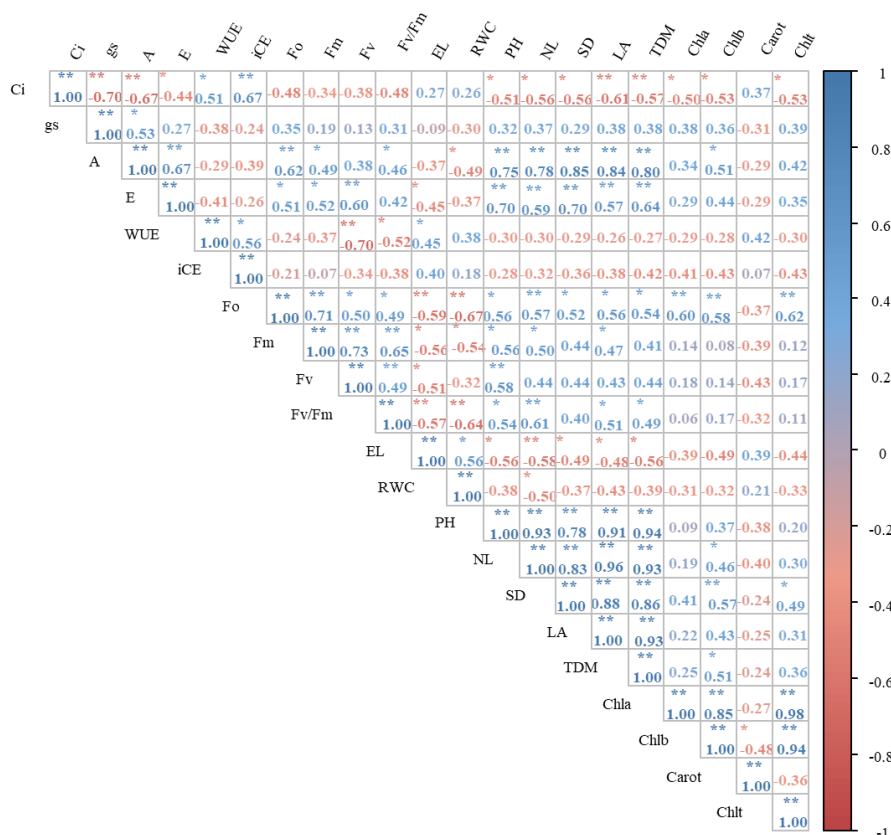
Plant height, stem diameter, number of leaves, leaf area, and total dry mass were higher in the 'Crioula' genetic material without water restriction (100% ETr) and with application of 2.4 mM of SA, while the lowest values were observed in plants under water deficit (50% ETr) and without application of SA, with decreases of 42.25, 35.5, 50.64, 60.42, and 56.58% when compared to plants under full irrigation, respectively (Table 4).

The growth of plants under water restriction is inhibited due to stomatal closure, which is a strategic mechanism used by

plants to avoid excessive water loss, resulting in a lower influx of CO₂ into plant cells, reducing photosynthetic efficiency and causing oxidative damage (Lobato et al., 2021). Souza et al. (2023), when investigating the impact of water deficit on the physiology of *Psidium myrtilloides*, observed that plants reduced gas exchange and growth when subjected to water restriction.

Despite the reductions observed in plants under water deficit, the application of SA resulted in increases in the mean values of these variables compared to seedlings that did not receive SA under the same conditions. For the 'Crioula' genetic material, increases in leaf area, number of leaves, and total dry mass were observed up to the concentration of 1.2 mM of SA. In the case of the 'Paluma' material, the highest values were obtained at the following SA concentrations: 4.8 mM for total dry mass, 3.6 mM for stem diameter, 2.4 mM for number of leaves, and leaf area, and 1.2 mM for plant height. This is due to the fact that SA plays an important role in modulating cellular redox balance and protecting plants from oxidative damage (Zafar et al., 2021). In addition, SA acts to reduce the osmotic potential of the root, contributing to the absorption of water and nutrients and to cell turgor, facilitating growth (Fatima et al., 2023).

According to the correlation matrix, guava plants with higher growth and dry mass accumulation have a higher positive correlation with CO₂ assimilation rate, transpiration and chlorophyll *b* content. Simultaneously, these variables are negatively correlated with internal CO₂ concentration and electrolyte leakage (Figure 3).



A - CO₂ assimilation rate, E - Transpiration; gs - Stomatal conductance; Ci - Internal carbon concentration; WUE - Instantaneous water use efficiency; iCE - Instantaneous carboxylation efficiency, Chl a - Chlorophyll a; Chl b - Chlorophyll b; Chl t - Chlorophyll total; Carot - Carotenoids; Fo - Initial fluorescence; Fm - Maximum fluorescence; Fv - Variable fluorescence; Fv/Fm - Quantum efficiency of photosystem II; RWC - Relative water content; EL - Electrolyte leakage; PH - Plant height; SD - Stem diameter; NL - Number of leaves; LA - Leaf area; TDM - Total dry mass

Figure 3. Pearson's correlation between growth and physiological variables of guava genetic materials under water conditions and salicylic acid concentrations

CO₂ assimilation rate is fundamental for photosynthesis, so plants with a higher CO₂ assimilation rate exhibit more robust growth, as this implies greater biomass production and consequently less membrane damage (Silva et al., 2022). Therefore, the attenuation of water deficit by SA is related to the maintenance of gas exchange, which implies a lower reduction in plant growth, corroborating the results reported by Fatima et al. (2023). In general, the results obtained in this study meet the expectations and confirm the hypothesis that foliar application of SA is capable of inducing tolerance to water deficit in guava plants.

CONCLUSIONS

1. Foliar application of salicylic acid at concentration of 2.4 mM increases the growth, gas exchange, and chlorophyll a fluorescence of 'Crioula' guava under irrigation with 50% of the reference evapotranspiration (ET_r).

2. Water deficit (50% ET_r) reduced growth, gas exchange, chlorophyll contents, and chlorophyll a fluorescence of guava.

3. The genetic material 'Paluma' showed greater growth, gas exchange, chlorophyll content, and chlorophyll a fluorescence compared to 'Crioula' under irrigation with 50% of ET_r without foliar application of salicylic acid.

4. Foliar application of salicylic acid at concentration of 1.2 mM increases leaf area, number of leaves, and total dry mass in the 'Crioula' genetic material under 50% ET_r.

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LITERATURE CITED

- Abrar, M. M.; Sohail, M.; Saqib, M.; Ahtar, J.; Abbas, G.; Wahab, H. A.; Mumtaz, M. Z.; Mehmood, K.; Memon, M. S.; Sol, N.; Xu, M. Interactive salinity and water stress severely reduced the growth, stress tolerance, and physiological responses of guava (*Psidium guajava* L.). *Scientific Reports*, v.12, e18952, 2022. <https://doi.org/10.1038/s41598-022-22602-5>
- Ahanger, M. A.; Aziz, U.; Alsahli, A. A.; Alyemeni, M. N.; Ahmad, P. Influence of exogenous salicylic acid and nitric oxide on growth, photosynthesis, and ascorbate- glutathione cycle in salt stressed *Vigna angularis*. *Biomolecules*, v.10, p.1-16, 2019. <https://doi.org/10.3390/biom10010042>
- Angulo-López, J. E.; Flores - Gallegos, A.C.; Torres-León, C.; Ramírez-Guzmán, K. N.; Martínez, G. A.; Aguilar, C. N. Guava (*Psidium guajava* L.) fruit and valorization of industrialization by - products. *Processes*, v.9, e1075, 2021. <https://doi.org/10.3390/pr9061075>
- Arnon, D. I. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiology*, v.24, p.1-15, 1949. <https://doi.org/10.1104/2Fpp.24.1.1>
- Chavouski, M.; Najafi, F.; Salimi, A.; Angaji, S. A. Effect of salicylic acid and sodium nitroprusside on growth parameters, photosynthetic pigments and secondary metabolites of safflower under drought stress. *Scientia Horticulturae*, v.259, e108823, 2020. <https://doi.org/10.1016/j.scienta.2019.108823>
- Fatima, R. T.; Lima, G. S. de; Soares, L. A. dos A.; Veloso, L. L. S. A.; Silva, A. A. R.; Lacerda, C.N. de; Silva, F. A.; Nobrega, J. S.; Ferreira, J. T. A.; Pereira, W. E. Salicylic acid concentrations and forms of application mitigate water stress in sour passion fruit seedlings. *Brazilian Journal of Biology*, v.83, e270865, 2023. <https://doi.org/10.1590/1519-6984.270865>
- Govaerts, B.; Sayre, K.D.; Lichter, K.; Dendooven, L.; Deckers, J. Influence of permanent raised bed planting and residue management on physical and chemical soil quality in rainfed maize/wheat systems. *Plant and Soil*, v.291, p.39-54, 2007. <http://dx.doi.org/10.1007/s11104-006-9172-6>
- Hotelling, H.; Eisenhart, C.; Hastay, M.W.; Wallis, W.A. *Multivariate quality control: techniques of statistical analysis* New York: John Wiley & Sons, 1947. 73p.
- IBGE - Instituto Brasileiro de Geografia e Estatística. Produção agrícola - lavoura permanente, safra - 2023. Available at: < <https://www.ibge.gov.br/explica/producao-agropecuaria/goiaba/br> >. Access on: 22 Oct. 2024.
- Kang, J.; Chu, Y.; Ma, G.; Zhang, Y.; Zhang, X.; Wang, M.; Lu, H.; Wang, L.; Kang, G.; Ma, D.; Xie, Y.; Wang, C. Physiological mechanisms underlying reduced photosynthesis in wheat leaves grown in the field under conditions of nitrogen and water deficiency. *The Crop Journal*, v.11, p.638-650, 2023. <https://doi.org/10.1016/j.cj.2022.06.010>
- Kaur, G.; Tak, Y.; Asthir, B. Salicylic acid: A key signal molecule ameliorating plant stresses. *Cereal Research Communications*, v.50, p.617-626, 2022. <https://doi.org/10.1007/s42976-021-00236-z>
- Lacerda, C. N. de; Lima, G. S. de; Soares, L. A. dos A.; Arruda, T. F. de L.; Silva, A. A. R. da; Gheyi, H. R.; Dantas, M. V.; Ferreira, J. T. A. Foliar application of ascorbic acid in guava cultivation under water replacement levels. *Revista Caatinga*, v.38, e12595, 2025. <https://doi.org/10.1590/1983-21252025v3812595rc>
- Lacerda, C. N. de; Lima, G. S. de; Soares, L. A. dos A., Fátima, R. T. de; Gheyi, H. R., Azevedo, C. A. de. Morphophysiology and production of guava as a function of water salinity and salicylic acid. *Revista Brasileira de Engenharia Agrícola e Ambiental*, v.26, p.451-458, 2022. <https://doi.org/10.1590/1807-1929/agriambi.v26n6p451-458>
- Lobato, A. K. da S.; Barbosa, M. A. M.; Alsahli, A. A.; Lima, E. J. A.; Silva, B. R. S. da. Exogenous salicylic acid alleviates the negative impacts on production components, biomass and gas exchange in tomato plants under water deficit improving redox status and anatomical responses. *Physiologia Plantarum*, v.172, p.869-884, 2021. <https://doi.org/10.1111/plpl.13329>

- Lucena, C. Y. de S.; Santos, D. J. R. dos; Silva, P. L. S. da; Costa, E. D. da; Lucena, R. L. O reuso de águas residuais como meio de convivência com a seca no semiárido do Nordeste brasileiro. *Revista de Geociências do Nordeste*, v.4, p.1-17, 2018. <https://doi.org/10.21680/2447-3359.2018v4n0ID13321>
- Macedo, J. G. F.; Rangel, J. M. L.; Santos, M. de O.; Camilo, C. J.; Costa, J. G. M. da; Souza, M. M. de A. Therapeutic indications, chemical composition and biological activity of native Brazilian species from *Psidium* genus (Myrtaceae): A review. *Journal of Ethnopharmacology*, v.278, e114248, 2021. <https://doi.org/10.1016/j.jep.2021.114248>
- Medyouni, I.; Zouaoui, R.; Rubio, E.; Serino, S.; Ahmed, H. B.; Bertin, N. Effects of water deficit on leaves and fruit quality during the development period in tomato plant. *Food Science & Nutrition*, v.9, p.1949-1960, 2021. <https://doi.org/10.1002/fsn3.2160>
- Melo, F. dos S. N. de; Silva, S. de M.; Sousa, A. S. B. de; Rodrigues, A. A. M.; Melo, R. de S.; Lima, R. P.; Silva, M. C. A.; Santos, E. F. da S. Functional potential and food safety of fresh-cut 'Paluma' guava under edible coatings. *Semina: Ciências Agrárias*, v.42, p.2313-2326, 2021. <https://doi.org/10.5433/1679-0359.2021v42n4p2313>
- Novais, R. F.; Neves, J. C. L.; Barros, N. F. Ensaio em ambiente controlado. In: Oliveira, A. J. (ed.). *Métodos de pesquisa em fertilidade do solo*. Brasília: Embrapa SEA, 1991. p.189-253.
- Oliveira, A. P. da S.; Melo, Y. L.; Alencar, R. S. de; Viégas, P. R. A.; Dias, G. F.; Ferraz, R. L. de S.; Sá, F. V. da S.; Dantas Neto, J.; Magalhães, I. D.; Gheyi, H. R.; Lacerda, C. F. de; Melo, A. S. de. Osmoregulatory and antioxidants modulation by salicylic acid and methionine in cowpea plants under the water restriction. *Plants*, v.12, p.1-16, 2023. <https://doi.org/10.3390/plants12061341>
- Queiroga, C. M. de; Lima, G. S. de; Torres, R. A. F.; Paiva, F. J. da S.; Soares, L. A. dos A.; Gheyi, H. R. Formation of guava seedlings under irrigation with water of different cationic natures and salicylic acid. *Revista Caatinga*, v.36, p.650-662, 2023. <https://doi.org/10.1590/1983-21252023v36n318rc>
- R Core Team. R: A language and environment for statistical computing Vienna: R Foundation for Statistical Computing, 2022. Available on: < <https://www.r-project.org/> >. Accessed on: 20 Jan. 2024.
- Sá, V. K. N. O. de; Lima, G. S. de; Soares, L. A. dos A.; Mendonça, A. J. T. de; Torres, R. A. F.; Dantas, M. V.; Guedes, M. A.; Gheyi, H. R. Physiological indices and growth of hydroponic cucumber under saline nutrient solutions and salicylic acid. *Revista Caatinga*, v.37, e12148, 2024. <http://dx.doi.org/10.1590/1983-21252024v37i12148rc>
- Saheri, F.; Barzin, G.; Pishkar, L.; Boojar, M. M. A.; Babaekhou, L. Foliar spray of salicylic acid induces physiological and biochemical changes in purslane (*Portulaca oleracea* L.) under drought stress. *Biologia*, v.75, p.2189-2200, 2020. <https://doi.org/10.2478/s11756-020-00571-2>
- Scotti-Campos, P.; Pham-Thi, A. T.; Semedo, J. N.; Pais, I. P.; Ramalho, J. C.; Matos, M. do C. Physiological responses and membrane integrity in three Vigna genotypes with contrasting drought tolerance. *Emirates Journal of Food and Agriculture*, v.25, p.1002-1013, 2013. <https://doi.org/10.9755/ejfa.v25i12.16733>
- Silva, A. A. R. da; Lima, G. S. de; Azevedo, C. A. V. de; Gheyi, H. R.; Soares, L. A. dos A.; Veloso, L. L. de S. A. Salicylic acid improves physiological indicators of soursop irrigated with saline water. *Revista Brasileira de Engenharia Agrícola e Ambiental*, v.26, p.412-419, 2022. <https://doi.org/10.1590/1807-1929/agriambi.v26n6p412-419>
- Silva, L. de A.; Soares, L. A. dos A.; Lima, G. S. de; Roque, I. A.; Fátima, R. T. de; Lima, A. S. Morphophysiology and water relations of *Spondias* rootstocks under different irrigation frequencies. *Revista Caatinga*, v.36, p.865-874, 2023. <https://doi.org/10.1590/1983-21252023v36n414rc>
- Soares, L. A. dos A.; Felix, C. M.; Lima, G. S. de; Gheyi, H. R.; Silva, L. A.; Fernandes, P. D. Gas exchange, growth, and production of cotton genotypes under water deficit in phenological stages. *Revista Caatinga*, v.36, p.145-157, 2023. <https://doi.org/10.1590/1983-21252023v36n116rc>
- Souza, I. L.; Santos Júnior, R. N. Faria-Silva, L.; Silva, D. M. Drought, heat, and their combined effect on the photosynthetic performance of *Psidium myrtoides* O. Berg (Myrtaceae). *Revista Ceres*, v.70, e70502, 2023. <https://doi.org/10.1590/0034-737X202370050002>
- Souza, L. de P.; Nobre, R. G.; Silva, E. M. da.; Lima, G. S. de.; Pinheiro, F. W.; Almeida, L. L. de S. Formation of 'Crioula' guava rootstock under saline water irrigation and nitrogen doses. *Revista Brasileira de Engenharia Agrícola e Ambiental*, v.20, p.739-745, 2016. <https://doi.org/10.1590/1807-1929/agriambi.v20n8p739-745>
- Teixeira, P. C.; Donagemma, G. K.; Fontana, A.; Teixeira, W. G. Manual de métodos de análise de solo. Rio de Janeiro: Embrapa, 2017. 573p.
- Usman, M.; Bokhari, S. A. M.; Fatima, B.; Rashid, B.; Nadeem, F.; Sarwar, M. B.; Nawaz-ul-Rehman, M. S.; Shahid, M.; Ayub, C. M. Drought stress mitigating morphological, physiological, biochemical, and molecular responses of guava (*Psidium guajava* L.) cultivars. *Frontiers in Plant Science*, v.13, e878616, 2022. <https://doi.org/10.3389/fpls.2022.878616>
- Weatherley, P. E. Studies in water relations of cotton plant. I: The field measurement of water deficits in leaves. *New Phytology*, v.49, p.81-97, 1950. <https://doi.org/10.1111/j.1469-8137.1950.tb05146.x>
- Xavier, A. V. O.; Lima, G. S. de; Gheyi, H. R.; Silva, A. A. da; Lacerda, C. N. de; Soares, L. A. dos A.; Fernandes, P. D. Salicylic acid alleviates salt stress on guava plant physiology during rootstock formation. *Revista Brasileira de Engenharia Agrícola e Ambiental*, v.26, p.855-862, 2022. <https://doi.org/10.1590/1807-1929/agriambi.v26n11p855-862>
- Zafar, Z.; Rasheed, F.; Atif, R. M.; Javed, M. A.; Maqsood, M.; Gailing, O. Foliar application of salicylic acid improves water stress tolerance in *Conocarpus erectus* L. and *Populus deltoides* L. saplings: Evidence from morphological, physiological, and biochemical changes. *Plants*, v.10, p.1-16, 2021. <https://doi.org/10.3390/plants10061242>