

Brazilian Journal of Agricultural and Environmental Engineering v.28, n.4, e278566, 2024

Campina Grande, PB – http://www.agriambi.com.br – http://www.scielo.br/rbeaa

DOI: http://dx.doi.org/10.1590/1807-1929/agriambi.v28n4e278566

ORIGINAL ARTICLE

# Salicylic acid reduces harmful effects of salt stress in Tropaeolum majus<sup>1</sup>

# Ácido salicílico reduz os efeitos prejudiciais do estresse salino em Tropaeolum majus

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# HIGHLIGHTS:

Salicylic acid reduces the impacts of salt stress in nasturtium. Application of 1 mM salicylic acid increases photosynthetic capacity of salt-stressed plants. Salicylic acid improves plant growth under severe salt stress.

**ABSTRACT:** Salt stress hampers the growth and physiology of nasturtium (*Tropaeolum majus*), due to biochemical, physiological, and anatomical disruptions. The application of salicylic acid stands as an alternative to alleviate the detrimental effects of salt stress, but studies on nasturtium are scarce. Thus, the aim of present study was to assess the effects of foliar application of salicylic acid on nasturtium cultivated under salt stress. The experiment followed a completely randomized design in a 3 x 3 factorial scheme, with 0 (no stress), 50 (moderate salt stress), and 100 (severe salt stress) mM of NaCl, and application of 0, 0.5, and 1 mM of salicylic acid, each with six replications. Growth (plant height, stem diameter, and number of leaves), gas exchange (stomatal conductance, photosynthesis, transpiration, internal CO<sub>2</sub> concentration, intrinsic water use efficiency, instantaneous water use efficiency, and intrinsic carboxylation efficiency), as well as chlorophyll indices and chlorophyll a fluorescence were evaluated. Salt stress of severe salt stress, resulting in a significant increase in the number of leaves. The most effective dose was 1 mM, also leading to notable improvements in water use efficiency and photochemical efficiency. However, other combinations of salinity and salicylic acid reduced growth and gas exchange in nasturtium plants.

Key words: nasturtium, phytohormone, salinity, Tropaeolaceae

**RESUMO:** O estresse salino limita o crescimento e a fisiologia da capuchinha (*Tropaeolum majus*), devido a distúrbios bioquímicos, fisiológicos e anatômicos. A aplicação de ácido salicílico é uma alternativa para mitigar os efeitos prejudiciais do estresse salino, no entanto, em capuchinha os estudos são escassos. Assim, objetivou-se avaliar os efeitos da aplicação de ácido salicílico via foliar em capuchinha cultivada sob estresse salino. O experimento foi realizado em delineamento inteiramente casualizado, em esquema fatorial 3 x 3, referente a 0 (sem estresse) 50 (estresse salino moderado) e 100 (estresse salino severo) mM de NaCl e aplicação de 0, 0,5 e 1 mM de ácido salicílico, com seis repetições. O crescimento (altura de planta, diâmetro do caule e número de folhas), trocas gasosas (condutância estomática, fotossíntese, transpiração, concentração interna de CO<sub>2</sub>, eficiência intrínseca do uso da água, eficiência instantânea do uso da água e eficiência intrínseca de carboxilação) e os índices de clorofila e fluorescência da clorofila a foram avaliados. O estresse salino afetou as variáveis analisadas neste estudo. A aplicação de ácido salicílico teve um efeito positivo na mitigação dos efeitos do estresse salino severo, resultando em um aumento significativo no número de folhas, sendo a dose mais eficaz a concentração de 1 mM, levando ainda, a melhorias notáveis na eficiência no uso da água e na eficiência fotoquímica. No entanto, as demais combinações de salinidade e ácido salicílico reduziram o crescimento e as trocas gasosas nas plantas de capuchinha.

Palavras-chave: capuchinha, fitormônio, salinidade, Tropaeolaceae

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• Accepted 18 Nov, 2023 • Published 04 Jan, 2024

Editors: Geovani Soares de Lima & Hans Raj Gheyi

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# INTRODUCTION

Nasturtium (*Tropaeolum majus* L. - Tropaeolaceae), also known as chagas flower and Mexican cress, is a versatile plant used for ornamental, medicinal, and culinary purposes (Jakubczyk et al., 2018). It is considered one of the primary edible flowers, playing a significant economic and social role (Silva et al., 2023a, b). In addition to its culinary value, nasturtium is rich in bioactive compounds, including fatty acids, flavonoids, benzyl isothiocyanate, and vitamin C (Bazylko et al., 2013).

Salt stress can induce growth disturbances in nasturtium plants, negatively affecting flower production and impairing plant metabolism (Silva et al., 2023a, b). Furthermore, under salt stress, the first target of damage is the plant's physiology, inducing stomatal closure, which represents a primary plant response to salt stress, reducing water loss through transpiration and decreasing  $CO_2$  uptake. This, in turn, can overload photosystems and induce photorespiration, resulting in the overproduction of reactive oxygen species (ROS) (Silva et al., 2022). However, there are few studies involving salinity in this crop (Silva et al., 2022; Silva et al., 2023a, b).

Salicylic acid (SA) plays an important role in osmoregulation, contributing to the accumulation of proline and glycinebetaine, which aids in enhancing antioxidant enzyme activity and plant tolerance (Henschel et al., 2022). SA attenuates salt stress by inducing the expression of genes involved in defense responses, in addition to stimulating the antioxidant system (Sofy et al., 2020). There is a lack of studies using SA to mitigate salt stress in nasturtium, and more research is needed under different conditions. Therefore, the aim of this study was to assess the effects of salicylic acid application on the photosynthetic processes and growth of nasturtium cultivated under salt stress.

#### **MATERIAL AND METHODS**

The experiment was conducted in a greenhouse at the Department of Biosciences, Center of Agricultural Sciences, Universidade Federal da Paraíba (6° 57' 42" S and 35° 41' 43" W, at an elevation of 573 m), Areia, Paraíba, Brazil. During the experiment (June to July 2023), minimum and maximum daily temperature and relative humidity of air were recorded using a digital thermo-hygrometer (AKSO° AK28new) installed inside the greenhouse, with average values of 24.7 °C, 19.8 °C and 92% (maximum temperature, minimum temperature, and humidity, respectively) (Figure 1).

The experiment was conducted using a completely randomized design in a  $3 \times 3$  factorial arrangement (irrigation water salinity levels × salicylic acid application), totaling nine treatments, six replicates and one plant per replicate. The salinity levels included 0 (no stress), 50 (moderate salt stress), and 100 (severe salt stress) mM of NaCl, while the salicylic



**Figure 1.** Maximum and minimum temperatures, and relative humidity of air in the greenhouse during the experimental period

acid (SA) treatments consisted of 0 (distilled water - control), 0.5, and 1.0 mM of SA. The values were based on the study of Silva et al. (2022) with nasturtium.

Seeds of nasturtium (*T. majus* 'Anã Sortida', ISLA') were sown in a polystyrene tray using commercial substrate (Mecplant<sup>\*</sup>, Telêmaco Borba, Brazil), at a depth of 2 cm. The electrical conductivity of the substrate used was 1.2 dS m<sup>-1</sup> (saturated extract). The chemical attributes of the substrate used are presented in Table 1.

The seedlings were transplanted into pots containing 5 dm<sup>3</sup> of the same commercial substrate 10 days after planting, with four true leaves and 12 cm tall. Basal fertilization was performed using NPK 4-14-8, suitable for initial application, with 10 g of the formulation applied to each pot. The 0.5 and 1 mM SA solutions were prepared by diluting the product in heated distilled water (67 °C) to ensure complete dissolution of the crystals in the solution. Subsequently, seven drops of the surfactant Tween 80 (0.05%) were added in 1 L of SA per concentration to enhance plant absorption. The control treatment consisted of distilled water and Tween 80 (0.05%) (Silva et al., 2022).

The plants were sprayed with SA on a weekly basis after the start of the treatments, which began 5 days after transplanting. This weekly application was based on the study by Silva et al. (2022) with nasturtium. In the first application, all plants were sprayed with 2.5 mL, in the second application with 4 mL, in the third with 5.5 mL, and in the fourth and final application with 7.5 mL of each solution, totaling 19.5 mL per plant during the experimental period. The applications were performed in the early morning, between 06:00 and 07:00 a.m., as this is the period in which plants have the greatest number of open stomata.

The brackish water solutions, corresponding to the two salinity levels (moderate and severe stress), were prepared by dissolving sodium chloride (NaCl at 50 or 100 mM) using 292.2 and 584.4 g in 100 L of water (per treatment) in the water from the local supply (ECw = 0.4 dS m<sup>-1</sup>, non-saline water) to achieve concentration. After the complete dilution of salts, the electrical

Table 1. Chemical attributes of the substrate

nU	P	Na+	H++Al <sup>3+</sup>	Al <sup>3+</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>	<b>K</b> +	TOC
μ <b>Π</b> (H20, 1:2.5)	(mg kg⁻¹)	(cmol <sub>e</sub> kg <sup>-1</sup> )						(g kg⁻¹)
5.00	233.27	14.69	20.62	0.0	8.9	8.9	97.59	35.25
and K. Mahlich Extractant, TOC. Total arganic carbon, Al. Co. and Mg. KCh H LAI. Calcium acctate								

P and K- Mehlich Extractant; TOC- Total organic carbon; Al, Ca and Mg - KCl; H+Al- Calcium acetate

conductivity of the 50 (5.7 dS m<sup>-1</sup>) and 100 mM NaCl (10.8 dS m<sup>-1</sup>) solutions was measured using a digital conductivity meter. Irrigation with distilled water was carried out for five days after transplanting, maintaining the plants at 80% of the pot retention capacity. After this, treatments with NaCl began. This irrigation level was determined based on the water needs of the plants, established using the drainage lysimetry method (Bernardo et al., 2019). For this, two additional pots per treatment, containing collector drains, were irrigated with a known volume until reaching the saturation point. 48 h after substrate saturation, the difference between the applied and drained water volumes was determined, being considered as the dynamic field capacity (100% of retention capacity). Then, each treatment was irrigated with the volume corresponding to 80% of the water retention capacity of the substrate, which was calculated proportionally to the 100% of retention capacity.

Twenty-four days after the start of brackish water irrigation, nitrogen topdressing was conducted through fertigation, using urea as the nitrogen source (45% N) and applying 1 g of N per liter of water. 100 mL of the solution was used per pot (0.1 g).

The chlorophyll a and b indices were measured using a digital chlorophyll meter (ClorofiLOG<sup>\*</sup>, model CFL 1030, Porto Alegre, RS, Brazil). Initial fluorescence ( $F_0$ ), maximum fluorescence ( $F_m$ ), variable fluorescence ( $F_v$ ), conversion of absorbed energy ( $F_v/F_0$ ), and the quantum yield of photosystem II ( $F_v/F_m$ ) were measured using a modulated fluorometer (model OS-30p, OptiSciences Inc., Hudson, USA), on an intermediate leaf per plant that had been pre-adapted to darkness for 30 min. Data collection was carried out 31 days after the initiation of saline water irrigation (DAI).

Gas exchange measurements were determined using an infrared gas analyzer (IRGA, LCpro-SD Portable Photosynthesis System, ADC BioScientific, Hoddesdon, ENG). Measurements were conducted from 8:00 to 10:00 a.m., based on the study by Silva et al. (2022) with this species, with ideal weather conditions, no clouds, using fixed artificial light at 1000  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>, a reference CO<sub>2</sub> concentration of 385  $\mu$ mol  $\rm CO_2\ mol^{-1}$  air, 200.5  $\mu mol\ s^{-1}$  air flow, chamber size of 6.25 cm<sup>2</sup> and relative humidity (ambient temperature). Stomatal conductance (gs - mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>), transpiration (E - mmol  $H_2O m^{-2} s^{-1}$ ), net CO<sub>2</sub> assimilation rate (A - µmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>), and internal CO<sub>2</sub> concentration (Ci - µmol CO<sub>2</sub> mol<sup>-1</sup> air) were measured. Based on these data, instantaneous water use efficiency (WUE) was calculated as the ratio of photosynthetic rate to transpiration (A/E), intrinsic water use efficiency (iWUE) was calculated as the ratio of photosynthetic rate to stomatal conductance (A/gs), and instantaneous carboxylation efficiency (iCE) was calculated as the ratio of photosynthetic rate to internal CO<sub>2</sub> concentration (A/Ci). Data collection was carried out at 31 DAI.

The data underwent analysis of variance (ANOVA), and when significant effects were observed, means were compared using the Tukey's test ( $p \le 0.05$ ). The ExpDes package (Ferreira et al., 2021) was employed for these statistical analyses. Additionally, a Principal Component Analysis (PCA) and Pearson's correlation were performed. All statistical analyses were performed using the R software (R Core Team, 2022).

# **RESULTS AND DISCUSSION**

The indices of chlorophyll a, b, and total chlorophyll in nasturtium plants were impacted by increasing salt concentrations, and lower indices of chlorophyll a, b, and total chlorophyll were found under moderate and severe salt stress (Figures 2A, C, and D). This response is attributed to



Means followed by the same letters do not differ according to the Tukey's test ( $p \le 0.05$ ). The values are expressed as mean ± standard deviation (n = 5) **Figure 2.** Chlorophyll a (A - B), chlorophyll b (C), and total chlorophyll (D - E) in nasturtium (*Tropaeolum majus*) cultivated under salt stress and salicylic acid application

salinity promoting increased activity of chlorophyllase, an enzyme responsible for chlorophyll degradation, likely due to the enhanced translocation of chloride rather than nitrate in the plant, resulting from the high salt concentration, ultimately leading to a reduction in chlorophyll content (Ibrahim et al., 2018).

The application of SA at 0 and 0.5 mM doses resulted in higher chlorophyll a and total chlorophyll indices compared to the 1 mM dose (Figures 2B and E). This can be attributed to its capacity to stimulate chlorophyll synthesis and inhibit its degradation, aiding in the dissipation of excess excitation energy in photosystem II and promoting photosystem protection (Moustakas et al., 2022). Additionally, SA plays a role in chlorophyll regulation, with a direct impact on photosynthesis concentration, proportion of carotenoids present, and stomatal closure control (El-Esawi et al., 2017).

Moderate and severe salt stresses caused a decrease in variable fluorescence (Figure 3B), quantum yield of photosystem II (Figure 3C), and conversion of absorbed energy (Figure 3D). Maximum fluorescence (Figure 3A)



Means followed by the same lowercase letters do not differ for salicylic acid, and those with the same uppercase letters do not differ for salt stress according to the Tukey's test ( $p \le 0.05$ ). The values are expressed as mean  $\pm$  standard deviation (n = 5)

**Figure 3.** Variable fluorescence -  $F_m$  (A), maximum fluorescence -  $F_v$  (B), quantum yield of photosystem II -  $F_v/F_m$  (C), conversion of absorbed energy -  $F_v/F_0$  (D), stomatal conductance - gs (E), net photosynthesis - A (F), transpiration - E (G), internal CO<sub>2</sub> concentration - Ci (H), intrinsic water use efficiency - iWUE (I), instantaneous water use efficiency - WUE (J), and intrinsic carboxylation efficiency - iCE (K) in nasturtium (*Tropaeolum majus*) cultivated under salt stress and salicylic acid application

decreased only under severe stress, being reduced by 27.14%. The application of 1 mM of SA reduced  $F_v$ ,  $F_m$ ,  $F_v/F_m$ , and  $F_v/F_0$  in the absence of stress. However, under severe stress, this dose resulted in the highest values for these variables. Under moderate stress, 1 mM of SA reduced  $F_v$ ,  $F_v/F_m$ , and  $F_v/F_0$  compared to the 0.5 mM dose, which, in turn, yielded the highest values for these variables. The application of SA increased  $F_v$ ,  $F_m$ ,  $F_v/F_m$ , and  $F_v/F_0$  in nasturtium plants under severe salt stress but not under moderate salt stress.

Regarding the fluorescence results observed in this study, Lotfi et al. (2020) observed similar results when investigating the use of SA in *Vigna radiata* plants under salt stress. They observed a positive response to SA application in the same variables as this study under saline conditions. In nonstressed plants, SA application decreased the variables as the doses increased. Thus, this study was able to assess the integrity of the photosynthetic apparatus based on chlorophyll fluorescence, which is a rapid and effective method for detecting and quantifying the resistance of nasturtium plants to environmental stressors such as salinity.

Salicylic acid exhibits inhibitory effects on the amount of chlorophyll, stomatal function, photosynthetic parameters, and carboxylating enzymes, playing a regulatory role associated with the electron transport and photochemical reactions of photosynthesis (Cheng et al., 2020). However, further investigations are needed to analyze the energy partitioning concerning exogenous foliar spraying of SA in plants under salt stress (Hamani et al., 2020).

The foliar application of 0.5 and 1 mM SA increased stomatal conductance (gs) by 12.5 and 4.16%, respectively, in nasturtium plants in the absence of stress. However, it was decreased under moderate stress and had no significant effect under severe stress (Figure 3E). Net photosynthesis (A) was decreased under both moderate and severe stress. SA application increased A in the absence of stress but reduced it under moderate stress, with no significant effect on A under severe stress (Figure 3F). Transpiration (E) was higher in plants under moderate stress and lower under severe stress when compared to plants without stress (Figure 3G). The application of 1 mM of SA increased transpiration (E) by 24.41% in the absence of stress but reduced it under moderate stress, with no significant effect on E under severe stress. Internal CO<sub>2</sub> concentration (Ci) was negatively affected by SA only under moderate stress, where the highest dose of SA (1 mM) reduced Ci (Figure 3H).

Despite the application of SA increasing gas exchange in the absence of stress, it was not able to reverse the effects of moderate and severe salt stress (Figure 3E, F, G, and H). Possibly, SA behavior in non-stressed plants may be associated with its impact on photosynthetic performance, promoting RuBisCO activity and reducing carbohydrate accumulation to enhance the photosynthetic rate. It also acts directly on thylakoids within chloroplasts (Song et al., 2021; Aamer et al., 2022). The increase in Ci (Figure 3H) under SA application under conditions of moderate stress may indicate  $CO_2$ accumulation in the leaf due to low assimilation, which results in a low A/Ci ratio (Aires et al., 2022). In plants under salt stress, intrinsic water use efficiency (iWUE) was higher (24.33%), while instantaneous water use efficiency (WUE) was lower under stress conditions (Figure 3J). The application of SA increased iWUE by 71.33% under both moderate (1 mM of SA) and severe (0.5 and 1 mM of SA) salt stress (Figure 3I). In all salt conditions studied, the application of 0.5 mM of SA resulted in the highest WUE. However, 1 mM of SA reduced WUE by 20.85% in the absence of stress (Figure 3J). Instantaneous carboxylation efficiency (iCE) was significantly reduced by 75% under severe salt stress, and SA did not reverse this effect (Figure 3K). On the other hand, the application of 0.5 mM of SA resulted in the highest iCE (25%) in the absence of stress, but both doses of SA reduced iCE under moderate stress (Figure 3K).

The application of SA increased iWUE and WUE in nasturtium plants under severe and moderate salt stress. iCE and WUE were enhanced in non-stressed plants with SA applications. The stomatal conductance of the plants is immediately impacted by salinity, leading to disruptions in water relations and a reduction in abscisic acid (ABA) synthesis (Silva et al., 2019). The positive results observed in iWUE and WUE can be attributed to the exogenous application of SA, which provides protection against heavy metal toxicity. This protection leads to physiological and molecular changes that create a conducive environment for adaptation or tolerance to stress (Wani et al., 2017). Furthermore, the application of SA also increases the concentration of methionine and glycine betaine in plants due to its ability to inhibit ethylene formation by suppressing the activity of 1-aminocyclopropane carboxylic acid synthase (Wani et al., 2020). This increase in glycine betaine accumulation, coupled with reduced ethylene production, is associated with elevated glutathione levels and reduced oxidative stress. Consequently, it results in improved photosynthetic efficiency and water relations in plants under salt-stress conditions (Khan et al., 2014).

The detrimental effect of severe salt stress on iCE is related to the reduction in water supply, which affects the ability of *T. majus* leaves to efficiently perform photosynthesis, reducing the availability of  $CO_2$  within the cells (Farhangi-Abriz & Ghassemi-Golezani, 2018). However, in the present research, this was due to the harmful effect of salt stress. In summary, lower iCE values indicate that, in addition to diffusion-related effects (related to stomatal conductance and  $CO_2$  availability), salinity also affects the activity of RuBisCO. This results in lower carbon assimilation due to biochemical limitations, as evidenced by the high Ci values. Despite the high availability of  $CO_2$ , carbon assimilation is not occurring (low A).

Salt stress reduced all the growth variables evaluated in this study. Severe stress resulted in the lowest values for plant height, stem diameter, and number of leaves in nasturtium plants (Figure 4). In the absence of stress (0 mM NaCl), the application of 0 (control) and 1 mM SA resulted in the greatest plant height (26.08 and 25.75 cm, respectively). Conversely, SA application did not mitigate the effects of moderate (50 mM NaCl) and severe (100 mM NaCl) salt stress on plant height (Figure 4A). The greatest stem diameter (3.69 mm) was observed in plants under no stress (0 mM NaCl) (Figure 4B). The number of leaves was decreased with the increasing NaCl concentration in the irrigation water, with severe stress (100 mM NaCl) resulting in the lowest values. The application of 1 mM SA, on the other hand, led to the highest number



Means followed by the same lowercase letters do not differ for salicylic acid, and those with the same uppercase letters do not differ for salt stress according to the Tukey's test ( $p \le 0.05$ ). The values are expressed as mean  $\pm$  standard deviation (n = 5) Figure 4. Plant height (A) stem diameter (B) and number of

**Figure 4.** Plant height (A), stem diameter (B), and number of leaves (C) of nasturtium (*Tropaeolum majus*) cultivated under salt stress and application of salicylic acid

of leaves in the absence of stress and under severe stress conditions (Figure 4C).

In this study, the application of 1 mM of SA reduced the harmful effects of severe salt stress on the growth of nasturtium, particularly in terms of the number of leaves, while the results also demonstrated that SA led to an increase in the number of leaves in non-stressed plants. This behavior is linked to the role of SA in cell division (Wang et al., 2021), hyperaccumulation of osmolytes (including glycine betaine, and proline), and reinforcement of the antioxidant defense system in plants under salt stress (Khan et al., 2015). Growth was negatively affected by salt stress due to reduced turgor pressure and tissue water content, resulting in a decreased growth rate (Paul & Roychoudhury, 2019).

A principal component analysis (PCA) explained 80.6% of the original data variance in its first two axes (PC1 and PC2) (Figure 5). Three groups were formed, with the grouping of N100S0.5 and N100S0 standing out, exhibiting the highest values for iWUE, chlorophyll a/b, and F<sub>0</sub>. In nasturtium plants not subjected to salinity, the addition of salicylic acid up to 0.5 mM resulted in higher pigment concentrations, SD, PH, NL, and better gas exchange performance. A third group was formed with treatments involving moderate NaCl doses, as well as 100 mM NaCl and 1 mM SA, along with the maximum SA dose in the absence of salinity, which showed intermediate performance for the evaluated variables. The application of SA can reduce the devastating effect of salt stress on plant growth, as well as inducing the accumulation of glycine betaine by increasing the methionine content, consequently increasing plant biomass (Khan et al., 2015).

The correlation matrix revealed that nasturtium plants with greater height, stem diameter, number of leaves, and



Ci- internal CO<sub>2</sub> concentration, E- transpiration, gs- stomatal conductance, A- net photosynthesis, WUE- instantaneous water use efficiency, iWUE- intrinsic water use efficiency, iCE- instantaneous carboxylation efficiency, PI- plant height, SD- stem diameter, NL- number of leaves, Chla- chlorophyll a, Chlb- chlorophyll b, tChl- total chlorophyll, Chla/b- chlorophyll a/b, F<sub>0</sub>- initial fluorescence, F<sub>m</sub>- maximum fluorescence, F<sub>v</sub>- variable fluorescence, F<sub>v</sub>/F<sub>m</sub>- quantum yield of photosystem II, F<sub>v</sub>/F<sub>0</sub>- conversion of absorbed energy, S- salicylic acid, N0S0- plants without stress and without salicylic acid application, N0S05.5 and N0S1- plants without stress and with salicylic acid application, N50S0.5 and N50S1- plants with moderate stress and salicylic acid application, N100S0- plants with severe stress and without salicylic acid application, N100S1- plants with severe stress and salicylic acid application, N100S1- plants with salicylic acid application, N100S1- plants with salicylic acid application, N100S0- plants with severe stress and salicylic acid application, N100S1- plants with severe stress and salicylic acid application, N100S1- plants with severe stress and salicylic acid application, N100S1- plants with severe stress and salicylic acid application, N100S1- plants with severe stress and salicylic acid application, N100S1- plants with severe stress and salicylic acid application, N100S1- plants with severe stress and salicylic acid application, N100S1- plants with severe stress and salicylic acid application, N100S1- plants with severe stress and salicylic acid application, N100S1- plants with severe stress and salicylic acid application, N100S1- plants with severe stress and salicylic acid application, N100S1- plants with severe stress and salicylic acid application, N100S1- plants with severe stress and salicylic acid application, N100S1- plants wi

Figure 5. Principal component analysis (PCA)



Ci- internal CO<sub>2</sub> concentration, E- transpiration, gs- stomatal conductance, A- net photosynthesis, WUE- instantaneous water use efficiency, iWUE- intrinsic water use efficiency, iCE- instantaneous carboxylation efficiency, PH- plant height, SD- stem diameter, NL- number of leaves, Chla- chlorophyll a, Chlb- chlorophyll b, tChl- total chlorophyll, Chla/b- chlorophyll a/b, F0- initial fluorescence, Fm- maximum fluorescence, Fv- variable fluorescence, Fv/Fm - quantum yield of photosystem II, Fv/F0 - conversion of absorbed energy. (n = 5) **Figure 6.** Pearson's correlation between growth and physiology of *Tropaeolum majus* under salt stress and salicylic acid application

pigment content exhibit a higher positive correlation with gas exchange (gs, A, and E). Simultaneously, these variables are negatively correlated with intrinsic water use efficiency (iWUE) (Figure 6).

The correlation analysis was crucial in understanding the relationship between the variables analyzed in this study. The positive correlation between gas exchange and the growth of nasturtium is related to the fact that osmoprotectants play vital roles in improving hyperosmolarity caused by salt stress and in establishing cellular ionic homeostatic conditions, which helps enhance gas exchange and consequently plant growth (Saleem et al., 2021). Furthermore, the decrease in growth in various plant species subjected to stress situations is often linked to a reduction in photosynthetic efficiency (Xiaotao et al., 2013). Therefore, maintaining gas exchange through the use of phytohormones is closely connected to plant growth and production (Silva et al., 2022).

# Conclusions

1. The application of salicylic acid mitigated the effects of severe salt stress in nasturtium plants, increasing their number of leaves.

2. Foliar application of 1 mM SA was the most effective, resulting in higher iWUE, WUE, and photochemical efficiency.

3. The application of salicylic acid was effective at higher salinity and higher concentration; however, the other combinations reduced the growth and gas exchange in nasturtium plants.

4. Studies with higher doses of salicylic acid under normal conditions are needed.

#### **ACKNOWLEDGEMENTS**

The authors would like to thank the Universidade Federal da Paraíba, Campus II (CCA-UFPB) for their support during the experiment.

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