Application of phytohormones as attenuators of salt stress in *Tropaeolum majus* L. (Tropaeolaceae)

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**Abstract** – The salinity of the soil and irrigation water is one of the great challenges of agriculture. Salinity can have harmful effects on physiological processes and plant growth, including *Tropaeolum majus* L. (Tropaeolaceae). The application of phytohormones can be a strategy to mitigate these effects. The aim of this study was to evaluate the application of jasmonic acid, salicylic acid, cytokinin and polyamine as attenuators of salt stress in *T. majus*. Three levels of salt stress were used: 0 mM NaCl (no stress), 50 mM NaCl (moderate stress) and 100 mM NaCl (severe stress). Four phytohormones and a control treatment were used: control (deionized water), jasmonic acid (200 µM), salicylic acid (2 mM), cytokinin (6-benzylaminopurine – 10 µM) and polyamine (spermine – 1 mM). Growth and gas exchange parameters were evaluated. Applied in conditions of moderate salt stress, all the phytohormones were efficient in improving plant height and leaf area (except salicylic acid); cytokinin and polyamine improved the number of flowers as well as gs, A and iCE; jasmonic acid improved the stem dry mass and total dry mass. In relation to severe salt stress, applications of jasmonic acid and polyamine were efficient in improving plant height; cytokinin improved leaf dry mass as well as gs, A, E, WUE, iWUE and iCE. The application of cytokinin, polyamine and jasmonic acid can be used to mitigate moderate salt stress in *T. majus*.

**Keywords:** abiotic stress, edible flowers, gas exchange, growth regulators

**Introduction**

Edible flowers have recently been substantially publicized due to changes in eating styles and the return to previous lifestyles, in which edible flowers played an important role (Rop et al. 2012). *Tropaeolum majus* L. (Tropaeolaceae), popularly known as nasturtium, is an edible, ornamental and medicinal plant, with showy flowers (Melo et al. 2018) and widely known as an unconventional food plant.

Plants can be subjected to various environmental stresses such as extreme temperatures, drought, salinity, flooding, the toxicity of pollutants and oxidative stresses (Forni et al. 2017). Abiotic stresses can cause losses of up to 50% to agricultural productivity worldwide and limit food security for the growing human population (Kumar and Verma 2018, Phour and Sindhu 2020). Abiotic stresses, such as soil salinity, are challenging obstacles to agricultural production and are directly related to changes in various plant metabolic pathways (Feng et al. 2020).

Soil salinity affects 800 Mha, or about 20%, of irrigated agricultural land worldwide, mainly in arid, semi-arid and coastal regions and tends to be particularly prevalent in irrigated land, due to inadequate irrigation and drainage management, low precipitation, high evaporation and irrigation with salt water (Ibrahim 2016, Negrão et al. 2017, Phour and Sindhu 2020). Most plant species are unlikely to survive at 100 mM NaCl, due to ionic toxicity, osmotic pressure, oxidative damage and nutritional shortages that affect seed germination, seedling establishment, vegetative growth and flower fertility (He et al. 2018, Trifunović-Momčilov et al. 2020). High levels of Na⁺ are toxic to plants because they interfere with cell metabolism and ionic homeostasis (Wang et al. 2019). Salinity above 1 dS m⁻¹ decreased the rooting and growth of *T. majus* seedlings (Xu et al. 2021). Salt application slightly increased biomass production of *T. majus* (Bloem et al. 2014).

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Salt stress conditions result in biochemical changes, such as the accumulation of reactive oxygen species (ROS) that can inhibit plant growth and development, if not regulated (Nxelle et al. 2017). The application of phytohormones can attenuate the deleterious effects of salt stress on plants. Phytohormones are molecules derived from several essential metabolic pathways that regulate and mediate growth, development, differentiation, responses to biotic and abiotic stresses, apical dominance, bulb formation, cell division, flowering, seed germination, dormancy and senescence (Bosco et al. 2014) through synergetic or antagonistic actions. Cytokinins, jasmonates, salicylates and polyamines are some of the phytohormones used to mitigate the harmful effects of salt stress on plants.

Polyamines are aliphatic polyamines of low molecular weight present in all living organisms, occurring in free, conjugated (associated with small molecules, such as phenolic acids) or linked (associated with several macromolecules) forms (Liu et al. 2015, Pál et al. 2015). Cytokinins are the main regulators of plant growth and development, performing the functions of cell expansion and differentiation, seed germination, leaf senescence and controlling plant adaptation to abiotic and biotic stresses (Wu et al. 2014).

Jasmonic acid and its derivatives are signaling molecules that regulate the formation of reproductive organs, seed germination, nutrient storage, transport of assimilates, root growth, tuber formation, flowering, fruit ripening, senescence and defense of plants against biotic and abiotic stresses (Avalbaev et al. 2016, Tavallali and Karimi 2019). Salicylic acid plays an important role in plant development, disease resistance, fruit production and as an activator of enzymes in the antioxidant system of plants under stress (El-Esawi et al. 2017).

Salinity causes various injuries to plants, such as reduced growth and gas exchange, as observed in Mesosphaerum suaveolens (L.) Kuntze (Figueiredo et al. 2021). NaCl inhibits net photosynthesis due to osmotic stress caused by the accumulation of salts, which leads to reduced water potential and stomatal conductance. Photosynthesis is the basis for ensuring the normal growth and development of plants under stress. Saline stress inhibits plant growth by influencing the photosynthetic capacity of plants. Saline stress can damage the photosynthetic system of plants, forcing a decrease in the photosynthetic capacity of the leaves and damage to photosystem II (Xu et al. 2021), leading to reduced growth. The aim was to evaluate the application of jasmonic acid, salicylic acid, cytokinin and polyamine as attenuators of salt stress in T. majus.

**Materials and methods**

**Experimental design and seedlings production**

The experiment was carried out in a greenhouse at the Floriculture Sector of the Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil. The average maximum and minimum temperatures were 35.8 and 19.7 °C; the average maximum and minimum relative air humidity were 87.8% and 38.1%. *Tropaeolum majus* seeds (semi-folded variety) were planted in a 128-cell styrofoam tray with a commercial substrate (Topstrate). The seedlings were transplanted to size 15 pots with a commercial substrate (Topstrate) at 15 days after planting (DAP).

The experiment was carried out in a completely randomized design in a 3 × 5 factorial scheme with four replications. Three levels of salt stress were used: 0 mM NaCl (no stress), 50 mM NaCl (moderate stress) and 100 mM NaCl (severe stress). Four phytohormones and control treatment were used: control (deionized water), jasmonic acid (200 µM; Bloem et al. 2014), salicylic acid (2 mM; Silva et al. 2018), cytokinin (6-benzylaminopurine – 10 µM; Chang et al. 2016) and polyamine (spermine – 1 mM; Baniasadi et al. 2018).

**Application of salt stress and phytohormones**

The plants were irrigated with 0, 5 and 10 mM NaCl between 20 and 27 DAP to adapt to salt stress. This initial application of NaCl was necessary to prevent plants with severe stress from dying earlier than expected. The daily irrigation with 0, 50 and 100 mM NaCl was applied between 28 and 65 DAP. Phytohormones were diluted in deionized water. 0.05% Tween 20 was used as a surfactant to increase absorption by plants. The control was prepared with deionized water and 0.05% Tween 20. Each plant was sprayed with about 3 mL of each solution. Two applications of phytohormones were performed during the experiment, at 28 and 38 DAP, according to preliminary and unpublished studies. The plants were fertigated with 2 g L⁻¹ of NPK 20-20-20 fertilizer with micronutrients (Peters), once a week.

**Variables analyzed**

Growth and gas exchange were evaluated at 65 DAP, 37 days after the beginning of irrigation with saline water. Plant height (cm), number of branches, number of leaves, stem diameter (mm), number of flowers, number of buds, leaf dry mass (g), stem dry mass (g), total dry mass (g), leaf area (cm²), leaf area ratio (cm² g⁻¹), specific leaf area (cm² g⁻¹), stem mass ratio (g g⁻¹), root mass ratio (g g⁻¹), robustness quotient, sclerophilia index and root/shoot ratio were evaluated. The plants were divided into root, stem, leaves and flowers/buds and dried in an oven with forced air circulation at 65 °C for 72 hours. Then, the plant parts were weighed on a precision scale (0.001 g).

The leaf area was measured with a leaf area integrator (LI-3100, Li-COR, Inc., Lincoln, NE, USA). The leaf area ratio was obtained using the formula: leaf area (LA)/total dry mass (TDM). The specific leaf area was obtained using the formula: LA/leaf dry mass (LDM). The stem mass ratio was obtained using the formula: stem dry mass/TDM. The root mass ratio was obtained using the formula: root dry mass/TDM. The robustness quotient was obtained using the formula: plant height/stem diameter. The sclerophilia index was obtained using the formula: LDM/LA.

Gas exchanges were measured with an infrared gas analyzer (IRGA - model LCPRO, ADC BioScientific Ltd.), with measurements taken between 8 and 10 hours. Two readings were taken.
were taken for each experimental plot. Net photosynthesis (\(A = \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}\)), stomatal conductance (\(g_s = \text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}\)), internal carbon concentration (\(C_i = \mu\text{mol CO}_2 \text{ mol air}^{-1}\)), transpiration (\(E = \text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}\)), instantaneous water use efficiency (\(\text{WUE} = A/E\)), intrinsic water use efficiency (\(\text{iWUE} = A/g_s\)) and intrinsic carboxylation efficiency (\(\text{iCE} = A/C_i\)) were evaluated.

Statistical analysis

The data were submitted to the normality test (Shapiro-Wilk) and homogeneity of variances (Bartlett). The means were compared using the Bonferroni t-test (\(P \leq 0.05\)) using the ExpDes statistical package (Ferreira et al. 2018). Canonical variables analysis and confidence ellipses (\(P \leq 0.01\)) were performed to study the interrelationship between variables and factors using the candisc package (Friendly and Fox 2017), while Pearson’s correlation analysis was performed using the package corrplot (Wei and Simko 2017). The statistical program R (R Core Team 2020) was used to perform the statistical analyses.

Results

Severe salt stress caused damage to all growth variables evaluated. Plant height was affected by moderate salt stress, but the number of flowers was not affected (Fig. 1).
harmful effects of severe salt stress (100 mM NaCl) on plant height of *T. majus* plants were mitigated with the exogenous application of jasmonic acid and polyamine. All phytohormones were efficient in attenuating the effects of moderate salt stress (50 mM NaCl) on plant height. The application of polyamine provided a greater number of branches in plants under moderate stress. This variable did not change when stress was increased and jasmonic acid was applied. The application of jasmonic acid in plants under moderate stress did not cause difference in plant height, number of leaves, number of flowers and total dry mass compared to plants without stress (control).

Regarding stem diameter, the application of polyamine and cytokinin were more efficient in mitigating moderate stress, while all hormones alleviated severe stress effects. The application of cytokinin and polyamine provided the highest number of flowers in plants under moderate stress. The harmful effects of moderate stress on the number of buds and leaf dry mass were mitigated by the application of jasmonic acid, cytokinin and polyamine (On-line Suppl. Fig. 1). The application of cytokinin favored the largest leaf dry mass in plants under severe stress. The harmful effects of moderate stress on the stem dry mass (On-line Suppl. Fig. 1) and total dry mass were mitigated by the application of...
jasmonic acid. Plant dry masses under moderate stress were not different from plants without stress when jasmonic acid was applied.

The leaf area and stem mass ratio were negatively affected by severe salt stress, even with the application of phytohormones. The harmful effects of moderate salt stress on leaf area were mitigated with the application of jasmonic acid, cytokinin and polyamine and by all the phytohormones in severe salt stress (Fig. 2). The deleterious effects of severe salt stress on the leaf area ratio, specific leaf area, robustness quotient and sclerophilia index were mitigated by the application of all the phytohormones. The effects of severe salt stress on the root mass ratio and root/shoot ratio were attenuated by the application of jasmonic acid, salicylic acid and polyamine (On-line Suppl. Fig. 2).

Severe salt stress and moderate salt stress decreased stomatal conductance (gs), net photosynthesis (A) and transpiration (E) of *T. majus* plants in relation to the control. The harmful effects of moderate salt stress on gs, A and intrinsic carboxylation efficiency (iCE) were mitigated with the application of cytokinin and polyamine (Fig. 3; On-line Suppl. Fig. 3). The application of cytokinin attenuated the harmful effects of severe stress on gs, A, E, instantaneous water use efficiency (WUE) (Fig. 3) as well as intrinsic water use effi-
The greatest positive correlations were observed between root mass ratio (rmr) and root/shoot ratio (rms) \((r = 1.0)\), leaf area (la) and total dry mass (tdm) \((r = 0.94)\), ICE and A \((r = 0.98)\), ICE and E \((r = 0.92)\), ga and A \((r = 0.91)\), E and A \((r = 0.86)\), E and gs \((r = 0.84)\), la and E \((r = 0.81)\), tdm and E \((r = 0.75)\), ln and E \((r = 0.67)\), tdm and A \((r = 0.66)\), tdm and gs \((r = 0.69)\) (Fig. 5). The greatest negative correlations were observed between the specific leaf area (sla) and the sclerophillia index (ief) \((r = -0.94)\), leaf area ratio (lar) and ief \((r = -0.79)\), la and Ci \((r = -0.72)\), tdm and Ci \((r = -0.68)\), plant height (ph) and Ci \((r = -0.63)\), Ci and E \((r = -0.66)\), Ci and iCE \((r = -0.63)\).

**Discussion**

The accumulation of salts near the root zone causes osmotic stress, reducing plant growth and yield, altering membrane stability, water status of cells, enzymatic activity, protein synthesis and gene expression due to the accumulation of toxic saline ions in tissues of plants (Ghalati et al. 2019). Plants develop several physiological and biochemical mechanisms to survive in soils with high levels of salts, such as ion transport and absorption, homeostasis and compartmentation, activation of antioxidant system enzymes and biosynthesis of antioxidant compounds, osmoprotectors, compatible solutes, polyamines, generation of nitric oxide and hormonal modulation (Gupta and Huang 2014). These mechanisms reduce the deleterious effects of salinity on plant growth and gas exchange.

The attenuation of the deleterious effects of salinity on the growth of *T. majus* is due to the ability of jasmonic acid to induce the production of antioxidants, proteins and secondary metabolites that play a crucial role in the defense of this plant against free radicals, preventing the lipid peroxidation caused by the excess of reactive oxygen species (Qiu et al. 2014, Dar et al. 2015, Tang et al. 2020). This is related to the fact that jasmonates interact with other hormones, such as salicylic acid (Mur et al. 2013) and cytokinins (Schäfer et al. 2015) in the regulation of differentiation, growth and development of plants (Tavallali and Karimi 2019). The application of methyl jasmonate had a protective effect on *Prunus dulcis* Mill. (Tavallaliand Karimi 2019) and *Brassica napus* L. under salt stress.

In addition, the effect of cytokinin application in attenuating salt stress may be related to its effect on the production of protective osmolytes and antioxidant enzymes and induction of cell division and differentiation that delay the senes-
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The presence of leaves and induce flowering and production of new leaves (Bielach et al. 2017, Höning et al. 2018). The increase in the number of flowers was due to free endogenous polyamines being able to regulate floral opening mainly through gene expression and activity of the enzyme SAMDC (S-Adenosylmethionine decarboxylase) (Gómez-Jiménez et al. 2010). The damage caused by salt stress on growth is related to biochemical changes, such as the accumulation of reactive oxygen species (ROS) that inhibit plant growth and development, if not carefully regulated (Nxele et al. 2017).

The higher sclerophilia index observed in plants under severe stress and without the application of phytohormones can be explained by the decrease in the use of water by the plant, causing reduced development of the leaf area as compared to the total growth of the plant, allowing the maintenance of soil moisture for the avoidance of high levels of salt absorption from the soil. The application of polyamine favored this variable, with no change when the salinity increased due to the action of this phytohormone in the activities of ATPase, in addition to being involved in the regulation of various cationic channels that may be involved in toxic Na⁺ compartmentation at the cellular level (Lutts et al. 2013).

The attenuation of the effects of salt stress on the gas exchanges of *T. majus* plants are related to the high positive charges of spermine that allow a strong connection with negative charges, stabilizing the membranes more effectively (Fu et al. 2016), causing the stomata to maintain their stability and gas exchange even under stress. Besides, this action is related to the role of cytokinins and jasmonic acid in regulating the gas exchange of plants under stress conditions (Trifunović-Momčilov et al. 2020), mainly by regulating the metabolism of EROs (Wu et al. 2014). Cytokinin ap-

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The application of jasmonic acid reduced the photosynthetic inhibition induced by salinity in *Prunus dulcis* (Tavallali and Karimi 2019).

*Tropaeolum majus* plants had higher Ci when grown under severe stress and without phytohormone application due to the inhibition of photosynthetic capacity under stress that leads to stomatal closure, limiting photosynthetic CO₂ assimilation (Xiaotao et al. 2013). The largest WUE was observed in plants under moderate stress and application of polyamine. The increase in WUE and decrease in Ci during salt stress can be explained by the action of polyamines in the cross-talk with hormonal pathways, mainly in the regulation of ABA and ethylene under stress, which promoted the maintenance of stomata opening, hindered oxidative stress and stabilized photosynthesis (Fariduddin et al. 2013, Gupta et al. 2013, Dar et al. 2015). The mitigating effect of cytokinin is related to the action of this phytohormone in protecting cell membranes and the photosynthetic system from damage, in addition to being an abscisic acid antagonist, increasing stomatal conductance (Hönig et al. 2018).

The damaging effect of salt stress on gas exchange is related to the disturbance in the water balance and ionic homeostasis that affects hormonal status (mainly abscisic acid), transpiration, photosynthesis and other metabolic processes (Ilangumaran and Smith 2017). In addition, high salinity mainly damages the integrity of the cell membrane and, consequently, the functioning of the photosynthetic apparatus (Farhangi-Abriz and Ghassemi-Golezani 2018).

The effect of salicylic acid on iWUE is related to its strong regulation of photosynthesis, influencing chlorophyll content, carotenoid composition and stomatal closure (El-Esawi et al. 2017). The effect of polyamine on iWUE may be related to its function as an antioxidant, free radical scavenger and membrane stabilizer that can to some extent alleviate the salinity-induced decline in photosynthetic efficiency, but this effect depends on the concentrations or types of polyamines and levels of stress (Fariduddin et al. 2013, Gupta et al. 2013).

Increased cytokinin production can delay leaf senescence and a reduction in its endogenous level accelerates senescence (Liu et al. 2016). However, the actual step in which cytokinin could be involved in the chlorophyll metabolic pathway and the mechanisms by which it could retard senescence are still not known (Talla et al. 2016). The positive effect of the application of cytokinin (6-benzylaminopurine – BAP) on salt stress may be related to the delay of leaf senescence and induction of protective accumulation of osmolytes, such as proline (Bielach et al. 2017), as well as, the induction of energy absorption, dissipation, trapping and flow of electron transport under stress during photosynthesis (Wu et al. 2014) promoted by cytokinin. Moreover, cytokinin and jasmonic acid act as a signaling molecule that activates the cellular antioxidant system (Ogweno et al. 2010, Tang et al. 2020). The application of cytokinin relieved the physiological damage and the growth of *Lolium perene* L., *Cucumis sativus* L. (Xiaotao et al. 2013) and *Solanum melongena* (Ogweno et al. 2010) subjected to stress.

Canonical variables analysis reveals the variation between treatments and variables, considering the residual dispersion of each variable, and is similar to principal components analysis, but it should be preferred when using an experimental design in which there are repetitions in each treatment (Flores et al. 2020). The high relationship of the variables analyzed under moderate salinity and the application of polyamine, jasmonic acid and cytokinin (Fig. 4) can be related to the action of polyamines as stabilizers of proteins, nucleic acids, membrane phospholipids and constituents of the cell wall of plants under salt stress (Baniasadi et al. 2018), causing the plant to adapt to stress without much damage. In addition, exogenous application of jasmonic acid probably alleviated the toxicity of saline ions through stimulating antioxidant activities, induction of ROS-eliminating enzymes and ion absorption (Farhangi-Abriz and Ghassemi-Golezani 2018), causing the plant to be able to grow even in salt stress conditions. Also, the positive effects of exogenous application of cytokinin on plants under salt stress may be associated with the reduction of excess Na⁺ accumulation, endogenous ABA content and ROS accumulation, in addition to activating the antioxidant defense systems.

Correlation analysis is important to understand the relationship between the variables analyzed. The greatest positive correlations between growth and gas exchange were observed between leaf area and total dry mass and A, E, gs and Ci. The biggest negative correlations were between leaf area, total dry mass and plant height and Ci. The decline in growth of many species of plants subjected to stress is often associated with a reduction in photosynthetic capacity (Xiaotao et al. 2013); thus, maintaining gas exchange through the application of phytohormones is intrinsically related to growth and production.

**Conclusion**

Exogenous application of cytokinin (6-benzylaminopurine), polyamine ( spermine) and jasmonic acid are effective in the mitigation of the deleterious effects of moderate salt
stress (50 mM NaCl) on the growth and gas exchange of *Tropaeolum majus*. In severe salt stress, application of jasmonic acid, polyamine and cytokinin were less efficient.

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