ISSN 1807-1929
Revista Brasileira de Engenharia Agrícola e Ambiental
Brazilian Journal of Agricultural and Environmental Engineering
v.28, n.7, e279042, 2024
Campina Grande, PB – http://www.agriambi.com.br – http://www.scielo.br/rbeaa

DOI: http://dx.doi.org/10.1590/1807-1929/agriambi.v28n7e279042

ORIGINAL ARTICLE

Phytohormones mitigate salt stress damage in radish[1]

Fitormônios diminuem os danos causados pelo estresse salino em rabanete

Toshik I. da Silva[2*], Marlon G. Dias[3], Samuel D. O. Lannes[3], Paloma Domingues[4], Giuliana N. B. Sales[4], Jackson S. Nóbrega[4], João E. da S. Ribeiro[5], Franciscleudo B. da Costa[4], Lauriane A. dos A. Soares[4] & Geovani S. de Lima[4]

[1] Research developed at Universidade Federal de Viçosa, Departamento de Agronomia, Viçosa, MG, Brazil
[2] Universidade Federal do Recôncavo da Bahia/Centro de Ciências Agrárias, Ambientais e Biológicas, Cruz das Almas, BA, Brazil
[3] Universidade Federal de Viçosa/Departamento de Agronomia, Viçosa, MG, Brazil
[4] Universidade Federal de Campina Grande/Centro de Ciências e Tecnologia Agroalimentar, Pombal, PB, Brazil
[5] Universidade Federal Rural do Semi-Árido/Departamento de Ciências Agronômicas e Florestais, Mossoró, RN, Brazil

HIGHLIGHTS:

Phytohormones mitigate the reduction of chlorophyll in salt-stressed radish, sustaining photosynthesis.
Stomatal regulation by phytohormones enhances radish photosynthesis under salt stress.
Phytohormones influence radish tuber, promoting growth and improving shape.

ABSTRACT: Phytohormones play a pivotal role in regulating plant growth and responding to salt stress, aiding in signal perception and defense system mediation. With this, the objective of the present study was to assess the impact of phytohormone application in mitigating the harmful effects of salt stress on radish. Three levels of NaCl (0, 50, and 100 mM) and five phytohormones (jasmonic acid, salicylic acid, cytokinin, gibberellin, and polyamine) plus a control treatment (deionized water) were studied. The application of phytohormones such as jasmonic acid and cytokinin improved photosynthetic efficiency, and diameter, length, and total soluble solids content of tuber. Under salt stress conditions, plants showed adaptations in gas exchange, varying their rates of photosynthesis and transpiration. Furthermore, an effective balance between carbon assimilation and water loss was observed in some plants. The application of phytohormones counteracted salt stress, safeguarding chlorophyll, sustaining gas exchange, and promoting plant growth of radish. Consequently, use of phytohormones represents an alternative for radish cultivation under salt stress.

Key words: *Raphanus sativus*, chlorophyll indices, gas exchange, growth

RESUMO: Os fitormônios desempenham um papel fundamental na regulação do crescimento das plantas e na resposta ao estresse salino, auxiliando na percepção de sinais e mediação do sistema de defesa. Com isso, o objetivo do presente estudo foi avaliar o impacto da aplicação de fitormônios na mitigação dos efeitos prejudiciais do estresse salino em rabanete. Três níveis de NaCl (0, 50 e 100 mM) e cinco fitormônios (ácido jasmônico, ácido salicílico, citocinina, giberelina e poliamina) e tratamento controle (água deionizada) foram estudados. A aplicação de fitormônios, como ácido jasmônico e citocinina melhorou a eficiência fotossintética, diâmetro, comprimento e teor de sólidos solúveis totais de tubérculo. Sob condições de estresse salino, as plantas demonstraram adaptações nas trocas gasosas, variando suas taxas de fotossíntese e transpiração. Além disso, foi observado um equilíbrio eficaz entre a assimilação de carbono e a perda de água em algumas plantas. A aplicação de fitormônios contrabalançou o estresse salino, protegendo a clorofila, mantendo as trocas gasosas e promovendo o crescimento das plantas de rabanete. Consequentemente, o uso de fitormônios representa uma alternativa para o cultivo de rabanete sob estresse salino.

Palavras-chave: *Raphanus sativus*, índices de clorofila, trocas gasosas, crescimento

• Ref. 279042 – Received 29 Sept, 2023
* Corresponding author - E-mail: iarley.toshik@gmail.com
• Accepted 08 Feb, 2024 • Published 05 Mar, 2024
Editors: Ítalo Herbet Lucena Cavalcante & Hans Raj Gheyi

Introduction

Environmental stressors exert a detrimental impact on the overall growth performance of plants (Qin et al., 2024). Salinity exerts detrimental effects on the photosynthetic machinery, transpiration, and gas exchange by diminishing chlorophyll and carotenoid levels, disrupting chloroplast ultrastructure and the PSII system, and decreasing stomatal conductance (Pan et al., 2021). Furthermore, soil salinity reduces both soil water potential and leaf water potential, disrupting plant water relations, diminishing plant turgor, and ultimately leading to osmotic stress (Navada et al., 2020). Precise control of leaf gas exchange plays a pivotal role in enhancing tolerance to abiotic stresses, such as salt exposure (Montanaro et al., 2022). Amongst the myriad of biochemical processes, photosynthesis stands out as highly susceptible to environmental stress, with the photosynthetic apparatus ranking among the most stress-sensitive components in plants (Hudeček et al., 2023).

Phytohormones play a vital role in how plants respond to salt stress, as they oversee the regulation of plant growth and facilitate adaptations in development because they contribute to perceiving salt stress signals and mediating the plant's defense system (Zhao et al., 2021). Jasmonic acid, salicylic acid, cytokinin, and polyamine have been reported as mitigators of damage caused by salt stress in plants (Naeem et al., 2020; Azzam et al., 2022; Silva et al., 2022a; Hudeček et al., 2023; Henschel et al., 2023).

Radish (*Raphanus sativus* L. – Brassicaceae) is classified as moderately sensitive to salt stress, and when exposed to high salt levels, it experiences a decline in photosynthetic capacity, growth, and ultimately yield (Henschel et al., 2023). Salicylic acid (Ulukapi et al., 2020; Mahdavian, 2023), methyl jasmonate (Henschel et al., 2023), gibberellic acid (Mishra et al., 2019), and polyamine (Çavuşoğlu et al., 2008) have been reported as mitigators of salt stress-induced damage in radish plants. Thus, the objective of this study was to assess the impact of phytohormone application in mitigating the harmful effects of salt stress on radish.

Material and Methods

The experiment was conducted in a greenhouse with an upper covering of 150-micron polyethylene plastic film, and the sides were covered with plastic film and shade cloth (from the base up to 1.5 m in height), located at the Department of Agronomy of the Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil. The average temperature and relative humidity inside the greenhouse were 25 °C and 59.5%, respectively.

The study employed a completely randomized design in a 3 × 6 factorial scheme, with four replicates. The factors were three levels of NaCl (0, 50, and 100 mM) representing varying degrees of salt stress (no stress, moderate stress, and severe stress (Silva et al., 2022a) and five phytohormones plus a control treatment: control (deionized water), jasmonic acid (200 μM – Silva et al., 2022a), salicylic acid (1 mM – Mahdavian, 2023), cytokinin (6-benzylaminopurine – 10 μM – Silva et al., 2022a), gibberellin (GA_3 – 50 mM – Mishra & Nagaich, 2019), and polyamine (spermine – 1 mM – Silva et al., 2022a).

Radish seeds, variety 'Cometa' (Isla®), were planted in 1.2 L pots filled with commercial substrate (Topstrato, EC = 0.5 ± 0.3 dS m^{-1}; pH = 5.8 ± 0.3). Three seeds were placed in each pot, and after emergence, only the most vigorous seedling was kept.

Between 8 and 35 days after planting (DAP), daily irrigation was carried out using solutions of 0, 50, and 100 mM NaCl. Phytohormones were dissolved in deionized water, and 0.05% Tween 20® was added as a surfactant to enhance plant absorption. The control group received a solution of deionized water and 0.05% Tween 20®. Each plant was sprayed with approximately 3 mL of the respective solution according to treatment. The applications of phytohormones were carried out weekly, in a total of four. Additionally, the plants received weekly fertigation with a solution containing 2 g L^{-1} of NPK 20-20-20 fertilizer enriched with micronutrients (Peters®).

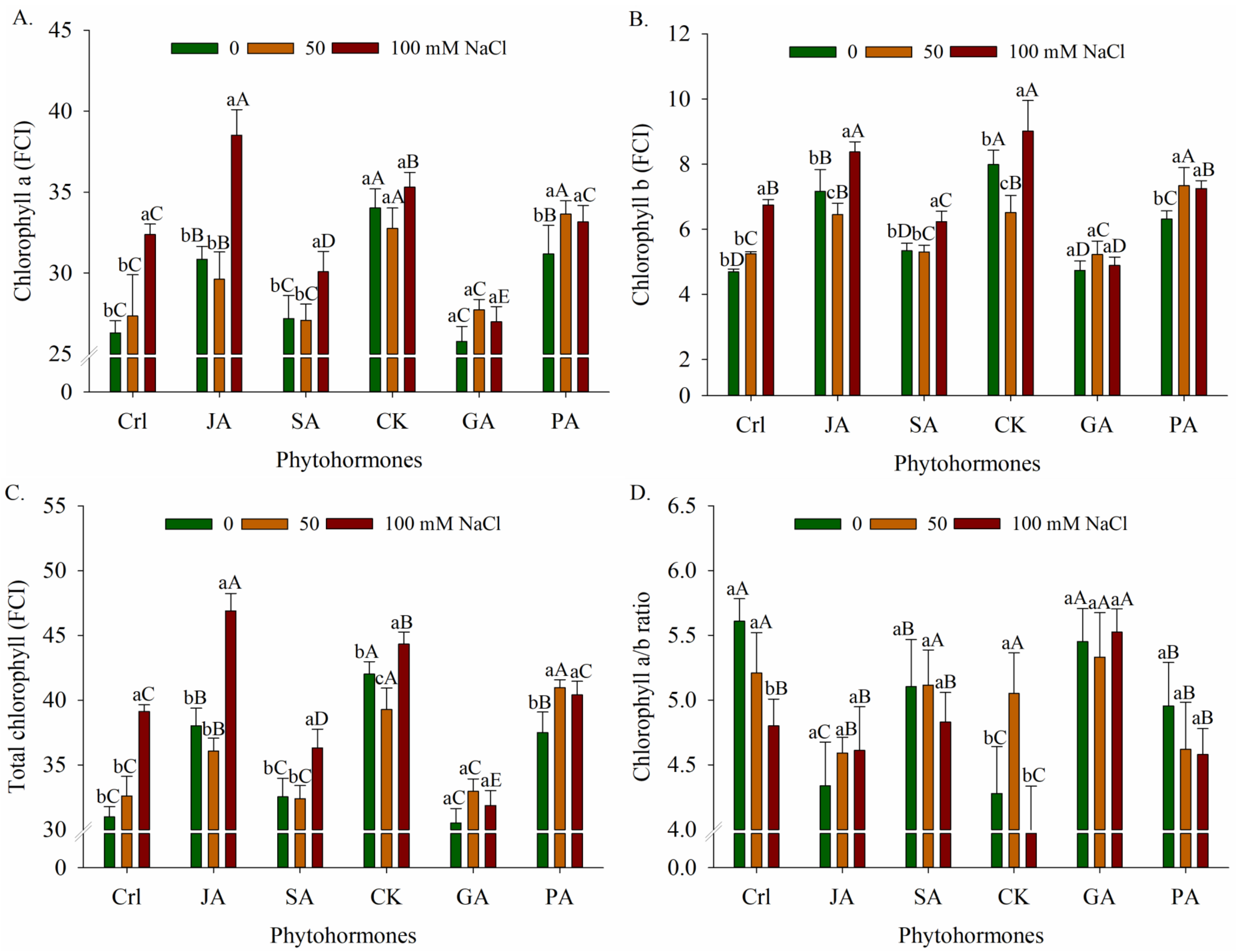
Chlorophyll indices were measured using a non-destructive chlorophyll analyzer (Clorofilog, Falker®), quantified in the form of Falker Chlorophyll Index (FCI). Gas exchange and chlorophyll indices were analyzed at 35 DAP. Gas exchange was assessed using an infrared gas analyzer (IRGA – model LCPro, ADC BioScientific Ltd.®), and measurements were taken between 8:00 and 9:00 a.m. on a specific day. Stomatal conductance (gs - mol H_2O m^{-2} s^{-1}), net photosynthesis (A = μmol CO_2 m^{-2} s^{-1}), transpiration (E = mmol H_2O m^{-2} s^{-1}), internal CO_2 concentration (Ci = μmol CO_2 mol^{-1} air), instantaneous water use efficiency (WUE = A/E), and intrinsic water use efficiency (iWUE = A/gs) were evaluated.

All data were collected at 35 DAP. The measurements were taken on the fully expanded uppermost leaf, with three readings per leaf at different points. Tuber diameter (mm), tuber length (mm), shape, tuber fresh mass (g), and total soluble solids (°Brix) were evaluated. Tuber diameter and length were assessed using a digital caliper, while the shape was determined based on the tuber's length-to-diameter ratio. Soluble solids were obtained by analyzing the samples using a digital refractometer (Hanna Hi96801 model®) with automatic temperature compensation (AOAC, 2005).

The data were subjected to analysis of variance (ANOVA), and when statistical significance was observed ($p \leq 0.05$), a comparison of means was conducted using Tukey's test. Principal component analysis with clustering was performed to explore the interrelationships between variables and factors. The statistical analyses were carried out using the R statistical program (R Core Team, 2023).

Results and Discussion

Application of jasmonic acid (JA) resulted in a 24.28% increase in chlorophyll a content in radish plants cultivated under severe salt stress, compared to the control (Figure 1A). Remarkably, severe salt stress elevated chlorophyll a index in all treatments, except for plants subjected to cytokinin (CK) and gibberellin (GA) application. This increase demonstrates the potential of jasmonic acid to mitigate the adverse effects of salt stress on the production of chlorophyll a, a critical pigment for photosynthesis and overall plant performance (Choudhary et al., 2021a). This behavior



Same uppercase letters do not differ for phytohormones, and same lowercase letters do not differ for salt stress according to the Tukey's test ($p \leq 0.05$). Crl- control, JA- jasmonic acid, SA- salicylic acid, CK- cytokinin, GA- gibberellic acid, PA- polyamine; Vertical bars represent standard deviation of mean (n= 4)

Figure 1. Chlorophyll a (A), chlorophyll b (B), total chlorophyll (C) indices, and chlorophyll a/b ratio (D) of radish cultivated under salt stress and phytohormone application

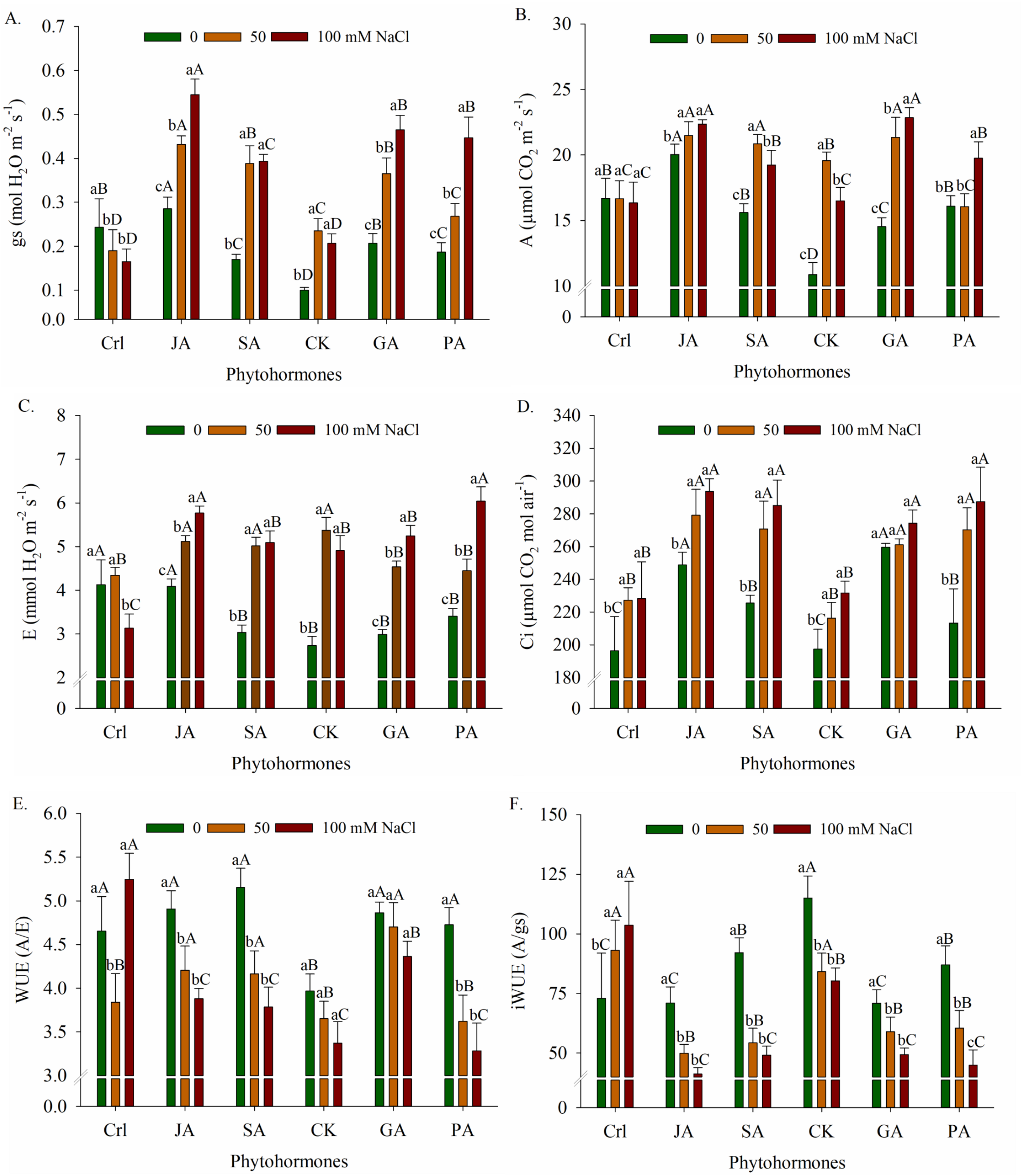
may be linked to the potential of jasmonic acid to enhance the content of intermediates in the chlorophyll biosynthetic pathway, including glutamate 1-semialdehyde (GSA), δ-ALA, protoporphyrin, Mg-protoporphyrin, and protochlorophyllide, resulting in a significant increase in total chlorophyll content (Qin et al., 2024). Stress conditions trigger the activation of chlorophyllase, leading to reduction in magnesium absorption and a decline in RuBisCO activity and synthesis, disrupting plant photosynthetic performance (Jan et al., 2018; Qin et al., 2024).

Cytokinin promoted a 12.83% increase in chlorophyll b index, compared to the control (Figure 1B). Furthermore, severe salt stress increased these indices in all treatments, except when gibberellin was applied. The total chlorophyll index exhibited a similar pattern to the chlorophyll a index (Figure 1C). Cytokinin, even under severe salt stress conditions, stimulates chlorophyll production in plants (Liu et al., 2020). This occurs because cytokinins play a central role in chloroplast development and function, as well as in chlorophyll biosynthesis, by regulating genes associated with photosynthesis and protecting plant photosynthetic machinery and productivity in the face of various environmental stresses, acting in the safeguarding of photosynthesis at both levels of photosynthetic reactions (Hudeček et al., 2023). This behavior underscores that the application of this phytohormone provides a more significant balance between chlorophyll production and maintenance in radish plants under salt stress conditions.

The lowest chlorophyll a/b ratio was observed in plants subjected to cytokinin application and exposed to severe salt stress (Figure 1D). This could be related to the ability of this phytohormone to promote a more efficient balance between chlorophyll production and maintenance under adverse conditions. Consequently, these results indicate that the application of jasmonic acid and cytokinin can play fundamental roles in the adaptation of radish plants to salt stress, contributing to the maintenance of chlorophyll production and, consequently, to photosynthetic efficiency and overall plant performance under adverse conditions.

The application of jasmonic acid resulted in a notable increase in stomatal conductance (gs) in plants subjected to salt stress, both moderate (51.46%) and severe (91.23%, Figure 2A). A similar trend was observed for other phytohormones, such as salicylic acid (with increases of 128.43 and 131.37%, respectively), cytokinin (with increments of 147.37 and 117.54%, respectively), gibberellin (with increases of 76.61 and 125.00%, respectively), and polyamine (with increases of 43.75 and 139.29%, respectively). In contrast, salt stress induced a reduction in stomatal conductance in plants that did not receive phytohormone application (control).

During abiotic stress, jasmonic acid assumes a multifaceted role involving various plant responses, including gene regulation, synthesis of specific proteins, and secondary metabolism (Singh et al., 2022). It orchestrates the mitigation of adverse effects induced by environmental stress through a series of plant responses (Choudhary et al., 2021a). Its application may have stimulated stomatal opening, leading to an increase in gs. Phytohormones such as salicylic acid (SA), cytokinin (CK), gibberellin (GA), and polyamine (PA) can also influence stomatal regulation, affecting gs (Choudhary et



Vertical bars with the same uppercase letter do not differ for phytohormones, and vertical bars with the same lowercase letter do not differ for salt stress according to the Tukey's test ($p \leq 0.05$). Crl- control, JA- jasmonic acid, SA- salicylic acid, CK- cytokinin, GA- gibberellic acid, PA- polyamine; Vertical bars represent standard deviation of mean (n= 4)

Figure 2. Stomatal conductance (gs - A), net photosynthesis (A - B), transpiration (E - C), internal CO_2 concentration (Ci - D), instantaneous water use efficiency (WUE - E), and intrinsic water use efficiency (iWUE - F) of radish cultivated under salt stress and phytohormone application

al., 2021b). They can directly act on the physiology of guard cells, promoting their opening and density, thereby enhancing gs (Montanaro et al., 2022). Moreover, exogenous cytokinins often inhibit abscisic acid-induced stomatal closure in various species (Hudeček et al., 2023).

The application of any phytohormone also led to an increase in net photosynthesis (A) in plants exposed to salt stress, whereas no changes were observed in plants without phytohormone application (Figure 2B). Specifically, jasmonic acid, salicylic acid, cytokinin, and gibberellin enhanced this variable in plants subjected to both moderate salt stress, with increments of 7.20, 33.59, 80.21, and 46.95, respectively, and severe salt stress, with increments of 11.53, 23.29, 51.90, and 57.29%, respectively, and polyamine increased it by 22.82%.

The increase in gs can result in a higher intake of carbon dioxide (CO_2) into the plant's leaves (Taiz et al., 2017). This CO_2 is essential for photosynthesis, where it is converted into sugars and other organic compounds. Therefore, the elevated gs facilitated by phytohormones may have led to greater CO_2 availability for photosynthesis. Phytohormones can also directly influence the activity of enzymes involved in photosynthesis (Li et al., 2020). The application of phytohormones may have stimulated these enzymes, making photosynthesis more efficient even under salt stress conditions. Severe salt stress can induce oxidative stress in plants, impairing photosynthesis. Phytohormones possess antioxidant properties and can help reduce oxidative stress, enabling more effective photosynthesis (Ali et al., 2020).

Furthermore, phytohormone application had a positive impact on the transpiration rate (E) of plants subjected to stress, with jasmonic acid, salicylic acid, cytokinin, gibberellin, and polyamine increasing this variable in both moderate salt stress-exposed plants (with increments of 25.15, 65.38, 96.35, 52.01, and 30.63%, respectively) and severe salt stress-exposed plants (with increments of 41.05, 67.86, 79.35, 75.56, and 77.35%, respectively, Figure 2C). It is important to note that severe salt stress, represented by a concentration of 100 mM NaCl, significantly reduced this variable in plants cultivated without phytohormone application. Another pertinent point is the increase in internal CO_2 concentration in response to salinity, a trend further accentuated with phytohormone application (Figure 2D).

Phytohormones stimulated stomatal opening in plant leaves (Figure 2A). This stimulation may have resulted in increased water loss through transpiration, which is beneficial for plants under salt stress conditions as it helps regulate temperature and maintain cell turgidity. However, it can also lead to greater uptake of ions, such as Na^+ and Cl^-. Furthermore, severe salt stress can cause excessive stomatal closure as a response to water shortage (Taiz et al., 2017). Phytohormone application may have alleviated this excessive closure, allowing stomata to remain more open and thus increasing the transpiration rate (Arif et al., 2020). The down-regulation of photosynthesis under salt stress is primarily attributed to a limitation in RuBisCO activity, which has been identified as one of the major constraints (Singh et al., 2022).

The increase in gs, as observed with phytohormone application, may have allowed a more efficient influx of CO_2 into the leaves. This would result in a higher internal CO_2 concentration, which is essential for photosynthesis. Greater CO_2 availability in the leaves can lead to more effective photosynthesis, which is crucial for energy and biomass production in plants (Pan et al., 2021). However, higher intracellular CO_2 rates might indicate that the plants may not be efficiently utilizing CO_2 in photosynthetic processes.

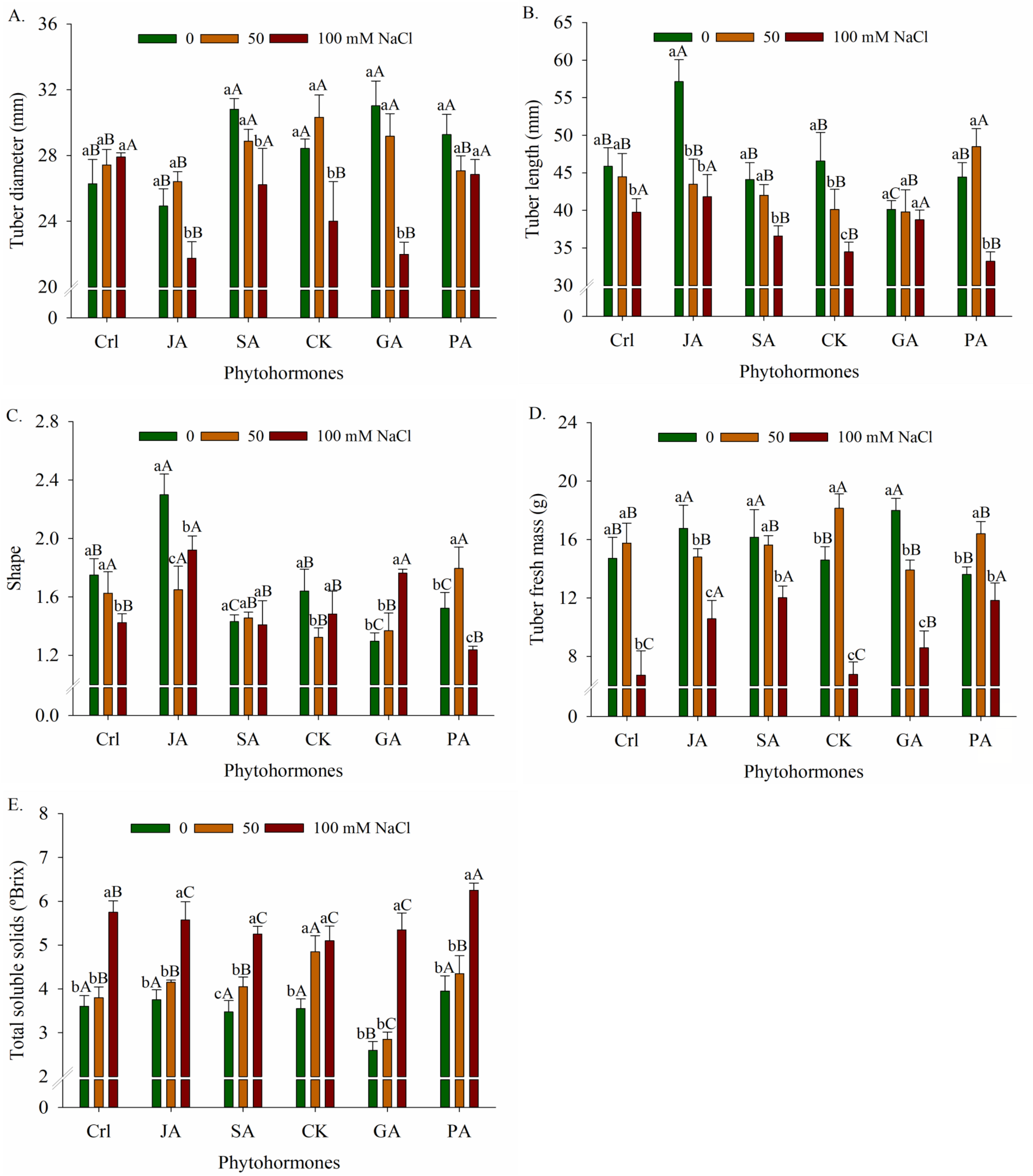
The instantaneous water use efficiency (WUE) decreased with the application of jasmonic acid, salicylic acid, and polyamine in plants cultivated under both moderate (14.31, 19.18, and 23.42%) and severe (20.95, 26.56, and 30.52%) salt stress conditions, respectively (Figure 2E). The reduction in WUE with the application of these phytohormones in plants under both moderate and severe salt stress might be related to their influence on gas exchange and stomatal regulation. A possible explanation is that the application of these phytohormones led to increased transpiration (E) in plants, as mentioned earlier. This could result in greater water loss relative to the photosynthesis rate (A), leading to a decrease in WUE.

The intrinsic water use efficiency (iWUE) decreased with the application of jasmonic acid, salicylic acid, cytokinin, gibberellin, and polyamine in plants subjected to both moderate (29.63, 41.08, 26.89, 16.83, and 30.48%) and severe (41.92, 46.79, 30.25, 30.39, and 48.42%) salt stress, compared to the control, respectively (Figure 2F). This decrease may be related to the action of these phytohormones in maintaining stomatal conductance and net photosynthesis, keeping them in balance (Silva et al., 2022a).

Severe salt stress had a negative effect on tuber diameter in radish plants that received phytohormone application, with the exception of polyamine (Figure 3A). The average commercial diameter of radish 'Cometa' is 20-30 mm (Isla®), with all treatments reaching this value. This could be attributed to polyamines serving a protective function due to their capacity to function as osmolytes or to trigger the generation and accumulation of additional osmolytes, such as proline, glycine betaine, and γ-Aminobutyric acid (GABA, Silva et al., 2022b). Remarkably, the application of salicylic acid, cytokinin, and gibberellin kept this variable stable in plants subjected to moderate salt stress compared to the control.

Regarding the length of radish tubers, severe salt stress led to a significant decrease, except in plants that were subjected to gibberellic acid application (Figure 3B). The average tuber length of radish 'Cometa' is 27 mm (Teixeira et al., 2019), with all treatments reaching this value. Polyamine application also increased this variable in plants subjected to moderate salt stress. It is important to note that the application of salicylic acid did not influence the shape of radish tubers (Figure 3C). However, plants cultivated under moderate salt stress and subjected to jasmonic acid and cytokinin application had tubers with more reduced shapes compared to the respective controls. This result suggests that these substances promoted a more oval shape of the tubers, which could be visually appealing for this vegetable.

The primary cause of reduced plant growth under salt stress is a decrease in chlorophyll content, which subsequently diminishes the photosynthetic capacity of the plants (Singh et



Vertical bars with the same uppercase letter do not differ for phytohormones, and vertical bars with the same lowercase letter do not differ for salt stress according to Tukey's test ($p \leq 0.05$). Crl- control, JA- jasmonic acid, SA- salicylic acid, CK- cytokinin, GA- gibberellic acid, PA- polyamine; Vertical bars represent standard deviation of mean (n= 4)

Figure 3. Tuber diameter (A), tuber length (B), shape (C), tuber fresh mass (D), and total soluble solids (E) of radish cultivated under salt stress and phytohormone application

al., 2022). Salt stress affects plants through osmotic and ionic effects, reducing soil water potential and causing osmotic stress. This, in turn, lowers water uptake by plants, decreasing plant water potential, resulting in reduced turgor, stomatal closure, reduced photosynthesis, and inhibited plant growth (Hajihashemi et al., 2021). The application of salicylic acid, cytokinin, and gibberellin may have counteracted the negative effects of salt stress, potentially by stimulating water uptake and promoting radial tuber growth. Severe salt stress can also inhibit longitudinal tuber growth, as reduced water uptake due to decreased soil osmotic potential caused by salt stress leads to cellular dehydration and loss of turgor pressure, ultimately halting growth (Zhao et al., 2021). Salicylic acid application may have acted as a protective agent against the negative effects of salt stress, allowing cells to continue elongating even under high salt concentrations (Ulukapi et al., 2020).

Severe salt stress led to a significant reduction in the fresh mass of tubers in all treatments (Figure 3D). The average fresh mass of radish 'Cometa' is 5 g (Teixeira et al., 2019), with all treatments reaching this value. However, the application of cytokinin and polyamine increased this variable in plants under moderate salt stress, while the application of salicylic acid maintained it stable compared to the respective control for each phytohormone. The reduction in tuber fresh mass in response to severe salt stress can be attributed to the challenging nature of this type of stress on plants. The high salt concentration in the soil affects water absorption by the roots, leading to a reduction in cell turgidity and plant growth (Zhao et al., 2021). This results in smaller tubers with lower fresh mass due to decreased cell expansion and compromises organ development. However, the application of cytokinin and polyamine seemed to have a beneficial effect as it increased the fresh mass of tubers compared to the control group, which did not receive these phytohormones. This suggests that these substances may possess properties that help mitigate the negative effects of salt stress on plant growth and development. They may have promoted water and nutrient absorption as well as cell expansion in the tubers (Zhou et al., 2023), resulting in larger tubers with higher fresh mass. On the other hand, salicylic acid appeared to maintain the fresh mass of tubers stable compared to the control, even under severe salt stress. This indicates that salicylic acid may have the ability to sustain tuber growth under challenging conditions, contributing to its stability in the face of salt stress (Naeem et al., 2020).

The average total soluble solids content of radish 'Cometa' is 3.37 °Brix (Sena et al., 2015), with all treatments achieving this value, except for plants grown without salt stress and under moderate stress with the application of salicylic acid. Regarding the total soluble solids content in the tubers, the application of polyamine led to a significant increase in this variable in plants subjected to severe salt stress compared to the other phytohormones and the control (Figure 3E). This suggests that polyamine plays a crucial role in regulating the accumulation of soluble sugars under adverse conditions (Silva et al., 2023). On the other hand, the application of cytokinin kept this variable constant in tubers subjected to both moderate and severe salt stress, with values higher than those observed in the control treatment, indicating its stabilizing effect on the maintenance of total soluble solids, especially under moderate and severe salt stress (Azzam et al., 2022).

A principal component analysis with clustering was conducted (Figure 4). The first two components were able to account for a total of 64.6% of the variance in the data. From this analysis, three distinct groups of treatments were identified. In the first group, treatments included the control without phytohormone application (Crl0), plants not subjected to salt stress (0 mM NaCl) with the application of salicylic acid (SA0), cytokinin (CK0), and polyamine (PA0), as well as plants under moderate salt stress (50 mM NaCl) with jasmonic acid application (JA50), and plants under severe salt stress (100 mM NaCl) with jasmonic acid (JA100), salicylic acid (SA100), and cytokinin (CK100) application. In the second group, plants subjected to moderate salt stress (50 mM NaCl) with cytokinin (CK50), polyamine (PA50), and gibberellin (GA50) application

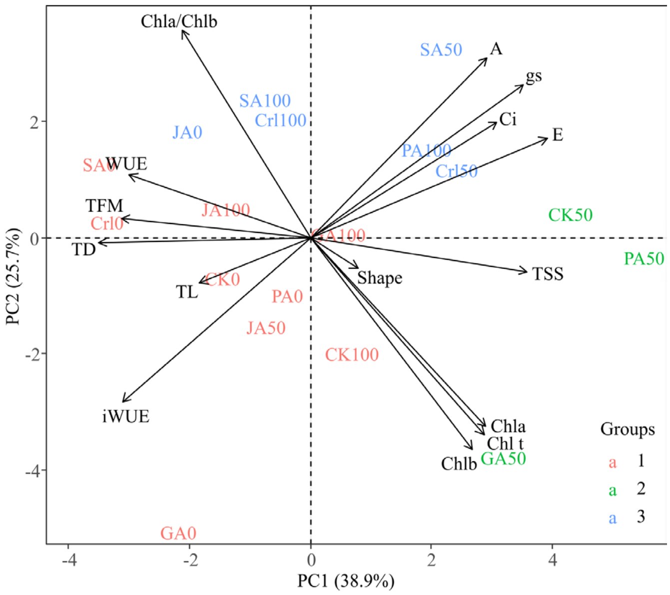


Figure 4. Principal component analysis with clusters of radish grown under salt stress and phytohormone application

were grouped. Finally, the third group consisted of plants that grew with jasmonic acid application without stress (JA0), plants under moderate salt stress (50 mM NaCl) with salicylic acid (SA50) application and without phytohormone application (Crl50), as well as plants under severe salt stress (100 mM NaCl) with salicylic acid (SA100), polyamine (PA100), and without phytohormone application (Ctl100).

The chlorophyll a (Chla), chlorophyll b (Chlb), and total chlorophyll (Chl t) indices had higher values when plants were subjected to treatments from Group 2, which indicates that plants in this group had a higher concentration of these chlorophylls, contributing to greater photosynthetic efficiency. Additionally, the shape and total soluble solids (TSS) content were more pronounced in this group, suggesting a positive relationship between these variables. Gas exchange parameters, represented by A (net photosynthesis), gs (stomatal conductance), Ci (internal CO_2 concentration), and E (transpiration), were more closely associated with treatments from Group 3. This indicates that plants in this group exhibited different responses in gas exchange compared to the other groups due to the influence of salt stress or phytohormone application. In contrast, instantaneous water use efficiency (WUE) and intrinsic water use efficiency (iWUE) were more related to Group 1. This suggests that plants in this group may have achieved a better balance between carbon assimilation and water loss, indicative of an adaptive response to salt stress or phytohormone application. Finally, tuber fresh mass (TFM), tuber diameter (TD), and tuber length (TL) were also more closely related to Group 1, indicating that tuber characteristics were more influenced by these treatments.

The analysis of treatment groups reveals significant patterns in the responses of radish plants. In Group 2, the application of phytohormones such as jasmonic acid and cytokinin resulted in higher levels of chlorophyll, suggesting an improvement in photosynthetic efficiency. Additionally, tuber characteristics, such as shape and total soluble solids (TSS) content, were also more pronounced, indicating a possible positive relationship. Group 3 exhibited distinct responses in gas exchange, highlighting plant adaptations to salt stress or phytohormone application. Plants in this group showed variations in photosynthesis and transpiration rates, possibly as response mechanisms to stresses. Group 1 displayed a stronger relationship with water use efficiency (WUE) and intrinsic water use efficiency (iWUE), indicating an effective balance between carbon assimilation and water loss. Furthermore, tuber characteristics were more influenced by these treatments. These results underscore the complex responses of radish plants to phytohormone application and salt stress, providing valuable insights for optimizing cultivation under challenging conditions.

Conclusions

1. Under severe salt stress, jasmonic acid and cytokinin increased chlorophyll contents, mitigating salt-induced reductions in chlorophyll production.

2. Phytohormones boosted stomatal conductance and net photosynthesis, even under salt stress and influenced radish tuber variables.

3. Polyamine increased total soluble solids content in tubers under severe salt stress, while cytokinin maintained this content under both moderate and severe salt stress.

Literature Cited

Ali, Q.; Shahid, S.; Nazar, N.; Hussain, A. I.; Ali, S.; Chatha, S. A. S.; Perveen, R.; Naseem, J.; Haider, M. Z.; Hussain, B.; Murtaza, S.; Hussain, S. M. Use of phytohormones in conferring tolerance to environmental stress. In: Hasanuzzaman, M. (ed.). Plant ecophysiology and adaptation under climate change: mechanisms and perspectives II: mechanisms of adaptation and stress amelioration, v.1, Singapore: Springer, 2020. p.245-355. https://doi.org/10.1007/978-981-15-2172-0_11

AOAC – Association of Official Analytical Chemists. Official methods of analysis of the association of agricultural chemists. 18.ed. Gaithersburg: AOAC, 2005.

Arif, Y.; Singh, P.; Siddiqui, H.; Bajguz, A.; Hayat, S. Salinity induced physiological and biochemical changes in plants: An omic approach towards salt stress tolerance. Plant Physiology and Biochemistry, v.156, p.64-77, 2020. https://doi.org/10.1016/j.plaphy.2020.08.042

Azzam, C. R.; Zaki, S. N. S.; Bamagoos, A. A.; Rady, M. M.; Alharby, H. F. Soaking maize seeds in zeatin-type cytokinin biostimulators improves salt tolerance by enhancing the antioxidant system and photosynthetic efficiency. Plants, v.11, e1004, 2022. https://doi.org/10.3390/plants11081004

Çavuşoğlu, K.; Kılıç, S.; Kabar, K. Effects of some plant growth regulators on stem anatomy of radish seedlings grown under saline (NaCl) conditions. Plant, Soil and Environment, v.54, p.428-433, 2008.

Choudhary, K. K.; Singh, S.; Agrawal, M.; Agrawal, S. B. Role of Jasmonic and salicylic acid signaling in plants under UV-B stress. In: Aftab, T.; Yusuf, M. (eds.). Jasmonates and salicylates signaling in plants. Singapore: Springer, 2021b. p.45-63. https://doi.org/10.1007/978-3-030-75805-9_3

Choudhary, P.; Pramitha, L.; Rana, S.; Verma, S.; Aggarwal, P. R.; Muthamilarasan, M. Hormonal crosstalk in regulating salinity stress tolerance in graminaceous crops. Physiologia Plantarum, v.173, p.1587-1596, 2021a. https://doi.org/10.1111/ppl.13558

Hajihashemi, S.; Skalicky, M.; Brestic, M.; Pavla, V. Effect of sodium nitroprusside on physiological and anatomical features of salt-stressed *Raphanus sativus*. Plant Physiology and Biochemistry, v.169, p.160-170, 2021. https://doi.org/10.1016/j.plaphy.2021.11.013

Henschel, J. M.; Moura, V. S.; Silva, A. M. O.; Gomes, D. D. S.; Santos, S. K.; Batista, D. S.; Dias, T. J. Can exogenous methyl jasmonate mitigate salt stress in radish plants? Theoretical and Experimental Plant Physiology, v.35, p.51-63, 2023. https://doi.org/10.1007/s40626-023-00270-8

Hudeček, M.; Nožková, V.; Plíhalová, L.; Plíhal, O. Plant hormone cytokinin at the crossroads of stress priming and control of photosynthesis. Frontiers in Plant Science, v.13, e1103088, 2023. https://doi.org/10.3389/fpls.2022.1103088

Jan, S.; Alyemeni, M. N.; Wijaya, L.; Alam, P.; Siddique, K. H.; Ahmad, P. Interactive effect of 24-epibrassinolide and silicon alleviates cadmium stress via the modulation of antioxidant defense and glyoxalase systems and macronutrient content in *Pisum sativum* L. seedlings. BMC Plant Biology, v.18, 146, 2018. https://doi.org/10.1186/s12870-018-1359-5

Li, X.; Sun, P.; Zhang, Y.; Jin, C.; Guan, C. A novel PGPR strain *Kocuria rhizophila* Y1 enhances salt stress tolerance in maize by regulating phytohormone levels, nutrient acquisition, redox potential, ion homeostasis, photosynthetic capacity and stress-responsive genes expression. Environmental and Experimental Botany, v.174, 104023, 2020. https://doi.org/10.1016/j.envexpbot.2020.104023

Liu, Y.; Zhang, M.; Meng, Z.; Wang, B.; Chen, M. Research progress on the roles of cytokinin in plant response to stress. International Journal of Molecular Sciences, v.21, 6574, 2020. https://doi.org/10.3390/ijms21186574

Mahdavian, K. Application of salicylic acid on chlorophyll, carotenoids, and proline in radish under salinity stress. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences, v.93, p.809-818, 2023. https://doi.org/10.1007/s40011-023-01484-1

Mishra, P.; Nagaich, K. N. Response of gibberellic acid on growth and yield of radish (*Raphanus sativus* L.) cv. Japanese white. Journal of Pharmacognosy and Phytochemistry, v.8, p.1521-1523, 2019.

Montanaro, G.; Briglia, N.; Lopez, L.; Amato, D.; Panara, F.; Petrozza, A.; Cellini, F.; Nuzzo, V. A synthetic cytokinin primes photosynthetic and growth response in grapevine under ion-independent salinity stress. Journal of Plant Interactions, v.17, p.789-800, 2022. https://doi.org/10.1080/17429145.2022.2102259

Naeem, M.; Basit, A.; Ahmad, I.; Mohamed, H. I.; Wasila, H. Effect of salicylic acid and salinity stress on the performance of tomato plants. Gesunde Pflanzen, v.72, p.1-10, 2020. https://doi.org/10.1007/s10343-020-00521-7

Navada, S.; Vadstein, O.; Gaumet, F.; Tveten, A. K.; Spanu, C.; Mikkelsen, Ø.; Kolarevic, J. Biofilms remember: Osmotic stress priming as a microbial management strategy for improving salinity acclimation in nitrifying biofilms. Water Research, v.176, e115732, 2020. https://doi.org/10.1016/j.watres.2020.115732

Pan, T.; Liu, M.; Kreslavski, V. D.; Zharmukhamedov, S. K.; Nie, C.; Yu, M.; Kuznetsov, V. V.; Allakhverdiev, S. I.; Shabala, S. Non-stomatal limitation of photosynthesis by soil salinity. Critical Reviews in Environmental Science and Technology, v.51, p.791-825, 2021. https://doi.org/10.1080/10643389.2020.1735231

Qin, C.; Lian, H.; Alqahtani, F. M.; Ahanger, M. A. Chromium mediated damaging effects on growth, nitrogen metabolism and chlorophyll synthesis in tomato can be alleviated by foliar application of melatonin and jasmonic acid priming. Scientia Horticulturae, v.323, e112494, 2024. https://doi.org/10.1016/j.scienta.2023.112494

R Core Team. R: A language and environment for statistical computing. Viena: Austria, 2023.

Sena, C. C.; Silva, V. M.; Araujo, K. K. S.; de Campos, A. J. Atmosfera modificada passiva na qualidade póscolheita do rabanete 'Cometa' in natura armazenado em diferentes embalagens. Revista Agrotecnologia, v.6, p.81-96, 2015.

Silva, T. I.; Dias, M. G.; Araújo, N. O.; Santos, M. N. S.; Cruz, R. R. P.; Dias, T. J.; Ribeiro, W. S.; Grossi, J. A. S.; Barbosa, J. G. Spermine reduces the harmful effects of salt stress in *Tropaeolum majus*. Physiology and Molecular Biology of Plants, v.28, p.687-696, 2022b. https://doi.org/10.1007/s12298-022-01165-9

Silva, T. I.; Dias, M. G.; Barbosa, L. B.; Araújo, N. O.; Ferreira, F. D.; Grossi, J. A. S.; Costa, F. B.; Marco, C. A.; Ribeiro, D. M. Spermine decreases ethylene and increases sugars and phenolic compounds in nasturtium flowers grown under drought and salt stress. Bragantia, v.82, e20230041, 2023. https://doi.org/10.1590/1678-4499.20230041

Silva, T. I.; Dias, M. G.; Grossi, J. A. S.; Ribeiro, W. S.; Moraes, P. J.; Araújo, F. F.; Barbosa, J. G. Application of phytohormones as attenuators of salt stress in *Tropaeolum majus* L. (Tropaeolaceae). Acta Botanica Croatica, v.81, p.51-60, 2022a. https://doi.org/10.37427/botcro-2022-001

Singh, P.; Choudhary, K. K.; Chaudhary, N.; Gupta, S.; Sahu, M.; Tejaswini, B.; Sarkar, S. Salt stress resilience in plants mediated through osmolyte accumulation and its crosstalk mechanism with phytohormones. Frontiers in Plant Science, v.13, e1006617, 2022. https://doi.org/10.3389/fpls.2022.1006617

Taiz, L.; Zeiger, E.; Moller, I. M.; Murphy, A. Fisiologia e desenvolvimento vegetal. 6.ed. Porto Alegre: ArtMed, 2017. 888p.

Teixeira, A.; Blind, A.; Silva Filho, D.; Sprey, M.; Dias Junior, L. Desempenho de cultivares de rabanete na condição edafoclimatica de Manaus–AM. Agrarian Academy, v.6, p.123-132, 2019.

Ulukapi, K.; Nasırcılar, A. G.; Kurt, Z. Determination of salt tolerance levels of some radish cultivars and evaluation of the effectiveness of salicylic acid on germination in saline conditions. Turkish Journal of Agriculture-Food Science and Technology, v.8, p.632-637, 2020. https://doi.org/10.24925/turjaf.v8i3.632-637.3109

Zhao, S.; Zhang, Q.; Liu, M.; Zhou, H.; Ma, C.; Wang, P. Regulation of plant responses to salt stress. International Journal of Molecular Sciences, v.22, e4609, 2021. https://doi.org/10.3390/ijms22094609

Zhou, H.; Cheng, L.; Wang, Z. Current understanding of unlocking the power of melatonin and other phytohormones to boost abiotic stress tolerance in solanaceous vegetable crops. Scientia Horticulturae, v.321, e112313, 2023. https://doi.org/10.1016/j.scienta.2023.112313