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Article

Effect of Hormonal Priming on Mitigating Salt Stress in Tomato Plants

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Abstract: Salinity is one of the major factors affecting crop plants growth and productivity. Hormonal seed priming using salicylic acid (SA) has been shown to improve plant tolerance to salinity. This study aims to definen the effect of 1mM SA addition under different NaCl levels (0, 50, 100, 150 and 200mM) on tomato plants (*Solanum lycopersicum* L. Mill.). The results of this study revealed that the salicylic acid addition had no significant effect on emergence rate and stems heights of tomato plants stressed to salinity. On the other hand, a positive effect was reported on roots lengths. Indeed, the values recorded in salinity conditions and in the presence of SA were higher than those recorded in its absence (2.83±0.5cm at 50mM). The data from the study of the biochemical aspect seems to indicate a significant effect of salicylic acid on leaves sugar content. A maximum content estimated at 70.822±9.887 mg/g MF was recorded at 150mM. No significant effect was revealed in the presence of SA on proline content. SA can provide sustainable solution in mitigating salt stress in tomato plants.

Keywords: *solanum lycopersicum*; salicylic acid; NaCl; mitigating.

1. Introduction

One of the most important environmental issues facing arid and semi-arid regions today is the increasing accumulation of mineral salts in soils, reducing plant growth and yield through various physiological stresses [1]. According to [2], more than 20% of agricultural land worldwide is affected by salinization, and this proportion will increase to 50% by 2050. In general, crops are sensitive to salt stress. Germination, emergence and early seedling growth are the stages in which plants are most sensitive to salt conditions [3]. Salinity reduces germination rates [4]. Excessive absorption of ions can lead to toxicity and reduce the amount of water available to the seeds, thereby inhibiting the formation of primary roots [5].

To combat salt stress, desalination strategies can be used. Also, salt-tolerant plants can be developed by conventional or advanced molecular techniques, but these techniques are time-consuming and expensive. Recent research focuses on identifying alternative approaches to improve plant productivity under stressful conditions [6]. Seed priming techniques offer a cost-effective solution to address this need [7]. Seed priming is a widely adopted method to improve seed germination and plant development [8]. This alternative approach can enhance plants tolerance to abiotic stresses by promoting rapid and uniform seed germination [9]. Priming facilitates the rapid absorption of water and essential nutrients, reducing the seeds' sensitivity to external environmental factors [10]. Various priming methods are available, including hydropriming, osmopriming, nutrient priming, thermopriming, biopriming, chemical priming, and hormonal priming [10].

In hormonal priming, Salicylic acid (SA) can be used as priming agent in mitigating abiotic stresses such as salt stress; it controls several cellular processes in plants [11]. SA is an endogenous phenolic compound that acts as a signal molecule to regulate plant response to various environmental stresses [12]. It protects plant cells from ion toxicity and cell death by several processes management such as antioxidant defense system, nitrogen metabolism, photosynthesis, water stress [13] and expression of genes involved in defense responses [14]. Plant tolerance to salt can also be induced by exogenous SA application [15].

Salicylic acid (SA) have been showed to decrease the deleterious effects of abiotic stress on tomato plants [16]. Tomato (*Solanum lycopersicum* L.) is an important crop with high economic value and is widely cultivated in areas where salt stress is particularly severe [17].

To enhance sustainable agriculture, this research demonstrates the possibility of application of salicylic acid (SA), to support tomato growth under salt stress. The ability of (SA) to increase tomato salt stress tolerance was assessed, *in vivo*, through measuring growth and osmoregulation under both saline and non-saline conditions.

2. Materials and Methods

Plant material, salt treatments and SA application

The experiment was conducted in a greenhouse at Higher School of Agronomy, Mostaganem, Algerial. During the experiment (May to June 2024), daily temperature and relative humidity were noted, with average values of 25 °C, 18 °C maximum temperature, minimum temperature and 56% humidity. The experiment was carried out with 12 hours of light.

The experiment was conducted using a completely randomized design with five replicates and three plants per replicate. The salinity levels included 0 (no stress/distilled water), 50 (moderate salt stress), 100, 150 and 200mM (severe salt stress) of NaCl, while the salicylic acid (SA) treatments consisted of 0 (distilled water), and 1mM of SA. So, the treatments included, controls without SA and no stress (0 mM), controls for salt stress (50, 100, 150, and 200 mM) and combinations of SA × NaCl (20, 50, 100, 150, and 200 mM).

Tomato seeds (*Solanum lycopersicum* L. Mill. Var. SISILIA F1) were blotted dry onto sterile filter paper after being surface sterilized for five minutes using a sodium hypochlorite solution and rinsed three times, for one minute each time, in sterile distilled water.

The seedlings were transplanted into pots (15cm/diameter and 25cm/length) containing soil with 1.2 dS/m. The 1mM SA solutions was prepared by diluting the product in heated distilled water (67 °C) to ensure complete dissolution of the crystals in the solution. Irrigation with different treatments was carried at 100% of water retention capacity of the substrate.

Growth measurements

Emergence rate, Root lengths, and Stem height were estimated.

Analyses of proline accumulation in tomato plants

Proline accumulation was calculated using [18] methodology. One gram of fresh plant material was homogenized in 2mL of 40% methanol. Then, 1 mL of the prepared extract was mixed with 2 ml of glacial acetic acid and 2 ml of ninhydrin reagent (1.25 g ninhydrin liquefied in 20 ml of 6 mol l^{-1} phosphoric acid and 30 ml glacial acetic acid). After 60 minutes of boiling in a water bath at 100°C, 5 ml of toluene was added to the mixture. Utilizing a Jenway 67155 UV/Vis spectrophotometer (Stone, UK), absorbance was measured at 528 nm. Proline concentration was calculated as mg g^{-1} of fresh weight (FW) by using a calibration curve.

Determination of total soluble sugar content

Tomato plants' soluble sugar content was measured according to [19]. One gram of fresh plant material was extracted in 5.25 mL of 80% ethanol for 24 hours. The resulting extract was diluted ten times with ethanol 80%. Subsequently, 2 mL of the extract were added to 4 mL of an anthrone reagent (2 g anthrone + 1000 mL sulfuric acid). Using a Jenway 67155 UV/Vis spectrophotometer (Stone, UK), the developed blue-green color was measured at 585 nm. Since glucose was used to create a standard curve, the results are expressed in mg g^{-1} of fresh weight (FW).

Statistical analysis

Data were analyzed using STATBOX v6.4 software via a two-way ANOVA. Means were compared using the LSD test ($P < 0.05$).

3. Results

Emergence rate

Our results showed that emergence rate decreases significantly with increasing NaCl concentration ($F_{4,1}$=10.5, P=0.00011; Figure 1). Whether in the absence or presence of salicylic acid, a total (100%) emergence rate was observed for at 0mM NaCl (Figure 1). On the other hand, a significant reduction was obtained under salinity conditions without SA treatment; 83.33% of seeds emerged under 50mM saline treatment. Seed emergence was further affected by increasing NaCl concentration. Rates in the order of 58.3, 25 and 16.6% were recorded respectively by tomato seeds treated with 100, 150mM and 200mM NaCl.

However, the combination SA x salinity had no impact on emergence ($p > 0.05$). A non-significant increase in emergence was observed in tomato seeds subjected to a combined salicylic acid/salinity treatment. For tomato seeds treated with 150 and 200mM NaCl ith SA application, emergence rates were lower than 50%.

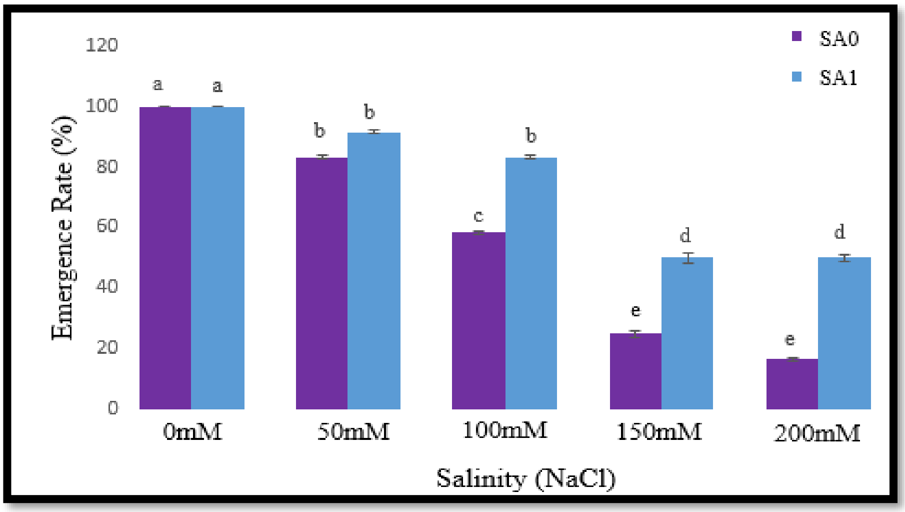

Figure 1. Effect of NaCl, SA application, and their interactions on emergence rate of tomato plants (%). Means followed by the same letter are not significantly different ($p < 0.05$) according to LSD test. SA: Absence of SA application. SA1: SA application

Root length and stem height

Data for root length (Figure 2A) and plant height (Figure 2B) showed that these two parameters were strongly influenced by different NaCl concentrations in tomato. Root length underwent a significant reduction with increasing saline concentration, reaching 0.5±0.08cm under 200mM NaCl treatment.

Salicylic acid shows a significant effect on root length in salt-stressed tomato plants.

The addition of salicylic acid to the different salt treatments allowed an increase in root length; tomato plants subjected to 50mM registered the maximum root length with 2.83±0.5cm.

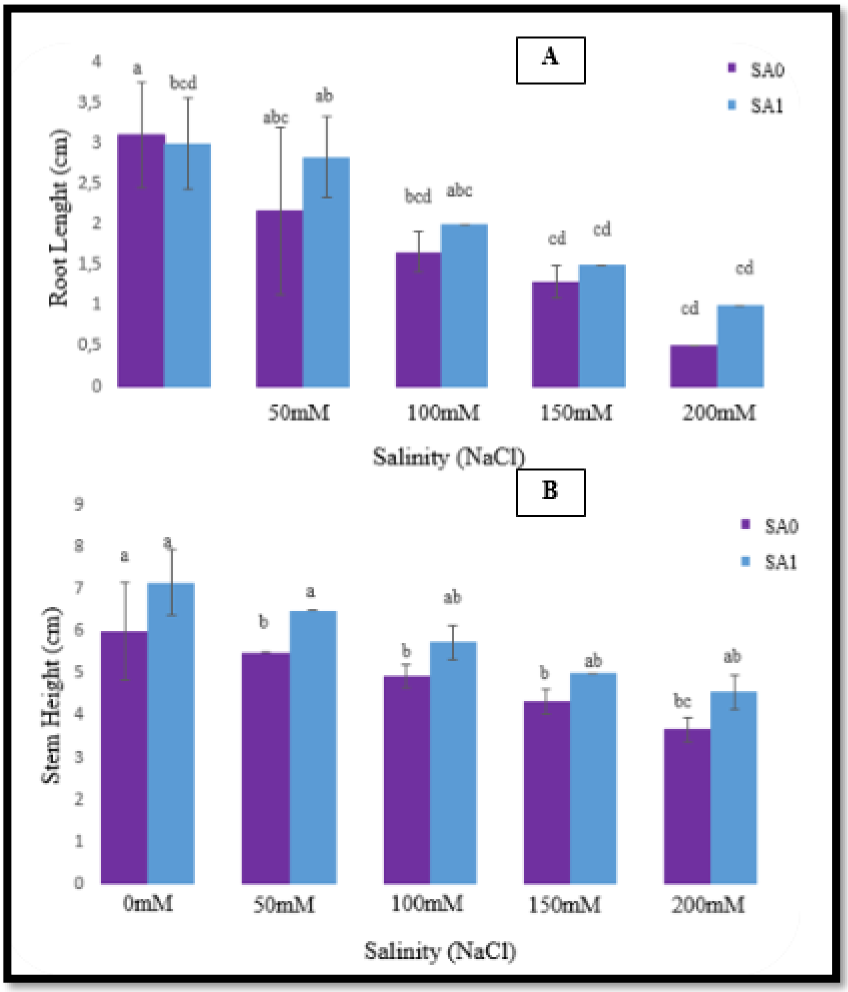

Figure 2. Effect of NaCl, SA application, and their interactions on Root Length (A) and Stem Height (B) of tomato plants (%). Means followed by the same letter are not significantly different ($p < 0.05$) according to LSD test. SA: Absence of SA application. SA1: SA application

Sugar and Proline Contents

The obtained results revealed a significant effect of salinity on tomato leaves sugar contents (Figure 3A). An important decrease in sugar content was observed in salinity-stressed tomato compared to the control.

A significant increase in tomato leaves soluble sugar levels was observed in the presence of the combined salt stress/salicylic acid treatment compared with salt-stressed tomato without SA application.

Our results showed that salinity has a significant impact on proline contents (Figure 3B). Indeed, proline levels increase with increasing substrate salinity. In fact, tomato plants conducted at 100 and 150mM recorded the maximum values with 93.1±13.1 and 87.9±12.5 mg/g MF, respectively.

However, no significant effect was observed by salicylic acid on proline accumulation.

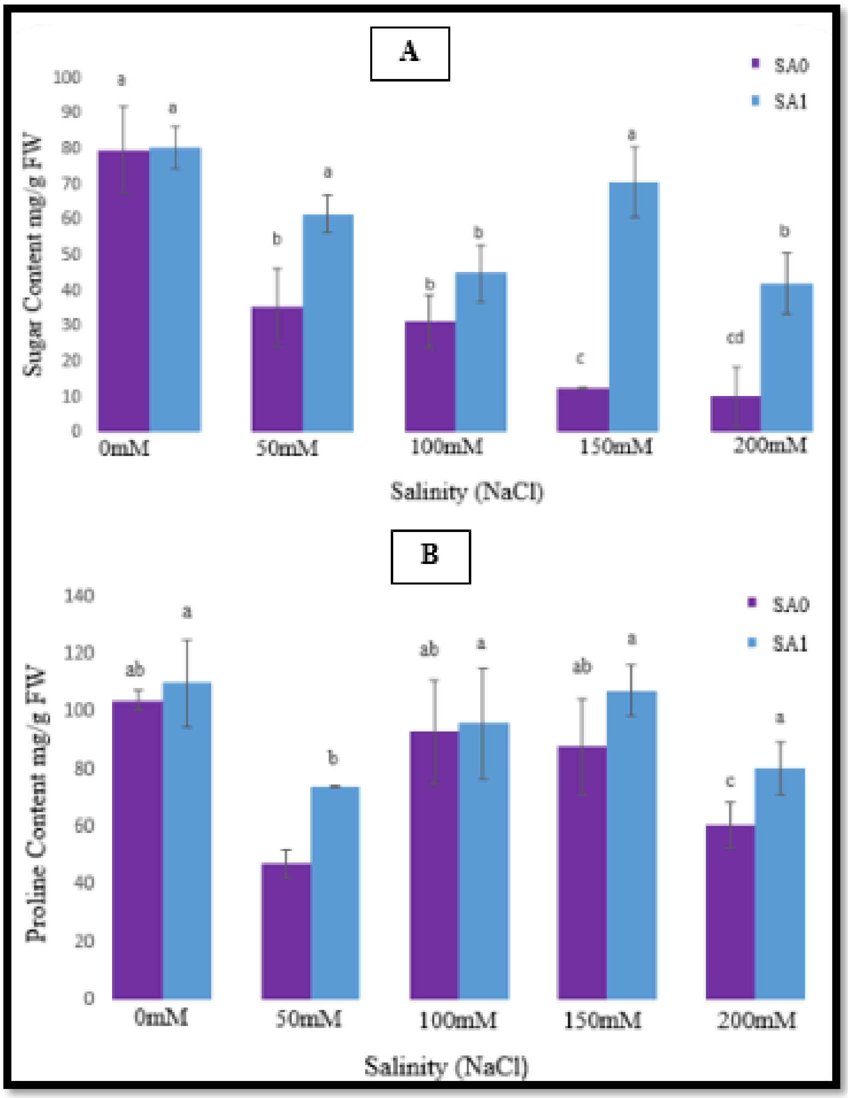

Figure 3. Effect of NaCl, SA application, and their interactions on Sugar (A) and Proline (B) contents of tomato plants (%). Means followed by the same letter are not significantly different ($p < 0.05$) according to LSD test. SA: Absence of SA application. SA1: SA application

4. Discussion

Salinity is one of the main causes of decreasing crop yields. The obtained results showed that the emergence percentage decreases significantly with increasing NaCl concentration in tomato. These results confirm those of [20], who concluded that salt prolongs the emergence process duration. In fact, salt and osmotic stress are responsible for both inhibiting and delaying plant emergence. The inhibition of seedling emergence is related to a cellular accumulation of Na^+ and Cl^- ions that disrupt enzymatic activities crucial to plant physiology, preventing dormancy lifting and leading to a reduction in germination capacity [21]. Similarly, [22] have shown that salinity reduces plants emergence percentage.

Under NaCl conditions, salicylic acid application had no significant effects on tomato plants emergence. [23] reported similar results, indicating that the addition of salicylic acid, whatever the salt concentration tested, had no influence on the emergence rate of durum wheat. On the other hand, [24] observed that pre-treatment with 0.05mM salicylic acid reduced salinity damage in wheat plants.

The results of stem height and root length showed that these two parameters were strongly influenced by the different concentrations of NaCl in tomato. Our results supported previous reports, which explained the negative impact of salinity on stem height and root system development, resulting in delayed germination and seedling emergence [25]. [26] revealed that reduced growth of the aerial vegetative apparatus is an adaptive form necessary for the survival of plants exposed to abiotic stress. In addition, [27] have shown that salinity has a negative impact on morphological characteristics such as plant height, number of leaves and root length. This reduction in growth observed in tomato seedlings can be explained by the action of NaCl, which increases the osmotic pressure of the medium and prevents the absorption of water by the root system, and by the reduction in cell divisions number [28]. Different results were obtained by [23], who found that the NaCl-enriched medium significantly increased final plant height compared with the control, and that saline water did not have clearly negative effects on plant growth.

Salicylic acid exhibited a significant effect on root length in tomato plants subjected to salt stress. [29] reported similar results, signaling that salicylic acid application improved root length and root biomass of maize plants subjected to salt stress conditions. [30] indicated that salicylic acid offers protection against salinity stress. However, salicylic acid was found to have no significant effect on the final height of tomato plants subjected to salt stress. Similar results were obtained by [31] who stated that no significant difference was observed in terms of final height of plants treated with salicylic acid under salt stress conditions compared to control plants and that its ability to influence vertical plant growth remains limited. However, [32] observed that in the presence of the NaCl/salicylic acid combination, plant height, leaf number, stem diameter and dry weight of beans were significantly higher than those of salt-stressed plants non-subjected to salicylic acid treatment.

A significant decrease in sugar content was observed in salinity stressed tomato compared to the control. Similar results were reported by [33], who demonstrated that plants exposed to salinity showed a significant decrease in soluble sugars amounts compared to non-exposed plants. Indeed, salinity delays tomato cells division or elongation, deterring sugar metabolism and reducing water uptake [34]. According to [35], this reduction results from the remobilization of sugars to the stems. Many species exposed to salinity have been reported to accumulate more sugars, such as barley [36]. This accumulation has been related to drought and salinity tolerance mechanisms [37].

A significant increase in the soluble sugar levels in tomato leaves was observed in the presence of the combined salt stress/salicylic acid treatment compared to those recorded in salt-stressed tomato without salicylic acid treatment. According to [38], previous and recent studies have confirmed that salicylic acid could significantly influence plant growth and salt stress resistance, as well as sugar production and metabolism. Also, [39] showed that enrichment of the medium with salicylic acid can influence sugar accumulation. Several studies suggest that salicylic acid offers plants protection against environmental stresses by reducing oxidative stress caused by salinity, promoting photon production and sugar biosynthesis [40].

Regarding the evaluation of the proline content in tomato leaves, the results showed that salinity has a significant impact on this quantity. Indeed, proline levels increase when the salinity of the substrate increases. Similar results were observed in barley [41], durum wheat [42], and rice [43]. According to [17], the accumulation of osmoregulators such as proline under of environmental stresses been shown to maintain ROS scavenging, cellular osmolarity, bio-macromolecule function in plants, as well as a significant improvement in plant adaptation to stressful conditions.

Salicylic acid had no significant effect on proline accumulation. These findings are not comparable to those of [44], who observed an increase in proline levels in salt-stressed maize plants treated with salicylic acid, helping to mitigate the negative effects of salt. Therefore, [45] showed that exogenous application of salicylic acid at 0.5mM increases proline content in tomato plants subjected to salinity stress, thus mitigating the deleterious effects of salinity. From the comparison of the obtained results with those already reported by previous studies, it can be concluded that salicylic acid can mitigate the negative effects of salt stress when used in low doses particularly 0.5mM [46]; consequently it acts at low dose.

5. Conclusions

The obtained results indicated that SA can be useful for sustainable agriculture to improve yield quantity and quality under salinity conditions. The effectiveness of SA depends on its concentration, method of application and the state of development of the plant. It is therefore possible to suggest further experimental tests on concentrations lower than 1mM of AS accompanied by a biometric study to understand plants responses under abiotic stresses.

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