

## Research Article

## The study of the effect of salicylic acid on some morphophysiological characteristics of *Salicornia persica* under salinity stress

Mahsa Sadat Khorsandi Moghadam<sup>1</sup>, Masoume Maleki<sup>1\*</sup> and Zahra Movahedi<sup>2</sup>

<sup>1</sup> Department of Biology, Faculty of Basic Sciences, Malayer University, Malayer, Iran

<sup>2</sup> Department of Plant Production and Genetics, Faculty of Agriculture, Malayer University Malayer, Iran

(Received: 2023/12/12-Accepted: 2024/03/11)

### Abstract

Salinity stress is one of the most important environmental stresses limiting plant growth and performance. *Salicornia persica* is a species of *Chenopodiaceae* family; it is a halophyte and resistant to salinity. In addition, salicylic acid is known as an important molecule for the adaptation of plant responses to environmental stress. In the present study, the effect of salinity and salicylic acid on some morphophysiological traits of plants treated with three sodium chloride concentrations (zero, 200, and 500 ppm) and two salicylic acid concentrations (zero and 0.1 mM) and three harvest durations (1, 7, and 14 days) was investigated. This experiment was conducted as a factorial experimental design in the form of a completely randomized experimental design with three replicates. The initial results of the petridish trials showed that during salinity stress with increase in shoot length and decrease in the malondialdehyde levels compared to the control, the 0.1 mM salicylic acid treatment was more effective than other concentrations. The potting results showed that salinity stress caused a significant decrease in shoot and root dry weight. In addition, salt stress let to an increase in the amount of flavonoids and soluble sugars in most crops. The effect of salinity stress on the amount of pigments showed no particular trend. The amount of potassium increased at a salinity of 200 ppm and decreased at 500 ppm. The use of 0.1 mM salicylic acid increased the dry weight of the roots in all harvests. In addition, the use of salicylic acid increased the amount of chlorophyll b at salinity levels of 200 and 500 ppm. Also, the application of salicylic acid let to an increase in the amount of flavonoids at a salinity of 500 ppm, while the amount decreased at a salinity of 200 ppm. The application of 0.1 mM salicylic acid increased the content of potassium and soluble sugars in most harvests at 200 and 500 salinity levels. In general, the modulating effect of 0.1 mM salicylic acid on salinity stress was observed in *Salicornia persica* in most cases.

**Keywords:** Flavonoid, Salinity stress, Salicylic acid, *Salicornia persica*

### Introduction

In the most regions of the world, salinity is the most important stress factor, limiting plant growth and its performance by reducing the osmotic potential and interfering with the uptake of water and some nutrients. When the amount of sodium and chlorine ions increases, the absorption of essential ions such as potassium, calcium, ammonium and nitrate ions decreases, and they reduce the activity of enzymes and disrupt the structure of membranes (Ahmad *et al.*, 2020). Salinity affects plant growth in two ways: Osmotic tension, which occurs when the amount of salt increases around the environment of the root, and ion toxicity, caused when the amount of salt on the surface of the leaf's increases (Bimurzayev *et al.*, 2021). *Salicornia* is a halophyte with very small leaf-like appendages, pale flowers and fruits and belongs to the *Chenopodiaceae* family (Golshan and Keshavarzi,

2007). Halophytes are adapted to salinity and prevent oxidative stress through osmotic regulation and accumulation of various organic substances such as proline and soluble sugars (Aghaleh *et al.*, 2009). *Salicornia* is a C<sub>4</sub> plant and has mesophilic tissue with large cells that can store some salt, to remove toxic ions from the tissue, the ATPase activity of the tissue must be increased (Yildiz *et al.*, 2020). Salicylic acid is one of the most important signaling molecules that alleviate environmental stress. Salicylic acid has a protective function in plants that are under environmental stress. As a non-enzymatic antioxidant, this substance plays an important role in the regulation of physiological processes in plants (Arfan *et al.*, 2007). In addition, salicylic acid is known as a messenger molecule in acquired systemic resistance (Raskin, 1992). This substance plays an important role in the defense reactions of plants against abiotic stresses (Yuan and

\*Corresponding Author, Email: masoumemaleki@gmail.com

Lin., 2008). The mechanism of action of salicylic acid against stresses is related to its role in regulating antioxidant enzymes and compounds with reactive oxygen species in the plant (Shi and Zhu, 2008; Khan and Gulzar, 2003). External application of salicylic acid has been reported to improve the rate of growth of sunflower and corn under salinity stress (Khan and Gulzar, 2003; Noreen and Ashraf, 2008). Since Iran is a semidesert Country with salty soils, the use of halophytic plants such as *Salicornia* is very useful for the improvement of saline areas and desertification. Salinity modifiers can reduce this damage, due to the lack of studies on the effect of salicylic acid on *Salicornia* plant and the moderating feature of this compound, the need to study in this regard is evident. Also, due to the low cost of such a modifier, it can help to be Practical in agriculture. The purpose of this research is to investigate the moderating effect of salicylic acid in *Salicornia persica* under salinity stress.

### Materials and methods

Initially, this experiment was conducted to study the positive or negative effect of salicylic acid and to determine the best concentration at the stage of germination in the germinator. The stress applied in this experiment included three levels of salinity with concentrations of zero, 200 and 500 ppm sodium chloride and the salicylic acid treatment included two levels with concentrations of zero and 0.1 mM. Prior to conducting this experiment, the seeds of *Salicornia* were placed in a 10% sodium hypochlorite solution for one minute for disinfection and washed three times with distilled water. The equipment of this experiment was disinfected in an autoclave at 121°C for 20 minutes. Then, the seeds were placed in petridishes that contained Whatman paper. To each petridish were added the desired stress and treatment in different concentrations. The petridishes containing the studied seeds were placed inside the germinator at a temperature of 25 °C with a light cycle of 16 hours of light and 8 hours of darkness for 10 days. During this experiment, tension and the desired treatment were applied to each petridish every day. The seeds of *Salicornia persica* were obtained from Ala Avazhan Company. In the second stage of this research, the seeds were planted in plastic pots containing light soil in the green house of Malayer University. Plants were treated with salicylic acid and salt stress after initial growth. This research was carried out as a factorial experiment in the form of a completely randomized design with three repetitions, which includes salinity level (zero, 200 and 500 ppm), and two levels of salicylic acid leaf treatment (zero and 0.1 mM) and the time of harvest (the first day, the seventh day and the fourteenth day) on shoot parts.

**Measurement of photosynthetic pigments:** The measurement of chlorophyll content was performed by using the method of Lichtenthaler and wellburn (1983) in the second stage of this research. The optical absorption intensity of the extract was read by

spectrophotometric method at wavelengths of 663.2, 646.8, and 470 nm (Lichtenthaler and wellburn, 1983).

**Malondialdehyde (MDA) measurement:** First, 0.5 g of fresh tissue (petridish plant) was ground in 5 mL of 0.1% trichloroacetic acid (TCA). The resulting extract was centrifuged for 5 minutes at 10000 g. And 4 mL of a 20% TCA solution containing 0.5% TBA was added to one milliliter of the centrifuged supernatant solution. The resulting mixture was heated in a Hot Water Bath for 30 minutes at 95°C. Then, it was immediately chilled in ice, and it was centrifuged again for ten minutes at 10000 g. The absorption intensity of this solution was read by using spectrophotometry at a wavelength of 532 nm (Heath *et al.*, 1986).

**Phenol measurement:** 0.5 gr of the fresh tissue (pot plant) and 5 mL of 80% methanol were combined and placed in a dark place. After 24 hours, the tubes were centrifuged at 3400 revolutions per minute (rpm) for 20 minutes. And 2 mL of Folin-Dennis reagent and 2 mL of 7% sodium carbonate solution were added to 1 mL of the supernatant extract, and finally the volume reached 12.5 with deionized water, and the absorption of each sample was measured with a spectrophotometer in the wavelength at 760 nm (Boonyuen *et al.*, 2016).

**Flavonoid essay:** First, the methanolic extract of the shoot was evaporated, and redissolved in methanol were used for flavonoid essay, 1.5 mL of 80% methanol, 100 microliters of 10% aluminum chloride solution, 100 microliters of 1 M potassium acetate solution, and 2.8 mL of distilled water were added to 500 microliters of each extract. The absorption of the mixture was measured after 40 minutes at 415 nm wavelength. (Chang *et al.*, 2002).

**Measurement of sodium and potassium:** Hamada and EL-enany (1994) method was chosen for the measurement of sodium and potassium. 0.1 g of shoot in the pots was weighted and placed inside the test tube. Then, 10 mL of 0.1 normal glacial acetic acid was poured on each of the samples, and it's kept for 24 hours in the laboratory environment. Then, the samples were placed inside a Ben-Marie at a temperature of 70 to 90 °C for 2 hours. After 2 hours, the resulting extract was ready to be read on the photometric film JENWAY pfp. 7. About 15 to 20 minutes before reading, turn on the film photometer until it is completely warmed up. (Hamada and EL-enany, 1994).

**Soluble and insoluble sugar measurement:** 0.1 g of the dry substance of the shoot in the pots and 10 mL of ethanol were combined, and it's kept in the refrigerator for 1 week. After one week, 0.5 mL of the supernatant solution of the samples was removed, and the volume of each of them was increased to 2 mL, and it was used to measure soluble carbohydrates. For measuring insoluble carbohydrates, first the sediment containing insoluble carbohydrates was obtained by filtering the extract obtained from the dry substance of the plant and ethanol. 15 mL of distilled water was added to the resulting precipitate and placed at 100 °C for 20 minutes to dissolve insoluble carbohydrates.

Then the volume of the resulting solution was increased to 25 mL, and 2 mL of each sample was taken. To each of the solutions prepared for soluble and insoluble carbohydrates, 1 mL of 5% phenol was added, and 5 mL of concentrated sulfuric acid was added under pressure. A yellow-colored solution was obtained, which changed color over time and became light brown. Then it was read using a spectrophotometer at a wavelength of 485 nm. The amount of absorption will be proportional to the color intensity of the solution, and it will be proportional to the concentration of dissolved sugars (Dubois *et al.*, 1956). Statistical calculation was done by using SPSS Software version 2016, and graph drawing was done by using Excell1 Software. Also, a ruler and digital caliper were used to check the morphological factors.

### Result and discussion

Based on the results of the analysis of variance in the petridish study (Table 1), the interaction effect of salinity stress and the application of salicylic acid was significant. The results of the mean comparison interaction showed that the highest shoot length occurred at a salinity of 200 ppm and the application of 0.1 mM salicylic acid, and the lowest shoot length occurred at a salinity of 500 ppm and no application of salicylic acid (Figure 1). Application of 0.1 mM salicylic acid at zero salinity had no significant effect compared to no-application of salicylic acid, but at salinity levels 200 and 500 ppm, it increased shoot length compared to no-application of salicylic acid. The use of 0.2 mM salicylic acid increased shoot length in all three salinity levels of zero, 200 and 500 ppm compared to the non-application of salicylic acid. It was observed that the seeds of wheat treated with salicylic acid showed increased tolerance to salt stress. Salicylic acid increased tolerance to salinity in *Triticum aestivum* (Sakhabut dinova, 2003). The results of a study on *Satureja hortensis* showed that irrigation with saline water leads to a decrease in some morphological characteristics of the plant, such as the height of the plant compared to the control (Sodaeizadeh *et al.*, 2016). In general, irrigation with salt water led to a deterioration in the morphological characteristics of the plant, one of the reasons for this may be that as the concentration of dissolved substances increases, the osmotic pressure of the soil solution increases, thereby increasing, the amount of energy that the plant has to expend to absorb water from the soil (Molavi *et al.*, 2001).

According to the analysis of variance results, only the simple effect of salicylic acid was significant for the trait of shoot weight. Increasing the concentration of salicylic acid caused a decrease in the weight of the shoot, so that at the concentration of 0.2 mM salicylic acid, the weight of the shoot was reduced compared to the concentration of 0.1 mM. However, both concentrations of salicylic acid (0.1 and 0.2 mM) caused an increase in the weight of shoot compared to control (Figure 2). It has been reported that

**Table 1. Variance analysis table for pot test**

low concentrations of salicylic acid increased the fresh and dry weight of (*Triticum aestivum* L.) but these characteristics decreased in high concentrations (Hayat *et al.*, 2005). Also, salicylic acid caused an increase in the fresh and dry weight of *Dracocephalum* (Pourakbar and Abedzadeh, 2014). Salicylic acid plays a role in the synthesis of certain proteins called kinases. These proteins play an important role in the regulation of cell division, differentiation, and morphogenesis (Rahimi tashi and Niknam, 2015). The effect of exogenous SA on plant resistance to abiotic stress depend on a set of factors: (a) applied SA concentration (Brito *et al.*, 2018); the dose for maximum stress tolerance ranging between 0.1 mM and 0.5 mM (Hara *et al.*, 2012); (b) method of SA administration, including pre-soaking, addition to the growth medium or foliar spray (Poshtdar *et al.*, 2015); (c) plant species and cultivars (Khalil *et al.*, 2012); (d) plant developmental stage (Poshtdar *et al.*, 2015); (d) stress level and system in which the study was carried out (Pal *et al.*, 2013).

Based on the results of the analysis of variance, the interaction effect of salinity and salicylic acid was significant for malondialdehyde trait (Table 2). The content of malondialdehyde in all three levels of salinity zero, 200 and 500 ppm and the non-application of salicylic acid had a significant increase compared to its use and this increase in 500 ppm salinity was more than the other two levels. Application of both levels of salicylic acid (0.1 and 0.2) in all three salinity levels of zero, 200 and 500 ppm caused a decrease in the content of malondialdehyde compared to its non-application. It seems that the concentration of 0.2 mM salicylic acid has a better effect on the content of malondialdehyde, and it has reduced the amount of malondialdehyde compared to the concentration of 0.1 mM salicylic acid at 200 ppm and 500 ppm salinity (Figure 3). In this research, according to the results Yousefvand in 2022 (Yousefvand *et al.*, 2022), the treatment of salicylic acid acted as a resistance process, and by increasing the power of antioxidants in the cell, including carotenoids, it reduced the amount of lipid peroxidation under salinity stress. An increase in the production of malondialdehyde and its decrease due to the use of salicylic acid have also been observed under salinity stress in blue lentil (Panda *et al.*, 2004) and under drought stress in barley (Habibi *et al.*, 2012). One of the effects of environmental stress such as drought and salinity is increasing the production of reactive oxygen species and inducing oxidative stress (Souza *et al.*, 2018). Reactive oxygen species lead to peroxidation of membrane lipids and changes in membrane permeability (Ion leakage) and damage to cells, therefore, measuring malondialdehydes produced during lipid peroxidation is a good indicator to measure the amount of oxidative damage to the membrane (Sheikhalipour *et al.*, 2021). An increase in the peroxidation of lipid has been observed in different cultivars and wild cultivars of tomato under salinity

source of variance	df	Diameter of root	Diameter of shoot	Shoot Weight	Chl a	Na	K	Flavonoids	Soluble sugars
a	2	0/079**	0/056 <sup>ns</sup>	0/194**	<0/0000**	0/06**	0/009**	92/33**	7856/3**
b	1	0/91**	3/7 <sup>ns</sup>	<sup>ns</sup> 0/036	<0/0000**	0/082**	0/002**	2/32**	3663/4**
c	2	0/045**	0/95 <sup>ns</sup>	0/44**	<0/0000**	0/481**	0/045**	18/1**	34/9**
a×b	2	0/038**	0/25 <sup>ns</sup>	0/059 <sup>ns</sup>	<0/0000**	0/022**	0/013**	108/3**	2368/8**
a×c	4	0/011 <sup>ns</sup>	0/16 <sup>ns</sup>	0/367**	<0/0000**	0/022**	0/022**	1/67**	75/1**
b×c	2	0/017 <sup>ns</sup>	0/37 <sup>ns</sup>	0/017 <sup>ns</sup>	<0/0000**	0/008**	0/001**	0/844**	**39/4
a×b×c	4	0/011 <sup>ns</sup>	0/195 <sup>ns</sup>	0/053 <sup>ns</sup>	<0/0000**	0/083**	0/029**	1/23**	63/6**
Error	54	0/009	1/75	0/031	<0/0000	<0/0000	<0/0000	<0/0000	2/3
C.V%	-	22.49	30.5	24.3	17.51	1.46	1.78	1.03	2.16

\* and \*\* significant at  $P \leq 0.05$ ,  $P \leq 0.01$ , respectively. a= salinity, b= salicylic acid

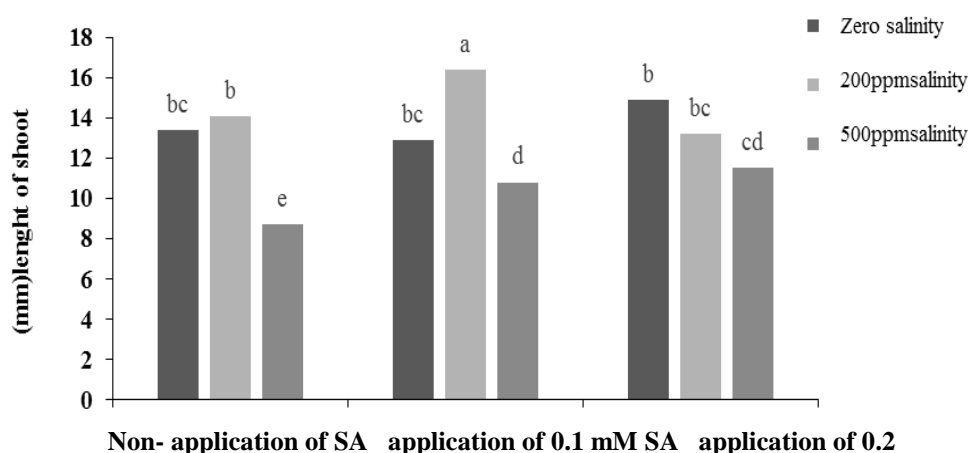


Figure 1. The interaction effect of different concentrations of salinity and the application of SA on the length of the shoot of *Salicornia persica* in petridish

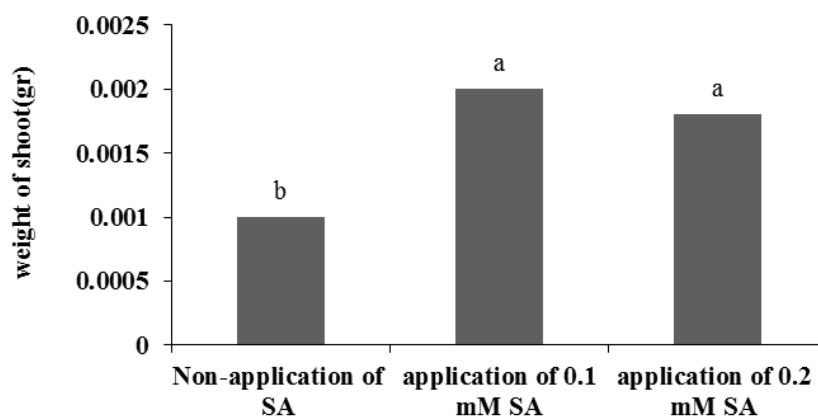


Figure 2. The effect of SA application on the weight of *Salicornia persica* shoot in petridish

Table 2. Variance analysis table for petri dish test

MDA	df	Source of variation
0/005 **	2	Salinity
0/005 **	2	Salicylic acid
0/001 **	4	Salinity × salicylic acid
<0/0000	18	Error
2.11	-	C.V%

\* and \*\* significant at  $P \leq 0.05$ ,  $P \leq 0.01$ , respectively.

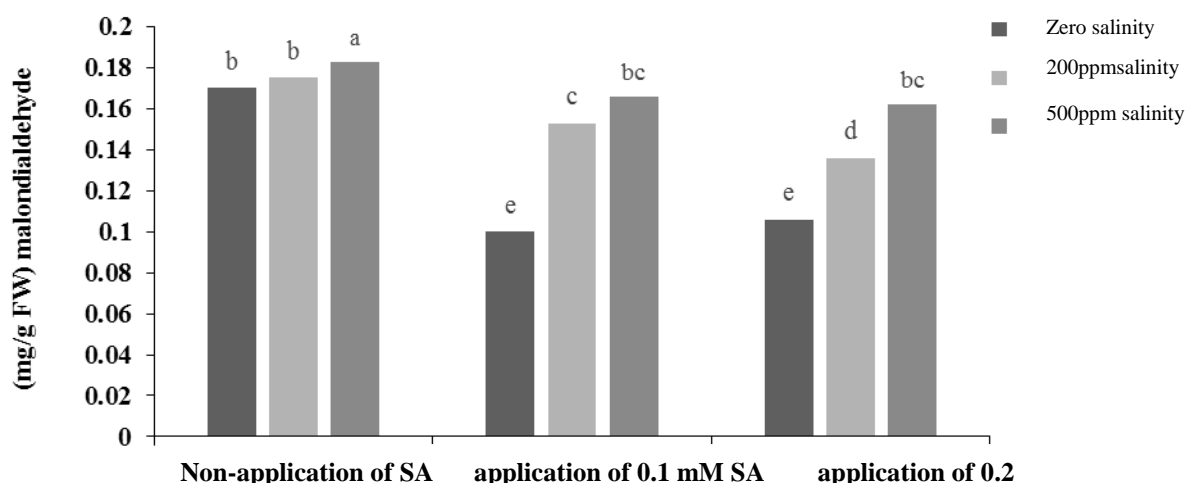


Figure 3. Interaction effect of different concentrations of salinity and application of SA on the amount of malondialdehyde of *Salicornia persica* in petridish

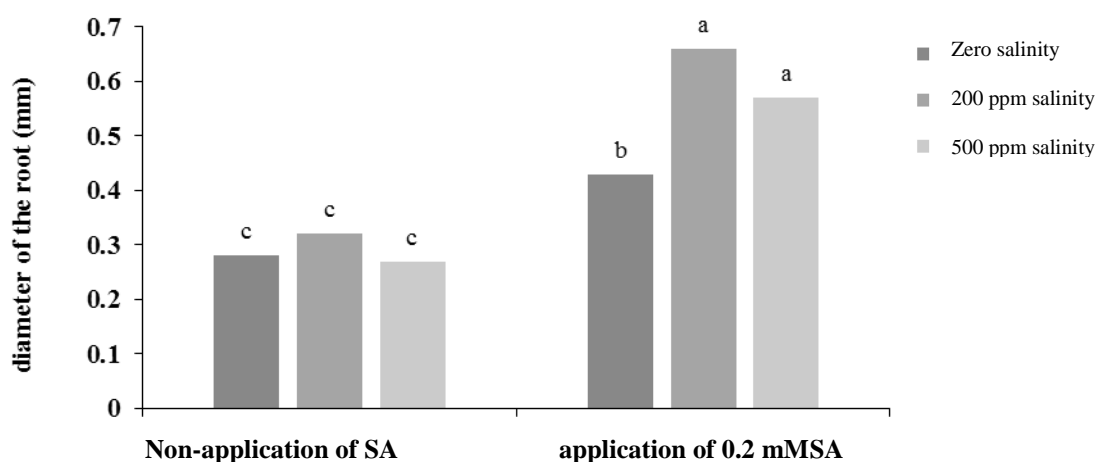


Figure 4. Interaction effect of different salinity concentrations, SA application on root diameter of *Salicornia persica* in pot

stress conditions (Juan *et al.*, 2005).

Initial studies in petridish confirmed the positive effect of SA application during salinity stress in *Salicornia persica*. In the next steps, studies were carried out in plant pots in which it's done with more harvests. Summarizing the morphophysiological results in the petri dish was chosen in favor of the use of 10 mM SA to modulate the salinity stress. According to the results of the analysis of variance for the trait of root diameter in plant potted conditions, the simple effects of salinity, SA and the time of harvest, as well as the interaction effect of salinity and SA, were significant. The comparison of the average effect of the simple harvest time showed that the largest root diameter (0.46) cm was related to the third harvest and the lowest root diameter (0.37) cm was related to the first harvest. (Figure 5). It seems that with the passage of time, the

growth of *Salicornia persica* plant has increased, and for this reason, the diameter of root has increased in the third harvest compared to the second and first harvests. The results of the comparison of the average interaction effect of salinity and SA showed that the largest root diameter was found in salinity of 200 ppm and the application of 0.1 mM SA, and the smallest root diameter was found in salinity of 500 ppm and without the use of SA (Figure 4). At 200 ppm salinity, the root diameter increased significantly with the use of 0.1 mM SA and also without its use compared to the control. This is probably due to the fact that *Salicornia persica* is a halophyte, and the maximum growth of this plant occurs in the presence of sodium. It has been reported that halophyte plants from *Chenopodiaceae* family, such as *Chenopodium album* and *Salicornia* show maximum growth in salinity of 100–300 ppm (Amor *et*

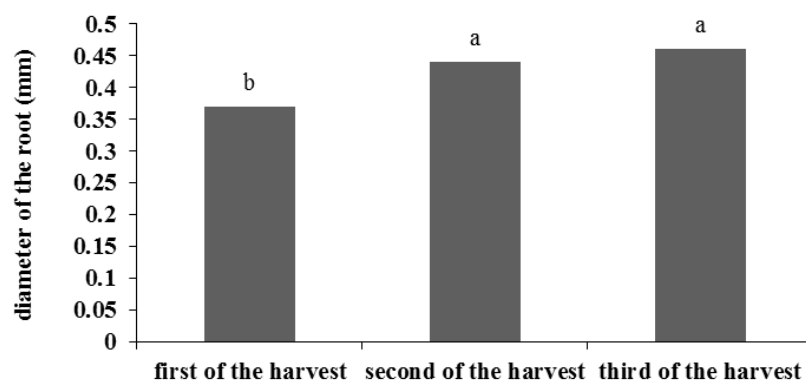


Figure 5. The effect of harvesting time on root diameter of *Salicornia persica* in pot

*al.*, 2005). The diameter of the root in salinity 500 ppm and no application of SA was reduced compared to the control. In agreement with our results, (Dawood and El-Awadi, 2015) with studying the *Pelargonium graveolens* plant, they showed that the diameter of the branch decreased significantly at three levels of salinity (zero, 30 and 60 ppm) with increasing salinity. Under salt stress, root growth reduction and poisoning are caused by the accumulation of toxic ions. Under these concentrations, the stoma is closed and the amount of photosynthesis is reduced, and finally, salinity can stop the growth of the roots, in which it causes a decrease in the capacity of water absorption and the transfer of water and nutrients from the soil to the aerial parts (Munns and Tester, 2008). Application of 0.1 mM SA caused an increase in the diameter of the root in all three salinity levels of zero, 200 and 500 ppm. It is reported that the treatment of bean seeds and shoots with SA under salinity stress has increased the parameters related to vegetative growth (Rady and Mohammad, 2015). It has also been reported, the positive and significant effect of SA treatment in increasing the growth and performance of *Peppers* plants (Changli and Chanyou, 2010), and corn (Gunes *et al.*, 2007) under stress.

According to the results of the analysis of variance, the interaction effect of salinity, SA and harvesting was significant, so the average comparison was made for the interaction effect. The results of comparing the averages showed that in 200 ppm salinity and without the use of SA, the amount of chlorophyll b increased in the first and third harvests and its amount decreased in the second harvest. Also, the application of 0.1 mM SA caused an increase in the amount of chlorophyll b in the second harvest compared to its non-application (Figure 6). At low levels of salinity stress, due to the activation of stress tolerance mechanisms, such as reducing the area of the leaf and increasing its thickness, it can increase the amount of chlorophyll per leaf area unit (Rajcan *et al.*, 1999). In this regard, the researchers attributed the increase in the amount of chlorophyll as a result of mild stress to the increase in the specific weight of the leaf, and they stated that with an excessive increase in stress and its adverse effects on the structure of chlorophyll and as a result of the destruction of

chloroplasts, the amount of chlorophyll decreases (Cramer, 2002). In 2004, Misra *et al.* reported that stress causes chloroplast destruction and a decrease in chlorophyll in *Mentha* plants. It seems that the decrease in the amount of chlorophyll is due to the lack of synthesis of this substance and the increase of ethylene under stress conditions. Also, the activity of chlorophyllase enzyme increases with the application of salt stress, so it can be said that the decrease of chlorophyll in salt conditions is due to both the reduction of its synthesis and the increase of chlorophyll destruction (Misra *et al.*, 2004). Khan's studies in 2005 showed that in the conditions of salinity stress, the leaves first become chlorosis, and then they start to fall (Khan, 2005). The reduction in the amount of photosynthetic pigments in plants is due to the closing of the stoma and prevention of chlorophyll biosynthesis. Salinity stress increases oxygen free radicals in chloroplasts, and it causes damage to the membrane of the chloroplast (Zhang *et al.*, 2003). At low levels of salinity stress, due to the activation of stress tolerance mechanisms, such as reducing the area of the leaf and increasing its thickness, it can increase the amount of chlorophyll per unit of leaf area. (Rajcan *et al.*, 1999).

For the amount of flavonoids, the results of analysis of variance showed that the interaction effect of salinity, SA, and harvest was significant. Based on this, the results of the average comparison indicated that in zero salinity and without the use of SA, the amount of flavonoids increased in the second harvest compared to the first one and the third harvest compared to the second one. The application of 0.1 mM SA had no significant effect on the amount of flavonoids. In salinity of 200 ppm and no application of 0.1 SA, the amount of flavonoids increased in all three harvests compared to the control (Figure 7). The main role of leaf flavonoids is to protect photosynthetic cells against harmful UV rays. At mild levels of salinity, the amount of flavonoids increases to deal with stress and prevent oxidative damage. However, at higher salinity levels, the number of flavonoids has decreased in most cases (Manai *et al.*, 2014). In the salinity of 500 ppm and without the use of SA, the amount of flavonoid was reduced compared to the control. The antioxidant

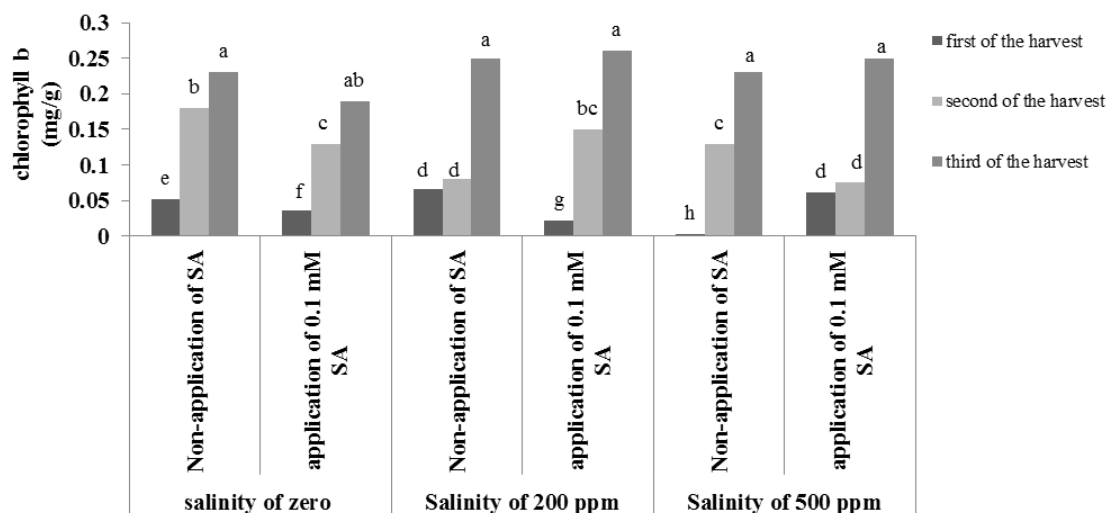


Figure 6. The interaction effect of different concentrations of salinity, application of SA and the time of harvest of chlorophyll b of *Salicornia persica* in petridish

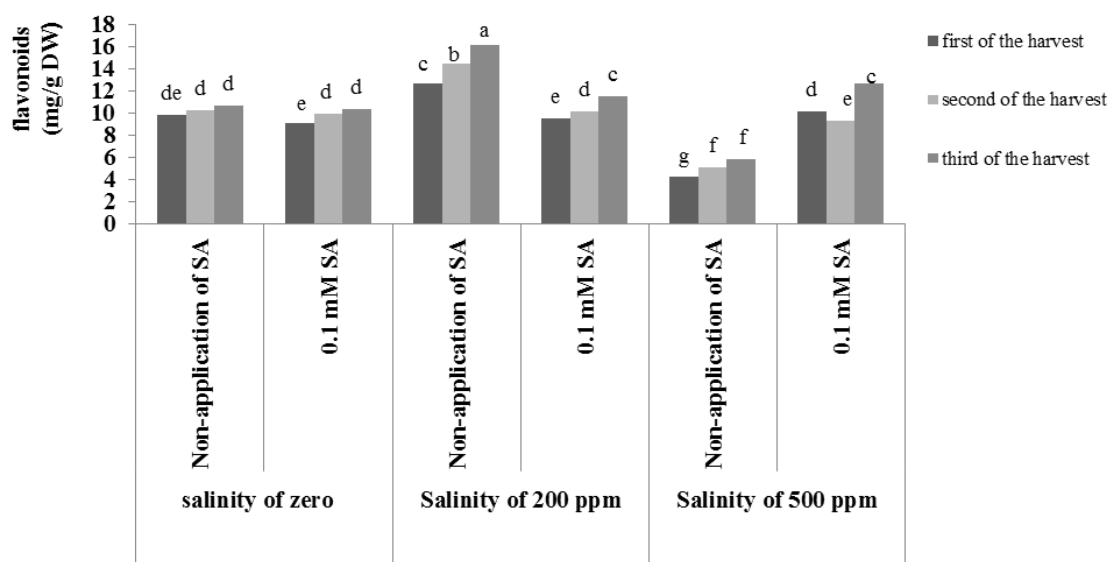


Figure 7. The interaction effect of different concentrations of salinity, application of SA and harvesting time on the concentration of flavonoid of *Salicornia persica* in pot

capacity of flavonoids is not the same, and at high salinity levels, they will not succeed in producing compounds with high antioxidant capacity (Calvo *et al.*, 2014). At a salinity of 500 ppm, the application of 0.1 mM SA caused a significant increase in the amount of flavonoids compared to its non-application. Due to its antioxidant properties, SA increases the amount of flavonoid and thus increases its antioxidant power.

The results of the analysis of variance of the interaction effect of salinity, SA and harvesting time were significant for sodium content. With the increase in salinity, especially in the third harvest, an increase in sodium was observed. In the salinity of 200 ppm and no use of SA in the second harvest, the amount of sodium increased compared to the control, and the use of SA caused a decrease in the amount of sodium. In general, there was no significant increase in the amount of sodium at salinity of 200 and 500 ppm (Figure 8).

Probably, because *Salicornia persica* is a halophyte plant, it has resisted the increase of sodium and the imbalance of ions such as sodium and potassium inside the cell. At salinity of 200, especially in the first and second harvests, sodium was reduced compared to the control, which may indicate the ability of halophytes to remove or adjust and use sodium optimally. In various studies, there are contradictory reports about the effect of SA on ion absorption. The use of SA had no effect on the amount of sodium in carrot (Eraslan *et al.*, 2007) and spinach (Eraslan *et al.*, 2007). Gunes *et al.* reported in 2007 that SA decreased the concentration of sodium and chlorine and increased cations, including potassium, in corn plants under different stresses. In our studies, the application of SA had no significant reduction effect on the amount of sodium, and the modulating effect of SA on salinity stress uses another mechanism.

For the amount of potassium, the results of the

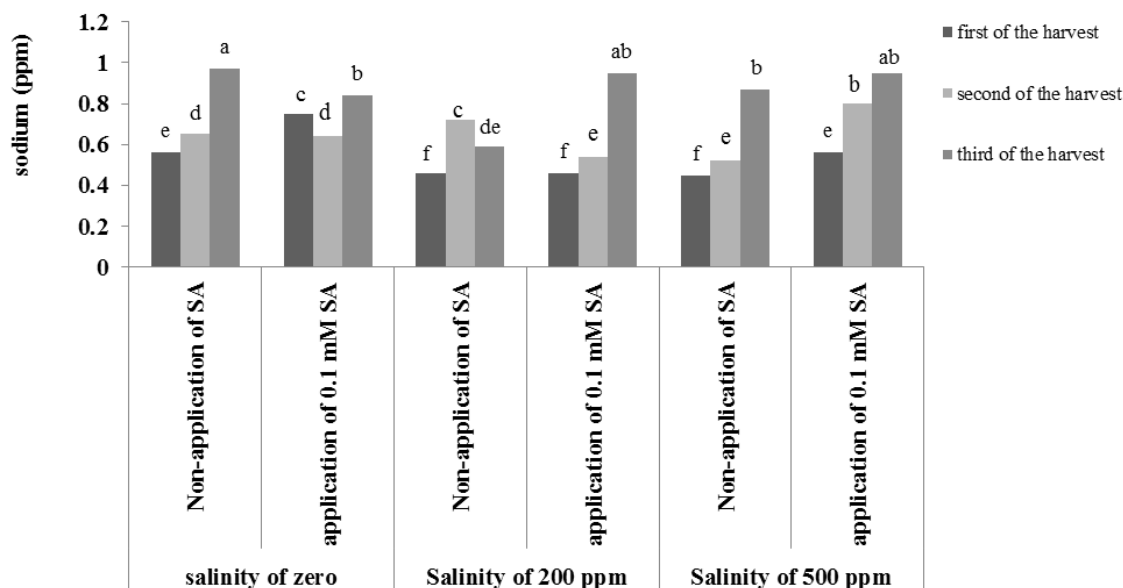


Figure 8. The interaction effect of different concentrations of salinity, the application of SA and the harvesting time on the amount of sodium element of *Salicornia persica* in pot

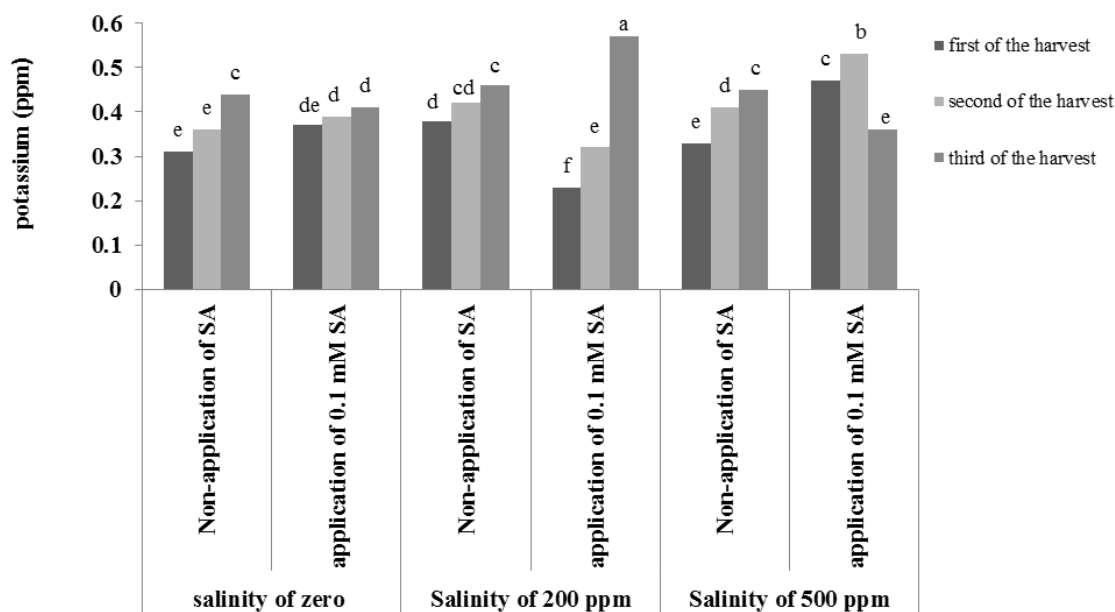
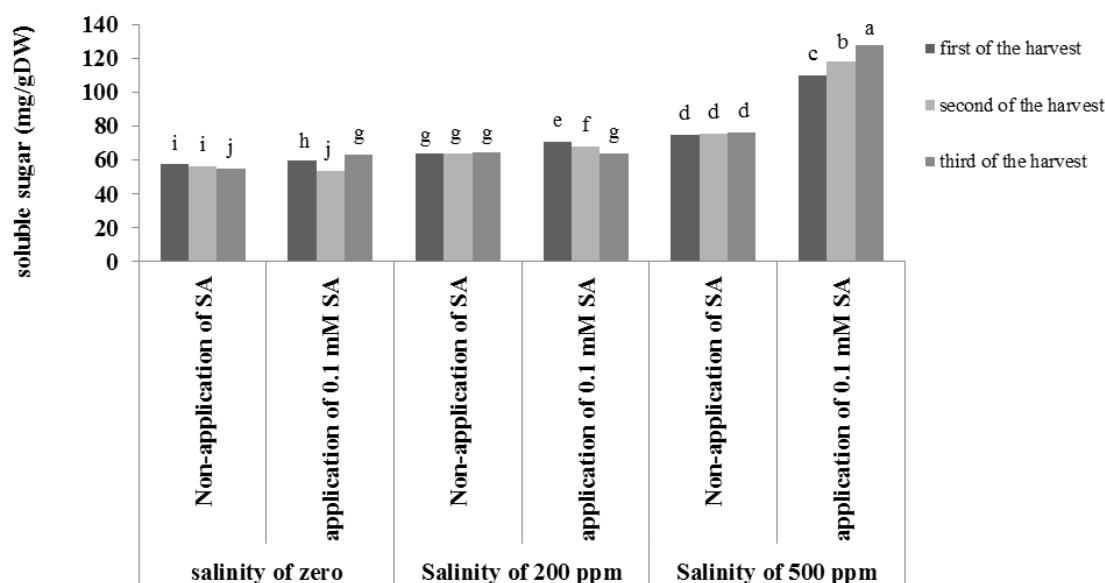


Figure 9. The interaction effect of different concentrations of salinity, the application of SA and the time of harvest on the amount of potassium element of *Salicornia persica* in pot

analysis of variance showed the interaction effect of salinity, SA and harvesting is significant in that, according to the results of the mean comparison, at zero salinity, the application of 0.1 mM SA increased the amount of potassium in the first and second harvests, and its amount decreased in the third harvest compared to the absence of SA. In salinity of 200 ppm and no application of SA, the amount of potassium increased compared to the control in all three harvests, and the use of 0.1 mM SA caused a decrease in the amount of potassium in the first and second harvests, and its amount decreased in the third harvest. In salinity of 500 ppm and no application of SA, the amount of potassium

increased in all three harvests compared to the control, and the use of 0.1 mM SA increased the amount of potassium in the first and second harvests, and its amount decreased in the third harvest compared to no application of SA. Contrary to our idea that high salinity reduces the amount of potassium, there was no reduction in potassium content at 500 ppm salinity. Probably due to the resistance of *Salicornia persica* Akani, which is a halophyte, and it shows resistance to salinity, we do not observe this reduction (Figure 9). The decrease in the amount of potassium and the increase in the amount of sodium is one of the most obvious effects of salinity stress. Its replacement by





**Figure 10.** The interaction effect of different concentrations of salinity, the application of SA and the time of harvest on the amount of soluble sugar of *Salicornia persica* in pot

sodium can cause damage to the plant because sodium is not able to perform the roles of potassium. Accumulation of sodium and change of  $K^+/Na^+$  ratio in cytoplasm can effect the processes of bioenergetics. Substituting sodium instead of potassium can deactivate enzymes and lead to reduction of growth or even death of the cell or plant (Wu *et al.*, 2008). In general, salinity stress reduces the concentration of potassium in plant organs through disruption of potassium absorption mechanisms by roots (Ashraf and Oleary, 1999). Gunzo *et al.* reported in 2007 that those different concentrations of SA decreased the amount of potassium in corn, both under saline and non-saline conditions. (Gunes *et al.*, 2007).

According to the results of the analysis of variance, the interaction effect of salinity, harvesting time and SA was also significant for the amount of soluble sugar. With the increase of salinity in most harvests, the amount of soluble sugar increased compared to the control, and at zero salinity, the application of 0.1 mM SA increased the amount of soluble sugar in the first and third harvests. In salinity of 200 ppm, the application of 0.1 mM SA increased the soluble sugar in the first and second harvests. In salinity of 500 ppm, the application of 0.1 mM SA increased the amount of soluble sugar in all three harvests. The increase of soluble sugar in the salinity of 500 is higher than other treatments. Also, at the stress of 500, SA had a greater effect on the amount of soluble sugar (Figure 10). In *Nigella sativa* plant, with increasing sodium chloride concentration, the content of soluble sugar in the aerial parts increased compared to the control, and in the presence of SA, a significant increase in the soluble

sugar content of the aerial parts was observed, in comparison with the control. (Ghorbanli *et al.*, 2010). The same results have been observed in tomato (Poor *et al.*, 2011). In some plants, including pepper, it has been reported that the treatment of SA by increasing the activity of invertase has increased the amount of soluble and insoluble sugar. It has also been observed that SA stimulates the hydrolysis of carbohydrates, and by increasing compounds such as soluble sugars, while creating an osmotic source, it reduces environmental stress damage (Hayat and Ahmad, 2007).

### Conclusion

Due to the importance of salinity in current conditions and the possibility of using the positive effect of a salinity modulating such as SA, this research was carried out. Also, the use of native halophyte plants (*Salicornia persica*) in this study can be significant. In this research, it was possible to obtain a more effective concentration of SA (0.1 mM) for the adjustment of salinity. Also, with the morphophysiological study in petridish and pot conditions, it was found that the application of SA has a significant positive effect in modulating salinity stress. The results of the effect of salinity showed an increase in the growth (optimal growth) of *Salicornia persica* plant at 200 ppm sodium chloride. At the same time, the results showed that SA could increase the growth of plant, the amount of flavonoids, soluble sugar and potassium, and its decrease the amount of malondialdehyde. In general, it increases the resistance to salinity stress in the plant.

### References

- Aghaleh, M., Niknam, V., Ebrahimzadeh, H., & Razavi, V. (2009). Salt stress on growth, pigments, proteins and lipid peroxidation in *Salicornia persica* and *S. europaea*. *Journal of Biological Plant*, 53, 243-248. <https://doi.org/10.1007/s10535-009-0046-7>
- Ahmad, M., Wang, X., Hilger, T. H., Luqman, M., Nazli, F., Hussain, A., Zahir, Z. A., Latif, M., Saeed, Q., & Malik, H. A. (2020). Evaluating biochar-microbe synergies for improved growth, yield of maize, and post-harvest soil characteristics in a semi-arid climate. *Agronomy*, 10, 1055. <https://doi.org/10.3390/agronomy10071055>
- Amor, N. B., Hamed, K. B., & Debez, A. (2005). Physiological and antioxidant response of the perenial halophyte *Crithmum maritimum* to salinity. *Plant Sciences*, 168, 889-889. <https://doi.org/10.1016/j.plantsci.2004.11.002>
- Arfan, M., Athar, H. R., & Ashraf, M. (2007). Does exogenous application of salicylic acid through the rooting medium modulate growth and photosynthetic capacity in two differently adapted spring wheat cultivars under salt stress. *Plant Physiology*, 164, 685-694. 10. DOI: 1016/j.jplph.2006.05.010
- Ashraf, M. & Oleary, (1999). Interactive effect of salt (NaCl) and nitrogen form on growth, water relations and photosynthetic capacity of sunflower (*Helianthus annuus* L.). *Applied Biology*, 35, 509-513. <https://doi.org/10.1111/j.1744.7348.1999.tb00881.x>
- Boonyuen, U. Chamchay, K. Swangsri, T. Saralamba, N. Day, N. P. Imwong, M. (2016). Detailed functional analysis of two clinical glucose-6-phosphate dehydrogenase (G6PD) variants, G6PDViangchan and G6PDViangchan + Mahidol, decreased stability and catalytic efficiency contribute to the clinical phenotype. *Molecular Genetics and Metabolism*, 118:84-91. doi: 10.1016/j.ymgme.2016.03.008.
- Brito, C. L. T., Dinis, M., Meijon, N., Meijon, M., Ferreira, H., & Pinto, G. (2018). Salicylic acid modulates olive tree physiological and growth responses to drought and rewatering events in a dose dependent manner. *Journal of Plant Physiology*, 230, 21-32. 10.1016/j.jplph.2018.08.004
- Bimurzayev, N., Sari, H., Kurunc, A., Hayri Doganay, K., & Asmamaw, A. (2021). Effects of different salt sources and salinity levels on emergence and seedling growth of faba bean genotypes. *Scientific Reports*, 11, 18198. <https://doi.org/10.1038/s41598-021-97810-6>
- Calvo, P., Nelson, L., & Kloepper, J. W. (2014). Agricultural uses of plant bio stimulants. *Plant and Soil*, 383, 3-41. DOI 10.1007/s11104-014-2131-8
- Chang, C., Yang, M., Wen, H., & Chern, J. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Food and Drug Analysis*, 10, 178-182. <https://doi.org/10.38212/2224-6614.2748>
- Changli, Z. & Chanyou, C. (2010). Physiological effect of exogenous salicylic acid on decreasing salt damage to pepper seedlings. *Acta Botanica Boreali-Occidentalia Sinica*, 3, 111-115.
- Cramer, G. R. (2002). Response of abscisic acid mutant of *Arabidopsis* to salinity. *Functional Plant Biology*, 29, 561-567. DOI: 10.1071/PP01132
- Dawood, M. G. & El-Awadi, M. E. (2015). Alleviation of salinity stress on *Vicia faba* L. plants via seed priming with melatonin. *Acta Biologicaiana Colombiana*, 20(2), 223-235. doi: <http://dx.doi.org/10.15446/abc.v20n2.43291>
- Dubois, M., Gills, K., Hamilton, J., Rebers, P., & Smith, F. (1956). Sugar estimation by phenol -sulphuric acid method. *Analytical Chemistry*, 28, 350-356. <http://dx.doi.org/10.1021/ac60111a017>
- Eraslan, F., Inal, A., Gunes, A., & Alpaslan, M. (2007). Impact of exogenous salicylic acid on the growth, antioxidant activity and physiology of carrot plants subjected to combined salinity and boron toxicity. *Scientia Horticulturae*, 113(2), 120-128. DOI:10.1016/j.scienta.2007.03.012
- Ghorbanli, M., Adib Hashemi, N., & Peyvandi, M. (2010). Study of salinity and ascorbic acid on some physiological responses of *Nigella sativa* L. *Iranian Journal of Medical and Aromatic Plants*, 26(3), 70-380. <https://civilica.com/doc/1286527>
- Golshan, Z. & Keshavarzi, M. (2007). Morphological study of *persica* (*Chenopodiaceae*) native to Iran. *Journal of Biological Sciences*, 10(6), 852\_860. DOI: 10.3923/pjbs.2007.852.860
- Gunes, Y., Inal, A., Alpaslan, M., Eraslan, F., Bagci, E. G., & Cicek, G. N. (2007). Salicylic acid induced changes on some physiological parameters symptomatic for oxidative stress and mineral nutrition in maize (*Zea mays* L.) grown under salinity. *Journal of Plant Physiology*, 164, 728-736. DOI: 10.1016/j.jplph.2005.12.009
- Habibi, D., Ooroojnia, S., Fatollah Taleghani, D., Pazoki, A., & Davoodifard, M. (2012). Antioxidants and yield evaluation of sugar beet genotypes under drought stress. *Iranian Journal of Agronomy and Plant Breeding*, 8(4), 63-82. <https://sid.ir/paper/190291/en>
- Hamada, A. M. & EL-enany, A. E. (1994). Effect of NaCl salinity on growth, pigment and mineral element contents and gas exchange of broad bean and pea plants. *Biologia Plantarum*, 36, 75-81. <https://doi.org/10.1007/BF02921273>
- Hara, I. J., Furukawa, A., Sato, T., Mizoguchi, V., & Miura, K. (2012). Abiotic stress and role of salicylic acid in plants In abiotic stress responses in plants metabolism, *Scientific Research*, 235-252. <https://www.springer.com/gp/book/9781461406334>
- Hayat, S., Fariduddin, Q., Ali, B., & Ahamad, A. (2005). Effect of salicylic acid on growth and enzyme activities of wheat seedlings. *Acta Agronomica Hungarica*, 53(4), 433-437. DOI:10.1556/AAgr.53.2005.4.9

- Hayat, S. and Ahmad, A. (2007). *Salicylic Acid: A Plant Hormone*. Springer, Netherlands.  
<https://doi.org/10.1007/1-4020-5184-0>.
- Heath, M. T. Laub, J. A. Paige, C. C. Ward, R. C. (1996). Computing the singular value decomposition of a product of two matrices. *SIAM Journal on Scientific and Statistical Computing*, 7: 1147–1159.
- Juan, M., Rivero, R. M., Romero, L., & Ruiz, J. M. (2005). Evaluation of some nutritional and biochemical indicators in selecting salt-resistant tomato cultivars. *Environmental and Experimental Botany*, 54, 193-201. DOI: 10.1016/j.envexpbot.2004.07.004
- Khalil, F., Qureshi, M., Khan, A., Fakharul, H., & Bibi, N. (2012). Effect of girdling and plant growth regulators on productivity in olive (*Olea europaea*). *Pakistan Journal of Agricultural Research*, 25, 30-38.
- Khan, M. A. & Gulzar, S. (2003). Germination responses of *Sporobolus ioclados*: A saline desert grass. *Journal of Arid Environments*, 55, 453-464. <https://doi.org/10.1006/jare.2002.1045>
- Khan, N. A. (2005). NaCl inhibited chlorophyll synthesis and associated changes in ethylene evolution and antioxidative enzyme activities in wheat. *Plant Biology*, 47(3), 437-440. DOI: 10.1023/B:BIOP.0000023890.01126.43
- Lichtenthaler, H. & Wellburn, A. R. (1983). Determination of total carotenoids and chlorophylls *a* and *b* in leaf extracts in different solvents. *Biochemical Society Transaction*, 11, 591-592. <https://doi.org/10.1042/bst010591>
- Manai, J., Kalai, T., Gouia, H., & Corpas, F. (2014). Exogenous nitric oxide (NO) ameliorates salinity-induced oxidative stress in tomato (*Solanum lycopersicum*) plants. *Journal of Soil Science and Plant Nutrition*, 433-446. <http://dx.doi.org/10.4067/S0718-95162014005000034>
- Misra, A. & Srivastava, N. K. (2004). Influence of water stress on Japanese Mint. *Journal of herbs, Spices and Medicinal Plant*, 7(1), 51-58. DOI:10.1300/J044v07n01\_07
- Molavi, H., Mohammadi, M., Liaghat, A., Hoekstra, F. A., Golovina, E. A., & Buitink, J. (2001). Mechanisms of plant desiccation tolerance. *Plant Biology*, 6, 431-438. DOI: 10.1016/s1360-1385(01)02052-0
- Munns, R. & Tester, M. (2008). Mechanisms of salinity tolerance. *Annual Review Plant Physiology*, 59, 651-681. <https://doi.org/10.1146/annurev.arplant.59.032607.092911>
- Noreen, S. & Ashraf, M. (2008). Alleviation of adverse effects of salt stress on (*Helianthus annuus* L.) by exogenous application of salicylic acid growth and photosynthesis. *Pakistan Journal of Plant Biology*, 40(4), 1657-1663.
- Panda, S. K. & Upadhyay, R. K. (2004). Salt stress induces oxidative alterations and antioxidative defense in the roots of *Lemna minor*. *Journal of Plant Biology*, 48(2), 249-253. <https://doi.org/10.1023/B:BIOP.0000033452.11971.fc>
- Pal, M. G., Salat, V., Kovacs, O., Gondor, K., & Junda, T. (2013). Salicylic acid-mediated abiotic stress tolerance. *Plant Growth and Development*, 183-248. DOI:10.1007/978-94-007-6428-6\_10
- Pourakbar, L. & Abedzadeh, M. (2014). Effects of UV-B and UV-C radiation on antioxidative enzymes activity of *Melissa officinalis* and influences of salicylic acid in UV-stress ameliorations. *Iranian Journal of Plant Biology*, 21, 23-34.
- Poshtdar, A., Abdali Mashhadi, A., & Monjezi, F. (2015). Response germination and vigor index seed of *Milk Thistle* to priming with salicylic acid under salt stress. The 2<sup>nd</sup> Conference on New Findings in the Environment and Agricultural Ecosystems. *Institute New Energies and Environment of Tehran University*, 9(33), 11-19.
- Poor, P., Gemes, K., Horvath, F., Szepesi, A., Simon, M. L., & Tari, I. (2011). Salicylic acid treatment via the rooting medium interferes with stomatal response, CO<sub>2</sub> fixation rate and carbohydrate metabolism in tomato, and decreases harmful effects of subsequent salt stress. *Plant Biology*, 13, 105-114. DOI: 10.1111/j.1438-8677.2010.00344.x
- Rady, M. M. & Mohamad, G. F. (2015). Modulation of salt stress effects on the growth, physio-chemical attributes and yields of *Phaseolus vulgaris* L. plant by the combined application of salicylic acid and *Moringa oleifera* leaf extract. *Plant Biology*, 193, 105-113. <https://doi.org/10.1016/j.scienta.2015.07.003>
- Rahimi Tashi, T. & Niknam, V. (2015). The Effect of SA on physiological and biochemical responses of wheat (*Triticum aestivum* L.) to salinity stress. *Journal of Plant Research (Iranian Journal of Biology)*, 28(2), 10.
- Raskin, I. (1992). Salicylate, a new plant hormone. *Plant Physiology*, 99(3), 799-803. DOI: 10.1104/pp.99.3.799
- Rajcan, I., Dwyer, L. M., & Tollenaar, M. (1999). Note on relationship between leaf soluble carbohydrate and chlorophyll concentration in maize during leaf senescence. *Field Crops Research*, 63, 13-17. [https://doi.org/10.1016/S0378-4290\(99\)00023-4](https://doi.org/10.1016/S0378-4290(99)00023-4)
- Sheikhalipour, M., Esmailpour, B., Behnamian, M., Gohari, G., Giglou, M. T., Vachova, P., Rastogi, A., Brestic, M., & Skalicky, M. (2021). Chitosan–selenium nanoparticle (Cs–Se NP) foliar spray alleviates salt stress in bitter melon. *Nanomaterials*, 11(3), 684-706. DOI: 10.3390/nano11030684
- Shi, Q. & Zhu, Z. (2008). Effects of exogenous salicylic acid on manganese toxicity, element contents and antioxidative system in cucumber. *Environmental and Experimental Botany*, 63, 317-326. DOI:10.1016/j.envexpbot.2007.11.003
- Sodaeizadeh, H., Tajamolian, M., & Rafiei Hoseini, M. (2016). Effect of composing of sweet and salty water on some morphological index of (*Satureja hortensis* L.) under NaCl stress. *Crop and Environmental*, 595-606. DOI: 10.22077/ESCS.2019.1419.1308
- Souza, M. M., Mendes, C. R., Doncato, K. B., Badiale-Furlong, E., & Costa, C. S. (2018). Growth, phenolics, photosynthetic pigments, and antioxidant response of two new genotypes of sea asparagus (*Salicornia neei* Lag.) to

- salinity under greenhouse and field conditions. *Agriculture*, 8(7), 115-133. DOI: 10.3390/agriculture8070115
- Wu, Y., Hu, Y., & Xu, G. (2008). Interactive effects of potassium and sodium on root growth and expression of K/Na transporter genes in rice. *Plant Growth Regulation*, 57, 271-280. DOI: 10.1007/s10725-008-9345-9
- Yildiz, M., Poyraz, I., Cavdar, A., Ozgen, Y., & Beyaz, R. (2020). Plant responses to salt stress. *Plant Breeding-Current and Future Views*. DOI: 10.5772/intechopen.93920
- Yousefvand, P. Sohrabi, Y. Heidari, GH. Weisany, W. Mastinu, A. (2022). Salicylic Acid Stimulates Defense Systems in *Allium hirtifolium* Grown under Water Deficit Stress. *Molecules*, 1; 27(10): 3083. doi: 10.3390/molecules27103083.
- Yuan, S. & Lin, H. H. (2008). Role of salicylic acid in plant abiotic stress. *Plant Physiology*, 63, 313-320. DOI: 10.1515/znc-2008-5-601
- Zhang, S., Weng, J., Pan, J. T., Yao, S., & Xu, C. (2003). Study on the photogeneration of superoxide radicals in photosystem II with EPR spin trapping techniques. *Photosynthesis Research*, 75, 41-48. DOI: 10.1023/A:1022439009587