Glycine betaine aldehyde as a promising new source of valuable physiological indices of Prosopis spp.

Sara Ansari and Hossein Sadeghi*

Department of Natural Resources and Environmental Engineering, School of Agriculture, Shiraz University, Shiraz, Iran

Abstract

Soil salinity has become a very serious issue in many parts of the world. Salinity is the most important factor limiting plants growth in these areas. Therefore, in order to make better use of saline soils, it is necessary to identify salinity-resistant plants. The aim of this study was to evaluate the effect of salinity on the response of two *Prosopis* species to salt stress at arid and semi-arid lands. The study was done as a factorial experiment based on a completely randomized design. The first factor was salinity at 1.8 (control), 5.8, 10.2, and 16.4 dS/ m, and the second factor was two genotypes (*P.cineraria and P.juliflora*). Results showed a gradual decrease in stem height, total leaf area, root and shoot dry weights, and number of leaves per seedling of both species by increasing salinity of the root zone. Low salinity levels (5.8 dS/ m) did not cause a substantial inhibition of growth, but increasing salt concentration gradually reduced the vegetative characteristics. In both genotypes, proline, total nitrogen, glycine betaine aldehyde, and calcium (Ca) increased with increasing salt concentration. Also, these factors were significantly higher in *Prosopis juliflora* than *Prosopis cineraria*. Potassium (K) and organic carbon (OC%) decreased significantly in both genotypes with increasing salt concentration. Applying glycine betaine aldehyde is one of the effective strategies in salt stress tolerance and increasing carbon storage in soil.

Keywords: organic carbon, proline, properties, Prosopis, salinization, vegetative

Ansari, S. and H. Sadeghi. 2023. 'Glycine betaine aldehyde as a promising new source of valuable physiological indices of Prosopis spp.'. *Iranian Journal of Plant Physiology 1*3 (4),4765- 4775.

Introduction

Salinity stress and methods of dealing with it are among important issues that human beings have been struggling with for thousands of years (Soleimani et al., 2011). Abiotic environmental stresses reduce global production more than any other factor. The harmful effects of salinity on plants are manifested in various ways such as mortality or reduced plant production (Jahanbazi et al., 2015). On the other hand, soil salinity has become a very serious issue in many parts of the world (FAO 2005). Today, with the lifestyle of contemporary humankind and ensuing the climate change we witness the worse situation especially in arid and semi-arid regions ((Hassani et al., 2021) et al., 2021). Approximately 4 million square kilometers of land around the world have some kind of salinity at different levels (Corbishley and Pearce, 2007).

* *Corresponding Author*
E-mail Address: sadeghih@shirazu.ac.ir
Received: November, 2022
Accepted: May, 2023

Salinity poisoning includes physiological and biochemical disturbances of food deficiency, cell membrane destruction, altered enzymatic and metabolic activity (Hasegawa et al., 2000; Neumann, 1997). In many studies on salinity tolerance of various plants, sodium chloride has been studied as the predominant salt (Hesami et al., 2020). More than 1,500 species of salt-tolerant plants have been identified until now, some of which can tolerate higher salt concentrations than seawater (Holguin Pena et al., 2021). In recent decades, researchers have identified the most resistant to stress crops by studying the adaptation of plants and have tried to rehabilitate rangelands by developing them (Zerga et al., 2018).

Saline and alkaline soils in arid and semi-arid regions of Iran are spread over an area of 25 million hectares. These soils are rich in soluble salts and their organic matter is very low. In general, anions in these regions are mostly chloride and to some extent sulphate. The predominant cation is also sodium. Therefore, salts are mostly sodium chloride and sodium sulphate (Soleimani et al., 2011). The study of germination of different plant species at different salinity concentrations is important in selecting the appropriate species for cultivation in barren and saline lands and also selecting the most suitable species for breeding and regenerating rangeland ecosystems (Nafees et al., 2019). Therefore, due to the development of salinity in agricultural lands and the existence of saline water resources, it is necessary to determine the salinity tolerance of different plants, and the use of salinity tolerant plants as a management factor in saline water or soil conditions is recommended.

Salinity can affect seed germination by reducing the osmotic potential of the growing medium, the toxicity of certain ions such as sodium and chlorine, and the reduction of required food ions such as calcium and potassium (Fathi-Sadabadi et al., 2022). Plants usually use different defense mechanisms against salinity stresses. One of these strategies is to increase compatible osmolytes in different parts of the plant. Osmolytes contain the amino acids proline and glycine betaine, as well as soluble sugars, which perform functions such as protecting the intracellular structure, reducing oxidative damage through the production of free radicals, and regulating osmosis in response to drought and salinity stress (Soleimani et al., 2011). The genus *Prosopis* is one of the most important genera under the genus Mimosoideae of the extended family Fabaceae (Keshavarzi et al., 2016).

There are 45 species of this genus in the world, most of which are native to the United States (Sherry et al., 2011). These plants are known by their adaptation to drought and heat condition. This plant is highly resistant to drought and able to withstand less than 100 mm of rainfall and soil salinity up to 16 dS/m (Hughes et al., 2022). Rapid growth and salinity resistance are prominent features of these species (Abdulraouf et al., 2023). In Iran, there are three native species called *P. cineraria*, *P. fracta*, *P. koelziana* and a non-native species called *P. juliflora*. Increasing salinity significantly reduces the salinity tolerance index and germination percentage of the plant (Keshavarzi et al., 2016). Seed germination of *P. juliflora* decreased significantly with increasing NaCl concentration and temperature. Also, light germination was significantly higher than darkness at low salinity and high temperature levels (El-Keblawy and Al-Rawai, 2005).

Salinity reduces the amount of mucilage, potassium and calcium in the plant tissue of *Echium amoenum* and increases the amount of sodium and chlorine in the plant (Makizade Tafti et al., 2008). NaCl plays an important role in germination by inhibiting the activity of some enzymes. Salinity slows down the absorption of water and with this process, prevents the germination and longitudinal growth of the roots (Katembe et al., 1998). The results of research on Aloe Vera showed that salinity stress had a significant effect on leaf dry weight and gel, sodium, potassium, magnesium, and calcium and leaf K/Na and Ca/Na ratios (Mahmoudi et al., 2016). According to research, one of the solutions to reduce cell damage caused by abiotic stresses and increase tolerance to it, is the use of osmotic substances such as proline, betaine glycine, trehalose, etc. in the form of foliar application, which significantly reduces the destructive effects of stress. Among them, the use of proline was an effective method to reduce the adverse effects of

stress. The effect of proline foliar application on plants depends on the plant species, plant growth stage, time of use, and the amount of proline concentration. According to research, proline has a wide range of adaptations to drought and salinity stress in many glycophytes (Sudhakar et al., 1993). There are numerous studies have shown that GB plays an effective role facing abiotic stresses, including salinity, drought, and low temperature by acting as a compatible osmolyte in plant (Annunziata et al., 2019; Bai et al., 2022; Munns and Tester, 2008). The phenomenon of plants accumulating compatible organic solutes, such as proline, soluble sugars, betaine glycine, and polyols, is frequently observed when they are subjected to salt-stress conditions. These soluble compounds, with their relatively low molecular weight, function as osmoprotectants (Balasubramaniam et al., 2023). An investigation revealed that the external application of glycine betaine on common beans (*Phaseolus vulgaris* L.) yielded significant reductions in Na+ uptake, while promoting the uptake of K+. Consequently, this resulted in an increased potassium to sodium ratio (K+/Na+), ultimately enhancing the salt tolerance of common beans (Sofy et al., 2020).

Increasing salinity stress reduces the absorption of copper, zinc, iron, manganese, and potassium and increases the absorption of magnesium, sodium, chlorine, nitrogen, phosphorus, and calcium (Jahanbazi Gojani et al., 2014). In a study on salinity resistance of *P. koelziana, P. juliflora*, and *P. cineraria*, the amount of proline, chlorophyll, and soluble sugars of seedlings in different treatments in these three species was investigated. The results showed that *P. juliflora* has more adaptation mechanisms with salinity compared to the two native species. The aim of this study was to evaluate the effect of salinity on the response of two *Prosopis* species to salt stress at arid and semi-arid land.

Materials and Methods

This research was conducted to investigate the effect of salinity on *P. cineraria* and *P. juliflora*, located at '29° 38' north latitude and '52° 35' east longitude at 1810 m above sea level (Fig. I). It was also conducted as a factorial experiment based on

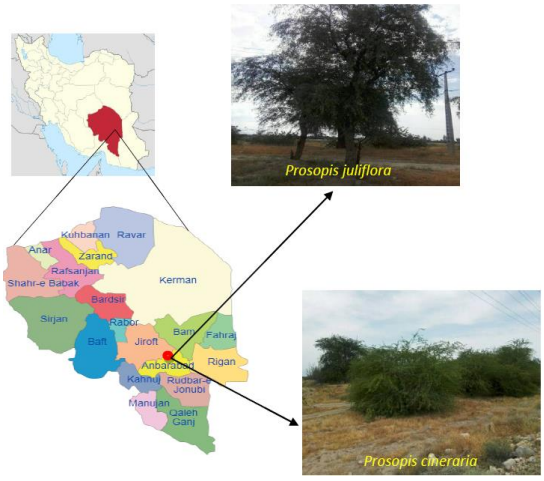

Fig. I. The study site and two Prosopis spp. Genotypes under investigation

a completely randomized design with three replications. The first factor was salinity of 1.8 (control), 5.8, 10.2, and 16.4 dS/m and the second factor was two genotypes (*P. cineraria and P. juliflora).* Preliminary experiments were conducted based on which these salinity levels were selected for the salt tolerance study of these species. Soil acidity was determined by potentiometric method with metelectric pH in saturated extract (Pauwels et al., 1992). In this study, a saturated extract solution and a digital EC meter were used to measure EC (Pauwels et al., 1992). Titration method was used to measure chlorine concentration (Frankenberger Jr et al., 1996). Organic matter (OM%) was estimated using Walkley and Black method (Walkley and Black, 1934).

Plant calcium concentration was measured using plant extract with an AA670J absorption atomic absorption device. Potassium and sodium were calculated by using JENWAY PEP-7 film

Table 1
Chemical and physical properties of plant growing conditions

Soil properties	Amount
Soil texture	Sandy Loamy
OC (%)	1.73
N (%)	0.13
K (%)	229.41
Na (%)	0.10
pH	8.78
EC (dS/m)	1.61

Table 2
Analysis of variance of the vegetative characteristics of *Prosopis* seedlings under different salinity levels

Variation Source	Df	Stem height	Total leaves area	Number of leaves per seedling	Shoot dry weight	Root dry weight
Genotype	1	148**	4624.13ns	10065.82**	2.03ns	11.01ns
Salinity	3	72.3**	2931.26**	12876.03**	16.82**	27.63**
Genotype × Salinity	3	7.12**	21455.87ns	809.95**	0.05ns	0.45**
Error	16	0.08	6.68	21.06	0.003	0.05

* Significant at (P≤0.05); ** Significant at (P≤0.01); ns: non- significant

Table 3
Analysis of variance of the proline, glycine betaine aldehyde, Na, K, Ca, total N, and OC of two genotypes of *Prosopis juliflora and Prosopis cineraria* under different salinity levels

Variation source	Df	Proline	Na	K	Ca	OC	Glycine betaine aldehyde	N
Genotype	1	173.26ns	0.33**	0.52**	0.09**	0.52ns	4.58**	0.09**
Salinity	3	711.60**	7.52**	2.12**	3.21**	0.61**	2.84**	8.24**
Genotype × Salinity	3	58.69**	0.48**	0.03**	0.4**	0.03**	2.31**	0.06**
Error	16	4.38	0.03	0.3	0.66	3.91	6.0.3	2.47

* Significant at (P≤0.05); ** Significant at (P≤0.01); ns: non-significant

Table 4
The interaction effect of salinity and genotypes on stem height (cm), leaf number, and root dry weight of two genotypes of *Prosopis juliflora and Prosopis*

Plant	Salinity (dS/ m)	Stem height (cm)	Number of leaves per seedling	Root dry weight (g)
P. cineraria	Control (1.8)	28.64a	274.55b	3.41a
	5.8	20.40b	212.01d	3.25a
	10.2	16.91c	177.22f	2.10b
	16.4	14.03c	85.23h	1.54c
P. juliflora	Control (1.8)	29.21a	320.01a	4.39a
	5.8	23.15b	258.63c	4.01a
	10.2	17.89c	194.88e	2.85b
	16.4	15.84c	110.25g	2.40b

In each column, means having the same letter are not significantly different at 5% level of probability (Duncan's multiple range test).

photometer (Black, 1965). The amount of total nitrogen was determined using the Kjeldahl method (Jm, 1982). Table 1 shows the physical and chemical properties of the soil used in this study. After collection, the topsoil was air-dried and passed through a 4 mm filter paper. Soils were mixed to a certain extent with sodium chloride to achieve electrical conductivity of 5.8, 10.2 and 16.4 dSm^{-1}, and no NaCl was added to the control soil sample to show an electrical conductivity of 1.8 dS/m. Also, pots with a height of 30 and a diameter of 15 cm and filled with humus, sand, and agricultural soil were used. Each pot contained 3 kg of soil.

Proline

Bates method (Bates et al., 1973) was used to measure proline concentration. According to this method, 0.1 g of leaves of each sample was placed in 10 ml of aqueous solution of 3% sulfosalicylic acid and the resulting mixture was completely homogenized in a porcelain mortar and then with Whatman 2 filter paper smoothed. In the next

Table 5
The interaction effect of salinity and genotypes on proline, glycine betaine aldehyde, Na, K, Ca, OC concentrations (%) and total N in leaves of two genotypes of *Prosopis juliflora and Prosopis cineraria*

Plant	Salinity (dS/m)	Proline Concentration (µmol g−1 F.W)	Glycine betaine aldehyde	Na (%)	K (%)	Ca(%)	OC(%)	Total N
Prosopis cineraria	Control (1.8)	4.58d	40.87d	2.01e	2.47b	0.37e	428.74a	2.39c
	5.8	5.23 c	42.12cd	3.48d	2.11bc	0.47d	408.45ab	2.71bc
	10.2	7.08c	56.55bc	4.22b	1.93bc	0.65c	215.11b	2.94b
	16.4	8.32bc	58.83b	4.89b	1.56c	0.92b	110.94bc	3.12ab
Prosopis juliflora	Control (1.8)	18.26b	47.22c	3.91c	2.72a	0.83c	647.59a	2.58bc
	5.8	18.16b	58.66b	4.17c	2.53ab	0.59c	532.96a	2.89b
	10.2	22.18ab	60.01ab	4.46b	2.21b	0.83c	325.11ab	3.17ab
	16.4	25.03a	61.28a	5.14a	1.82bc	1.15a	220.71b	3.26a

In each column, means having the same letter are not significantly different at 5% level of probability (Duncan's multiple range test).

step, 2 ml of this solution was mixed with 2 ml of ninhydrin reagent (1.25 g of ninhydrin, 30 ml of acetic acid, and 20 ml of 6 M phosphoric acid); then 2 ml of acetic acid was added to each tube. In the next step, samples were placed in a Ben Marie bath at 100 ℃ for one hour. Immediately after taking out of the bath, they were placed on ice for a few minutes. After this step, 8 ml of toluene was added to each test tube and the samples were stirred with a mixer for 15-20 seconds to become completely uniform.

The tubes were then placed in the laboratory for some time. During this period, the upper and lower phases inside the test tube were completely distinguishable and the upper phase was used to determine the proline concentration according to the standard curve of proline in the spectrophotometer at 520 nm (Shimadzu Corporation, Kyoto, Japan). Concentrations of 0, 12.5, 25, 50, and 100 µM L-proline were used to plot the standard proline curve, and toluene was used as a control (zero level). After making standard solutions, instead of leaf extract, each of the standard solutions was added to zero to one hundred and other steps such as proline measurement method were performed and according to the light absorption values and concentrations of the storage solution, the standard curve was drawn. The following equation was used to calculate proline:

$$\text{Proline } (\mu M\ g^{-1} \text{ fresh wt.}) = \frac{M \times T \times W}{115.5} \times 1000$$

M= the value read with spectrophotometer,

W= the weight of the leaf sample used (in these samples it was equal to 0.1 g), and

T= Volume of toluene used (8 ml).

Glycine betaine

Grieve and Grattan (1983) method was used to measure the concentration of glycine betaine. Based on this method, leaf samples were homogenized in a porcelain mortar with 20 ml of ionized water. Then, samples were placed on a vibrating device at 25 ℃ for 48 hours. In the next step, samples were passed through filter paper and mixed with 2 molar sulfuric acids in a ratio of 1: 1; then, samples were placed in ice water for 2 hours. In the next step, potassium iodide solution was added to each seedling and after mixing, the samples were kept at 4 ℃ for 16 hours. The samples were then centrifuged at 15,000 rpm for 20 minutes at 0 ℃. Periodite crystals were dissolved at the bottom of the container in 9 ml of 1 and -2 dichloroethane and the adsorption of the samples was measured at 365 nm. The concentration of glycine betaine in the samples was calculated using a standard glycine betaine graph.

Leaf sodium, potassium, nitrogen, organic carbon and calcium content

The leaves were turned to ash at 550 ℃ for 6 h. The Na and K contents of the samples were measured by JENWAY, PEP-7 flame gauge (Chapman and Pratt, 1962). Organic carbon was estimated using the Walkley and Black method (Walkley and Black, 1934)**.** The Kjeldahl method was used to determine total nitrogen (Jm, 1982). Calcium was measured using an atomic absorption device (AA670J, Japan)**.**

Statistical Analysis

The present study was done as a factorial experiment based on a completely randomized design with three replications. The obtained results were analyzed using Excel and SAS software. Statistical analyses were done based on analysis of variance (ANOVA). Mean's comparison was done based on Duncan's multiple range test at 5% level of probability.

Results

The effect of genotype on all measured traits except total leaves area, shoot and root dry weights, proline, and organic carbon was significant at 1% level. Different salinity levels had a significant effect (P≤0.01) on stem height, total leaves area, number of leaves per seedling, shoot dry weight, root dry weight, proline, Na, K, Ca, OC, Glycine betaine aldehyde, and N (Tables 2 and 3).

The interaction effect of salinity and genotype on stem height, total leaves number, root dry weight, proline, sodium, potassium, calcium, organic carbon, betaine glycine, and total nitrogen showed a significant difference (P≤0.01).

The results showed that with increasing soil salinity, a significant decrease in stem height was recorded in both genotypes, so that the control treatment in comparison with other treatments had the highest and the treatment with 16.4 ds/m had the lowest stem height (Table 4).

Salinity had a significant effect on the number of leaves per seedlings at P≤0.01. Increasing salinity levels resulted in a significant decrease in the number of leaves per seedlings. Also, the highest number of leaves after the control treatment was related to 5.8 dS/m treatment and the lowest number was reported in 16.4 dS/m treatment (Table 3). In general, the lowest number of leaves per seedling was observed for *P. cineraria* at 16.4 salinity level. Overall, *P. juliflora* had higher number of leaves at all salinity levels compared to *P. cineraria* (Table 4).

An indirect relationship was reported between salinity level and root dry weight, so with increasing salinity level in both genotypes, root dry weight decreased significantly. The highest and the lowest root dry weights were observed in control and 16.4 dS/m treatments in both plant species, respectively (Table 4).

Total leaf area gradually decreased with increasing soil salinity. The highest and lowest values belonged to 1.8 and 16.4 dS/m, respectively, equal to 650.44 and 347.61 cm^2 (Table 6).

Shoot dry weight decreased significantly in response to the increase of salinity level (P≤0.05). The shoot dry weight varied from 2.87 to 0.52 g at the lowest and highest salinity levels, respectively (Table 6).

Effect of salinity on proline, glycine betaine aldehyde, sodium, potassium, calcium, organic carbon and total nitrogen

Increased salinity level caused a significant increase in proline content in both genotypes so that the highest amount of proline was observed in 16.4 dS/m for *Prosopis juliflora* and equal to 25.03 and the lowest amount was observed in control treatment of *Prosopis cineraria* (Table 5).

Results of the interaction effect of salinity and genotype showed that the highest (61.28) and lowest (40.87) levels of glycine betaine aldehyde were related to the salinity level of 16.4 in *Prosopis juliflora*, and 1.8 ds/m salinity in Prosopis *cineraria* genotype, respectively (Table 5).

In general, the concentration of sodium in all treatments performed in this study was higher in *Prosopis juliflora* than *Prosopis cineraria*. The highest amount of sodium was reported at a concentration of 16.4 dS/m in both genotypes (Table 5).

Concentration of K decreased with increasing salinity level. Potassium concentration in 16.4 dS/m treatment was equal to 1.56 and 1.82 % in *Prosopis cineraria* and *Prosopis juliflora*, respectively (Table 5).

When the level of salinity increased, the percentage of calcium increased gradually as well. Totally, the lowest and highest Ca contents were observed in control for *Prosopis cineraria* and 16.4 salinity for *Prosopis juliflora*, respectively (Table 5).

The highest amount of organic carbon was recorded with *Prosopis juliflora* at control and the lowest amount was reported in 16.4 dS/m salinity level in *Prosopis cineraria* equal to 647.59 and 110.94, respectively (Table 5).

A significant increase in plant nitrogen content was observed in both genotypes in response to the increase in salinity, so that the highest amount of nitrogen was observed in 16.4 dS / m for *P. juliflora* equal to 3.26 percentage and the lowest amount (2.39%) was recorded in the control treatment of *P. cineraria* (Table 5).

Discussion

The effect of different salinity levels on proline content of *Trachyspermum ammi* L. was significant at 1% probability level. Proline plays several roles, including osmotic regulation, hydroxyl sweeping, cell pH regulation, stabilizing protein structure, and protecting macromolecules from hydration (Ashraf and Foolad, 2007). During salinity stress, the cytoplasm produces and accumulates low molecular weight substances called compatible substances including proline, glycine betaine, polyols, and sugars.

These substances maintain osmotic balance by affecting osmotic regulation, followed by continued water absorption and preservation of cellular and molecular structures such as protein and enzymatic compounds (Nazarpoor et al., 2020). During periods of salinity stress, compatible solutions such as glycine betaine and proline increase (Düring, 2016). Salinity stress increases sugar and proline in *P. koelziana* and *P. juliflora* and increases proline in *P. cineraria*. Furthermore, *P. juliflora* absorbs more sodium and potassium in its tissues than the other two species (Soleimani et al., 2011). Adding proline and glycine betaine aldehyde to *Triticeae. PP* can increase the weight of stem in saline conditions (WynJones, 1984). The application of glycine betaine (GB) at an optimal concentration improved photosynthesis, antioxidant activity, and overall plant growth in maize in saline conditions. GB reduced the accumulation of Na+ in shoots and roots by limiting Na+ uptake and increasing Na+ efflux from leaf cells, thereby enhancing salt tolerance in maize (Zhu et al., 2022).

Table 6
Mean comparisons of total leaf area (cm^2) and shoot dry weight (g) of two genotypes of *Prosopis juliflora and Prosopis cineraria* under different salinity levels

Salinity (dS / m)	Total leaf area (cm^2)	Shoot dry weight (g)
Control (1.8)	650.44a	2.87a
5.8	574.31b	2.17b
10.2	480.14c	1.74c
16.4	347.61d	0.52d

In each column, means having the same letter are not significantly different at 5% level of probability (Duncan's multiple range test).

Regulation of Na content and the ability to maintain high concentrations of K in leaves by reducing the toxic effect of salts have been suggested as important mechanisms in plant adaptation to salinity (Volkmar et al., 1998). In one study, without salinity, species type did not affect the emergence of final seedlings (Villagra and Cavagnaro, 2000). Ca reduces the negative effect of Na by controlling the consumption of toxic ions through selecting cell membranes. Increasing the capacity of Ca content can be one of the compatible mechanisms of *P. alpataco* to cope with salinity (Villagra and Cavagnaro, 2005). The amount of calcium in the leaves of seedlings of *Prosopis cineraria* increased under salt stress.

In the case of *Prosopis juliflora*, calcium may also increase during salt stress (Ramoliya et al., 2006). Comparison of means showed that increasing salinity stress decreased copper, zinc, iron, manganese, and potassium uptake while it increased magnesium, sodium, chlorine, nitrogen, phosphorus, and calcium uptake (TESTER and DAVENPORT, 2003). Low salt concentrations do not appear to have a detrimental effect on *Salicornia persica* growth. However, high salinity reduces plant growth and many species significantly reduce their biomass under severe salinity stress (Abshenas et al., 2019).

In *P. juliflora*, the results showed that the levels of sodium chloride and calcium in roots as well as leaves increased with increasing salinity in the soil. Potassium levels in leaves and roots of seedlings grown under NaCl stress as well as stem and root dry weight decreased (Patil and Karadge, 2012). The effects of salinity on *Prosopis* seedling germination have been investigated by several authors using the hydroponic method or potting soil.

All saline treatments applied to *Prosopis flexuosa* seedlings reduced growth (Catalán et al., 1994). It has been shown that salinity has a greater effect on the emergence and early growth of *P. argentina* than *P. alpataco* ((Villagra and Cavagnaro, 2005). In studies on *Prosopis glandulosa* and *Prosopis tamarugo* in sand cultivation, it was found that after irrigating the plants for 200 days with 0.6 M NaC1 solution, there was a 50% to 40% reduction in the final stem height, respectively (CAZEBONNE et al., 1999). The number of plant stem leaves in all concentrations of NaCl increased during 8 weeks of treatment with a statistical difference in relation to control plants. The size of the leaf area also decreased with increasing NaCl. Also, the effect of NaCl on stem length and number of leaves of plants indicated the negative effect of salt on these factors (CAZEBONNE et al., 1999). Increased vigor index, stem length, and dry weight of roots and stems were reported under polyamine treatment, which like glycine betaine aldehyde application is an effective strategy in stress tolerance (Khan et al., 2012)

In saline conditions, the reduction of seedling shoot length and root length is a common phenomenon in many plants, because roots are the first organs that are exposed to salinity and are in direct contact with the soil, absorbing water from the soil to the shoot(Rajabi Dehnavi et al., 2020).

Conclusion

This study showed that *P. juliflora* and *P. cineraria* both are salinity tolerant, but their vegetation characteristics may be weakened or disrupted if the salinity increases. According to the present study, glycine betaine is a vital factor in salinity tolerance and plant survival even at higher levels of salinity in both genotypes. Glycine betaine aldehyde had a positive and compensatory effect on stem height. By increasing salinity, organic carbon, and potassium decreased significantly. Since the rate of plant decomposition is slower in soils with high values of electrical conductivity, planting salt-tolerant species in such soils, would be a wise policy to increase the storage of organic carbon. Furthermore, the increase in electrical conductivity may sometimes result in toxic effects, as caused by high concentrations of ions, which disturbs the regular absorption of vital elements and minerals by the plant and reduces the organic output of the plant into the soil.

Acknowledgements

This research Approved by the Education, Research, Technology, and Innovation Working Group of Fars Province. We would like to express our special thanks to the Education, Research, Technology, and Innovation Working Group of Fars Province and also the General Department of Natural Resources and Watershed Management **of Fars Province** for the **financial support** to conduct the present study with the Code number of **15512-217022**.

References

Abdulraouf, A., M. O. Mohamed and A. Al Ghamdi. 2023. Surveying, identification and characterization for the potential honeybee (*Apis mellifera* L.) pollen sources in the arid region of Riyadh-Saudi Arabia. *Bee Studies,* 15, (1) 1-11.

Abshenas, M., M. Esmaeili and A. Heydarzadeh, 2019. The effect of glycine betaine to increase resistance of Salicornia plant (*Salicornia persica*) to salinity. *Journal of Plant Process and Function*, 8(32): 177-194(Persian Language).

Annunziata, M. G., L. F. Ciarmiello, P. Woodrow, E. Dell'aversana and P. Carillo. 2019. Spatial and temporal profile of glycine betaine accumulation in plants under abiotic stresses. *Frontiers in plant science,* 10, 230.

Ashraf, M. and M. R. Foolad. 2007. Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environmental and experimental botany,* 59, (2) 206-216.

Bai, M., W. Zeng, F. Chen, X. Ji, Z. Zhuang, B. Jin, J. Wang, L. Jia and Y. Peng. 2022. Transcriptome expression profiles reveal response mechanisms to drought and drought-stress mitigation mechanisms by exogenous glycine betaine in maize. *Biotechnology Letters,* 44, (3) 367-386

Balasubramaniam, T., G. Shen, N. Esmaeili and H. Zhang. 2023. Plants' Response Mechanisms to Salinity Stress. *Plants,* 12, (12) 2253.

Bates, L. S., R. A. Waldren and I. Teare. 1973. Rapid determination of free proline for water-stress studies. *Plant and soil,* 39, 205-207.

Black, C. A. 1965. Method of soil analysis part 2. *Chemical and microbiological properties,* 9, 1387-1388.

Catalán, L., M. Balzarini, E. Taleisnik, R. Sereno and U. Karlin. 1994. Effects of salinity on germination and seedling growth of *Prosopis flexuosa* (DC). *Forest ecology and Management,* 63, (2-3) 347-357.

Cazebonne, C., A. Vega, D. A. Varela and L. A. Cardemil. 1999. Salinity effects on germination and growth of *Prosopis chilensis*. *Revista Chilena de Historia Natural,* 72, 83-91.

Chapman, H. D. and P. F. Pratt. 1962. Methods of analysis for soils, plants and waters. *Soil Science,* 93, (1) 68.

Corbishley, J. and D. Pearce. 2007. *Growing trees on salt-affected land*. Australian Centre for International Agricultural Research

Düring, H. 2016. Evidence for osmotic adjustment to drought in grapevines (*Vitis vinifera* L.). *VITIS-Journal of Grapevine Research,* 23, (1) 1.

El-Keblawy, A. and A. Al-Rawai. 2005. Effects of salinity, temperature and light on germination of invasive *Prosopis juliflora* (Sw.) DC. *Journal of Arid Environments,* 61, (4) 555-565.

Fathi-Sadabadi, M., G. Ranjbar, M. R. Zangi, S. K. Kazemitabar and H. Najafi-Zarrini. 2022. Assessment of Salinity Tolerance in the Selected Genotypes of Cotton. *Agrotechniques in Industrial Crops,* 2, (2) 65-73.

Frankenberger Jr, W., M. Tabatabai, D. Adriano and H. Doner. 1996. Bromine, chlorine, & fluorine. *Methods of Soil Analysis: Part 3 Chemical Methods,* 5, 833-867.

Hasegawa, P. M., R. A. Bressan, J.-K. Zhu and H. J. Bohnert. 2000. Plant cellular and molecular responses to high salinity. *Annual review of plant biology,* 51, (1) 463-499.

Hassani, A., A. Azapagic and N. Shokri. 2021. Global predictions of primary soil salinization under changing climate in the 21st century. *Nature communications,* 12, (1) 6663.

Hesami, A., L. Bazdar and M. H. Shahriari. 2020. Effect of Soil Salinity on Growth, Proline, and Some Nutrient Accumulation in Two Genotypes Seedlings of *Ziziphus Spina-christi*

(L.) Willd. *Communications in Soil Science and Plant Analysis,* 51, (6) 804-815.

Holguin Pena, R. J., D. Medina Hernandez, M. Ghasemi and E. O. Rueda Puente. 2021. Salt tolerant plants as a valuable resource for sustainable food production in arid and saline coastal zones. *Acta Biológica Colombiana,* 26, (1) 116-126.

Hughes, C. E., J. J. Ringelberg, G. P. Lewis and S. A. Catalano. 2022. Disintegration of the genus Prosopis L.(Leguminosae, Caesalpinioideae, mimosoid clade). *PhytoKeys,* 205, 147.

Jahanbazi Gojani, H., S. Hosseini Nasr, K. H. Safeb Talebi, and S. M. Hojati, 2014. Effect of salinity on growth factors, proline, pigments and absoption of elements in shoot of four wild *Almond*. Journal of Plant Research (Iranian Journal of Biology), 27(5): 777-787

Jahanbazi, H., S. M. Hosseini Nasr, K. Saghebtalebi and S. M. Hojjati. 2015. Effect of salinity on growth factors, proline, pigments and absoption of elements in shoot of four wild Almond. *Journal of Plant Research (Iranian Journal of Biology),* 27, (5) 777-787.

Jm, B. 1982. Nitrogen-total. *Methods of soil analysis,* 2, 595-624.

Katembe, W. J., I. A. Ungar and J. P. Mitchell. 1998. Effect of salinity on germination and seedling growth of two Atriplex species (Chenopodiaceae). *Annals of Botany,* 82, (2) 167-175.

Keshavarzi, M., F. V. Raeesi, and N. Farasat, 2016. Morphological study of the genus *Prosopis* (Fabaceae) in Iran. Journal of Plant Research (Iranian Journal of Biology), 29(2): 426-440.

Khan, H. A., K. Ziaf, M. Amjad and Q. Iqbal. 2012. Exogenous application of polyamines improves germination and early seedling growth of hot pepper. *Chilean Journal of Agricultural Research,* 72, (3) 429-433.

Mahmoudi, S., A. Mohammadkhani and V. Rouhi. 2016. Effects of sodium chloride and calcium chloride on growth, gel content and concentration of some nutrients in *Aloe vera* under greenhouse conditions. *Journal of Science and Technology of Greenhouse Culture,* 7, (26)

Makizade Tafti, M., R. Tavakoli Afshari, N. Majnoon Hosseini, and H. A. Naghdi Badi, 2008. Evaluation of salinity tolerance and solute uptake of *Borago officinalis* L. Iranian Journal of Medicinal and Aromatic Plants Research, 24(3): 253-262.

Mohamadi, N., G. H. Diyanati Tilaki, and A. Sadati, 2013. The effect of salinity stress with NaCl on germination and vigor of *avena sativa* seeds. The first national conference on salinity stress in plants and strategies for agricultural development in saline condition, Dissertation, Tabriz: Shahid Madani University of Azerbaijan, dissertation.

Munns, R. and M. Tester. 2008. Mechanisms of salinity tolerance. *Annu Rev Plant Biol,* 59, 651-681.

Nafees, M., M. A. Bukhari, M. N. Aslam, I. Ahmad, M. Ahsan and M. A. Anjum. 2019. Present status and future prospects of endangered Salvadora species: A review. *J Glob Innov Agric Soc Sci,* 7, 39-46.

Nazarpoor, S., A. Salimi, and S. Zeidi, 2020. Investigation of the effect of salinity stress related to different salts in Iranian soils on some physiological and biochemical responses of *Peganum harmala* L. *Journal of Plant Process and Function*, 9(39): 311-329(Persian Language).

Neumann, P. 1997. Salinity resistance and plant growth revisited. *Plant, Cell & Environment,* 20, (9) 1193-1198.

Patil, A. V. and B. A. Karadge. 2012. Effect of NaCl salinity on the growth and mineral nutrition of one month old *Prosopis juliflora* (Sw.) DC seedlings. *Pharmacognosy Journal,* 4, (31) 63-66.

Pauwels, J., E. Van Ranst, M. Verloo and Z. Mvondo. 1992. Analysis methods of major plants elements. Pedology Laboratory manual: Methods of plants and soil analysis. Stock management equipment of worms and chemical equipment. Agriculture Publications

Rajabi Dehnavi, A., M. Zahedi, A. Ludwiczak, S. Cardenas Perez and A. Piernik. 2020. Effect of salinity on seed germination and seedling

development of sorghum (*Sorghum bicolor* (L.) Moench) genotypes. *Agronomy,* 10, (6) 859.

Ramoliya, P., H. Patel, J. Joshi and A. Pandey. 2006. Effect of salinization of soil on growth and nutrient accumulation in seedlings of *Prosopis cineraria*. *Journal of Plant Nutrition,* 29, (2) 283-303.

Sherry, M., S. Smith, A. Patel, P. H. Harris, P. Hand, L. Trenchard, and J. Henderson, 2011. PAPD and microsatellite transferability studies in selected species of *prosopis* (section *Algarobia*) with emphasis on *Prosopis juliflora* and *P. pallid*. Indian Academy of Sciences, 90(2): 251-264.

Sofy, M. R., N. Elhawat and T. Alshaal. 2020. Glycine betaine counters salinity stress by maintaining high K^+/Na^+ ratio and antioxidant defense via limiting Na^+ uptake in common bean (*Phaseolus vulgaris* L.). *Ecotoxicology and Environmental Safety,* 200, 110732.

Soleimani, Z., A. Mosleh Arany and H. Sodaeizadeh. 2011. Investigation on the effect of salinity stress in Prosopis juliflora, P. cineraria and *P. koelziana* in two life cycles (germination and seedling). *Journal of Arid Biome,* 1, (3) 51-62.

Sudhakar, C., P. Reddy and K. Veeranjaneyulu. 1993. Effect of salt stress on the enzymes of proline synthesis and oxidation in greengram (*Phaseolus aureus* Roxb.) seedlings. *Journal of plant physiology,* 141, (5) 621-623.

Tester, M. and R. Davenport. 2003. Na^+ tolerance and Na^+ transport in higher plants. *Annals of Botany,* 503-527.

Villagra, P. E. and J. B. Cavagnaro. 2000. Effects of clayish and sandy soils on the growth of *Prosopis argentina* and P. alpataco seedlings. *Ecología Austral,* 10, (2) 113-121.

Villagra, P. E. and J. B. Cavagnaro. 2005. Effects of salinity on the establishment and early growth of *Prosopis argentina* and *Prosopis alpataco* seedlings in two contrasting soils: Implications for their ecological success. *Austral Ecology,* 30, (3) 325-335.

Volkmar, K., Y. Hu and H. Steppuhn. 1998. Physiological responses of plants to salinity: a review. *Canadian journal of plant science,* 78, (1) 19-27.

Walkley, A. and I. A. Black. 1934. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil science,* 37, (1) 29-38.

Wynjones, R. 1984. Organic and inorganic solute contents as selection criteria for salt tolerance in the Triticeae. *Salinity tolerance in plants,* 189-203.

Zerga, B., B. Workineh, D. Teketay and M. Woldetsadik. 2018. Rangeland degradation and rehabilitation efforts in the Somali National Regional State, Eastern Ethiopia: A review. *International Journal of Innovative Research and Development,* 7, (5) 84-100.

Zhu, M., Q. Li, Y. Zhang, M. Zhang and Z. Li. 2022. Glycine betaine increases salt tolerance in maize (*Zea mays* L.) by regulating Na+ homeostasis. *Frontiers in Plant Science,* 13, 978304.