chemistry proceedings

MDPI

Proceeding Paper

Study of seed germination and seedling growth of *Salicornia* species in different concentrations of sodium chloride †

Zahra Nouri Akandi [1], Farideh Goshasbi [2] and Raheb Mahforouzi [3,*]

1 PhD in Crop Ecology, Department of Agronomy, Shahrood University of Technology, Shahrood, Iran. noori11zahra@gmail.com
2 PhD in Crop Physiology, Department of Agronomy, Shahrood University of Technology, Shahrood, Iran. faridehgoshasbi@gmail.com
3 PhD student, Department of Water Engineering, Sari Agricultural Sciences and Natural Resources sUniversity (SANRU), Sari, Iran. r.mahforouzi@stu.sanaru.ac.ir
* Correspondence: r.mahforouzi@stu.sanaru.ac.ir; Tel.: +98 930 675 0158
† Presented at the 1st International Online Conference on Agriculture-Advances in Agricultural Science and Technology (IOCAG2022), 10–25 February 2022. Available online: https://iocag2022.sciforum.net/.

Abstract: *Salinity is one of the most important factors limiting the growth of plants in many parts of the world. A suitable mechanism in this field is the use of species that can have a favorable production in such environmental conditions. Salicornia is a plant of the Chenopodiaceae family that has many industrial and food benefits and is also used as an oily plant. In order to investigate the seed germination and seedling growth of Salicornia species in different concentrations of sodium chloride, a factorial experiment was done in a completely randomized design with three replications in the environmental stress laboratory of Sari University of Agricultural Sciences and Natural Resources, Iran. Experimental treatments included eight salinity levels (0, 50, 100, 200, 300, 400, 500, and 700 mM NaCl) and two species of Salicornia (Salicornia persica and Salicornia perspolitana). The results showed that with increasing salinity concentration, shoot length had a decreasing trend, while root length initially increased to the salinity of 285.2 mM and decreased with further increase. Shoots and root length in Persica species were significantly longer than Persepolitana species. However, in Persepolitana, the dry weight of roots and seedlings showed a better trend than Persica. In general, considering that the increase in root length indicates that the plant is more tolerant to salinity stress, it is recommended that in saline areas, Persica species be given priority for cultivation. However, in order to better understand the mechanisms involved in the growth of these two species in saline conditions, additional experiments are needed.*

Keywords: Germination, Iran, Regression, *Salicornia*, Salinity.

Citation: Nouri Akandi, Z.; Goshasbi, F.; Mahforouzi, R. Study of seed germination and seedling growth of *Salicornia* species in different concentrations of sodium chloride. *Chem. Proc.* **2021**, *3*, x. https://doi.org/10.3390/xxxxx

Published: date

1. Introduction

Salinity stress is of the most important obstacles to the development and expansion of agricultural production, which has excluded many areas from cultivation or reduced crop yields [1]. Salinity stress causes a wide range of reactions in plants and changes in growth rate and yield of crops [2]. Plants use several mechanisms to deal with salinity stress. Understanding the mechanism of tolerance to high concentrations of sodium chloride in salt tolerant plants can help increase tolerance in sensitive plants and, consequently, increase crop yield [3]. Recent studies have shown that understanding the components of stress as well as the signaling pathways play an important role in regulating plant response to salinity stress [4]. Undoubtedly, one of the most sensitive stages of plant growth to salinity stress is the germination stage [5]. Because this stage is the basis of the initial establishment of the plant and has a great impact on the final yield and the occurrence of stress at this stage can have irreparable consequences for the plant [6]. Salinity stress impairs plant growth by reducing water potential (osmotic stress), accumulation of sodium and chlorine ions (ion toxicity), damage to reactive oxygen groups, and disturbance of nutrient ion balance in the root environment [7, 8]. *Salicornia* plant of the family Chenopodiaceae is a plant with high halophyte as an oil plant. This plant has various industrial and food benefits. *Salicornia perspolitana* and *S. persica* are two valuable Iranian native species about which very little information is available. The height of *Salicornia persica* is higher and about 30 cm. *Salicornia perspolitana* is very unique in terms of flowering time and seems to have been caused by herbidation between *S. iranica* and *S. persica* and due to being triploid, its seeds have no potency [9]. There is an inverse relationship between increased salinity and germination rate of *Salicornia* seeds in several studies has been confirmed [10, 11]. Shoot and root length are the most important indicators of the intensity of environmental stresses, especially salinity stress; Because the root is in direct contact with the soil and absorbs water from the soil and the shoot transfers it to other parts of the plant, so the longitudinal changes of these two parameters of the shoot and root are important signs for plants to respond to salinity stress [12]. Considering that most of the different mechanisms of seed dormancy of these plants such as physiological, inductive factors and so on are due to the presence of areas under cultivation of *Salicornia* and the prevailing conditions in saline agro-ecosystems and also the percentage of seed germination of *Salicornia* species in concentration of different salts have shown significant differences, and it is important to consider different strategies to improve the germination of this plant. Therefore, the aim of this study was to investigate the different concentrations of salinity (sodium chloride) on the germination of seeds of two new species of *Salicornia Persica* and *S. Persepolitana*.

2. Materials and Methods

This experimental field was performed in 2019 in the Environmental Stress Laboratory of Agricultural Sciences and Natural Resources Sari University, in the form of factorial in a completely randomized design with three replications. The treatments consisted of salinity stress: eight salinity levels (0, 50, 100, 200, 300, 400, and 700 mmol NaCl), and two species of *Salicornia* (*S. perspolitana, S. persica*). To disinfect the seeds, they were first soaked in 70% alcohol for 1 minute and then immediately washed three times with sterile distilled water. It was disinfected for 15 minutes using sodium hypochlorite solution (1% concentration). Then, it was washed three times with distilled water. In the next step, 30 seeds were placed on filter paper in each petri dish. Then NaCl levels was applied to each treatment in form of solution. The Petri dishes were closed by parafilm to prevent evaporation and were stored at 25 ° C. Seedlings with a root length of two millimeters or more were counted as germinated seed in a daily basis [13]. After ten days, the number of normal seedlings was counted and 5 normal seedlings were randomly selected to measure the length of roots, stems, and seedlings, as well as the fresh and dry weight of roots, stems, and shoots. The roots and stems were dried in an oven at 70 °C for 48 hours and their dry weight was measured with a digital scale. To ensure the normality of the data, normality test was performed by the Kolmogorov-Smirnov method. Then the data were analyzed with SAS statistical software version 9.4. The changes of the studied parameters through different salinity levels were examined by regression analysis, fitting of linear equations (Equation 1) and two-piece linear equations (Equation 2) Used by Soltani et al. [14]. The curves were plotted using Microsoft Excel software.

$$y=b_1x + a \tag{1}$$

$$y=b_1x + a \quad \text{if} \quad x \le x_0 \tag{2}$$

$$y= (b_1x_0+a) + b_2 (x-x_0) \text{ if } \quad x > x_0$$

y: Predicted value for desired traits, a: Constant value at zero concentration of the desired treatment, x: Treatment concentration, x_0: Rotation point between two phases of the equation, b_1, b_2: The slope of trait changes (decreasing or increasing) in phase one and two of the equation, respectively.

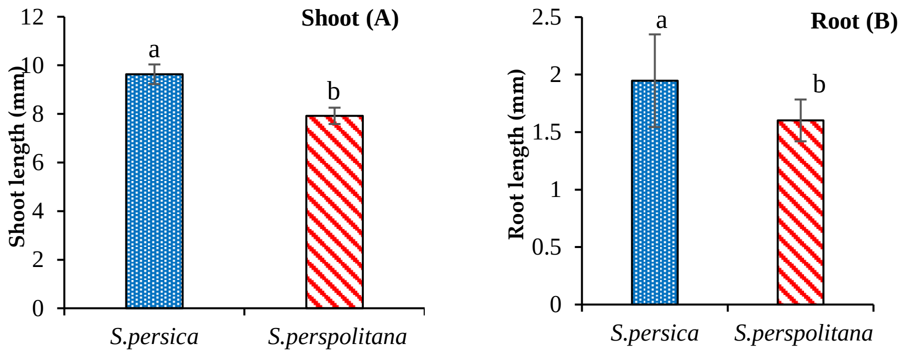

Figure 1. The shoot (A) and root length (B) difference of two *Salicornia* species

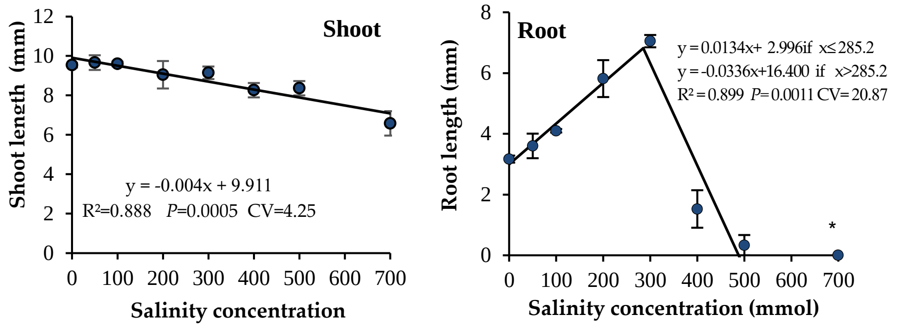

Figure 2. Shoot (A) and root (B) length response to salinity stress. *Data of 700 mmol were excluded.

3. Results

3.1. The length of roots, shoots, and seedlings

Analysis of variance showed a significant effect of salinity treatment on roots, shoots, and seedlings length of *Salicornia*. The difference in the amount of root, shoot, and seedling length in *Salicornia* species was very significant. However, the interaction effect of salinity treatments and *Salicornia* species was significant only for seedling length parameter. No difference was observed between root and shoot length (P>0.05, table 1).

Table 1. ANOVA result for stress's effect on the length and dry weight of *Salicornia* seedlings

Source of variance	df	Dry weight (mg)			Length (mm)		
		Seedling	Shoot	Root	Seedling	Shoot	Root
Salinity	7	0.0963**	0.030**	0.0092**	65.09**	6.42**	3.27**
Plant	1	0.0121^{ns}	0.0089^{ns}	0.00008^{ns}	113.62**	35.19**	1.42**
Salinity×Plant	7	0.0142*	0.0055^{ns}	0.0017**	1.64*	0.608^{ns}	0.057^{ns}
Error	32	0.006	0.0039	0.0004	0.665	0.602	0.052
C.V.(%)		27.60	30.10	2.86	6.81	8.89	12.93

^{ns}, *, **: no significant difference, significant differences at 5 and 1% of probability levels, respectively

According to the results, shoot and root length in *Persica* species were significantly higher and about 21.59% and 21.25%, respectively, than *Perspolitana* species (Figure 1). Examination of salinity regression and shoot length showed an inverse linear relationship with a slight slope of 0.004 and a correlation coefficient of R^2 =0.888 between these two parameters (Figure 2A). The fitted linear equation shows that the stem length decreases slightly with increasing salinity. The regression analysis showed that the trend

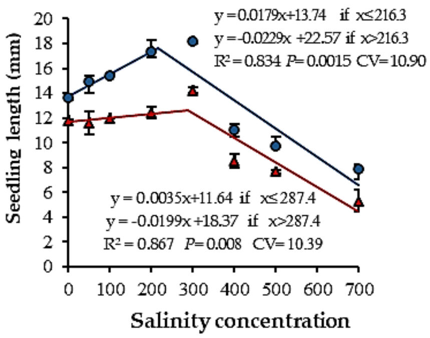

Figure 3. Response of seedling length of *Salicornia* species to salinity stress.

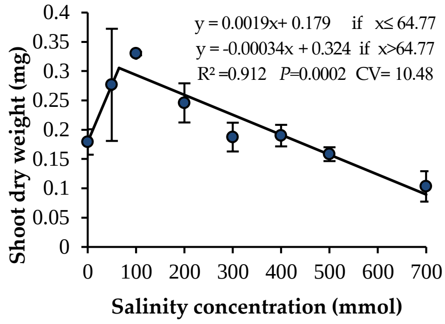

Figure 4. Response of shoot dry weight to salinity stress

of root length response to increasing salinity concentration was in two parts (R^2 =0.899). By increasing the salinity level from zero to 285.2 mmol, the amount of root length increased and then decreased. The slope of the line was calculated from zero to 285.2 salinity with a positive value of 0.0134 units and from 285.2 to 500 mmol salinity with a negative value of 0.0336 units, approximately 2.5 times the positive slope (Figure 2B). The salinity of 700 mmol caused root loss and was therefore not considered in the regression fit.

Seedling length in both *Salicornia* species increased at first with increasing salinity and then decreased. The fitting curves in both species were obtained in two pieces with correlation coefficient R_2=0.834 and R_2=0.876 for *S. persica* and *S. perspolitana*, respectively. Seedling length of *S.* persica was always greater than the seedling length of *S. perspolitana*. With increasing salinity level up to 216.3 mmol in *S. persica* species, seedling length increased with a slope of 0.0179 units and by 29.49% compared to the zero levels and at a higher salinity level showed a decreasing trend while in *Perspolitana* species, seedling length increased to the level of 287.4 mmol Salinity with an increasing slope of 0.0035 units and was 8.59% compared to the control level. The line slope in the decreasing trend was greater than the incremental slope in both species. This means that increasing the salinity from the point of rotation of the graph caused a sharp decrease in the studied parameter while increasing the salinity up to the point of rotation of the graph caused a milder increase in the parameter against salinity changes. Root length values at a salinity of 700 mmol were excluded from the calculations (Figure 3).

3.2. Dry weight of shoots, roots, and seedlings

The results of analysis of variance of the root, shoot, and total dry weight showed a very significant effect (P<0.01) of salinity treatment, but there was a difference between root, shoot dry weight and total dry weight of two *Salicornia* species was not observed (P>0.05). The interaction effect of salinity and species treatments on rootlet dry weight was very significant, on shoot dry weight without significant effect (P>0.05) and on total dry weight had a significant effect (Table 1). With increasing the salinity level, the dry weight of the shoot followed a two-part process, so that from the level of zero to 64.77 mmol of salinity, the dry weight of the stem increased with a slope of 0.0019 units and 68.72%, compared to the control level, and then showed a decreasing trend (with a slope of 0.0003 units) (Figure 4).

According to the results of regression analysis, the response of root dry weight response to increase in salinity level in *Persica* species was linear and decreasing with a coefficient of determination of 0.842 but in *Perspolitana* species was two-part with a coefficient of explanation of 0.894. In *Persica* species, with increasing salinity level up to 400 mmol, the dry weight of rootlet decreased by 82.75% compared to the control level with a slope of 0.0002 units. While in *Perspolitana*, the dry weight of the root increased

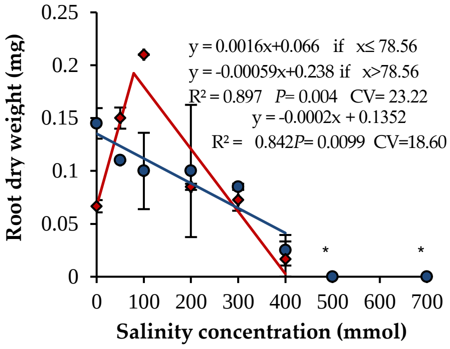

Figure 5. Response of root dry weight to salinity stress. *500 and 700 mmol data were excluded.

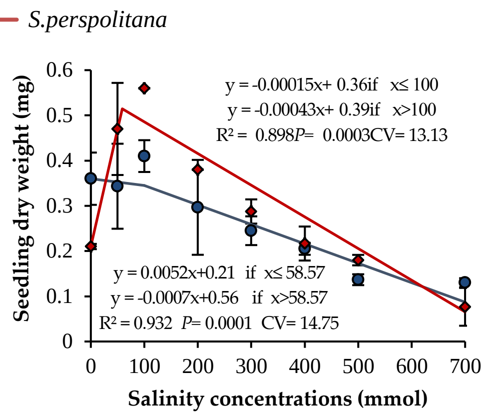

Figure 6. Response of seedling dry weight in two *Salicornia* species to salinity stress

from zero to 78.56, and with a further increase in salinity to the level of 400 mm, showed a decreasing trend (with a slope of 0.0005 units). The corresponding salinity values of 500 and 700 mmol were omitted due to zero due to non-survival (Figure 5).

The trend of seedling dry weight in both *Salicornia* species was two-part with increasing salinity, with the difference that in *Perspolitana* species, with increasing salinity level from zero to 58.57 mmol, seedling dry weight increased at first (with a slope of 0.0052 units) and decreased at levels higher than this (with a slope of 0.0007 units). While in *Persica* species with increasing salinity level up to 100 mmol, the amount of seedling dry weight decreased with a lower slope (0.00015 units) and with a further increase in salinity from 100 to 700 mmol with a higher slope (0.00043 units, Figure 6).

4. Discussion

The positive response of *Salicornia* too low to moderate salinity stresses by other researchers in different species such as *S. herbacea* [15], *S. virginica* [16], and *S. europeae* [17]. Also shown. Salicornia is believed to tolerate salinity stress by accumulation of organic solutions in cells and rapid germination [18]. Researchers report that the stem length growth rate in *S. europaea* at 300 mmol NaCl is greater than zero and 700 mmol NaCl [19]. It was also reported that the optimal growth of *S. dolichostachya* occurred at a salinity of 300 mmol and the growth decreased to 500 mmol when the salinity concentration increased [20]. Other researchers have reported that shoot growth of *S. persica* and *S. europaea* increased under low NaCl (100 mmol) and then decreased with increasing NaCl, and root length in both species increased steadily with increasing salinity [21]. Decreased height of *S. bijelovii*, at salinity levels of 5 to 200 mmol, has been reported by researchers. They attribute the reduced plant growth to the excessive toxic effects of potassium, magnesium and calcium ions by the stems to compensate for sodium deficiency [18]. *S. bijelovii* dry weight has been reported to reach its maximum with increasing salinity up to 200 mmol [22].

5. Conclusion

This experiment was statistically a good reflection of the actual germination conditions in the natural environment of two species of *Salicornia*, taking advantage of the range of salinity changes from 0 to 700 mmol. Changes in germination behavior around the rotation point were observed in both species with increasing salinity from zero to 700 mmol. According to the results of this study, shoots and root length in *S. persica* species were significantly longer than *S. perspolitana* species. However, in *S. perspolitana*, the dry weight of roots and seedlings showed a better trend than *S. persica*. In general, considering that the increase in root length indicates that the plant is more tolerant to salinity stress, it is recommended that in saline areas, *S. persica* species be given priority for cultivation. However, in order to better understand the mechanisms involved in the growth of these two species in saline conditions, additional experiments are needed.

Acknowledgments: Authors acknowledge all administrative and technical supports from Environmental Stress Laboratory members of Agricultural Sciences and Natural Resources Sari University.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Zhang, G.H.; Su, Q.; An, L.J.; Wu, S. Characterization and expression of a vacuolar Na antiporter gene the monocot halophyte Aeluropus littoralis. *Plant Physiol. Biochem.* **2008,** *46*, 117-127. DOI: 10.1016/j.plaphy.2007.10.022.
2. Reddy, A.R.; Chaitanya, K.V.; Viekanadan, M.V. Drought-induced responses of photosynthesis and antioxidant metabolism in higher plant. Plant Physiol., **2004**, 161, 1189-1202. DOI: 10.1016/j.jplph.2004.01.013.
3. Rozema, J.; Schat, H. Salt tolerance of halophytes, research questions reviewed in the perspective of saline agriculture. Environ. Exper. Bot., **2013**, 92, 83-95. DOI: 10.1016/j.envexpbot.2012.08.004.
4. Deinlein, U.; Stephan, A.B.; Horie, T.; Luo, W.; Xu, G.; Schroeder, J.I. Plant salt-tolerance mechanisms. *Trends. Plant. Sci.*, **2014**, 19, 371-379. DOI: 10.1016/j.tplants.2014.02.001.
5. Kader, M.A.; Jutzi, S.C. Effects of thermal and salt treatments during imbibition on germination and seedling growth of sorghum at 42/19°C. *J. Agron. Crop. Sci.,* **2004**, 190, 35-38. DOI: 10.1046/j.0931-2250.2003.00071.x.
6. Rauf, M.; Munir, M.M.; Hassan, U.; Ahmad, M.; Afzal, M. Performance of wheat genotypes under osmotic stress at germination and early seedling growth stage. *Afr. J. Biotechnol.*, **2007**, 6, 971-975.
7. Arzani, A. Improving salinity tolerance in crop plants: a biotechnological view. *In Vitro Cell. Develop. Biolo.Plant*., **2008**, 44, 373–383. DOI: 10.1007/s11627-008-9157-7.
8. Arzani, A.; Ashraf, A. Smart engineering of genetic resources for enhanced salinity tolerance in crop plants. *Crit. Rev. Plant Sci.,* **2016**, 35, 146–189. DOI: 10.1080/07352689.2016.1245056.
9. Akhani, H. *Salicornia persica* Akhani (Chenopodiaceae), a remarkable new species from central Iran. *Linzer Biologische Beitraege*., **2003**, 35, 607-612.
10. Khan, M.A.; Weber, D.J. Factors influencing seed germination in *Salicornia pacifica* var. utahensis. *Am. J. Bot*., **1986**. 73, 1163–1167.
11. Li, P.; Chun, H.; Zhou, Q.C.; Zhou, F.; Chen, Q.Z.; Zhao, M.D.; Li, J.L.; Zheng, Q.S.. Effects of exogenous GA_3 on seed germination and seedling growth of *Salicornia europaea* L. under salt stress. *J. Agric. Sci. Technol*., **2011**, 12, 217-221.
12. Jamil, M.; Lee, C.C.; Rehman, S.U.; Lee, D.B.; Ashraf, M.; Rha, E.S. Salinity (NaCl) tolerance of *Brassica* species at germination andearly seedling growth. *J. Agric. Food Chem*., **2005**, 4, 970-976.
13. Zabihi, H.R.; Savabeghi, K.; Khavazi, K., Ganjal, A. Effect of fluorescent *Pseudomonas* strains from different levels of soil salinity on yield and yield components of wheat. *J. Water Soil*, **2009**, *23*, 199-208. DOI: 10.22067/JSW.V0I0.1551 (in Persian)
14. Soltani, A; Hammer, G.L.; Torabi, B.; Robertson, M.J.; Zeinali, E. Modeling chickpea growth and development: phonological development. *Field Crops Res*., **2006**, *99*, 1-13. DOI: 10.1016/j.fcr.2006.02.004
15. Amiri, B.; Asareh, M.H.; Jafari, M.; Rassoli, B.; Jafari, A.A. Effect of NaCl & Na_2SO_4 on germination and seedling growth of *Salicornia herbacea* & *Alhagi persarum*. *Iranian J. Range Desert Res*., **2012**, *19*, 233-243. (in Persian)
16. Robert W.P.; Ustin, S.L. Effects of salinity on growth and photosynthesis of three Californiatidal marsh species. *Cell Bio. Int.*, **2004**, *17*, 839-845. DOI: 10.1007/BF00377375.
17. Todd P.E.; Ungar, I.A. Competition between *Salicornia europaea* and *Atriplex prostrate* (Chenopodiaceae) along an experimental salinity gradient. *Wet. Eco. Manag.*, **2004**, *9*, 457-461. DOI:10.1023/A:1012276510818.
18. Ayala, F.; O'Leary, J.W. Growth and physiology of *Salicornia bigelovii* Torr. at suboptimal salinity. *Int. J. Plant Sci.*, **1995**, *156*, 197–205. DOI: 10.1086/297241.
19. Parks, G.E.; Dietrich, M.A.; Schumaker, K.S. Increased vacuolar Na^+/H^+ exchange activity in *Salicornia bigelovii* Torr. In response to NaCl. *J. Exp. Bot*., **2002**, 53, 1055-1065. DOI: 10.1093/jexbot/53.371.1055.
20. Katschnig, D.; Broekman, R.; Rozema, J. Salt tolerance in the halophyte *Salicornia dolichostachya* Moss: growth, morphology and physiology. *Environ. Exp. Bot*., **2012**, 92, 32-42. DOI: 10.1016/j.envexpbot.2012.04.002.
21. Aghaleh, M.; Niknam, V.A.; Ebrahimzadeh, H.A.; Azavi, K.B. Salt stress effects on growth, pigments, proteins and lipid peroxidation in *Salicornia persica* and *S. europaea*. *Biol. Plant*., **2009**, 53, 243-248. DOI: 10.1007/s10535-009-0046-7.
22. Kong, Y.; Youbin, Z. Potential of Producing *Salicornia bigelovii* hydroponically as a Vegetable at Moderate NaCl Salinity. *J. Am. Soc. Hortic. Sci*., **2014**, 49, 1154-1157. DOI: 10.21273/HORTSCI.49.9.1154.