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Effect of Different Salinity Concentrations on Germination Parameters of Two Species of *Salicornia* †

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Abstract: *Salicornia persica* and *S. perspolitana* were investigated for the effect of eight salinity concentrations on germination parameters by a completely randomized factorial design. Seed germination was counted for 10 days. The parameters of coefficient of velocity of germination, Medium daily germination, index and germination uniformity were calculated. The results showed that with increasing salinity, the values of the studied parameters are not necessarily decreasing. Increased salinity up to a certain threshold improved germination parameters in both species. Generally, the response of germination indices in *S. persica* was better than *S. persepolitana* and probably tolerates higher salinity at the germination stage.

Keywords: Germination index; Iran; regression; *Persica*; *Perspolitana*; NaCl

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1. Introduction

Abiotic stresses are one of the factors reducing yield in crops. On average, they are the cause of 50% decline in major agricultural products [1]. Salinity stress is one of the most abiotic environmental stresses that affect crop production. This stress is one of the most important obstacles to the development and expansion of agricultural production, which has excluded many areas from cultivation or reduced crop yields [2]. Sodium and chlorine are the predominant ions in most saline regions which have negative effects on different stages of plant growth. Germination is a complex phenomenon involving physiological and biochemical changes resulting from fetal activation. Salinity, as an abiotic stress, causes many ailments for the seeds during the germination period. Salinity first reduces water uptake by seeds due to low osmotic potential of the environment and in the second stage causes toxicity and changes in enzymatic activities [3]. Research on the effect of salinity on germination and seedling growth has shown different results and indicates the fact that with increasing salinity, germination percentage, root length, shoot length and seedling dry weight is reduced significantly [4,5]. *Salicornia* plant of the Chenopodiaceae family, with its multiple uses such as oil production, forage, vegetables, biofuels, cleaning of oil fields and removal of heavy metals from the soil is an advisable candidate to introduce for cultivation marginal saline land or with saline and semi-saline waters. *Salicornia persepolitana* and *Salicornia persica* are two valuable Iranian native species which about them very little information is available. *S. persica* is the most well-known Iranian species that can be observed and cultivated

in different parts of the world. *S. perspolitana* is a new annual member of Persian Salicornia family which completely lying on the ground [6–8]. *Salicornia*'s physiological flexibility has led to its phenotypic diversity and has developed extensively in unfavorable environments [9]. Some researchers believe that *Salicornia* fights salinity through the accumulation of organic solutions in cells and rapid germination [10]. In the study of germination reaction of *Salicornia* seeds to salinity, it was reported that the germination of seeds produced by standard method (cultivation on filter paper at a temperature of 25 °C) and without any pretreatment, during the 15 days with a viability over 90%, *S. persica* has shown high tolerance to salinity during germination. After salinity of 10 dS/m, germination decreased by 2% for each salinity unit in *Persica* species. Also at 500 mM salinity, about 90% of *Persica* species germinated [11]. The use of halo priming, which is the treatment of seeds with mineral salt solution before planting, is an easy, low-cost and low-risk technique that may improve the number of plants established in salty conditions [12]. Therefore, the purpose 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 *Persepolitana*).

2. Materials and Methods

This experiment was performed in 2019 in the Environmental Stress Laboratory (ESL) of Sari Agricultural Sciences and Natural Resources University (SANRU), in the form of factorial in a completely randomized design with three replications. The treatments consisted of salinity stress with 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 min and then immediately washed three times with sterile distilled water. Seeds were disinfected for 15 min 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]. Germination components including Mean daily germination (MDG), coefficient of the velocity of germination (CVG) and germination index (GI) were calculated respectively using equations 1 to 3. Germination uniformity (GU) was determined using Jermaine software [14].

$$MDG = (FGP)/d \quad (1)$$

MDG = Mean daily germination, FGP = Final germination percentage, d = day of maximum germination (experiment period).

$$CVG = (G_1 + G_2 + \cdots + G_n)/((1 \times G_1) + (2 \times G_2) + \cdots + (n \times G_n)) \quad (2)$$

CVG = Coefficient of velocity of germination, G1 − Gn = Number of germinated seeds from the first day to the last day of test.

$$GI = (\sum TiNi)/S \quad (3)$$

GI = Germination index, Ti = Number of germinated seeds per day, Ni = days after planting, S= Total seeds.

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 (4)) and Piecewise linear equations (Equation (5)) from Soltani et al. [15]. The curves were plotted using Microsoft Excel software.

$$y = b_1x + a \quad (4)$$

$$y = b_1x + a \quad \text{if} \quad x \le x_0; \quad \text{and} \quad (5)$$

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

y: predicted value for desired traits, a: Constant value at zero concentration of the desired treatment, x: treatment concentration, x_0: knot value point between two pieces of the equation, b_1 and b_2: the slope of trait changes in phase one and two of the equation, respectively.

3. Results

3.1. Mean Daily Germination (MDG)

The effects of salinity treatment, plant species effect, and the interaction effect of salinity and species treatments on mean daily germination (MDG) were highly significant ($p < 0.0001$), significant ($p = 0.018$), and significant ($p = 0.030$) respectively (Table 1). MDG index in *S. perspolitana* was inversely related to salinity increase, and decreased linearly ($R^2 = 0.809$). MDG changes in *S. persica* increased from salinity of zero to 50 mmol (knot value) and then decreased with increasing salinity. The highest values of MDG index in *S. persica* and *S. perspolitana* were at the salinity of 50 and 0 mM, respectively. The lowest values of MDG were observed at the salinity of 700 mmol in both species (Figure 1).

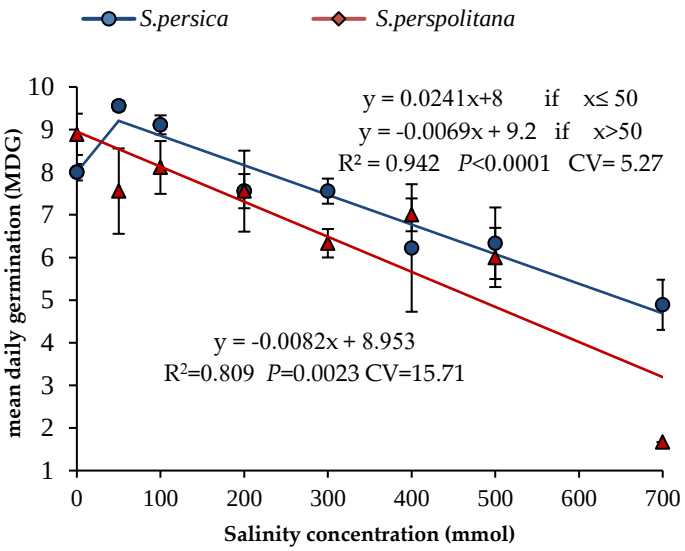

Figure 1. Trend response of the mean daily germination in two Salicornia species to increasing salinity concentration.

Table 1. Analysis of variance of the effect of treatments on germination parameters.

Source of Variance	GU	GI	CVG	MDG	df
Salinity	0.204 ^{ns}	1.70 **	23.99 ^{ns}	18.93 **	7
Plant	3885.4 **	0.204 ^{ns}	481.08 *	6.99 *	1
Salinity×Plant	3387.8 **	0.797 ^{ns}	116.5 ^{ns}	2.93 *	7
Error	342.06	0.501	69.62	1.13	32
C.V. (%)	18.25	29.91	26.38	15.15	-

ns, *, **: non-significantly difference, significantly differences at 5 and 1% of probability levels, respectively.

3.2. Coefficient of Velocity of Germination (CVG)

The results of statistical analysis of coefficient of velocity of germination (CVG) for the effect of salinity, plant, and their interactions were significant only for the effect of *Salicornia* plant species (p = 0.013) (Table 1). Comparison of CVG for two plant species also showed that the value of this coefficient in *S. persica* is significantly 22.24% higher than *S. perspolitana* (Figure 2).

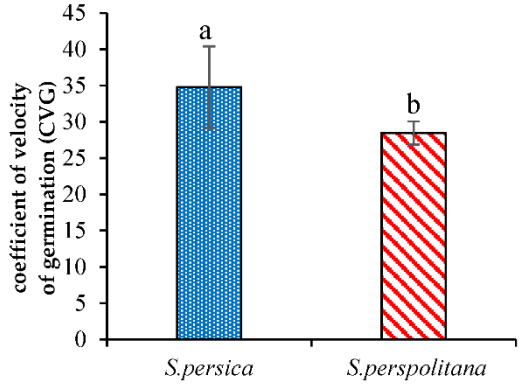

Figure 2. Diagram of the difference between two species of *Salicornia* in Coefficient of velocity of germination.

3.3. Germination Index (GI)

The effect of salinity treatment on germination index (GI) was very significant (p = 0.0079) (Table 1). The value of GI increased with increasing salinity to the knot value of 144.9 mmol (with a slope of 0.0026 units) and then with the continuation of increasing the salinity level. Its value decreased (with a slope of −0.0028 units). So, the lowest value at a salinity of 700 Mmol was observed. The species treatment and the interaction of salinity and species on this index had no significant effect (Figure 3).

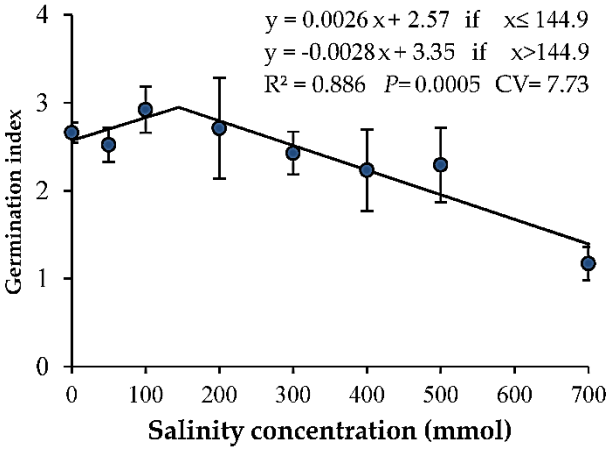

Figure 3. Trend response of germination index to increasing salinity concentration.

3.4. Germination Uniformity (GU)

Statistical analysis of germination uniformity index (GU) showed that there is no difference between different levels of salinity, but the effect of plant species and the interaction of salinity and species is very significant on GU (p = 0.002 and p < 0001, respectively) (Table 1). The GU index value in *S. persica* decreased at first with increasing salinity from zero to 400 mM (knot value). Then the GU value increased

with increasing salinity. So, the lowest GU was observed at 400 mM. In *S. perspolitana*, the changes were reversed. With increasing salinity from zero to 320 mmol (knot value), the GU index increased and then decreased with increasing salinity. So, the highest amount of GU was observed at the salinity of 300 mM. As shown in Although it may be expected that the GU increases with increasing salinity stress levels, but in *S. Persepolitana*, GU decreases at salinity above 400 mmol. However, it does not mean the positive effect of increasing salinity on *S. Persepolitana*. GU was affected by the time of reaching the maximum germination (data are not shown here). The high levels of salinity stress caused germination to stop in the early days of the experiment and as a result, GU decreased in *S. Persepolitana* through formula.

Regression relationships between salinity and GU levels in *Salicornia* species were inversely proportional to each other. Regression line were in two pieces with correlation coefficient of $R^2 = 0.889$ and $R^2 = 0.865$, and knot value points of 320 and 400 mmol for *S. persica* and *S. perspolitana*, respectively. (Figure 4).

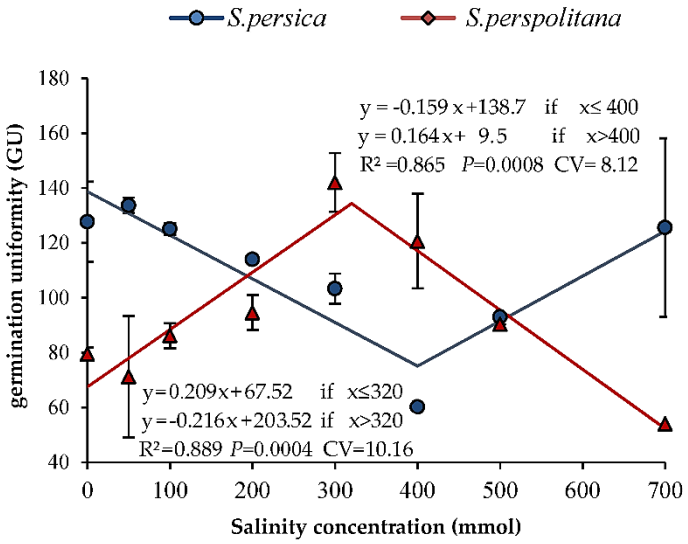

Figure 4. Trend response of germination uniformity in two *Salicornia* species to increasing salinity concentration.

4. Discussion and Conclusions

Examination of correlation equations showed that the *S. persica* in terms of most studied indicators, showed better results than *S. perspolitana* with increasing salinity. In general, *S. persica* was better at germination at equal salinity than *S. perspolitana* and probably has higher salinity resistance at germination. The positive response of Salicornia germination to low- to moderate salinity levels, is also reported by other researchers in other species such as *S. herbacea* [16], *S. virginica* [17], and *S. europeae* [18]. Salicornia is believed to tolerate salinity stress by rapid germination [19] which is in accordance with our results. It is also proved in our research that the seeds of *S. persica* and *S. perspolitana* are largely capable of germination under very high salinity conditions and they have different germination behaviors with increasing salinity from non-saline to high salinity level, as reported by Ayala and O'Leary for *Salicornia pacifica* var. utahensis [19], Keiffer and Ungar for *S. calceoliformis* and *S. europaea* [22] and other researchers [20–24]. Obtaining a two-piece linear regression model with a knot value point greater than zero (of salinity level) indicates the positive effect of low salinity values on *S. persica* and *S. perspolitana* germination. It is suggested to do more research on this case in future.

Institutional Review Board Statement:

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Data Availability Statement:

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