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## Effects of Salinity Stress and Drought Due to Different Concentrations of Sodium Chloride and Polyethylene Glycol 6000 on Germination and Seedling Growth Characteristics of lentil (*Lens culinaris Medik*)

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### ABSTRACT

Salinity and drought stresses are the common environmental stresses in Iran that effect on different stages of plant growth. In order to investigate the effects of drought and salinity stress on germination and seedling growth characteristics of lentil crops (including local masses of Oshnaviya and Shahindej) two separate factorial experiments in a completely randomized design with three replications was conducted in Agricultural and Natural Resources Research Center in West Azerbaijan in 2013. Drought and salinity stress treatments, including different levels of osmotic potential of sodium chloride and polyethylene glycol 6000 in the -4, -8, -12 bar and control treatment (distilled water), respectively. The results showed that drought and salinity stress exerted significant reduction effects on the traits of percentage and germination rate, radicle length, plumule length, radicle length to plumule length ratio, radicle fresh weight, plumule fresh weight and seedling fresh weight. The mean comparison showed that in relation to drought (except for radicle length to plumule length ratio) in all studied traits, the decrease in the traits were significant from control to 4 bar level, while with in salinity (except for the radicle length), in all of the traits, the decrease were not significant. However, the group comparison of the inhibitory effect of the salinity and drought stress showed no significant difference between the two stresses in relation to studied traits.

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### INTRODUCTION

Germination is one of the most important steps in the development of plants. Any plant germination in dire need of a specific range of environmental conditions [29]. However, the lack of germination and weak establishment of seedling due to drought or lack of adequate water and or the salinity of soil are the most important problems of crop production in arid and semi-arid regions such as Iran [28,35,25].

Soil salinity is one of the most important factors limiting crop is planted in the system which can be an important physiological process in plants influence [20]. The most sensitivity of the plant to salinity stress is known on the seed germination and early seedling growth stages. Salinity stress is mainly caused delay in germination and germination rate is reduced [23]. Drought stress may delay germination, reduce or completely prevent it [32].

The lentil as an important member of legume family has a particular place in food of developing countries due to its high protein level. It is necessary to conduct breeding programs due to the cultivation power of this plant and the differences in the average yield in Iran and the world. However, this plant has a cultivation Area of 225 thousand hectares in Iran and the average yield of 502 kg/ha has a production of 113 thousand tons yearly. One of the reasons of low yield of lentil was mentioned the sensitivity of it to the environmental stresses including drought stress [34]. Among the legumes, lentils can be considered as a sensitive plant to salinity that has low yield in relatively salinity farms [17]. A heuristic method of plant stress tolerance at the germination stage, the seeds of reaction in terms of artificial tension, in environments with a solution of polyethylene glycol

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(PEG) or sodium chloride (NaCl). Studies have shown that germination percentage was approximately equal in the solution of PEG 6000 and the soil with the same water potential [21].

Bukhtiar and Shakra [18] used PEG 6000 with potential levels of 0, -0.4, -0.8, -1.2, and -1.6 MPa on coarse and fine-grained varieties of lentils and reported that the reduction in the osmotic potential significantly decreased the total germination percentage and the radicle growth rate and also, the germination of both types of lentils hardly decreased at -1.6 MPa. Pour Amir and *et al.*, [2] resulted that increasing salinity levels and decreasing water osmotic potential decreased the percentage and rate of germination in two lentil genotypes that the decreasing rate in MLC56 was more than MLC261 so that, the highest (32.63 mm) and lowest (9 mm) length of radicles was observed under salinity condition in potential of 0 and -8 bar for MLC261 genotypes, respectively. Growth reduction of plant (radicle and plumule) in terms of drought and salinity in other research on lentil seeds [37], mung bean [19] and peas [31] have also been reported and in these experiments, the amount of reduction varies depending on the variety. Kazerooni Monfared and *et al.*, [8] in a study on the physiological effects of salinity and drought stress on germination of lentil and mung bean varieties reported that the drought and salinity stress significantly reduced the seed germination percentage in both studied plants.

Therefore, it must be considered that that proper germination and seedling establishment is primarily determined as a factor in the yield amount [17] so that the seeds with appropriate germination at stress condition would create vigorous seedling with better and stronger radicle system in the later stages of germination [33]. Based on this, this research was aimed to study the effects of different osmotic potentials of salinity and drought on related attributes to lentil crop germination and to compare and evaluate the sensitivity level of the studied local masses in relation to these stresses.

## MATERIALS AND METHODS

Two separate factorial experiments based on completely randomized design with three replications was carried out to study the effects of drought and salinity stress on two lentil local masses (including Ošnaviya and Shahindej local masses) in laboratory of Natural Resources and Agricultural Research Centre in western Azerbaijan.

Experimental treatments included different levels of osmotic potential due to different concentrations of sodium chloride and poly ethylene glycol 6000 in four levels (0, -4, -8, -12 bar). The Van't Hoff's law (Equation 1) was used to produce different salinity potentials from sodium chloride and the Michel and Kaufman method (Equation 2) was utilized to apply drought stress from poly glycol 6000 [30].

$$\psi_s = miRT \quad (1)$$

Where;  $\psi_s$  is the osmotic potential (bar),  $m$  is the solution molarity,  $i$  is ionization coefficient,  $R$  is gas constant (bar. l.  $0.0832 \text{ mol}^{-1} \cdot \text{K}^{-1}$ ), and  $T$  is the temperature (K).

$$\psi_s = -(1.18 \times 10^{-2})C - (1.18 \times 10^{-4})C^2 + (2.67 \times 10^{-4})CT + (8.39 \times 10^{-7})C^2T \quad (2)$$

Where;  $\psi_s$  is the osmotic potential (bar),  $C$  is the amount of poly ethylene glycol (g/l), and  $T$  is the temperature (°C).

Every experimental unit included one 9 cm diameter petri dish containing sterile filter paper and 20 aseptic seeds were placed in the dish. Disinfecting the seeds, first, they were washed with distilled water, second, they were dipped in solution of 5% sodium hypochlorite for one minute then were leaching with distilled water, and third, seeds were placed in a solution of 2 per thousand benomyl fungicide for one minute and again were leaching with distilled water for three times. 10 ml of produced solutions with definite levels of salinity and drought was added to every petri dish then the dishes were closed by parafilms and transferred to the incubator with  $25 \pm 1$  °C temperature.

Daily monitoring of the samples were done and the number of seeds germinated with radicle length of 1-2 mm were counted and recorded at the same time of the day and to the end of the experiments on the eighth day. Counting was continued until the number of the germinated seeds was constant in each sample during three consecutive days [12].

The equation 3 was utilized in order to determine the rate of seed germination [16].

$$R_s = \sum_{i=1}^m \frac{S_i}{D_i} \quad (3)$$

Where;  $R_s$  is the germination rate (number of seeds per day),  $S_i$  is the number of germinated seeds were counted on  $i$ -th day, and  $D_i$  is the number of days until  $i$ -th counting. The germination percentage was calculated by equation 4 [16].

$$GP = \frac{N_i}{N} \times 100 \quad (4)$$

Where;  $GP$  is the germination percentage (%),  $N_i$  is the number of germinated seeds up to  $i$ -th day, and  $N$  is the total number of seeds.

Finally, in every treatment 5 random samples were chosen and radicle length, plumule length, radicle to plumule length ratio, radicle fresh weight, and seedling fresh weight were measured and calculated. Before the analysis of the data, the normalization of data was done in required cases (transformation equation:  $\sin^{-1} \sqrt{x}$ ).

The statistical analysis of data was done by using SPSS software and the mean comparison was based on LSR test in probability level of 5%. Group comparison of drought and salinity stresses was conducted by t test in probability level of 5%.

## RESULTS AND DISCUSSION

### 1- The effects of drought stress:

Analysis of variance showed that the applied stress levels had a significant effects on the germination characteristics and all of the traits decreased with more negative osmotic potential (Table 1). These results were in line with other researcher's reports [13]. Kiani *et al* [10] suggested that a decrease in water entrance to the seeds led to a reduction in the amount or rate of the physiological and metabolic processes seed germination. The analysis of variance indicated that most of the characters (except the radicle) of the lentil study did not show a significant difference was observed masses of the same sensitivity.

The mean comparison analysis about germination percentage showed that increasing potential from control to -4 bar treatments and from -4 bar to -8 bar treatments, there was significant difference, so that the germination percentage was reduced (Table 2). Of course, in -12 bar, Germination percentage (and also in other traits) was zero due to the loss of germination. The study of the germination rate also showed that increase in water potential, the germination rate decreased from 14.43 (number per day) to 7.53 and 3.93 (number per day) at -4 and -8 bar, respectively. Kafy and *et al.*, [10] also examine the effects of stress caused by PEG on the lentil genotypes and reported that the highest percentage of germination rate was observed at zero potential (control) and the lowest yield was seen in potential of -12 bar.

Plumule length and radicle length showed similar decreasing trends at drought potential levels and had significant differences with control treatment so that, from control to -4 and -8 bar potential levels the plumule length decreased 59.24 % and 83.72 %, and the radicle length decreased 64.48 % and 89.28 %, respectively. Although, the ratio of radicle to plumule length, no significant difference was observed between the treated droughts that this showed more negative water potential the radicle and plumule length had the same sensitivity to the drought stress. Nezamy and *et al.*, [15] also studied the drought tolerance on lentil genotypes and showed that increasing potential levels the radicle and plumule length decreased so that the highest radicle length was occurred in no stress condition and the lowest one was observed in potential of -16 bar. Studies revealed that a decrease in water uptake by seeds under drought stress reduced the secretion of hormones and enzymes, resulted to the impairment in seedling growth (radicle and plumule) [3].

The mean comparison of radicle fresh weight, plumule fresh weight, fresh weight of seedlings revealed that the radicle fresh weight decreased 76.61 % and 70.84 %, and the fresh weight of plumule declined 52.5% and 75%, and seedling fresh weight reduced 55.4% and 78.4 %, in potential levels of -4 and -8 bar respectively, compared to control treatment. Rahimi and Rahimi [4] in a study on the effects of drought and salt stress on germination of mung bean and lentil genotypes reported The lentils and mung bean cultivars in both drought and salinity conditions, a significant decrease in fresh weight of seedlings of mung bean and lentil observed and Maximum reduction was observed in cultivars under drought stress.

### 2- The effects of salinity stress:

Analysis of variance showed that salinity levels exert significant effects on the germination traits (Table 3). These results are consistent with reports from other investigators [6]. Maybodi and Ghare Yazzy suggest that salt stress interferes with cell growth, All metabolic reactions of plants affected, leading to a reduction in the growth of plants in saline.

The mean comparison results showed that there was no significant difference between control and -4 bar with increasing levels of salinity on germination percentage characteristics and germination rate, but, increasing the potential from -4 bar to -8 bar treatment significantly decreased the germination percentage to 28.96% and also declined the germination rate to 58.72 % compared to control treatment (Table 4). In the potential of -12 bar the germination was not performed. It seemed that the reduction in germination percentage under salinity stress was due to decreasing osmotic potential with destroying the metabolic processes of germination and increasing ionic compounds, the increase in these compounds reduced the percentage and the rate of germination [24]. If the seed activity for various reasons such as salinity decreases, Period radicle out in the seed germination is increased, thereby reducing the rate of germination is affected more than the percentage of germination [36].

The mean comparison also showed that increasing potential from control to -4 bar treatment there was no significant difference between the two treatments for plumule length but increasing the potential up to -8 bar, the plumule length decreased 58.74% compared to control. But in the radicle length, increasing potential from control to -4 and -8 bar, the differences were significant, so that, the decreasing were 35.51% and 82.24% compared to control. Studies show that germinated seeds in saline soils had shorter plumule and radicles, and sodium chloride had more inhibitory effect than others in the emergence of fetal tissue [1]. Growth reduction of

seedling (plumule and radicle) in salinity condition on seed of lentil crop were consistent with the results of other researchers, of course, the amount of reduction was different depending on the studied varieties [11].

Analysis of the radicle to plumule length ratio showed that there were no significant difference between the control level and -4 and -8 bar treatments, although this ratio decreased from control to -12 bar level so that the decreasing was 24.38% and 52.34% in -4 and -8 bar, respectively, compared to the control which indicated the the same sensitivity of radicle and plumule to applied different levels of salinity in the studied local masses. This is despite the fact that Rahimi and Rahimi [4] in a study on lentil reported that this ratio increased with increasing osmotic potential because it could be related to the more growth sensitivity of plumule compared to radicle in the studied varieties.

The analysis of the plumule fresh weight, radicle fresh weight, and seedling fresh weight revealed that increasing the salinity stress decreased these traits that it was not significant between control and -4 bar but with increasing the potential from -4 to -8 bar the plumule fresh weight, radicle fresh weight, and seedling fresh weight decreased 44.75%, 64.14%, and 59.53%, respectively, compared to control, that the reduction was significant in fresh weight of seedlings. These results are consistent with observations of other researchers, the plant was cultivated lentil [1,6,4] Given the role of hormones Absizik acid as a growth regulator in plant adaptation to adverse environmental conditions such as salinity and plant organs and tissues respond to the interaction of hormones likely Absizik acid reduces the high level of oxcin and thus reduce plant growth and cell division [5].

### 3- Comparison and evaluation of inhibitory effect of salinity and drought stresses:

In order to compare and assess drought and salinity stresses an independent group comparisons between salinity and drought Germination percentage, germination rate, radicle length and plumule length were measured (Table 5). The comparison revealed that although the average of the traits were less in drought stress than salinity stress, the differences were not significant. Thus, the inhibitory effects of salinity and drought on lentil plant (in relation to the studied traits) had the same effects. Khajeh Hosseini and *et al.*, [27] found that the germination of soybean in sodium chloride solution was more than polyethylene glycol 6000 due to rapid absorption of water by seeds and receiving necessary moisture for germination in sodium chloride solution. Kaya and *et al.*, [26] also reported the same results.

According to the results of this experiment, it can be said that the mass of local lentils studied the germination stage, were relatively tolerant to Gently drought and salinity stresses and compare the effects of stress on lentil crops showed that the effect was almost identical. Yet, additional tests might be done in field and greenhouse conditions in order to more accurate evaluations of the stresses and better comparison of local masses.

**Table 1:** The ANOVA of germination traits of lentil in drought stress.

	df	Germination percentage	Germination rate	Plumule length (cm)	Radicle length (cm)	Radicle to plumule ratio	Radicle fresh weight (g)	Plumule fresh weight (g)	Seedling fresh weight (g)
Drought	3	102565.06**	216.57**	12.576**	26.983**	2.778**	0.001**	0.002**	0.006**
genotype	1	337.50 <sup>ns</sup>	0.952 <sup>ns</sup>	0.009 <sup>ns</sup>	1.215 <sup>*</sup>	0.084 <sup>ns</sup>	5.41E-6 <sup>ns</sup>	1.96E-5 <sup>ns</sup>	4.36E-5 <sup>ns</sup>
Drought* genotype	3	451.278 <sup>*</sup>	16.713 <sup>*</sup>	0.881 <sup>ns</sup>	2.47**	0.014 <sup>ns</sup>	5.56E-6 <sup>ns</sup>	0.000 <sup>*</sup>	000**
Error	16	85.833	4.030	0.088	0.212	0.094	4.103E-6	2.09E-5	3.046E-5

ns: not significant, \* and \*\*: significant in probability levels of 5% and 1%, respectively.

**Table 2:** Mean comparison and effect of various levels of drought on germination and growth characteristics of lentil seed.

Drought levels (bar)	Germination percentage	Germination rate	Plumule length (cm)	Radicle length (cm)	Radicle to plumule ratio	Radicle fresh weight (g)	Plumule fresh weight (g)	Seedling fresh weight (g)
Control	96.67 <sup>a</sup>	14.143 <sup>a</sup>	3.30 <sup>a</sup>	4.73 <sup>a</sup>	1.45 <sup>a</sup>	0.034 <sup>a</sup>	0.074 <sup>a</sup>	0.040 <sup>a</sup>
-4	67.67 <sup>b</sup>	7.53 <sup>b</sup>	1.345 <sup>b</sup>	1.68 <sup>b</sup>	1.435 <sup>a</sup>	0.013 <sup>b</sup>	0.033 <sup>b</sup>	0.019 <sup>b</sup>
-8	38 <sup>c</sup>	3.93 <sup>b</sup>	0.537 <sup>c</sup>	0.507 <sup>c</sup>	0.96 <sup>a</sup>	0.005 <sup>c</sup>	0.016 <sup>c</sup>	0.010 <sup>c</sup>
-12	000 <sup>d</sup>	000 <sup>c</sup>	000 <sup>d</sup>	000 <sup>c</sup>	000 <sup>b</sup>	000 <sup>d</sup>	000 <sup>d</sup>	000 <sup>d</sup>

In each column, means with different letters are significantly different, according to ANOVA and the LSR test.

**Table 3:** The ANOVA of germination traits of lentil in salinity stress.

	df	Germination percentage	Germination rate	Plumule length (cm)	Radicle length (cm)	Radicle to plumule ratio	Radicle fresh weight (g)	Plumule fresh weight (g)	Seedling fresh weight (g)
salinity	3	102242.67**	256.245**	14.116 <sup>*</sup>	27.627**	2.33**	0.001**	0.001**	0.006**
genotype	1	1944 <sup>*</sup>	57.258**	1.075 <sup>ns</sup>	3.420 <sup>*</sup>	0.076 <sup>ns</sup>	8.39E-5 <sup>ns</sup>	000 <sup>ns</sup>	000 <sup>ns</sup>
salinity* genotype	3	1188.44**	25.453**	0.443**	1.748 <sup>*</sup>	0.014 <sup>ns</sup>	2.07E-5 <sup>ns</sup>	000 <sup>ns</sup>	4.9E-5 <sup>ns</sup>
Error	16	44.667	2.380	0.814	0.497	0.059	5.002E-5	6.38E-5	0.011

ns: not significant, \* and \*\*: significant in probability levels of 5% and 1%, respectively.

**Table 4:** Mean comparison and effect of various levels of salinity on germination and growth characteristics of lentil seed.

salinity levels (bar)	Germination percentage	Germination rate	Plumule length (cm)	Radicle length (cm)	Radicle to plumule ratio	Radicle fresh weight (g)	Plumule fresh weight (g)	Seedling fresh weight (g)
Control	97.67 <sup>a</sup>	14.185 <sup>a</sup>	3.30 <sup>a</sup>	4.73 <sup>a</sup>	1.45 <sup>a</sup>	0.033 <sup>a</sup>	0.033 <sup>a</sup>	0.074 <sup>a</sup>
-4	94.67 <sup>a</sup>	13.45 <sup>a</sup>	2.93 <sup>a</sup>	3.05 <sup>b</sup>	1.105 <sup>ab</sup>	0.023 <sup>ab</sup>	0.031 <sup>ab</sup>	0.055 <sup>a</sup>
-8	68.67 <sup>b</sup>	8.33 <sup>b</sup>	1.26 <sup>b</sup>	0.84 <sup>c</sup>	0.691 <sup>b</sup>	0.0012 <sup>bc</sup>	0.018 <sup>b</sup>	0.030 <sup>b</sup>
-12	000 <sup>c</sup>	000 <sup>c</sup>	000 <sup>b</sup>	000 <sup>c</sup>	000 <sup>c</sup>	000 <sup>c</sup>	000 <sup>c</sup>	000 <sup>c</sup>

In each column, means with different letters are significantly different, according to ANOVA and the LSR test.

**Table 5:** Group mean comparison between drought and salinity stresses on decreasing germination percentage, germination rate, plumule length, and radicle length.

	Ave. germination percentage	Ave. germination rate	Ave. plumule length	Ave. radicle length
Drought	50.583	6.400	1.296	1.730
Salinity	65	8.990	1.873	2.154
Significant level between the two groups.	0.228	0.148	0.181	0.476

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