Effects of Salinity Stress and Drought Due to Different Concentrations of Sodium Chloride and Polyethylene Glycol 6000 on Germination and Seedling Growth Characteristics of Chickpea (Cicer arietinum L.)

1Rasoul Khodaverdivand Keshtiban, 2Vahid Carvani and 3Mojtaba Imandar

1Faculty member of Agricultural Department, payame Noor University (PNU), Tehran, Iran
2Faculty member of Statistics Department, Payame Noor University (PNU), Tehran, Iran
3MSC of Agriculture Department, Aras International Campus of Tehran University, Iran

ABSTRACT

Poor establishment and lack of seedling germination due to lack of adequate water and or salinity are the main problems of producing crops in country of Iran. In order to study the effect of drought and salinity stress on growth and germination characteristics of chickpea seedlings (including khoy and charcharh local masses), two separate factorial experiments based on completely randomized design with three replications was conducted in Agricultural and Natural Resources Research Center of West Azarbaijan Province in 2013. Drought and salinity stress treatments were including different levels of osmotic potential of sodium chloride and polyethylene glycol 6000 in three levels of -4, -8, -12 bar and control treatment (distilled water). The results revealed that applying different levels of drought and salinity stress had significant decreasing effects on most of the measured parameters such as the percentage and rate of germination, radicle length, plumule length, radicle length to plumule length ratio, and seedling fresh weight. The mean comparison showed that in drought stress and in all parameters except the radicle length to plumule length ratio and plumule fresh weight, the decreasing effects were significant between control and -4 bar treatment. In relation to salinity stress, except the germination percentage, the radicle length to plumule length ratio, the radicle length, and seedling fresh weight, the decreasing effects were significant in other parameters. Meanwhile, group comparison of inhibitory effects of salinity and drought stress indicated no significant differences between stresses in studied traits.

INTRODUCTION

Environmental stresses such as drought and salinity are the important stresses in decreasing growth and production of agricultural crops [25,5]. Plants, especially in germination and primary stages of growth, are more sensitive to environmental stresses like drought and salinity [18].

Salinity delays and declines the germination of most of the agricultural crops seeds so that low salinity delays the germination of the seeds and high salinity declines the germination percentage [10]. Salinity effects on the seeds germination and growth by reducing the water potential and toxicity of specific ions such as sodium and chloride and reducing required nutrients, such as calcium and potassium [19,44,29,20]. Meanwhile, it is revealed that among the indexes of seed germination, the percentage and rate of the germination are the most important factors that influenced by the salinity stress [41]. Poor germination in saline soils leads to lack of uniformity in emergence of chickpea seeds. Irrigation with saline water with electric conductivity of about 10 mmhos/cm decreases the crop yield up to 55%. Compared with common seeds, the chickpea seeds in saline soils, have less germination percentage [43]. Dashy and et al. [11], indicated that the effect of different levels of salinity in chickpea significantly declined the rate and percentage of seed germination and seed vigor index, so that the response of different chickpea genotypes to salinity stress in germination stage was different with seedling stage. They also reported that in FLIP95-64C genotype, which had a good germination power, the
salinity threshold was lower than FLIP95-50C genotype while in screening tests, FLIP95-50C genotype had the maximum threshold.

Drought stress might delay or decrease or completely prevent the germination [40]. Dutt and Sharma[14] showed that the water uptake by seeds, total percentage of germination, radicle length, plumule length were significantly decreased by using poly ethylene glycol 6000 in chickpea. Hadas [22] indicated that reducing soil water potential to less than -14 MPa, the chickpea germination was significantly decreased, so that only 20% of seeds germinated in potential of -1.5 MPa. The surveys showed that the germination percentage was approximately equal in poly ethylene glycol 6000 solution and soil with the same potential [45,15]. Poly ethylene glycol is a solution with high molecular mass that has the most application in drought resistance studies because it produces a solution similar to natural conditions [32].

Legumes and specially chickpea have high nutritional value due to their vegetable protein and essential amino acids, especially lysine [38]. Chickpea has the second place among the legumes because of its agricultural nutrition value and ecologic characteristics [35]. Worldwide, 1.3 million hectares are under cultivation of chickpea with the annual production of 7.5 million tons [16]. In Iran, chickpea is more important and has more cultivation area and production than other legumes [8] so that the cultivation area of this crop was 602558 ha in 2006-2007 cropping season with 13743.6 ha irrigated farming and 588815.5 ha dryland farming. Average yield in this cropping season was 1175.82 kg/ha for irrigated chickpea and 524.15 for dryland farming [2]. Low grain yield for chickpea is due to planting early yield cultivars and their sensitivity to various environmental stresses [8]. The drought has been expressed as the most important abiotic stresses in chickpea yield loss [21]. It is also revealed that the salinity could reduce the chickpea yield even in salinity tolerant cultivars [3]. Accurate study of the effect of negative potential on germination is hard in field and greenhouse condition because the physical and chemical properties of soil decrease the germination due to continuous changes in soil and capillary movement of water, so these studies commonly conducted in laboratory conditions [13]. Accordingly, this research was fulfilled to study the effects of various osmotic potential resulted by salinity and drought stress on the attributes associated with the germination of chickpea crop and to compare the sensitivity of the tested local masses in relation to these stresses.

MATERIALS AND METHODS

This research was carried out to study the effects of drought and salinity stress on two chickpea local masses (including khoy and chaipareh local masses) in two separate factorial experiments based on completely randomized design with three replications 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 [34].

$$\psi_s = \frac{mRT}{i}$$  \hspace{1cm} (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 mol$^{-1}$.K$^{-1}$), and $T$ is the temperature (K).

$$\psi_s = -1.18 \times 10^{-2}C - (1.18 \times 10^{-4})C^2 + (2.67 \times 10^{-4})CT + (8.39 \times 10^{-7})C^2T$$ \hspace{1cm} (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 10 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±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 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 (Mojab and Zamaney, 2010).

The equation 3 was utilized in order to determine the rate of seed germination (Agrawal, 1991).

$$R_s = \frac{S_i}{T_i - n}$$ \hspace{1cm} (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.

$$GP = \frac{S_i}{N_i} \times 100$$ \hspace{1cm} (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 3 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:

The analysis of variance (ANOVA) indicated that different levels of drought stress had a significant effect on all of the germination traits so that all of the studied traits decreased with decreasing (more negative) the osmotic potential of water (Table 1). These results were in line with other researcher’s results [33,23].

The mean comparison analysis about germination percentage showed that increasing osmotic potential the germination percentage decreased so that from control to -4 bar treatments and from -4 bar to -8 bar treatments the germination declined 41.78% and 94.5%, respectively and in -12 bar the germination percentage (and also other traits) was equal to zero due to loss of germination (Table 2). The analysis of germination rate indicated that more negative potential of water decreased the germination rate from 5.57 num/day in control treatment to 1.59 and 0.093 num/day in -4 and -8 bar, respectively.

Maasoumy and et al. [31] studied the physiologic effect of drought stress on germination of 12 chickpea genotypes and reported that the most and the least percentage and rate of germination were observed in control treatment (osmotic potential of 0) and -16 bar osmotic potential, respectively. Reducing water entrance to the seeds resulted by increasing drought stress, would decrease the electrical conductivity (EC) and effect the physiologic and metabolic processes of germination, so the rate and percentage of germination would decline [28].

The plumule and radicle length had the same decreasing trend in different levels of drought stress. The plumule length was reduced 79.94% and 97.96% in -4 and -8 potential levels compared to control treatment, respectively although there was no significant difference between -4 and -8 bar potential. The radicle length was also decreased in -4 and -8 potential compared to control and its reduction amount were 49.74% and 93.45%, respectively. The reduction in radicle and plumule length might be because the drought stress effected the meristem cells of these two organs and disordered elongation process of the cells. The elongation process influenced by drought stress more than cell distribution since the water absorption decreased in the condition of negative potential, so decreased the necessary turgescence pressure for elongation of cells and resulted in delaying or stopping the growth process [37]. Different experiments revealed that the drought stress decreased both radicle and plumule length but the reduction ratio in plumule length was more than radicle length [12,17]. Verslues and et al. [46], believed that the seed resources movement was not limited by the reduction of water potential so the decreasing ratio in radicle is less than plumule.

Assessment of radicle to plumule length ratio showed that the mean of this trait was more in potential levels of -4 bar compared to control treatment, however, there was no significant difference between control, -4 bar and -8 bar. Maasoumy and et al., reported in similar results that the radicle to plumule length ratio in -4 bar potential was significantly more than control treatment and there was no significant difference between -8, -12, and -16 bar treatments. De and Kar [13] observed that some chickpea genotypes had more radicle length in -0.4 MPa than 0 potential and found that this would induce the radicle growth because the increase in radicle growth is the first change to overcome the drought stress in order to absorb the maximum moisture.

Mean comparison of radicle fresh weight, plumule fresh weight and seedling fresh weight showed that more negative potential of water decreased the amount of these traits. However, there was no significant difference between the different levels of treatment in plumule fresh weight. Rahimi and Rahimi [42] studied the effects of drought and salinity stresses on germination of various genotypes of mung bean and lentil and reported the significant decrease in seedling fresh weight of these crops due to drought and salinity stresses while the maximum decrease was observed in drought stress.

2- The effects of salinity stress:

The ANOVA showed that the various levels of salinity stress had significant effects on all studied traits (except radicle fresh weight) while, the difference between studied local masses and interaction between salinity and local masses were not significant (Table 3). Reduction of studied germination components could be related to reduction of water absorption amount and rate and the negative effects of low osmotic potentials and the toxicity of Na+ and Cl ions on biochemistry processes of germination [36,39].

Mean comparison revealed that increasing salinity levels decreased the germination although this reduction was not significant from control treatment to -4 bar treatment but was significant from -4 to -8 bar (Table 4). However, there was no germination in -8 and -12 bar treatments that indicated the low levels of salinity tolerance of studied local masses. There were significant differences in germination rate and the reduction of germination rate were 24.6% and 100% in -4 and -8 bar compared to control treatment.
Behbodian and et al. [9] reported the impacts of salinity stress on percentage of germination of 4 Iranian chickpea genotypes (including Karaj, Kaka, Pirooz, and Jam), in 6th day of germination, increasing the salinity level from 0 to -3 bar there was no significant difference between the treatments, but the germination reduction was significant in more negative potential from -3 to -6 bar. They also concluded that the germination power of different cultivars depended on their genetic characteristics but this power would be influenced by salinity of the environment. Azary and et al. [6] also studied the effect of salinity stress on 6 chickpea cultivars (Bam, biosij, Hashem, Azad, Arman, and ILC482) in four levels of salinity (0,-4,-8, and -12 bar) and showed that increasing salinity level decreased the germination percentage and germination rate.

The mean comparison indicated that increasing the potential from control treatment to -4 bar, the plumule and radicle length were decreased 59.60% and 31.49% compared to control, respectively that was significant in plumule length. Khodabakhsh and et al. stated similar results for chickpea in salinity stress that the reduction in plumule length was more than radicle length in saline environment.

The assessment of radicle to plumule length ratio indicated that more negative potential decreased this ratio, but there was no significant difference between treatments that observed the same sensitivity of plumule and radicle to various levels of salinity stress in studied local masses.

The seedling fresh weight mean comparison showed that more negative potentials would decrease this trait. Although there was no significant difference between control and -4 bar treatments, the difference was significant between -4 and -8 bar treatments. The researchers believed that salinity stress decreased the seedling growth due to reduce the osmotic potential of growth environment and toxicity of special ions [30,4,24].

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

An independent group comparison was carried out for germination percentage, germination rate, radicle length, and plumule length in order to compare and evaluate the effect of salinity and drought stresses (Table 5). The results showed that although the average of these traits in drought stress were more than salinity stress, the differences were not significant. So the inhibitory effect (related to studied attributes) was the same for both stresses on chickpea. Mojab and Zamany studied the inhibitory effects of drought and salinity stresses on cardaria draba, which is a weed plant, and found that the seeds might absorb a part of sodium and chlorine ions in saline solution and keep their osmotic potential lower than the solution and thus water uptake continued in negative potential. Khajehosseini and et al realized that soybean germination in sodium chloride was more than polyethylene glycol 6000 and related it to faster water absorption by seeds and receiving sufficient moisture for germination in sodium chloride solution.

According to the results of this research it could be concluded that local masses of chickpea, in germination stage, were sensitive to drought and salinity stresses and the mean comparison indicated similar effects in both stresses. 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 chickpea in drought stress.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>df</th>
<th>Germination percentage</th>
<th>Germination rate</th>
<th>Plumule length (cm)</th>
<th>Radicle length (cm)</th>
<th>Radicle to plumule ratio</th>
<th>Radicle fresh weight (g)</th>
<th>Plumule fresh weight (g)</th>
<th>Seedling fresh weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drought</td>
<td>3</td>
<td>13437.62</td>
<td>40.818</td>
<td>16.25</td>
<td>19.37</td>
<td>11.23</td>
<td>0.003</td>
<td>0.032</td>
<td>0.049</td>
</tr>
<tr>
<td>genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>419.42*</td>
<td>0.202**</td>
<td>0.753**</td>
<td>0.012**</td>
<td>2.952**</td>
<td>1.204E-5**</td>
<td>0.005**</td>
<td>0.000**</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>172.54**</td>
<td>0.43**</td>
<td>0.486**</td>
<td>0.255**</td>
<td>1.890**</td>
<td>5.016E-6**</td>
<td>0.006**</td>
<td>0.000**</td>
</tr>
<tr>
<td>Error</td>
<td>16</td>
<td>138.45</td>
<td>0.137</td>
<td>0.326</td>
<td>0.073</td>
<td>1.872</td>
<td>7.374E-5</td>
<td>0.010</td>
<td>0.001</td>
</tr>
</tbody>
</table>

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 chickpea.

<table>
<thead>
<tr>
<th>Drought levels (bar)</th>
<th>Germination percentage</th>
<th>Germination rate</th>
<th>Plumule length (cm)</th>
<th>Radicle length (cm)</th>
<th>Radicle to plumule ratio</th>
<th>Radicle fresh weight (g)</th>
<th>Plumule fresh weight (g)</th>
<th>Seedling fresh weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100*</td>
<td>5.57*</td>
<td>3.49*</td>
<td>3.88*</td>
<td>1.74**</td>
<td>0.470*</td>
<td>0.145*</td>
<td>0.192*</td>
</tr>
<tr>
<td>-4</td>
<td>58.22*</td>
<td>1.59*</td>
<td>0.707*</td>
<td>1.95*</td>
<td>3.22**</td>
<td>0.193*</td>
<td>0.101*</td>
<td>0.498*</td>
</tr>
<tr>
<td>-8</td>
<td>5.50*</td>
<td>0.093*</td>
<td>0.071*</td>
<td>0.252*</td>
<td>0.900**</td>
<td>0.019*</td>
<td>0.005*</td>
<td>0.021*</td>
</tr>
<tr>
<td>-12</td>
<td>0.006*</td>
<td>0.000*</td>
<td>0.000*</td>
<td>0.000*</td>
<td>0.000*</td>
<td>0.000*</td>
<td>0.000*</td>
<td>0.000*</td>
</tr>
</tbody>
</table>

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

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 chickpea in salinity stress.

<table>
<thead>
<tr>
<th>Salinity* genotype</th>
<th>df</th>
<th>Germination percentage</th>
<th>Germination rate</th>
<th>Plumule length (cm)</th>
<th>Radicle length (cm)</th>
<th>Radicle to plumule ratio</th>
<th>Radicle fresh weight (g)</th>
<th>Plumule fresh weight (g)</th>
<th>Seedling fresh weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>salinity</td>
<td>3</td>
<td>19455.6*</td>
<td>4.670*</td>
<td>16.31*</td>
<td>22.97*</td>
<td>8.722*</td>
<td>0.015**</td>
<td>0.029*</td>
<td>0.040**</td>
</tr>
<tr>
<td>genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>0.006*</td>
<td>0.046*</td>
<td>0.346*</td>
<td>0.088*</td>
<td>0.251*</td>
<td>0.010**</td>
<td>0.001*</td>
<td>0.004**</td>
</tr>
<tr>
<td>salinity* genotype</td>
<td>3</td>
<td>0.001*</td>
<td>0.077*</td>
<td>0.526*</td>
<td>0.255*</td>
<td>0.979**</td>
<td>0.010**</td>
<td>0.001*</td>
<td>0.002**</td>
</tr>
<tr>
<td>Error</td>
<td>16</td>
<td>5.796*</td>
<td>0.133</td>
<td>0.620</td>
<td>1.252</td>
<td>0.042</td>
<td>0.007</td>
<td>0.001</td>
<td>0.003</td>
</tr>
</tbody>
</table>

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 chickpea.

<table>
<thead>
<tr>
<th>Salinity levels (bar)</th>
<th>Germination percentage</th>
<th>Germination rate</th>
<th>Plumule length cm</th>
<th>Radicle length cm</th>
<th>Radicle to plumule ratio</th>
<th>Radicle fresh weight (g)</th>
<th>Plumule fresh weight (g)</th>
<th>Seedling fresh weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100a</td>
<td>5.57a</td>
<td>3.49a</td>
<td>3.88a</td>
<td>2.35ab</td>
<td>0.047ab</td>
<td>1.145bc</td>
<td>0.166c</td>
</tr>
<tr>
<td>-4</td>
<td>97.22b</td>
<td>4.20bc</td>
<td>1.41bc</td>
<td>1.74bc</td>
<td>1.74bc</td>
<td>0.106c</td>
<td>0.068c</td>
<td>0.104c</td>
</tr>
<tr>
<td>-8</td>
<td>99.22c</td>
<td>4.00bc</td>
<td>0.00d</td>
<td>0.00d</td>
<td>0.00d</td>
<td>0.00d</td>
<td>0.00d</td>
<td>0.00d</td>
</tr>
<tr>
<td>-12</td>
<td>99.20c</td>
<td>4.00bc</td>
<td>0.00d</td>
<td>0.00d</td>
<td>0.00d</td>
<td>0.00d</td>
<td>0.00d</td>
<td>0.00d</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ave. germination percentage</th>
<th>Ave. germination rate</th>
<th>Ave. plumule length</th>
<th>Ave. radicle length</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drought</td>
<td>40.930</td>
<td>1.813</td>
<td>1.064</td>
<td>1.522</td>
</tr>
<tr>
<td>Salinity</td>
<td>49.305</td>
<td>2.442</td>
<td>1.225</td>
<td>1.624</td>
</tr>
</tbody>
</table>

Significant level between the two groups, 0.341 0.378 0.729 0.851

### REFERENCES
