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Different Salts Effects on the Germination of *Hordeum vulgare* and *Hordeum bulbosum*

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Abstract: The germination responses of *Hordeum ulgare* seeds to saline stress caused by different salt types was studied. For this, 25 seeds of mentioned species were placed on filter paper in Petri dishes containing distilled water (control), 60, 120, 180, 240, 300, 360 and 420 mM. saline solution of NaCl, $CaCl_2$ an KCl. The results indicated that saline levels effects were significant ($P < 0.05$) for seed germination percentage, seed germination velocity, mean time to germination, length of the stem and radicle and seed vigour. Seed germination decreased significantly by increasing salinity levels. Also, the results showed that *H. vulgare* is more tolerant than *H. vulgare* against salinity in germination stage.

Key words: Salinity stress, NaCl, $CaCl_2$, KCl, germination, *Hordeum vulgare* and *Hordeum bulbosum*

Introduction

Soil salinity is a major factor limiting plant productivity, affecting about 95 million hectares world wide (Szabolcs, 1994). The UNEP (United Nations Environment Program) estimates that 20% of the agricultural and 50% of the cropland in the world is salt stressed (Flowers and Yeo, 1995). Salinity imposes serious environmental problems that affect grassland cover and the availability of animal feed in arid and semi-arid regions (El-Kharbotly *et al.*, 2003). Salt stress unfavorably affected by plant growth and productivity during all developmental stages. For example Epstein *et al.* (1980) reported that salinity decreases seed germination, retards plant development and reduces crop yield. Shokohifard *et al.* (1989) reported that salt stress negatively affected seed germination; either osmotically through reduced water absorption or ionically through the accumulation of Na and Cl causing in imbalance in nutrient uptake and toxicity affect saline soils contain multiple types of soluble salt components, each of which has a different effect on the initial growth of plant (Redmann, 1974; Hardegree and Emmerich, 1990; Tobe *et al.*, 2002) and the composition of soluble salts in saline soils differ greatly among locations (Tobe *et al.*, 2002).

Although most of these reports are based on experiments with NaCl, it is hypothesized that other salts have similar effects on cellular function, but to different degrees, depending on the salt. Studies to examine salinity effects on the initial growth of plants have usually been carried out with individual salts (especially NaCl). Many authors have used NaCl solutions to study salinity tolerance in the germination of *H.vulgare* (Luque and Bingham 1981; Tsonev *et al.*, 1998; Flowers and Hajidagheri 2001; Neslihan *et al.*, 2002; Grant 2003; El-Tayeb 2005), but little information exists concerning the effect of other salts on the seed germination. However, there remains a question as to whether the results of experiments with individual salts simulate the actual initial growth behaviors of plants in saline soils. To clarify the responses of the seed germination and the initial growth of plants to salinity, examination of the effects of various salts is desirable. In the present study, we compared the effects of different types of salts on the seed germination of *H.vulgare* and *H. vulgare*. Because the main salt components of saline soils are Na^+, Mg^{2+} and Ca^{2+} cations and cl^- and SO_4^{2-} anions (Shainberg, 1975), we investigated the effect of individual salts, NaCl, $CaCl_2$ and KCl.

Materials and Methods

Study species: *H.vulgare* and *H. vulgare* belong to Graminae family. *H.vulgare* is widely grown in the arid and semi-arid regions of the Mediterranean for forage purposes and as grain crop (Al-Karaki, 2001). It is considered highly salt tolerant of the agriculturally important cereals and has been grown successfully in fields that irrigation has rendered unsuitable for other crops (Hopkins, 1995). Generally, it grown near soil surface where the salts accumulated and at this point of soil, the concentration of salt changes over time by continuous evapotranspiration gradually rising salt levels or rainfall leaching salts from the soil surface supplying water to seeds (Al-Karaki, 1997; Tobe *et al.*, 2000a,b). *H. vulgare* is a perennial grass with large distribution in Iran. It can endure high-temperature, aridity and cooling (Moghimi, 2005).

Effects of salinity on germination: To evaluate salt tolerance during germination, 25 seeds of each species were placed on filter paper in 9 cm petri dishes and submerged in 5 ml of each solution. Solutions of the NaCl, $CaCl_2$ and KCl were used at concentrations of 0 (control), 60, 120, 180, 240, 300, 360 and 420 mM.

Experiments were performed in a completely randomized design with 4 replicates in the seed laboratory of Natural Resources Faculty of Tehran University.

Germination counts were made daily and were considered to have germinated when the radicle emerged. The water level was adjusted at 2-d intervals with distilled water to avoid changes in salinity due to evaporation. At the end of the germination period, the germination percentage, germination velocity, the mean time to germination and length of the stem and radicle under salinity were calculated. Germination percentage was calculated using the equation:

$$\text{Final germination percentage} = \frac{\text{Number of germinated seeds}}{\text{total number of seeds planted}}$$

Also, germination velocity and the mean time to germination were calculated using the following equations:

$$\text{Germination Velocity (Maguire, 1962)} = \sum \frac{\text{number of germinated seeds}}{\text{day of count}}$$

Mean time to germination = ($\sum$ni x di) /N, where n is the number of seeds germinated at day I , d the incubation period in days and N is the total number of seeds germinated in the treatment (Brenchley and Probert, 1998). Also, seed vigour was obtained using the following equation (Abdul-Baki and Anderson, 1973):

$$\text{Seed vigour} = \frac{(\text{lenght of stem} + \text{lenght of root}) \times \text{germination percentage}}{100}$$

Statiscal analysis: A multivariate ANOVA was used to evaluate the effects of salinity on seed germination. Data were analyzed using SPSS 11.5 for windows (SPSS Inc., 1999). When significant main effects existed, differences were tested by a multiple comparison Tukey test at 95% confidence. Germination data were arcsine transformed before statistical analysis to ensure homogeneity of variance.

Results

Effects of salinity on germination: Significant differences were obtained for three considered factors (species, salts and concentrations) and their interactions regarding seed germination ($p < 0.05$) (Table 1). The velocity of germination was also significant for three factors (species, concentrations and theirs interaction) and for the other three factors (salts, salts-species and salts-concentrations interaction) was not significant (Table 2). The mean time to germination was also significantly affected by all the factors (except to species) and interactions except that between species and concentrations (Table 3). In the most of the treatments, *H.vulgare* showed higher germination percentage and germination velocity compared to *H. bulbosum* while its mean time to germination was less than *H. vulgare*. Generally, germination percentage and germination velocity were reduced by increased salt concentration. This condition was more intensity in *H. vulgare* than *H.vulgare*. For example, when the seeds were incubated with NaCl, concentrations higher than 120 mM, significantly reduced the germination of *H. vulgare*. This condition was similar to that of $CaCl_2$ and KCl (Table 1). When seeds incubated with NaCl, in the most of the salinity concentrations, for both *H.vulgare* and *H. vulgare*, the mean time to germination was less than two other salts ($CaCl_2$ and KCl). Generally, significant differences were obtained when comparing two similar salinity levels of different salts (for example, 60 mM solutions of NaCl and $CaCl_2$ and etc).

Table 1: Germination percentage of Hordeum vulgare and Hordeum bulbosum seeds in saline solutions of NaCl, $CaCl_2$ and KCl

Salts	Salinity (mM)	Germination in saline solutions(%) *H. vulgare*	*H. bulbosum*
NaCl	0	52±11.8aA	39±6.8aA
	60	51±13.2aA	27±3.8bB
	120	40±5.7abA	5±6cB
	180	51±15.4aA	0±0cB
	240	34±10.6abcA	0±0cB
	300	23±15.8bcdA	0±0cB
	360	13±8.9cdA	0±0cB
	420	0±0dA	0±0cA
$CaCl_2$	0	52±11.8aA	39±6.8aA
	60	44±17.0aA	28±8.6bA
	120	38±10.6aA	0±0cB
	180	12±5.7bA	0±0cB
	240	3±2.0bA	0±0cA
	300	0±0bA	0±0cA
	360	0±0bA	0±0cA
	420	0±0bA	0±0cA
KCl	0	52±11.8aA	39±6.8aA
	60	41±8.2aA	30±13.7aA
	120	37±12abA	12±8.6bA
	180	48±5.7aA	0±2bB
	240	36±13.9abA	0±0bB
	300	40±9.2aA	0±0bB
	360	26±17.4abA	0±0bB
	420	9±18.0bA	0±0bA

Values are mean±S.D. (n = 4). Means within a column that have a different small letter are significantly different from each other, and means within a row that have different capital letter are significantly different from each other (Tukey test; $P < 0.05$).

Effects of salinity on length of the stem and radicle: The results showed that *H.vulgare* stem length was significantly bigger than that of *H. vulgare* by both salts and salinity levels (Table 4). In both *H.vulgare* and *H. vulgare*, increase of each salt concentration caused

Table 2: Germination velocity of Hordeum vulgare and Hordeum bulbosum seeds in saline solutions of NaCl, $CaCl_2$ and KCl

Salts	Salinity (mM)	Germination velocity in saline solutions *H. vulgare*	*H. bulbosum*
NaCl	0	11.69±1.1aA	9.1±0.9aA
	60	10.99±2.2aA	7.14±1.9abA
	120	9.1±1.7abA	0.99±2.1bcB
	180	11.31±2.5aA	0±0cB
	240	7.04±1.4abA	0±0cB
	300	5.42±0.9abA	0±0cA
	360	2.04±1.5abA	0±0cA
	420	0±0bA	0±0cA
$CaCl_2$	0	11.69±1.1aA	9.1±0.9aA
	60	9.41±1.8abA	5.08±2.2abA
	120	9.13±2.1abA	0±0bB
	180	2.3±1.5abA	0±0bA
	240	0.25±0.9bA	0±0bA
	300	0±0bA	0±0bA
	360	0±0bA	0±0bA
	420	0±0bA	0±0bA
KCl	0	11.69±1.1aA	9.1±0.9aA
	60	9.38±3.1aA	7.23±1.5abA
	120	7.46±2.5abA	1.44±2.3bcB
	180	10.38±1.9aA	0.19±1.1cB
	240	6.93±2.2abA	0±0cB
	300	8.58±1.2abA	0±0cB
	360	4.95±1.1abA	0±0cA
	420	0±0bA	0±0cA

Values are mean±S.D. (n = 4). Means within a column that have a different small letter are significantly different from each other, and means within a row that have different capital letter are significantly different from each other (Tukey test; $P < 0.05$).

Table 3: Mean time to germination of Hordeum vulgare and Hordeum bulbosum seeds in saline solutions of NaCl, $CaCl_2$ and KCl

Salts	Salinity (mM)	Mean time to germination in saline solutions (day) *H. vulgare*	*H. bulbosum*
NaCl	0	1.39±0.31abA	3.69±0.73abB
	60	1.49±0.21aA	3.87±0.59abB
	120	1.43±0.21aA	5.4±0.9aB
	180	1.37±0.52abA	0±0bB
	240	1.15±0.44abA	0±0bB
	300	1.17±0.1abA	0±0bB
	360	2±0.5Aa	0±0bB
	420	0±0bA	0±0bA
$CaCl_2$	0	1.39±0.31abA	3.69±0.73abB
	60	1.57±0.18abA	4.64±0.65aB
	120	1.18±0.41abA	0±0bB
	180	1.92±0.25abA	0±0bB
	240	3±0.5aA	0±0bB
	300	0±0bA	0±0bA
	360	0±0bA	0±0bA
	420	0±0bA	0±0A
KCl	0	1.39±0.31abA	3.69±0.73abB
	60	1.37±0.35abA	4±0.59abB
	120	1.57±0.51abA	5.84±0.81aB
	180	1.4±0.3abA	4±0.62abB
	240	1.78±0.61abA	0±0bB
	300	1.4±0.53abA	0±0bB
	360	2.53±0.49aA	0±0bB
	420	0±0bA	0±0bA

Values are mean±S.D. (n = 4). Means within a column that have a different small letter are significantly different from each other, and means within a row that have different capital letter are significantly different from each other (Tukey test; $P< 0.05$).

decline of length of the stem. 60 mM treatment has shown the higher length of the stem following control (0 mM). Although, in this treatment significant difference was not obtained among three salts. *H. vulgare* has shown length of the radicle more than *H. vulgare* and significant difference was obtained by all the treatments except for the control treatment (Table 5).

In both *H. vulgare* and *H. bulbosum*, length of the radicle was more in NaCl than $CaCl_2$ and KCl by all the salinity levels except to the control treatment (Table 5).

Generally, significant difference was obtained for the salts. Similar to length of the stem, length of the radicle also reduced by increase salinity levels in both species *H. vulgare* and *H. bulbosum* and three salts (Table 5). Length of radicle was significantly different comparing with the control and other treatments in both species and three salts except to of NaCl salt in *H. bulbosum*. Length of the stem showed similar behavior for *H. vulgare*, but in *H. bulbosum*, significant difference was not obtained among control treatment with 60 mM in three salts.

NaCl, $CaCl_2$ and KCl concentrations of 120 mM and higher salinity levels sharply reduced the length of the stem of *H. vulgare*, but this condition (Sharp reduction) was obtained at a concentration of 60 mM and higher levels for *H. vulgare*. In spite of the length of the stem of *H. vulgare*, reduction of length of the radicle wasn't very clear (except to $CaCl_2$). On the other hand, reduction in length of the radicle due to increase of the salinity levels was gradually and nonclear. Adversely, this difference was clear. For this reason, significant and large difference was obtained in comparing control treatment with other treatments.

Seed vigor: The principal object of a seed vigor test is to differentiate a range of quality levels, for example, high, medium and low vigor seeds. The results obtained through germination test by way of evaluating likely seed performance (plant stand) under a wide range of field conditions (Rattan, 1998). As it has been represented in equation of seed vigor, seed vigor is obtained by germination percentage and length of stem and radicle, therefore the results of seed vigor is largely similar to them. However, as it is shown in Table 6, a significant difference is observed among species, salts and salinity levels. The seed vigor of *H. vulgare* was higher than *H. bulbsum*. In all of salts in different levels from 0-420 mM, the general trend is associated with seed vigor decrease. In *H. vulgare* and *H. bulbosum*, among three

Table 4: Stem length of seeds of Hordeum vulgare and Hordeum bulbosum seeds in saline solutions of NaCl, $CaCl_2$ and KCl

Salts	Salinity (mM)	Stem length of seeds in saline solutions (mm) *H. vulgare*	*H. bulbosum*
NaCl	0	44.4±7.17aA	12.2±4.60aB
	60	25.7±10.73bA	14.3±2.71aB
	120	22.6±7.71bcA	3.8±5.87bB
	180	9.3±7.60cdA	0±0bB
	240	5.4±5.82dA	0±0bB
	300	1.2±3.8dA	0±0bA
	360	0±0dA	0±0bA
	420	0±0dA	0±0bA
$CaCl_2$	0	44.4±7.17aA	12.2±4.60aB
	60	25.2±10.46bA	12.2±4.32aB
	120	17.6±7.06bA	0±0bB
	180	1.2±1.87cA	0±0bA
	240	0±0cA	0±0bA
	300	0±0cA	0±0bA
	360	0±0cA	0±0bA
	420	0±0cA	0±0bA
KCl	0	44.4±7.17aA	12.2±4.60aB
	60	26.2±4.90bA	12.4±3.1aB
	120	9.1±8.14cA	6.4±3.03bA
	180	1.9±2.85cA	0±0cA
	240	3.1±3.81cA	0±0cB
	300	8.2±6.61cA	0±0cB
	360	2.2±4.69cA	0±0cA
	420	0.3±0.49cA	0±0cA

Values are mean±S.D. (n=4). Means within a column that have a different small letter are significantly different from each other, and means within a row that have different capital letter are significantly different from each other (Tukey test; $P < 0.05$).

Table 5: Radicle length of seeds of Hordeum vulgare and Hordeum bulbosum seeds in saline solutions of NaCl, $CaCl_2$ and KCl

Salts	Salinity (mM)	Radicle length of seeds in saline solutions (mm) *H. vulgare*	*H. bulbosum*
NaCl	0	33.6±11.54aA	35.3±14.03aA
	60	32.4±5.69aA	10.4±7.65bB
	120	23±6.72abA	0±0bB
	180	18.1±2.80bcA	0±0bB
	240	11.5±3.24bcdA	0±0bB
	300	8.6±4.90cdA	0±0bB
	360	6.8±3.08cdA	0±0bB
	420	0±0dA	0±0bA
$CaCl_2$	0	33.6±11.54aA	35.3±14.03aA
	60	15.8±8.39bA	5.4±5.36bB
	120	12±4.78bcA	0±0bB
	180	2.2±3.70cA	0±0bA
	240	0±0cA	0±0bB
	300	0±0cA	0±0bA
	360	0±0cA	0±0bA
	420	0±0cA	0±0bA
KCl	0	33.6±11.54aA	35.3±14.03aA
	60	29.1±9.70abA	3.9±4.33bB
	120	14.4±8.83bcA	0.1±0.32bB
	180	10.7±4.55cA	0±0bB
	240	8.9±4.79cA	0±0bB
	300	9.4±6.60cA	0±0bB
	360	5.6±3.95cA	0±0bB
	420	2.1±3.58cA	0±0bA

Values are mean ± S.D. (n=4). Means within a column that have a different small letter are significantly different from each other, and means within a row that have different capital letter are significantly different from each other (Tukey test; $P < 0.05$).

salts, it seems that $CaCl_2$ has the largest effect on seed vigor, so that no seed vigor is considered offer 240 and 120 mM, respectively.

Discussion

As showed in results, *H. vulgare* was more salt-tolerant than *H. bulbosum*. For *H. vulgare*, although maximum germination was obtained under non-saline conditions (control treatment), its seeds had the ability to germinate at higher levels of salinity in NaCl and KCl, especially, but in *H. bulbosum*, intent reduction in germination was obtained at 180mM level and above. As referred in results, seed germination reduced by increasing salinity levels, except at salinity levels of KCl related to *H. Vulgare*.

Reduction in germination by an increase of salinity levels has been described by numerous authors (Othman *et al.*, 2006; El-Tayeb, 2005; Breen *et al.*, 1997; Ungar, 1982).

Considerable variations were observed between two species especially at higher salinity levels in response to salinity for germination percentage. These results were in agreement with Basalah (1991) who found that high levels of salinity can significantly inhibit seed germination. Further, Waisel (1972) found that increasing salinity concentration in germination often cause osmotic and/or specific toxicity which may reduce or retard germination percentage. Significant difference was obtained for salts regarding seed germination. The behavior (reduction or increase in seed germination by increase of salinity levels) of three salts which studied in this research, was different and partially intricated. As the results demonstrate, this significant difference was at higher levels, especially, while it wasn't significant for under levels such 0 mM (control treatment), 60 and 120 mM. In our research, at above levels maximum germination was obtained for KCl, *H. vulgare*, especially, thus we can tell that salt tolerance of seed to this salt is partially more than two other salts. It could be concluded that k^+ ion accumulation in the cytosolic solutes reduce more negative effect of cl^- than Na^+ ion. NaCl affects the permeability of the plasma membrane and increases influx of external ions and efflux of cytosolic solutes (Cramer *et al.*, 1985; Kent and Lauchli, 1985; Allen *et al.*, 1995) in plant cells. In addition, NaCl causes hardening of the cell wall (Neumann, 1993; Neumann *et al.*, 1994; Nabil and Coudret, 1995) and a decrease in water conductance of the plasma membrance (Azaizeh *et al.*,

Table 6: Seed vigor of Hordeum vulgare and Hordeum bulbosum seeds in saline solutions of NaCl, $CaCl_2$ and KCl

Salts	Salinity (mM)	Seed vigour in saline solutions *H. vulgare*	*H. bulbosum*
NaCl	0	41.4±17.53aA	20.53±10.04aB
	60	28.04±4.58abA	5.21±2.23bB
	120	18.08±5.71bcA	0±0.77bB
	180	15.64±4.81bcdA	0±0bB
	240	7.03±4.3cdA	0±0bB
	300	2.54±3.4cdA	0±0bA
	360	1.1±0.92dA	0±0bA
	420	0±0dA	0±0bA
$CaCl_2$	0	41.4±17.53aA	20.53±10.04aB
	60	19.57±12.94bA	6.14±4.98bB
	120	13.23±3.76bcA	0±0bB
	180	0.95±0.77cA	0±0bB
	240	0±0cA	0±0bB
	300	0±0cA	0±0bA
	360	0±0cA	0±0bA
	420	0±0cA	0±0bA
KCl	0	41.4±17.53aA	20.53±10.04aB
	60	23.63±4.0bA	4.84±1.89bB
	120	10.55±2.81bcA	0.73±0.69bB
	180	7.61±2.8bcA	0±0bB
	240	5.58±2.4cA	0±0bB
	300	7.56±3.7bcA	0±0bB
	360	2.88±3.9cA	0±0bA
	420	1.08±2.2cA	0±0bA

Values are mean±S.D. (n=4). Means within a column that have a different small letter are significantly different from each other, and means within a row that have different capital letter are significantly different from each other (Tukey test; $P < 0.05$).

1992; Cramer, 1992). These effects of NaCl on cellular function are alleviated by the addition of Ca^{2+} to the external medium (Cramer *et al.*, 1985; Kent and Lauchli, 1985; Azaizeh *et al.*, 1992; Cramer, 1992; Allen *et al.*, 1995). These effects of salts on the function of the cell membranes and the walls may affect the water potential of the cytosol and cellular extensibility and thus, may affect seed germination, while we found an unexpected result in our research and that is reduction in germination by $CaCl_2$ in both *H.vulgare* and *H. bulbosum*. Probably, we can conclude that large amount of Ca^{2+} caused an negative effect on germination. Generally, this differential behavior of seeds according to the salt type is also presumably due to the fact that the same concentration of salt generates different osmotic potentials and the osmotic effect may well have a greater influence on germination than specification toxicity, as has been suggested by several authors in other plants such much of halophytes (Unger, 1996; Pujol *et al.*, 2000).

In general, based on the obtained results it seems that *H. vulgare* is more tolerant than *H. bulbosum* against different salts. Therefore, between two mentioned species, using *H. vulgare* seeds in range reclamation and restoration projects could be lead to suitable results in arid and semi arid areas, where salinity affects vegetation development plans strongly.

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