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RESEARCH ARTICLE

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Effects of various salts on the germination of two cultivars of *Medicago sativa*

Abstract The germination responses of *Medicago sativa* (Yazdi and Hamedani cultivars) seeds to salinity stress by different salt solutions were studied in this project. To evaluate salt tolerance during germination, 25 seeds of each cultivar were placed on filter paper in 9 cm petri dishes containing distilled water (control), 30, 60, 90, 120, 150, 180, 210, 240, 270, and 300 mmol·L^{-1} saline solutions of NaCl, $CaCl_2$, and KCl, respectively. The results indicated that the effects of salinity levels were significant ($P < 0.05$) for percent seed germination, seed germination rate, mean time to germination, length of the stem, and radical and seed vigor. Seed germination decreased significantly by increasing salinity levels. These two cultivars of alfalfa were more resistant to KCl than NaCl and $CaCl_2$ salts, respectively. In addition, the results showed that the Hamedani cultivar was more tolerant than Yazdi cv. against salinity.

Keywords salinity stress, seed germination, *Medicago sativa* cv. Yazdi, *Medicagosativa* cv. Hamedani

1 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 agriculture and 50% of the cropland in the world are under the salt stress (Flowers and Yeo, 1995). Salinity imposes serious environmental problems that affect the grassland cover and the availability of animal feed in arid and semi-arid regions (El-Kharbotly et al., 2003). Salt stress unfavorably affects plant growth and productivity during all developmental stages. For example, Epstein et al. (1980) reported that salinity decreased seed germination, retarded plant development, and reduced crop yield. Shokohifard et al. (1989) reported that salt stress negatively affected seed germination and either osmosis through reducing water absorption or ionization through the accumulation of Na and Cl caused imbalance in nutrient uptake and toxicity. Saline soils contain multiple types of soluble salt components, each of which has a different effect on the initial growth of plants (Redmann, 1974; Hardegree and Emmerich, 1990; Tobe et al., 2002). On the other hand, the composition of soluble salts in saline soils differs greatly among different locations (Tobe et al., 2002). Although most of these reports are basing on experiments with NaCl, it have hypothesized that the other salts have similar effects on cellular function, but to different degrees, depending on salinity. Studies to examine salinity effects on the initial growth of plants have usually been carrying out with individual salts (especially NaCl). 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, the 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 *Medicago sativa*, Yazdi and Hamedani cultivars. Because the main salt components of saline soils are Na^+, K^+, Mg^{2+}, and Ca^{2+} cations along with Cl^- and SO_4^{2-} anions (Shainberg, 1975), we investigated the effect of individual salts of NaCl, $CaCl_2$, and KCl.

Alfalfa is a herbaceous perennial plant that can produce large amounts of nutritious forage materials. It is one of the leading forage crops in the world. It first originated in Asia before 700BC. It was thought to have been cultivated first in Iran (Wikipedia, 2007).

Alfalfa lives for 3 to 12 years, depending on varieties and climates. It is a cool season perennial legume, sometimes growing to a height of 1 meter. It resembles clover with clusters of small purple flowers. It also has a

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deep root system, sometimes stretching to 4.5 meters. This makes it more tolerant, especially to drought stress. This plant has a wide range of environmental adaptation and can be grown in very cold northern plains to high mountain valleys, from rich temperate agricultural regions to Mediterranean climates and searing hot deserts (Milius, 2006).

2 Materials and methods

2.1 Alfalfa cultivars

The alfalfa crop can be used as a pasture, hay, or silage crop. In addition, it cut and dehydrated to make protein-rich meal or pellets for livestock (Encyclopædia Britannica Article, 2007).

These two experimental cultivars, Hamedani cultivar and Yazdi cultivar, are two important cultivars in Iran. They are cultivated in the west and the center of Iran, respectively. Moreover, these two cultivars used for animal forage are very tolerant in different situations. Therefore, with various values of *Medicago sativa*, we studied the two cultivars in Iran to make a comparison between them in terms of their resistance against salinity stress in germination and initial growth phases.

2.2 Salinity test procedures

To evaluate salt tolerance during germination, 25 seeds of each cultivar were placed on filter paper in 9 cm petri dishes and submerged in 5 mL of each saline solution. Solutions of the NaCl, $CaCl_2$ and KCl were used at concentrations of 0 (control), 30, 60, 90, 120, 150, 180, 210, 240, 270, and 300 $mmol \cdot L^{-1}$. Experiments performed in a completely randomized block with a factorial arrangement with four replicates in the seed laboratory of Natural Resources College of University of Tehran.

Germination counts were making daily and seeds were considering germinating only when 2 mm radicles emerged. The water level was adjusting at 2-day intervals with distilled water to avoid changes in salinity due to evaporation. At the end of the germination period, the germination percentage, the germination rate, the mean time to germination and the length of the stem and radicle under salinity recorded. Germination percentage was calculated using the following equation:

$$\text{Final germination percentage} = \frac{\text{number of germinated seeds}}{\text{total number of seeds planted}} \times 100.$$

Also, germination rate and the mean time to germination were calculated using the following equations (Maguire, 1962):

$$\text{Germination rate} = \sum \frac{\text{number of germinated seeds}}{\text{day of count}},$$

Mean time to germination = $(\sum ni \times di)/N$, where n is the number of seeds that germinated at day i, d is the incubation period in days, and N is the total number of seeds that germinated in the treatment (Brenchley and Proberet, 1998). Furthermore, seed vigor obtains using the following equation (Abdul-baki and Anderson, 1973):

$$\text{Seed vigor} = (\text{length of stem} + \text{length of root}) \times \text{germination percentage}/100.$$

2.3 Statistical analysis

A multivariate ANOVA was used to evaluate the effects of salinity on seed germination. Data were analyzed using SPSS 15 for windows. When significant main effects existed, differences had tested by a multiple comparison Tukey test at 95% confidence. Germination data were arcsine transformed before statistical analysis to ensure homogeneity of variance.

3 Results

3.1 Effects of salinity on germination

Significant differences were obtained for three considered variables (cultivars, salt types, and concentrations) and their interactions on percent seed germination ($P < 0.05$) (Table 1). The effect of different variables of salt types and their interactions were also significant ($P < 0.05$) on germination rate (Table 1).

Salinity stress by NaCl solution had significantly different effects on the germination percentage of the two cultivars (Table 1). In 0, 30, and 60 $mmol \cdot L^{-1}$ solutions, no significant differences in germination percentage were observed for Yazdi cultivar. As the concentration of salinity increased, the germination percent followed a decreasing trend. In 210, 240, 270, and 300 $mmol \cdot L^{-1}$ solutions, the germination percentage were approximately equal with each other. In Hamedani cultivar, the germination percentage was similar in 0, 30, 60, 90, and 120 $mmol \cdot L^{-1}$ solutions. Also, not much variations of the germination percentage were observed in 210, 240, 270, and 300 $mmol \cdot L^{-1}$ solutions for Hamedani cultivar (Table 1).

For $CaCl_2$ salt, the germination percentage in two cultivars was similar except for the two treatments (120 and 180 $mmol \cdot L^{-1}$). In these two cultivars, by increasing salt concentration, germination percentage decreased. Only in Hamedani cultivar, an increasing trend in germination percentage observes from distilled water to

Table 1 Germination percentage, germination rate, and mean time to germination of *Medicage sativa*, Hamedani and Yazdi cultivar seeds in saline solutions of NaCl, $CaCl_2$, and KCl

salt solution /(mmol·L^{-1})		germination percentage		germination rate		mean time to germination	
		Yazdi	Hamedani	Yazdi	Hamedani	Yazdi	Hamedani
NaCl	0	99.0 ± 2.0 aA	92 ± 8.6 aA	21.7 ± 0.3 aA	20.4 ± 0.1 abA	1.3 ± 0.0 bA	1.4 ± 0.1 cA
	30	97.0 ± 2.0 aA	92 ± 5.7 aA	21.6 ± 0.2 aA	19.8 ± 0.1 abA	1.4 ± 0.1 bA	1.6 ± 0.1 cB
	60	91 ± 6.8 aA	97 ± 2 aA	19.5 ± 0.1 abA	21.3 ± 0.1 aA	1.5 ± 0.0 bA	1.6 ± 0.1 cA
	90	72.0 ± 8.0 bA	78 ± 7.7 aA	17.2 ± 0.1 bcA	17.5 ± 0.1 bA	1.2 ± 0.0 bA	1.3 ± 0.1 cA
	120	58 ± 14.8 bA	79 ± 8.2 aB	13.5 ± 0.1 cdA	17.4 ± 0.1 bB	1.3 ± 0.1 bA	1.3 ± 0.1 cA
	150	32 ± 5.7 cA	47 ± 13.6 bB	12.2 ± 5.7 dA	10.6 ± 0.1 cA	1.4 ± 0.1 bA	1.4 ± 0.1 cA
	180	24 ± 11.3 cdA	30 ± 12.4 bcA	5.2 ± 0.1 eA	6.6 ± 0.1 dA	1.6 ± 0.1 bA	1.5 ± 0.1 cA
	210	3 ± 2 eA	11.0 ± 5.0 cdA	0.3 ± 0.1 fA	1.5 ± 0.1 eA	3.7 ± 0.1 aA	2.6 ± 0.1 bcA
	240	10 ± 6.9 deA	9 ± 6.8 dA	0.0 ± 0.1 efA	2.2 ± 0.1eA	3.4 ± 0.1 aA	2.6 ± 0.1 bcA
	270	5 ± 7.6 eA	1 ± 2 dA	0.4 ± 0.1 fA	0.0 ± 0.0 eA	4.4 ± 0.1 aA	6.1 ± 0.1 aB
	300	0 ± 0 eA	2 ± 4 dA	0.1 ± 0.0 fA	0.1 ± 0.0 eA	0 ± 0 bA	4.0 ± 0.1 bB
$CaCl_2$	0	99 ± 2 aA	92 ± 8.6 aA	21.7 ± 0.3 aA	20.4 ± 0.1 aA	1.3 ± 0.0 cdA	1.4 ± 0.1 dA
	30	97 ± 3.8 aA	98 ± 2.3 aA	21.4 ± 0.1 aA	19.7 ± 0.0 aA	1.4 ± 0.1 cdA	1.5 ± 0.0 dA
	60	94 ± 5.2 aA	93 ± 6 aA	21.3 ± 0.1 aA	20.2 ± 0.1 aA	1.3 ± 0.0 cdA	1.6 ± 0.0 dA
	90	91 ± 6.8 aA	89 ± 3.8 aA	20.8 ± 0.1 aA	19.2 ± 0.2 abA	1.2 ± 0.0 cdA	1.6 ± 0.1 dA
	120	56 ± 13.5 bA	68 ± 11.8 bB	13.5 ± 0.1 bA	16.0 ± 0.1 bA	1.1 ± 0.1 cdA	1.2 ± 0.1 dA
	150	24 ± 11.8 cA	23 ± 8.9 cA	5.0 ± 0.1 cA	3.3 ± 0.1 cA	1.7 ± 0.0 cA	2.6 ± 0.1 cdA
	180	3 ± 2 dA	10 ± 5.2 cdB	0.2 ± 0.1 dA	0.9 ± 0.0 cdA	4.0 ± 0.1 bA	3.7 ± 0.1 bcA
	210	0 ± 0 dA	2 ± 2.3 dA	0 ± 0 dA	0.1 ± 0.0 dA	0 ± 0 dA	5 ± 0.1 abB
	240	0 ± 0 dA	1 ± 2 dA	0.1 ± 0 dA	0.1 ± 0.0 dA	7 ± 0.1 aA	6 ± 0 aA
	270	0 ± 0 dA	1 ± 2 dA	0.2 ± 0.3 dA	0 ± 0 dA	5.0 ± 0.1 bA	6 ± 0 aA
	300	0 ± 0 dA	1 ± 2 dA	0 ± 0 dA	0.1 ± 0.0 dA	0 ± 0 dA	5 ± 0 abB
KCl	0	99 ± 2 aA	92 ± 8.6 aA	21.7 ± 0.3 aA	20.4 ± 0.1 abA	1.3 ± 0 bA	1.4 ± 0.1 cA
	30	100 ± 0 aA	94 ± 5.2 aA	22.5 ± 0.1 aA	21.5 ± 0.1 aA	1.3 ± 0 bA	1.5 ± 0 cB
	60	96 ± 4.6 aA	86 ± 4 aB	21.8 ± 0.1 aA	20.4 ± 0.0 abA	1.3 ± 0 bA	1.5 ± 0 cB
	90	89 ± 6.8 aA	82 ± 8.3 aA	20.9 ± 0.1 aA	18.3 ± 0.1 bA	1.2 ± 0 bA	1.5 ± 0 cB
	120	87 ± 10.5 aA	56 ± 15.7 bB	20.5 ± 0.1 aA	17.8 ± 0.1 bA	1.2 ± 0 bA	1.2 ± 0 cA
	150	68 ± 5.7 bA	47 ± 15.1 bcB	14.2 ± 0.0 bA	9.8 ± 0.1 cB	1.7 ± 0 bA	2.1 ± 0 bcB
	180	55 ± 8.9 bA	17 ± 8.9 deB	11.2 ± 0.1 bA	5.4 ± 0.1 dB	1.6 ± 0 bA	3.1 ± 0 abcB
	210	22 ± 6.9 cdA	32 ± 5.7 cdB	3.0 ± 0.1 cdA	1.5 ± 0.1 eA	2.7 ± 0.1 abA	3.6 ± 0 abA
	240	32 ± 11.8 cA	7 ± 2 eB	4.8 ± 0.1 cA	5.1 ± 0.1 dA	2.7 ± 0 abA	2.3 ± 0 abcA
	270	13 ± 5.0 deA	4 ± 4.6 eB	0.8 ± 0.1 dA	0.3 ± 0.2 eA	4.1 ± 0.1 aA	4.2 ± 0 aA
	300	5 ± 3.8 eA	0 ± 0 eB	0.3 ± 0.0 dA	0.2 ± 0.0 eA	4.2 ± 0.1 aA	4.2 ± 0 aA

Note: Values are mean ± S.D. 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; $P < 0.05$).

30 and 60 mmol·L^{-1} salt solutions. However, in KCl solutions for Yazdi cultivar, the maximum germination have achieved in 30 mmol·L^{-1} solution. The germination percentage in Yazdi cultivar was higher than that in Hamedani cultivar especially in higher salinities (more than 120 mmol·L^{-1}) of KCl solutions.

The results of mean time to germination with NaCl salt showed similar reactions in Yazdi and Hamedani cultivars. Only in three treatments of 30, 270, and 300 mmo·L^{-1}, the results were different between the two cultivars. After 210 mmol·L^{-1}, the mean time to germination followed an increasing trend, while in lower salinity treatments; the mean time was similar and shorter.

In $CaCl_2$ salt solution, two cultivars were approximately similar in four treatments (60, 90, 210, and 300 mmol·L^{-1}). Like NaCl solution in $CaCl_2$, the maximum mean time to germination observes in 240 mmol·L^{-1} solution. For KCl salt solution, the concerning mean time to germination between two cultivars observed was more different than that of other salt types. We found that the mean time to germination in our study increased with increasing in salinity of solutions (Table 1).

The next factor we saw was that the germination rate was approximately similar in two studied cultivars of *Medicago sativa* except in 120 mmol·L^{-1} solution of NaCl, and in 150 and 180 mmol·L^{-1} solutions for KCl salt instead of $CaCl_2$ salt, the two cultivars showed an exactly similar result. Furthermore, we observed that the velocity of germinating had a remarkable decrease with an increase in salinity. Therefore, in both control and treatments, the germination rate had a decreasing trend (Table 1).

3.2 Effects of salinity on length of the hypocotyl (coleoptile) and radicle

Under salinity stress, the average length of coleoptiles was similar in all solutions of NaCl in the two cultivars. According to Table 2, both the Yazdi cultivar treated with 150, 180, 210, 240, 270, and 300 mmol·L^{-1} solutions of NaCl, and the Hamedani cultivar in all the above treatments except for the 150 mmol·L^{-1} solution had a small and similar length of coleoptiles to each other. In addition, in the other two salt types, we observed that in the last six treatments with high salinity, the length of the coleoptiles was very small and weak (Table 2).

Just as we saw in the other observed variables, the lengths of the radicles in two cultivars were close to each other except for 0, 90, 120, and 150 mmol·L^{-1} salt solutions, with a very small length in the radicle in the last six treatments. In short, the Hamedani cultivar had a bigger length of the radicle than the Yazdi cultivar in different salt solutions. With an increased salinity, the length of the radicle decreased (Table 2). Generally, the length of the radicle had a higher tolerance than that of the coleoptile.

3.3 Seed vigor

Seed vigor is one of the important variables. In our research, we found a similarity between the two cultivars in all treatments except for a few treatments. The maximum seed vigor was found both in the 30 mmol·L^{-1} solution of NaCl and in distilled water for the other two salt types (Table 2).

As for NaCl solutions, the Yazdi cultivar in all treatments except for 0, 30, and 60 mmol·L^{-1} solutions had similarly very low seed vigor with each other, while the Hamedani cultivar gradually decreased in seed vigor from 60 mmol·L^{-1} salt solution on. In the $CaCl_2$ salt treatments, the Yazdi cultivar lost seed vigor in the last five treatments, while the Hamedani cultivar lost seed vigor only in the last three treatments, wherein the seed could tolerate about 210 mmol·L^{-1} salt concentration. In terms of KCl salt treatments, Yazdi alfalfa and Hamedani alfalfa could resist 210 mmol·L^{-1} salinity and 240 mmol·L^{-1} salinity, respectively, and preserve their vigor. Significant differences existed in all variables ($P < 0.05$).

4 Discussion and conclusions

As shown in the results, *Medicago sativa* cv. Yazdi was more tolerant to salts in light salinity levels than *Medicago sativa* cv. Hamedani. However, the seeds of *Medicago sativa* cv. Hamedani germinate less than *Medicago sativa* cv. Hamedani, while *Medicago sativa* cv. Hamedani was more tolerant to salts in high salinity levels without germination. Although *Medicago sativa* had maximum germination under nonsaline conditions (CK), its seeds still had the ability to germinate at higher levels of salinity in NaCl and KCl not $CaCl_2$. Generally, in germination, the seeds of *Medicago sativa* were more resistant to KCl than NaCl and had a little tolerance to $CaCl_2$. Moreover, the seed germination could be reduced by increasing salinity levels, which was in accordance with the results by numerous authors (Breen et al., 1977; Ungar, 1982; Tayeb, 2005; Othman et al., 2006).

The germination in high salinity levels of 240, 270, and 300 mmol·L^{-1} solutions was very low, or no seed germinated. These results were in agreement with BaSalah (1991) who found that high levels of salinity could significantly inhibit and delay the seed germination and simultaneously affect the mean time to germination. Waisel (1972) found that increasing the salinity concentration in germination often caused osmotic and/or specific toxicity that may reduce or retard the germination percentage. Significant differences regarding seed germination existed among the salt types. The behaviors (reduction or increase in seed germination by increasing salinity levels) of three salt types in our research were different and partially intricated. As the results demonstrated, these significant differences were at higher levels.

In our research, the maximum germination was observed in KCl treatments, and hence, we suggested that the seed tolerance to KCl was partially more than that to the other two salt types. It can conclude that K^+ ion accumulation in the cytosolic solutes can reduce more negative effects of Cl^- than Na^+ ion. NaCl can affect the permeability of the plasma membrane and increase the influx of external ions and efflux of cytosolic solutes (Cramer et al., 1985; Kent and Lauchli, 1985; Allen et al., 1995) in plant cells to have higher germination in NaCl salt. In addition, NaCl causes the hardening of the cell wall (Neumann, 1993; Neumann et al., 1994; Nabil and Coudret, 1995) and decreases the water conductance of the plasma membrane (Azaizeh et al., 1992; Cramer, 1992). These effects of NaCl on cellular functions alleviates by the addition of Ca^{2+} to the external medium (Cramer et al., 1985; Kent and Lauchli, 1985; Kurth et al., 1986; Azaizeh et al., 1992; Cramer, 1992; Allen et al., 1995). These effects of salt types on the functions of the cell membranes and the walls may affect the water potential of the cytosol and cellular extensibility and hence may affect the seed germination. Although we found an unexpected result in our research that $CaCl_2$ in both Yazdi and Hamedani cultivars reduced the

Table 2 Length of coleoptile, length of radicle, and seed vigor of Medicage sativa, Hamedani and Yazdi cultivar in saline solutions of NaCl, $CaCl_2$, and KCl

salt solution /(mmol·L^{-1})		length of coleoptile		length of radicle		seed vigor	
		Yazdi	Hamedani	Yazdi	Hamedani	Yazdi	Hamedani
NaCl	0	12.4 ± 3.1 aA	14.9 ± 6 aA	22.4 ± 18.2 aA	27.4 ± 14.2 aA	34.1 ± 18.5 aA	38.7 ± 18.0 aA
	30	10.6 ± 1.6 abA	12.1 ± 3.5 abA	32.6 ± 14.3 aA	30.9 ± 15.9 aA	41.4 ± 14.2 aA	39.9 ± 15.0 aA
	60	9.2 ± 3.9 bcA	8.3 ± 2.2 bcA	23.3 ± 13.2 aA	24.7 ± 6.9 aA	29.6 ± 12.6 aA	31.7 ± 7.0 aA
	90	4.8 ± 2.7 dA	8.8 ± 3.1 bcB	4.0 ± 2.3 bA	13.2 ± 2.3 bB	6.3 ± 2.6 bA	17.3 ± 2.1 bB
	120	6.9 ± 1.8 cdA	8.4 ± 3.1 bcA	2.7 ± 1.6 bA	7.2 ± 6.0 bcA	5.2 ± 1.5 bA	12.1 ± 5.9 bcB
	150	1.7 ± 1.8 eA	6.6 ± 2.5 cB	0.8 ± 1.0 bA	4.6 ± 2.4 bcB	0.8 ± 0.6 bA	5.3 ± 2.0 cdB
	180	0.6 ± 1.1 eA	1.3 ± 1.4 dA	0.8 ± 1.3 bA	0.6 ± 0.7 cA	0.4 ± 0.6 bA	0.5 ± 0.6 dA
	210	0.5 ± 1.1 eA	0.5 ± 0.7 dA	0.5 ± 1.1 bA	0.2 ± 0.4 cA	0.0 ± 0.1 bA	0.1 ± 0.1 dA
	240	0.4 ± 0.8 eA	0.3 ± 0.5 dA	0.5 ± 1.3 bA	0.3 ± 0.5 cA	0.1 ± 0.1 bA	0.0 ± 0.1 dA
	270	0.4 ± 1.0 eA	0.2 ± 4 dA	0.3 ± 0.7 bA	0.2 ± 0.4 cA	0.1 ± 0.2 bA	0.0 ± 0.0 dA
	300	0.0 ± 0.0 eA	0.2 ± 4 dA	0.0 ± 0.0 bA	0.2 ± 0.4 cA	0.0 ± 0.0 bA	0.0 ± 0.0 dA
$CaCl_2$	0	12.4 ± 3.1 aA	14.9 ± 6.0 aA	22.4 ± 18.2 aA	27.4 ± 14.2 aA	34.1 ± 18.5 aA	38.7 ± 18.0 aA
	30	7.0 ± 2.4 bA	7.5 ± 3.2 bA	12.6 ± 5.5 bA	11.0 ± 3.2 bA	19.1 ± 7.0 bA	18.2 ± 3.6 bA
	60	5.8 ± 3.2 bA	7.6 ± 2.9 bA	15.9 ± 7.3 abA	6.5 ± 3.2 bcB	20.4 ± 7.6 bA	13.2 ± 3.6 bcB
	90	1.8 ± 1.1 cA	4.3 ± 3.0 bcB	2.0 ± 1.2 cA	5.9 ± 3.0 bcA	3.4 ± 0.8 cA	8.9 ± 4.2 cdB
	120	0.8 ± 0.8 cA	3.5 ± 2.9 bcB	0.9 ± 1.0 cA	4.6 ± 1.7 bcB	1.0 ± 0.8 cA	5.4 ± 1.8 cdeB
	150	0.2 ± 0.4 cA	2.4 ± 1.6 cA	0.3 ± 0.5 cA	3.0 ± 2.7 cB	0.1 ± 0.2 cA	1.3 ± 1.0 deA
	180	0 ± 0 cA	1.9 ± 1.7 cA	0.2 ± 0.4 cA	2.4 ± 2.1 cB	0 ± 0 cA	0.5 ± 0.3 deA
	210	0.1 ± 0.3 cA	1.5 ± 1.6 cA	0 ± 0 cA	1.0 ± 1.2 cA	0 ± 0 cA	0.0 ± 0.1 eA
	240	0 ± 0 cA	0.6 ± 1.1 cA	0 ± 0 cA	0.5 ± 1.0 cA	0 ± 0 cA	0 ± 0 eA
	270	0 ± 0 cA	0.4 ± 1.0 cA	0 ± 0 cA	0.5 ± 1.3 cA	0 ± 0 cA	0 ± 0 eA
	300	0 ± 0 cA	0.3 ± 0.7 cA	0 ± 0 cA	0.4 ± 1.0 cA	0 ± 0 cA	0 ± 0 eA
KCl	0	12.4 ± 3.1 aA	14.9 ± 6.0 aA	22.4 ± 18.2 aA	27.4 ± 14.2 aA	34.1 ± 18.5 aA	38.7 ± 18.0 aA
	30	7.9 ± 2.2 aA	6.7 ± 2.9 bA	4.6 ± 3.3 cA	18.5 ± 4.7 bB	12.5 ± 5.3 bcA	24.2 ± 5.7 bB
	60	6 ± 1.4 bA	6.0 ± 3.2 bA	13.9 ± 3.8 abA	15.4 ± 5.0 bA	19.0 ± 4.2 bA	19.5 ± 4.8 bA
	90	7 ± 2.2 bA	3.8 ± 3.3 bcB	7.6 ± 2.3 bcA	7.3 ± 4.5 cA	12.8 ± 2.9 bcA	9.2 ± 4.0 cB
	120	2.1 ± 1.4 cA	2.7 ± 3.0 bcA	4.1 ± 2.0 cA	2.2 ± 2.2 cA	5.3 ± 2.1 cdA	4.0 ± 4.3 cA
	150	0.5 ± 0.8 cA	0.6 ± 1.1 cA	0.7 ± 1.1 cA	0.4 ± 0.8 cA	0.8 ± 0.8 dA	0.5 ± 0.8 cA
	180	0 ± 0 cA	0.8 ± 1.5 cA	0.2 ± 0.4 cA	0.6 ± 1.1 cA	0.1 ± 0.2 dA	0.5 ± 0.6 cA
	210	0 ± 0 cA	0.6 ± 1.1 cA	0.3 ± 0.7 cA	1.3 ± 2.1 cA	0.1 ± 0.2 dA	0.3 ± 0.4 cA
	240	0 ± 0 cA	0.4 ± 0.8 cA	0.2 ± 0.4 cA	0.4 ± 1.0 cA	0.0 ± 0.1 dA	0.1 ± 0.1 cA
	270	0 ± 0 cA	0.2 ± 0.6 cA	0.1 ± 0.3 cA	0.4 ± 1.0 cA	0.0 ± 0.1 dA	0.0 ± 0.1 cA
	300	0 ± 0 cA	0 ± 0 cA	0 ± 0 cA	0 ± 0 cA	0 ± 0 dA	0 ± 0 cA

Note: Values are mean 6 S.D. Means with different small letters in the same column are significantly different from each other, and means with different capital letters in the same row are significantly different from each other (Tukey test; $P < 0.05$).

germination, a probable conclusion was that a large amount of Ca^{2+} caused a negative effect on germination. Generally, this differential behavior of seeds according to the salt types was also presumably because the same saline concentration generated different osmotic potentials, and the osmotic effect may well have a greater influence on germination than specification toxicity, as suggested by several authors in other plants, such as halophytes (Ungar, 1996; Pujol et al., 2000).

With increasing salinity concentration, the length of the coleoptiles and radicles had a decreasing trend, indicating that the additional salinity could cause weakening of seeds. It was very remarkable that the radicle had higher resistance to salts than the coleoptile, which is one of the mechanisms to tolerate easily the salinity stresses. The length of the alfalfa radicles, Yazdi and Hamedani cultivars from 90 mmol·L^{-1} solution to higher solutions was reduces, which poorly showed that these two cultivars could tolerate these levels of salinity.

In general, based on the obtained results, it seems that *Medicago sativa* cv. Hamedani has more vigor than *Medicago sativa* cv. Yazdi. Therefore, selecting *Medicago*

sativa cv. Hamedani seeds in reclamation and restoration projects could lead to suitable results in arid and semiarid areas, where salinity affects vegetation development strongly.

References

Abdul-baki A A, Anderson J D (1973). Vigor determination in soybean seed by multiplication. Crop Science, 3: 630–633

Allen G J, Wyn Jones R G, Leigh R A (1995). Sodium transport measured in plasma membrane vesicles isolated from wheat genotypes with differing K^+/Na^+ discrimination traits. Plant, Cell and Environment, 18: 105–115

Azaizeh H, Gunse B, Steudle E (1992). Effects of NaCl and $CaCl_2$ on water transport across root cells of maize (*Zea mays* L.) seedlings. Plant Physiology, 99: 886–894

Basalah M O (1991). Effect of salinity on seed germination and growth of sequash (*Cucubita pepo* L.). Arab Gulf J Sci Res, 9: 87–97

Breen C M, Everson C, Rogers K (1997). Ecological studies on *Sporobolus virginicus* (L.) Kunth with particular reference to salinity and inundation. Hydrobiologia, 54: 135–140

Brenchley J L, Probert R J (1998). Seed germination responses to some environmental variables in the sea grass *Zoostera capricorni* from eastern Australia. Aquat Bot, 62: 177–188

Cramer G R (1992). Kinetics of maize leaf elongation. II. Response of a Na-excluding cultivar and a Na-including cultivar to varying NA/CA salinities. Experimental Botany, 43: 857–864

Cramer G R, Lauchli A, Polito V S (1985). Displacement of Ca^{2+} by Na^+ from the plasmalemma of root cells. A primary response to salt stress? Plant Physiology, 79: 207–211

El-Kharbotly A, Mahgoub O, Al-Subhi A, Al-Halhali A (2003). Indigenous grass cultivars with potential for maintaining rangeland and livestock feeding in Oman. Agric Ecosyst Environ, 95: 623–627

Epstein E, Norlyn J D, Rush D W, Kinsbury R W, Kelly D B, Gunningham G A, Wrona A F (1980). Saline culture of crops: A genetic approach. Science, 210: 399–404

Flowers T J, Yeo A R (1995). Breeding for salinity resistance in crop plants. Aust J Plant Physiol, 22: 875–884

Hardegree S P, Emmerich W E (1990). Partitioning water potential and specific salt effects on seed germination of four grasses. Annals of Botany, 66: 587–595

Kent L M, Lauchli A (1985). Germination and seedling growth of cotton: salinity-calcium interactions. Plan, Cell and Environment, 8: 155–159

Maguire J D (1962). Speed of germination in selection and evaluation for seedling vigour. Crop Science, 2: 176–177

Nabil M, Coudret A (1995). Effects of sodium chloride on growth, tissue elasticity and solute adjustment in two *Acacia nilotica* subcultivars. Physiologia Plantarum, 93: 217–224

Neumann P M (1993). Rapid and reversible modifications of extension capacity of cell walls in elongating maize leaf tissues responding to root addition and removal of NaCl. Plant, Cell and Environment, 16: 1107–1114

Neumann P M, Azaizeh H, Leon D (1994). Hardening of root cell walls: a growth inhibitory response to salinity stress. Plant, Cell and Environment, 17: 303–309

Othman Y, A1-karaki G, Al-Tawaha A R, Alhorani A (2006). Variation in germination and ion uptake in barley genotypes under salinity conditions. J Agricultural Science, 2(1): 11–15

Pujol J A, Calvo J F, Ramirez-Diaz L (2000). Recovery of germination from different osmotic conditions by four halophytes from south-eastern Spain. Ann Bot, 85: 279–286

Redmann R E (1974). Osmotic and specific ion effects on the germination of alfalfa. Canadian Journal of Botany, 52: 803–808

Shainberg I (1975). Salinity of soils–effects of salinity on the physics and chemistry of soils. In: Poljakoff-Mayber A, Gale J, eds. Plants in Saline Environments. Berlin: Springer Verlag, 39–55

Shokohifard G, Sakagam K H, Matsumoto S (1989). Effect of amending materials on growth of radish plant in salinized soil. J Plant Nutr, 12: 1195–1294

Susan M (2007). Most Bees Live Alone: No hives, no honey, but maybe help for crops. Science News, 171(1): 11–13

Szabolcs I (1994). Soils and salinization. In: Pessarakli M, ed. Handbook of Plant and Crop Stress. New York: Marcel Dekker, 3–11

Tobe K, Li X, Omasa K (2002). Effects of sodium, magnesium and calcium salt types on salt types on seed germination and radicle survival of a halophyte, Kalidium capsicum (Chenopodiaceae). Australian Journal of Botany, 50: 163–169

Ungar I A (1982). Germination ecology of halophytes. In: Sen D N, Rajpurchit K S, eds. Contributions to the Ecology of Halophytes. The Hague: Junk, 143–154

Unger I A (1996). Effect of salinity on seed germination, growth, and ion accumulation of *Atriplex patula* (Chenopodiaeceae). Am J Bot, 83: 604–607

Waisel Y (1972). Biology of Halophytes. New York and London: Academic Press