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Effect of calcium on reducing salt stress in seed germination and early growth stage of *Festuca ovina* L.

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ABSTRACT

Festuca ovina L. is an important ornamental species used in urban landscapes and pastures reclamation. However, low resistance of this species to salt stress has hindered its extended application in low-water and salty regions. To study the effects of calcium on reducing salt stress injuries in seed germination and early growth of this species, a factorial experiment based on completely randomized design with three replications was conducted. The salt levels included NaCl solutions (5, 10, 15 and 20 dS/m). Calcium source was $CaCl_2$ with concentrations of 0, 10, 20 and 30 $mmol_+$/L. Germination percentage and mean germination time were significantly affected by interaction of salinity and calcium. As salinity increased, mean germination time increased. However, germination percentage, shootlet length, rootlet length, shootlet fresh and dry weight, rootlet fresh and dry weight and seed vigour index decreased following the increasing salinity. Concentrations of 10 and 20 $mmol_+$/L $CaCl_2$ had significant effects on reducing salt injuries on seed germination of *Festuca ovina*. This finding was even prominent in higher salinity levels of 15 and 20 dS/m. Therefore, to facilitate using this species in urban landscapes or in pastures, pre-treatment of the seeds using 10 and 20 $mmol_+$/L $CaCl_2$ is recommended.

Keywords: fertilizer applications; sheep fescue; morphological characteristics; abiotic stress; landscape designing

High soil salinity is a common problem in turfgrass management, especially in arid and semiarid regions (Zhang et al. 2013). In such regions, germination and seedling establishment constitute the most critical periods in the life-cycle of plants (Meloni et al. 2008). The process of germination commences with absorption of water (Wahid et al. 1998, Zehra et al. 2012) and finishes with the emergence and elongation of embryonic tissues. Soil salinity, being a serious environmental hazard, greatly prevents seed germination and its related processes (Wahid et al. 1998). Salinity problems are becoming more severe in turfgrass management (Gao and Li 2012). The need for salinity-tolerant turfgrass is increasing because of the increased use of effluent or other low quality waters for turfgrass irrigation (Alshammary et al. 2004). Rapid urban population growth has put enormous pressures on limited freshwater supplies. As water shortages continue to escalate worldwide, use of saline water for turf landscape irrigation will be increasingly necessary (Marcum 2006).

Recently, application of fertilizers for reducing plant growth restrictions under salinity stress conditions and for increasing fertility of saline soils have been considered (Astaraei and Forouzan Ghohar 2000). The calcium ion has important effects on physiological processes of plants and improves growth and biochemical factors of the plants under salt stress conditions (Mozafari et al. 2008). Calcium was reported to inhibit Na^+ uptake (Zehra et al. 2012) and thereby to reduce its negative effect on seed germination and to increase plant growth (Faiza et al. 2007, Zehra et al. 2012). Understanding the mechanism of the effects of calcium in saline soils is less clear (Faiza et al. 2007).

Festuca ovina L., commonly named as sheep fescue, is considered as one of the most important pasture grasses (Dianati-Tilaki et al. 2011). It is a perennial plant from the Poaceae family (Azarnivand et al. 2012) which has a stack and compact form (Dianati-Tilaki et al. 2011, Mirhaji et al. 2013) and rhizomes (Dianati-Tilaki et al.

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2011). Sheep fescue, in addition to being cultivated in pastures, is used in urban landscape as an ornamental grass without clipping and also as a quality sport turfgrass after clipping. This plant showed an adequate turf quality when it was evaluated over a wide range of climates and under low maintenance regimes such as little or no irrigation, pesticide or fertilizer applications and frequent mowing (Zhang et al. 2013) but is not highly resistant to salinity stress (Dianati-Tilaki et al. 2011, Zhang et al. 2013). Therefore, increasing salinity tolerance of this species is important in order to reform and recover pastures as well as for expanding this important species in urban landscape.

MATERIAL AND METHODS

This study was conducted to investigate the effect of calcium ion on seed germination and seedling growth of *Festuca ovina* L. in different levels of salinity, *in vitro* conditions, in Agricultural Faculty of Ferdowsi University of Mashhad, Iran. Seeds of *Festuca ovina* L. were provided by Agriculture and Natural Resources Researches Center of Mashhad, Iran. In this experiment, at first, some petri dishes with a diameter of 10 cm were selected, filter paper were placed within each of them and then they were sterilized in an oven at 110°C for 24 h (Astaraei and Forouzan Ghohar 2000). Seeds used in this experiment were immersed in sodium hypochlorite (2%) for 20 min and then were rinsed several times with distilled water. Then, 25 intact seeds were placed in each petri dish. In this experiment three replications for each treatment were considered. In order to examine salinity stress, 8 mL of NaCl solutions with electrical conductivities 5, 10, 15 and 20 dS/m was added in each petri dish (Dianati-Tilaki et al. 2011), so that each of the experimental treatments had 4 calcium chloride concentrations (0, 10, 20 and 30 $mmol_+/L$). Then petri dishes were placed at 20°C and a 12-h lighting period (Dianati-Tilaki et al. 2011). Petri dishes were checked for the number of germinated seeds and the seeds with a visible rootlet were recorded every day. The end of the germination date was considered when any germination was not observed after two consecutive days. This was 27 days after experiment beginning. Percentage of germination was calculated from the following equation:

$$(\text{number of germinated seed/total number of seeds}) \times 100$$

Average time for germination was calculated based on the following equation:

$$MGT = (A1D1 + A2D2 + AnDn)/A1 + A2 + An$$

Where: A – number of germinated seeds in the D time; n – number of germination days (Nichols and Heydecker 1968).

Length of shootlet and rootlet were measured with a millimetre ruler.

Seed vigour was calculated using the equation:

$$VI = (RL + SL) \times GP$$

Where: RL – rootlet length; SL – shootlet length; GP – percentage of seed germination (Dianati-Tilaki et al. 2011).

Fresh weight and dry weight of shootlet and rootlet were measured by putting these organs in the oven at 65°C until they get a constant weight (Dianati-Tilaki et al. 2011).

Experimental design was factorial, based on completely randomized design and the data were analysed using the JMP8 software package (SAS Institute, USA). *T*-student tests were performed to determine significant differences between means at probability levels of 0.01 and 0.05.

RESULTS AND DISCUSSION

The results of the analysis of variance showed that the interaction between calcium and salinity on germination percentage of the seeds of *Festuca ovina* was significant ($P < 0.01$) (Table 1). Means of interaction effects of salinity and calcium in Figure 1 showed that the effect of calcium on germination percentage in high concentrations of salinity (more than 10 dS/m) was improving. In other words, in salinity levels of 15 and 20 dS/m, calcium chloride at concentration levels of 10, 20 and 30 $mmol_+/L$, significantly increased germination percentage of the seeds compared to the control (the medium without calcium). However, in salinity levels of 5 and 10 dS/m, this positive effect was not significant compared to the control treatment. The largest germination percentage was observed when 10 $mmol_+/L$ $CaCl_2$ was used as a treatment along with salinity levels of 10 dS/m (71.66%) (Figure 1).

The germination reduction of plants in saline environments can occur for two reasons. One is reduction of effective uptake due to impairing osmotic balance that creates water stress for plant

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Table 1. Analysis of variance (mean of squares) related to germination of the seeds of *Festuca ovina*

Source of variance	*df*	Germination percentage	Time of germination	Shootlet length	Rootlet length	Fresh weight of shootlet	Dry weight of shootlet	Fresh weight of rootlet	Dry weight of rootlet	Seed vigour index
Salinity	3	2937/188**	1/56*	3/8**	0/58*	500/7**	6/83**	75/59*	0/36**	87087**
Calcium	3	592/188**	2/43**	6/46**	0/46*	96/7^{ns}	0/97^{ns}	22/28^{ns}	0/03^{ns}	40304/3**
Salinity × calcium	9	426/910**	1/07*	0/38^{ns}	0/34*	113/46*	1/87**	66/16**	0/2**	12154/6**
Error	32	29/396	0/36	0/29	0/15	41/8	0/47	20/45	0/05	1646/5

***$P < 0.01$; *$P < 0.05$; ns – non-significant

and the other is creation of ion toxicity because of absorption and accumulation of ions (Rengel 1992, Anvari et al. 2009). Salinity stress increases the ABA (abscisic acid) concentration in seed and increasing the concentration of this hormone prevents seed germination (Mokhtari et al. 2008). From this point of view, the results of this experiment correspond with the results of Marcum (2006), Dianati-Tilaki et al. (2011) and Zhang et al. (2013). Supplemental Ca^{2+} alleviates deleterious salt effects probably through mitigating the toxic effects of Na^+ ions rather than the osmotic effects associated with salt stress. Therefore, the ratio of Ca^{2+}/Na^+ in the rooting medium appears as a more reliable indicator of salt stress than the concentration of Na^+ alone. Addition of Ca^{2+} reduced Na^+ binding to cell walls, alleviated membrane leakiness, and prevented salt-induced decline in cell production and cell elongation (Rengel 1992).

Means of interaction effects of salinity and calcium on germination time, showed that in salinity levels of 5 and 10 dS/m there was not a significant difference between different treatments of $CaCl_2$ and the control, but in high concentration levels of salinity (15 dS/m) there was a significant reduction in average of germination time compared to the control treatment (Figure 2).

Salinity causes delay in germination and thus increases the germination time (Dianati-Tilaki et al. 2011). The primary effect of salinity is thought to take place in roots; it was argued that this primary effect is due to water deficit rather than a specific toxic effect of salt (Rengel 1992). For critical activities before seed germination, water should be absorbed sufficiently by seeds. If water absorption by seeds is slow or disturbed, activities inside the seed occur slowly and the time of rootlet emergence increases and rate of germination decreases (Anvari et al. 2009). Calcium with adjustment of transmission and permeability of ions and control of ion exchanges plays an important role in improving germination rate (Mokhtari et al. 2008).

The results of the analysis of variance also showed that shootlet length was significantly affected by salinity levels and $CaCl_2$ levels applied in *Festuca ovina* seeds ($P < 0.01$) (Figure 3). However, there were no significant interaction effects between these two factors (Table 1).

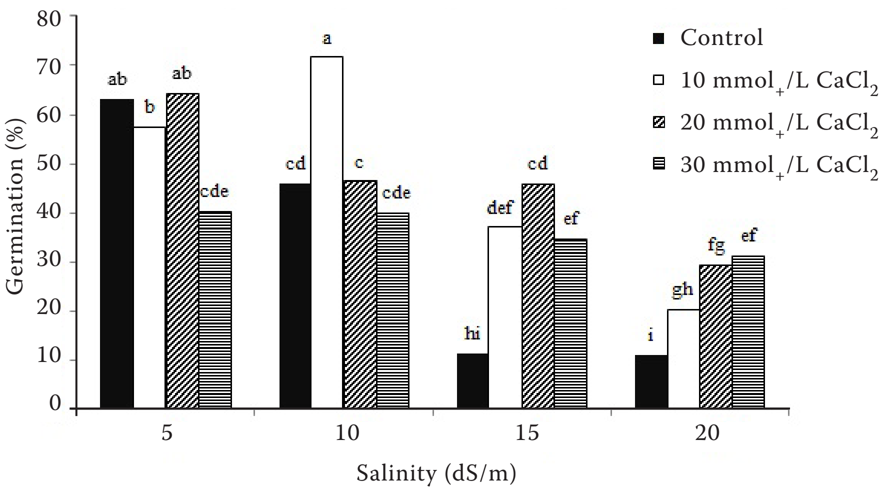

Figure 1. Comparison of the means of interactions between calcium and salinity on germination percentage of the seeds of *Festuca ovina* L.

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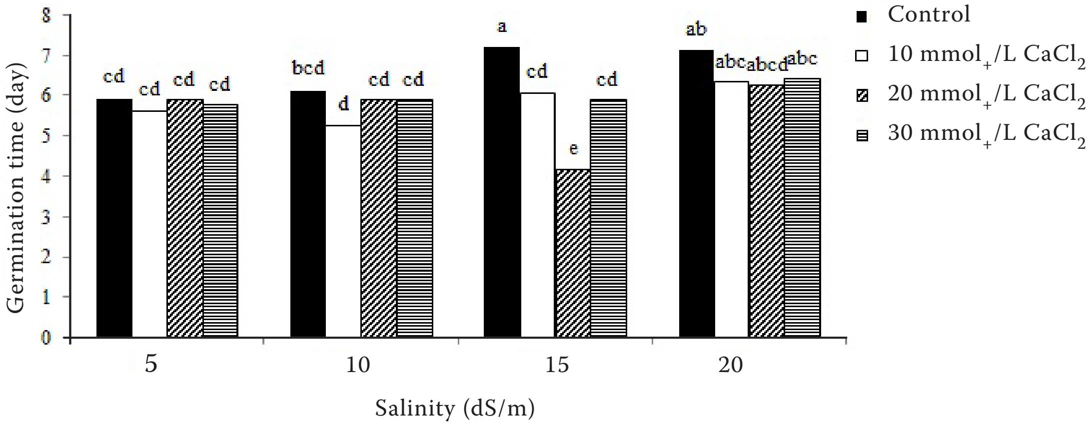

Figure 2. Comparison of the means of interaction between calcium and salinity on germination time of the seeds of *Festuca ovina* L.

The interaction between calcium and salinity on means of rootlet length of *Festuca ovina* was significant ($P < 0.05$) (Table 1). Increase of the levels of salinity was associated with decreasing length of rootlets. However, different levels of $CaCl_2$ did not provide a clear relationship between the best levels of $CaCl_2$ for achieving the largest rootlet length in each level of salinity applied. However, the largest rootlet length was achieved when 20 $mmol_+$/L $CaCl_2$ was used in salinity level of 15 dS/m and the shortest rootlet length was obtained when high salinity levels of 15 and 20 dS/m without using any $CaCl_2$ was applied (Figure 4a).

With increasing salinity, water absorption decreases, release of hormones and enzymes activity are disturbed and the final result of this is a reduction in rootlet and shootlet growth (Mokhtari et al. 2008). Sodium ions inhibit elongation of root cells. High activities of Na^+ in the apoplasm may disrupt ionic interactions between cell wall constituents such as pectin and extensin, and might have adverse effects on apoplasmic enzymes (Rengel 1992). Increasing in Na^+ flux competes with Ca^{2+} at the binding sites to plasma membranes. Therefore, addition of calcium may counter the adverse consequences by providing protection to the integrity and permeability of plasma membranes against Na^+ toxicity (Zehra et al. 2012).

The results showed that interaction between calcium and salinity on fresh and dry weight of shootlet of the seeds of *Festuca ovina* was significant (Table 1). In the highest salinity level (20 dS/m), increasing levels of $CaCl_2$ were associated with increase of shootlet fresh weight, but in the lowest salinity level (5 dS/m) this trend was opposite (Figure 4b). The same trend was observed for dry weight of shootlet (Figure 4c).

The interaction between calcium and salinity on rootlet fresh and dry weight of the seeds of *Festuca ovina* was significant ($P < 0.01$) (Table 1) and the best treatment for having the largest rootlet fresh and dry weight was obtained from a combination of 10 dS/m salinity and 10 $mmol_+$/L $CaCl_2$ (Figures 4d and 5a).

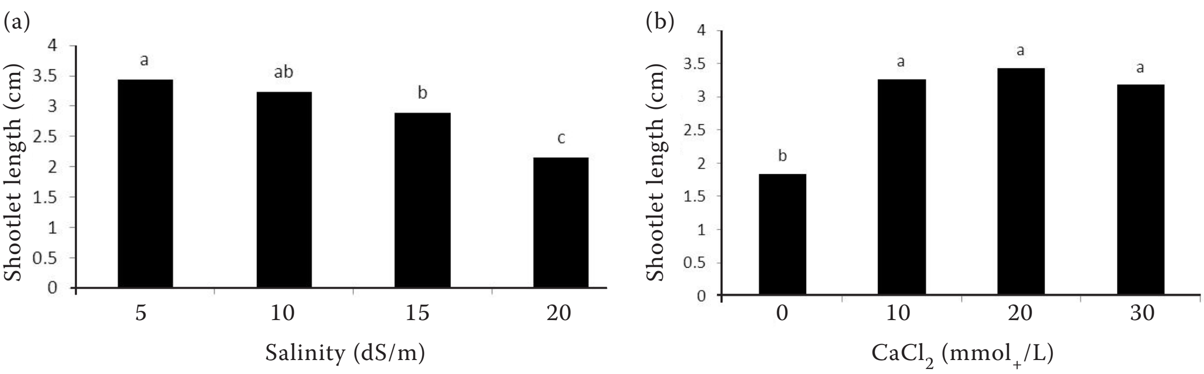

Figure 3. Simple effects of (a) salinity, and (b) calcium on mean of shootlet length of *Festuca ovina* L.

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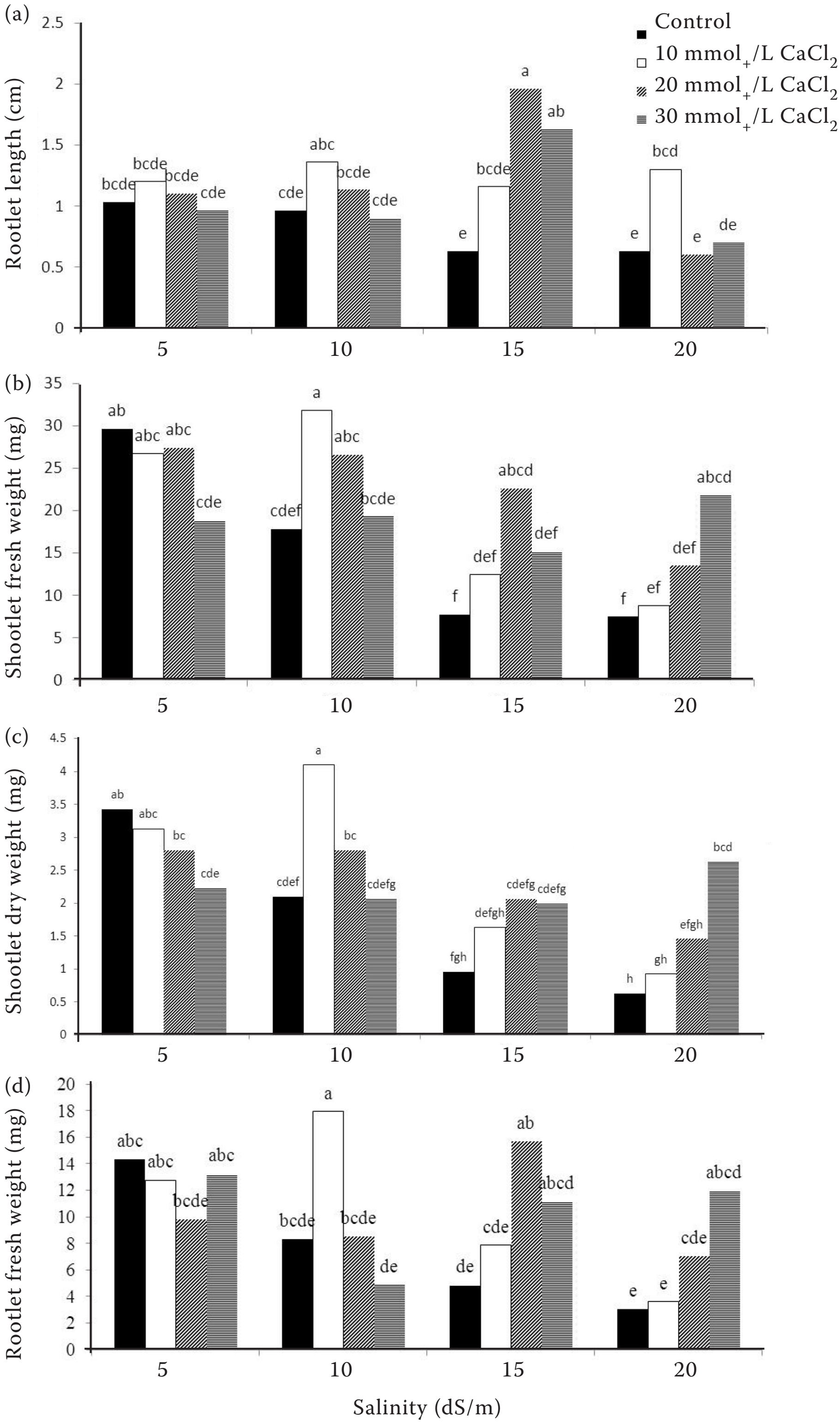

Figure 4. Comparison of the means of interaction between calcium and salinity (a) on rootlet length; (b) shootlet fresh weight; (c) shootlet dry weight, and (d) rootlet fresh weight of the seeds of *Festuca ovina* L.

It was observed that the interaction between calcium and salinity on seed vigour index of *Festuca ovina* was significant ($P < 0.01$) (Table 1) and combination of 10 dS/m salinity and 10 mmol$_+$/L $CaCl_2$ was associated with the largest seed vigour index (Figure 5b).

In general, negative effect of salinity on seed germination and growth could be due to osmotic effects (low osmotic potential), nutritional imbalance, effect of specific ions, ion toxicity or a combination of these four factors that occur due to compounds or concentrations of salinity in

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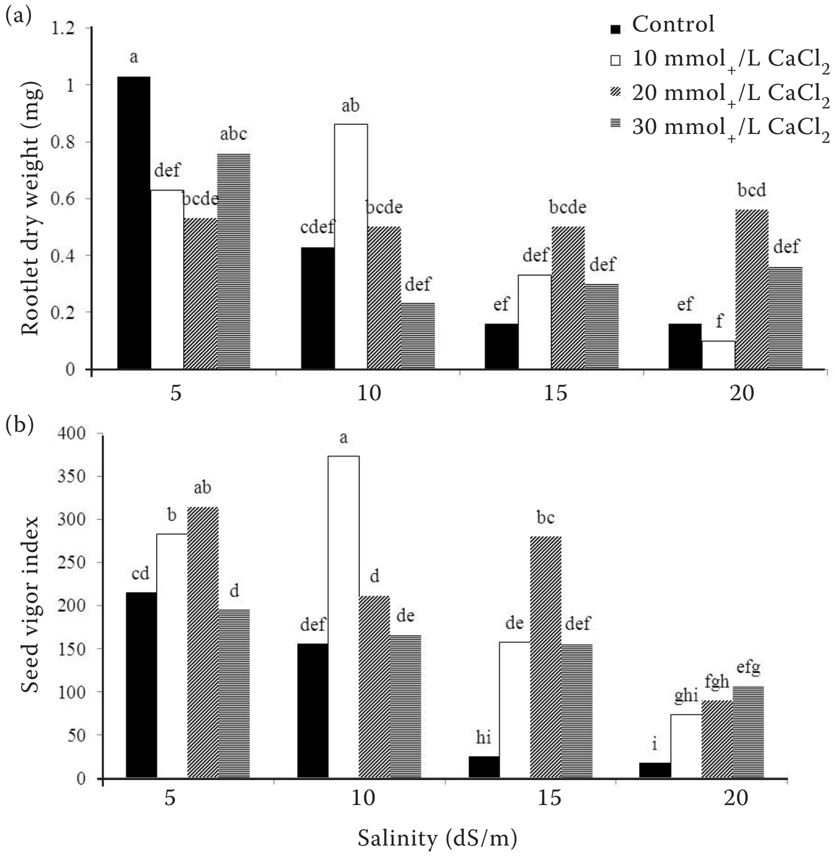

Figure 5. Comparison of the means of interaction between calcium and salinity on (a) rootlet dry weight of the seeds and (b) seed vigour index of *Festuca ovina* L.

seeds of plants (Dianati-Tilaki et al. 2011). Many studies indicate that calcium acts as a secondary messenger in abiotic stress and in response to hormones. An increase in cellular calcium ion in quick response to increasing concentration caused by abiotic stresses, the necessity of the presence of calcium ion for the expression of many genes involved in stress and finally increasing calcium ions in the activation of stress genes promotor are evidences that confirm positive and ameliorator role of calcium during encounter with various environmental stresses (Mokhtari et al. 2008).

According to this research 10 dS/m salinity and 10 $mmol_+/L$ $CaCl_2$ had positive effects in some germination and early growth characteristics of *Festuca ovina* L. It appears that $CaCl_2$ especially in the highest levels of salinity (15 and 20 dS/m) can show clear positive effects on germination characteristics of the seeds of *Festuca ovina* L. Therefore, it is necessary to conduct more research work to increase resistance of salt-sensitive plants such as sheep fescue by using suitable calcium fertilizers in soil and water or pretreating seeds with appropriate concentrations of calcium chloride in the stages of seed germination and early growth of seedlings. Such treatments may promise more stability of this plant species in saline environments.

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