Seed Science and Biotechnology *©2010 Global Science Books*

Response of Fescue (*Festuca rubra*) to Salinity Sources and Levels at Seed Germination and Seedling Stage

Neda Jalali[1] • Naghmeh Daneshvar[1] • Ali Shahi-Gharahlar[1*] • Jaime A. Teixeira da Silva[2] • Rozbeh Farhoudi[3]

[1] Department of Horticulture Science, Faculty of Agriculture, University of Tehran, Karaj, Iran
[2] Faculty of Agriculture and Graduate School of Agriculture, Kagawa University, Miki-cho, Ikenobe 2393, Kagawa-ken, 761-0795, Japan
[3] Department of Agronomy, Islamic Azad University, Shoshtar Branch, Iran

Corresponding author: * **ashahi.gh@gmail.com, shahia@ut.ac.ir**

ABSTRACT

Salinity is one of the major obstacles to increasing production in crop-growing areas throughout the world. Salinity also impairs seed germination. Due to increasing salinity problems, in this study *Festuca rubra* seeds were treated with different concentrations of salt solutions with different sources of salt to study the response of this plant during seed germination and at the seedling stage. Germination percentage, coleoptile, radicle and seedling length, and seedling fresh weight (SFW) were determined under various salt stresses. Salt treatments included: NaCl, KNO_3 and $CaCl_2$ at 50, 100 and 150 mmol/L. An increase in salt concentration caused a decrease in germination. Lowest germination was observed by $CaCl_2$ at 150 mmol/L while longest coleoptiles formed at 50 mmol/L $CaCl_2$, i.e., longer than the control. An increase in KNO_3 concentration from 0 to 50 mmol/L decreased radicle and seedling length more than the control (salt-free), or NaCl and $CaCl_2$ treatments at equal concentrations. Highest SFW was observed in control seedlings and lowest SFW in seedlings grown in 150 mmol/L NaCl.

Keywords: grass, osmotic stress, salinity, seed priming, seedling characteristics
Abbreviations: GP, germination percentage; **SFW**, seedling fresh weight

INTRODUCTION

Salinity is a common abiotic stress factor seriously affecting crop production in different parts of the world, particularly in arid and semi-arid regions (Karmoker *et al.* 2008). Over 400 Mha across the world are affected by salinity, i.e., about 25% of the world's total area (including 15% of Iran's lands) (Janmohammadi *et al.* 2008). During growth, plants are usually exposed to different environmental stresses which limit their growth and productivity. In a saline environment adaptation of plants to salinity during germination and early seedling stages is crucial for the establishment of a species. Seedlings are the most vulnerable stage in the life cycle of plants and germination determines when and where seedling growth begins (Kaymakanova 2009).

High concentrations of salts have pernicious effects on germination of seeds and plant growth (Rahman *et al.* 2008). Soil salinity may affect the germination of seeds either by creating an osmotic potential external to the seed preventing water uptake, or through the toxic effects of Na^+ and Cl^- ions on the germinating seed (Khajeh-Hosseini *et al*. 2003). Consequently, higher K^+/Na^+ or Ca^+/Na^+ ratios are typical in tissues of salt-tolerant varieties, and are often used as a screening parameter for identification of salt stress-tolerant varieties (Munns and James 2003; Song *et al*. 2006). Tavili and Biniaz (2009) reported that saline concentration effects seed germination percentage, length of the stem and radicle of *Hordeum* species. Seed germination decreased significantly by increasing salinity (NaCl, $CaCl_2$ and KCl) levels.

Fescues (*Festuca* spp.) are major cool-season forage and turf grasses grown in a wide range of soil and climatic conditions. Fescues are outcrossing species that belong to the grass family Poaceae, subfamily Pooideae, and tribe Poeae (Zhang *et al.* 2005). Selection of plants for landscapes requires consideration of a number of factors, including cold-hardiness, aesthetic characteristics, maintenance requirements, soil pH tolerance, drought tolerance, tolerance to soil salinity, saline irrigation water, and sodic soils (Kratsch *et al.* 2008).

Due to the existence of salinity and high temperature and sensitivity of some plants in most regions of Iran, there are many difficulties in the planting and husbandry of lawn.

Therefore the aim of the present study was to evaluate the effect of NaCl, KNO_3 and $CaCl_2$ salinity at germination and early seedling growth stage of *Festuca rubra*.

MATERIALS AND METHODS

The experimental design was two factors factorially arranged in a completely randomized design (CRD), with three replications and 20 *F. rubra* seeds per replicate. The first factor was salinity stress and the second factor was salinity concentration.

F. rubra seeds were disinfected with 0.5% sodium hypochloride solution (commercial bleach) for 5 min, rinsed with sterile distilled water several times, and then briefly blotted with sterile paper towels. Three replicates of 20 seeds were germinated on sheets of filter paper with 10 ml of respective test solutions in 9-cm plastic Petri dishes. Treatments were: NaCl, KNO_3 and $CaCl_2$ solution (all at 50, 100 and 150 mmol/L). The control treatment was distilled water. Seeds were placed in a growth chamber at 24 ± 1°C in continuous dark for 8 days.

A seed was considered germinated when the radicle was 2 mm long. Seed germination was recorded every 24 h for 7 days. Coleoptile, radicle and seedling length and seedling fresh weight (SFW) were measured on the 7^{th} day of the experiment.

Germination percentage was calculated using this formula:

Final germination percentage (GP) =

$$100 \times \frac{\text{Number of germinated seeds}}{\text{Total of number seeds}}$$

Received: 11 August, 2010. Accepted: 27 August, 2010.

Original Research Paper

Statistical analysis

For statistical analysis, the data of germinating percentage was transformed to arcsin $\sqrt{X/100}$ and experimental data was analyzed by SAS v. 9.1. Treatments means were compared using Duncan's multiple range test (DMRT) at the 5% level of probability and graphs were drawn using Excel 2007 software.

RESULTS AND DISCUSSION

Effects of salinity on germination

Salinity affected germination of *F. rubra* (**Table 1**). Increasing salt concentration decreased germination. A strong reduction in germination was observed mainly at high salt concentrations. Lowest germination was observed with 150 mmol/L $CaCl_2$ (**Fig. 1**). The highest germination percentage was observed by using KNO_3 and NaCl (100 mmol/L), although this result was not significant compared to the control and 50 and 100 mmol/L of $CaCl_2$. Increasing NaCl and KNO_3 concentrations decreased germination (**Fig. 1**). Khan *et al*. (2009) reported that increasing the level of salt concentration (i.e. EC increased from 10 to 30 ds/m) had a significant negative effect (at EC = 30 ds/m) on the germination of grasses (*Panicum antidotale*, *Cenchrus cilliaris* and *Dichanthium annulatum*). Under saline conditions, seed priming with KNO_3 (-1.27 MPa at EC=7 ds/m) was better than NaCl and PEG priming treatments because seedling fresh weight, root and shoot length increased in summer squash (*Cucurbita pepo*) (Shahi-Gharahlar *et al*. 2009). According to the results of this study, KNO_3 and NaCl at 100 mmol/L had the best effect on seed germination of *F. rubra*; however, when compared with the control, 50 mmol/L of any salt source, and 100 mmol/L of $CaCl_2$ showed no significant differences.

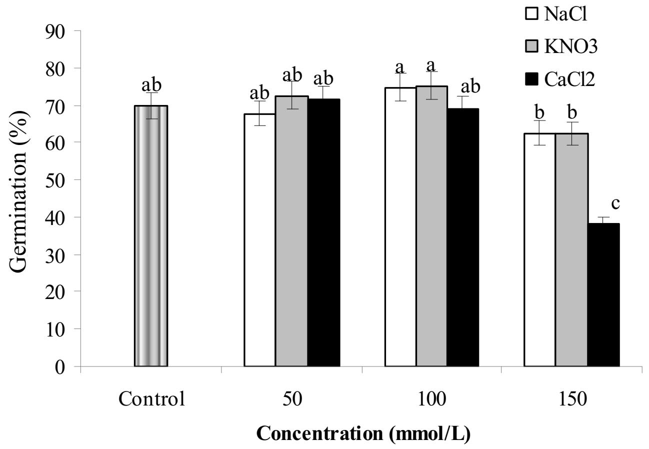

Fig. 1 Effect of salinity source on germination % of *Festuca rubra*.

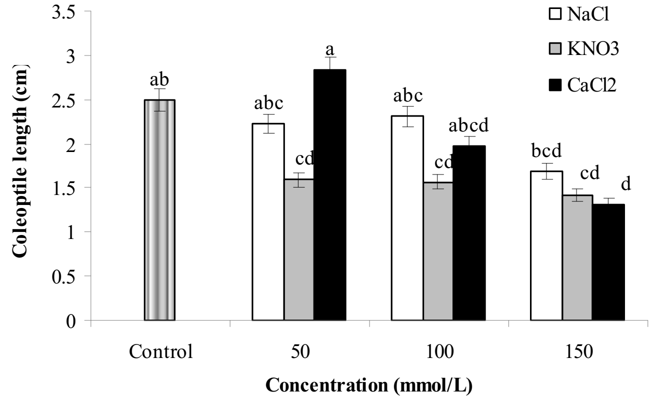

Fig. 2 Effect of salinity source on coleoptile length percentage of *Festuca rubra*.

Effects of salinity on coleoptile length

Coleoptile length of *F. rubra* was significantly affected by different salinity levels (**Table 1**). 50 mmol/L $CaCl_2$ resulted in higher coleoptile length in *F. rubra* than in other saline treatments. Moreover, these values decreased as salinity level rose from 50 mmol/L. This is the reason why higher salinity level (150 mmol/L) gave the shortest coleoptiles. $CaCl_2$ at 50 mmol/L resulted in the longest coleoptiles, but this result was not significantly different to the control, NaCl (50 mmol/L) and $CaCl_2$ (100 mmol/L) (**Fig. 2**). Calcium is a key element in the structure of primary cell walls. When calcium was omitted from oats (*Avena sativa* L.) growth media, there was an adverse effect on growth (Barker and Pilbeam 2007). Rudd and Franklin-Tong (2001) suggested the rapid increases in cytoplasmic Ca^{2+} concentration occur when the channels open and let calcium out of the vacuolar store and the magnitude, duration, and precise location of these increases give a series of calcium signatures that are part of the plant to a range of environmental signals. These responses enable the plant to respond to drought, salinity, cold shock, mechanical stress, ozone and blue light, ultraviolet radiation, and other stresses.

Effects of salinity on radicle and seedling length

Radicle and seedling length of *F. rubra* was significantly affected by different salinity sources and levels (**Table 1**). The salt-free treatment (control) gave longer radicles and seedlings than in saline treatments (**Figs. 3, 4**). Moreover, these values decreased as salinity increased. This is the reason why a higher salinity level (150 mmol/L) gave the shortest radicles (0.88 cm) and seedlings (3.27 cm). An increase in KNO_3 concentration from 0 to 50 mmol/L decreased radicle and seedling length more than NaCl and $CaCl_2$. Kaymakanova (2009) reported that bean cultivars (*Phaseolus vulgaris* L.) treated with NaCl and Na_2SO_4 showed decreased seedling growth. Also, Kaya and Day (2008) reported that increased salinity (NaCl) levels (from 0 to 20 ds/m) resulted in a decrease in root length of sunflower (*Helianthus annuus* L.) cultivars. Shoot length showed a progressive decrease with increasing salinity (Kaya and Day 2008). Tavili and Biniaz (2009) reported that an increase of three salt (NaCl, $CaCl_2$, and KCl) concentrations caused a decline in the length of the stem of *Hordeum* species (*H. vulgare* and *H. bulbosum*). They also observed that *H. vulgare* had a longer radicle than *H. bulbosum* under salt stress.

Effects of salinity on seedling fresh weight

The concentration of salt and the salt × concentration interaction significantly affected SFW (**Table 1**). Salt stress decreased SFW (**Fig. 5**). Highest SFW weight was obtained

Table 1 Analysis of variance of the effect of salinity source on *Festuca rubra* seed germination under study.

	Mean of square				
Source of variance	**Coleoptile length (cm)**	**Seedling length (cm)**	**Radicle length (cm)**	**Seedling fresh weight (g)**	**GP (%)**
Salt	0.64 ns	3.30 ns	1.64 **	0.00002 ns	213.62 **
Concentration	1.98 **	58.61 **	22.65 **	0.004 **	657.05 **
Salt × Concentration	0.36 ns	3.98 ns	0.43 ns	0.0003*	142.83 **
Error	0.22	5.31	0.23	0.00009	31.20
CV (%)	23.02	38.71	21.98	12.58	8.33

ns: non significant, *: significant at $P = 0.05$; **: significant at $P = 0.01$.

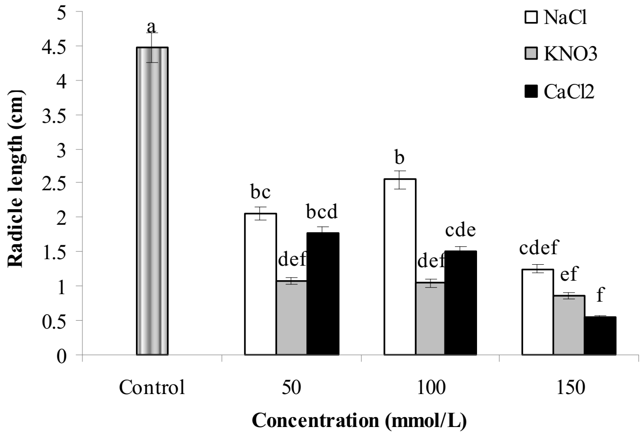

Fig. 3 Effect of salinity source on radicle length of *Festuca rubra*.

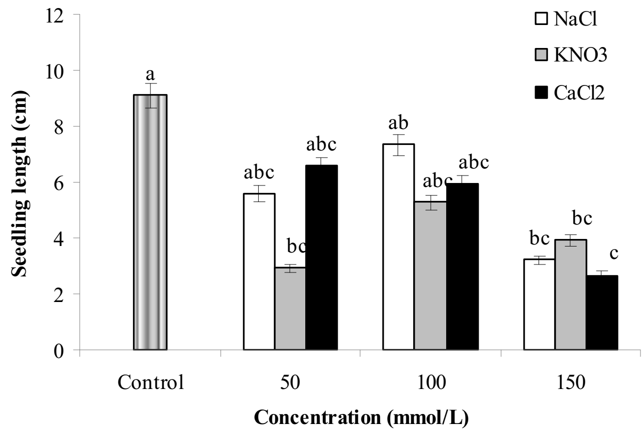

Fig. 4 Effect of salinity source on seedling length of *Festuca rubra*.

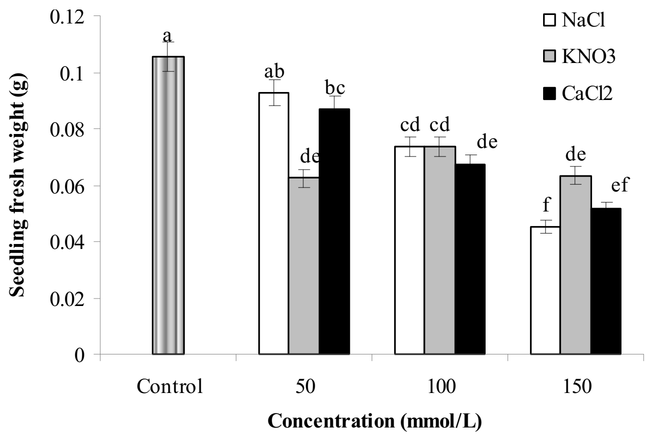

Fig. 5 Effect of salinity source on seedling fresh weight of *Festuca rubra*.

by control seedlings (salt-free) and lowest SFW was obtained in the NaCl (150 mmol/L) treatment (**Fig. 5**). Roughly, an increase of KNO_3 concentration from 50 to 150 mmol/L equally affected SFW of *F. rubra*. In fact, KNO_3 at 50, 100, and 150 mmol/L showed no significantly differences in SFW. Increasing concentration of both NaCl and $CaCl_2$ treatments decreased SFW. Nascimento (2003) reported that muskmelon (*Cucumis melo* L.) seed priming with KNO_3 increased SFW under low temperature (at 17°C) stress in compared to non-primed seeds. Shahi-Gharahlar *et al*. (2009) suggested that, under saline conditions, seed priming with KNO_3 was better than other priming treatments (NaCl and PEG), because SFW, root and shoot length increased in summer squash (*Cucurbita pepo*) seedlings. Okcu *et al.* (2005) observed a remarkable decrease in SFW of pea (*Pisum sativum* L.) caused by a decrease in water potential when NaCl and PEG were used. They suggested differences among cultivars, which showed different responses to each NaCl and PEG concentration. Kaya *et al.* (2006) reported that KNO_3 priming and hydro-priming increased sunflower (*Helianthus annuus* L.) SFW. They showed that KNO_3 did not have any toxic effect on sunflower seedlings. Our results showed that KNO_3 treatment at a high concentration (150 mmol/L) showed no significant differences with low concentrations (50 and 100 mmol/L). In contrast, NaCl and $CaCl_2$ showed that an increase in concentration caused a remarkable increase in SFW of *F. rubra*. Kaya *et al.* (2003) reported that increasing salinity (NaCl) levels (from 0.8 to 23.0 ds/m) caused a remarkable decrease in shoot dry weight in safflower (*Carthamus tinctorius* L.) seedlings. According to their result, varieties showed different responses to different salinity levels. Root dry weight of safflower diminished drastically with increasing salinity levels, while the effects of salinity varied with concentration.

CONCLUSION

During growth, plants are usually exposed to different environmental stresses which limit their growth and productivity. Among these, drought and salinity are the most severe (Bohnert *et al.* 1995). Good seed germination is important for horticulture and agriculture. Earlier growth stages are more sensitive to salinity than subsequent ones (Boem and Lavado 1996). In this study, seed germination and seedling growth of *F. rubra* was negatively affected by salinity stress at high levels. Our study showed increased salinity concentration, decreased seed germination and seedling growth of *F. rubra*. Salinity source significantly influenced the response of *F. rubra* to salt stress. At 50 mmol/L $CaCl_2$, *F. rubra* seeds had longest coleoptiles. Calcium is a key element in the structure of primary cell walls (Barker and Pilbeam 2007). $CaCl_2$ at 150 mmol/L also limited germination percentage compared with NaCl and KNO_3. Salinity may also affect the germination of seeds and seedling growth by creating an external osmotic potential that prevents water uptake or due to the toxic effects of Na^+ and Cl- ions on the germinating seed (Khajeh-Hosseini *et al.* 2003). Supplemental calcium had no effect on final germination in some plants like cotton (Suhayda *et al.* 1992) and barley (Huang and Redman 1995) but it did improve seedling growth of these plants.

REFERENCES

Barker AV, Pilbeam DJ (2007) *Handbook of Plant Nutrition*, CRC Press, Taylor and Francis Group, New York, pp 121-144

Boem FHG, Lavado RS (1996) The effects of soil sodicity on emergence, growth, development and yield of oil seed rape (*Brassica napus* L.). *Journal of Agriculture Science (Cambridge)* **126**, 169-173

Bohnert H, Nelson DE, Jensen RG (1995) Adaptations to environment stresses. *The Plant Cell* **7**, 1099-1111

Ellis RA, Roberts EH (1981) The quantification of ageing and survival in orthodox seeds. *Seed Science and Technology* **9**, 373-409

Huang J, Redman RE (1995) Salt tolerance of *Hordeum* and *Brassica* species during germination and early seedling growth. *Canadian Journal of Plant Science* **75**, 815-819

Janmohammadi M, Moradi Dezfuli P, Sharifzadeh F (2008) Seed invigoration techniques to improve germination and early growth of inbred line of maize under salinity and drought stress. *General and Applied Plant Physiology* **34**, 215-226

Karmoker JL, Farhana Sh, Rashid P (2008) Effects of salinity on ion accumulation in maize (*Zea mays* L. cv. Bari-7). *Bangladesh Journal of Botany* **37**, 203-205

Kaya MD, Day S (2008) Relationship between seed size and NaCl on germination, seed vigor and early seedling growth of sunflower (*Helianthus annuus* L.). *African Journal of Agricultural Research* **3**, 787-791

Kaya MD, Ipek A, Ozturk A (2003) Effects of different soil salinity levels on germination and seedling growth of safflower (*Carthamus tinctorius* L.). *Turkish Journal of Agriculture and Forestry* **27**, 221-227

Kaya MD, Okcu G, Atak M, Cıkılı Y, Kolsarıcı O (2006) Seed treatments to overcome salt and drought stress during germination in sunflower (*Helianthus annuus* L.). *European Journal of Agronomy* **24**, 291-295

Kaymakanova M (2009) Effect of salinity on germination and seed physiology in bean (*Phaseolus vulgaris* L.). *Biotechnology and Biotechnological Equipment* **23**, 326-329

Khajeh-Hosseini M, Powell AA, Bingham IJ (2003) The interaction between salinity stress and seed vigour during germination of soybean seeds. *Seed Science and Technology* **31**, 715-725

Khan ZH, Qadir I, Yaqoob S, Khan RA, Khan MA (2009) Response of range grasses to salinity levels at germination and seedling stage. *Journal of Agricultural Research* **47**, 179-184

Kratsch H, Olsen Sh, Rupp L, Cardon G, Heflebower R (2008) Soil salinity and ornamental plant selection. *Horticulture Cooperative Extension Utah State University* **02pr**, 1-8

Munns R, James RA (2003) Screening methods for salinity tolerance: A case study with tetraploid wheat. *Plant and Soil* **253**, 201-218

Nascimento WM (2003) Muskmelon seed germination and seedling development in response to seed priming. *Ciência Agrícola* **60**, 71-75

Okcu G, Kaya MD, Atak M (2005) Effects of salt and drought stresses on germination and seedling growth of pea (*Pisum sativum* L.). *Turkish Journal of Agriculture and Forestry* **29**, 237-242

Rahman MU, Soomro UA, Zahoor-ul-Haq M, Gul Sh (2008) Effects of NaCl salinity on wheat (*Triticum aestivum* L.) cultivars. *World Journal of Agricultural Sciences* **4**, 398-403

Rudd JJ, Franklin-Tong VE (2001) Unraveling response specificity in Ca^{2+} signaling pathways in plant cells. *New Phytologist* **151**, 7-34

Shahi-Gharahlar A, Farhoudi R, Mosavi M (2009) Effect of seed pretreatment on summer squash (*Cucurbita pepo*) seed germination and seedling characteristics under salinity condition. *Seed Science and Biotechnology* **3**, 60-63

Song JQ, Mei XR, Fujiyama H (2006) Adequate internal water status of NaCl-salinized rice shoots enhanced selective calcium and potassium absorption. *Soil Science Plant Nutrition* **52**, 300-304

Suhayda CG, Redman RE, Harvey BL, Cypywnky A (1992) Comparative response of cultivated and wild barley to salinity and calcium supply. *Crop Science* **32**, 154-163

Tavili A, Biniaz M (2009) Different salts effects on germination of *Hordeum vulgare* and *Hordeum bulbosum*. *Pakistan Journal of Nutrition* **8**, 63-68

Zhang Y, Mian MAR, Chekhovskiy K, So S, Kupfer D, Lai H, Roe BA (2005) Differential gene expression in *Festuca* under heat stress conditions. *Journal of Experimental Botany* **56**, 897-907