

Influence of Salinity Stress on Seed Germination and Seedling Early Growth Stage of Three *Secale* Species

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ABSTRACT

Soil salinity is one of the most important constraints that limit crop production in arid and semi-arid regions. Salinity also influences seed germination and it is crucial for the establishment of plants that grow in saline soils. This research was carried out in order to test the effects of different salinity levels on germination and early seedling growth traits of *Secale* species (*Secale montanum*, *Secale cereale* and *Secale ceremont*). Seed were treated with different salt solutions in an experiment using completely randomized design in three replications and 50 seeds/replicate. Experimental treatment included 6 levels of NaCl concentration (0, 50, 100, 200, 300 and 350 m mol/L). According to results the high levels of seed germination, germination rate, plumule length, and radicle length and seed vigor index were showed at control treatment. In 300 and 350 mM salinity concentration, the number of germinated seeds decreased significantly and there was not showed any germinated seed in *S. cereale* and *S. ceremont*. There was an evident decreasing in radicle and plumule length by increasing in NaCl concentration from 0 to 350 mmol/L. The highest germination rate obtained in control for *S. ceremont*. Mean germination time increased in *Secale* seeds by increasing salt concentrations. The highest radicle and plumule length were showed in control (3.53 and 7.42 cm, respectively). Vigor index decreased when salt concentration increased. The results indicated that appropriate treatment of the salt stress can improve the salt-tolerance in *Secale*.

Keywords: NaCl, *S. cereale*, *S. ceremont*, *S. montanum*, seed priming

Abbreviations: GP, germination percentage

INTRODUCTION

Salt stress is one of the major environmental stress factors that affect plant growth and development (Lu 2010). Seed germination is the most sensitive stage to abiotic stress (Khajeh-Hossaini *et al.* 2003; Patade *et al.* 2011). Over 400 million hectares of 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). Salt excess in soils and water has detrimental effect on crop yields and results in substantial losses of arable soils especially in the arid and semi-arid areas (Kennedy and de Filippis 1999). Salt and osmotic stresses are responsible for both inhabitation or delayed seed germination and seedling establishment (Al-Mansouri *et al.* 2001).

In vegetative plants, salt stress causes reduced cell turgor and depressed rates of root and leaf elongation (Werner and Finkelstein 1995), suggesting that environmental salinity acts primarily on water uptake.

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). Hence the ability of plants to tolerate and thrive in saline soils is of great importance in agriculture, since it indicates that the affected plants have genetic potential for salt tolerance, which is a highly desirable trait (Mahmood *et al.* 2000).

There has been considerable research on salt tolerance in natural species among ecotypes within species and in cultivars of crop plants. Rahman and Ungar (1990) reported that seed germination and seedling growth of *Echinochloa crusgalli* decreased due to salt stress. Also Shahi-Gharahlar *et al.* (2010) in *Cuminum cyminum* L. and Jalali *et al.* (2010) in *Festuca rubra* indicated that salt (NaCl) stress

decreased seed germination and seedling growth and increased mean germination time.

The genus *Secale* (Poaceae) is comprised of annual (rarely perennial) herbaceous species mainly growing in Mediterranean, Eastern Europe to central Asia, and South Africa (Sheidai and Ali-Jarrahei 2008).

Most regions in Iran are saline especially with NaCl (Hosseini 1994) so the aim of this study was to evaluate of salinity tolerance in three species of *Secale* (*S. montanum*, *S. cereale* and *S. ceremont*) to determine salinity tolerant species to improve and revival of some pastures in Iran.

MATERIALS AND METHODS

The seeds of *Secale montanum*, *S. cereale* and *S. ceremont* provided from Institute of Pakan Seed Isfahan, Isfahan, Iran. At first, the seeds were surface sterilized with sodium hypochlorite solution for 2-3 min separately and then washed with distilled water before utilization and finally disinfected by 75wp Thiram fungicide (0.09 Kg/25 Kg seeds) to prevent seeds from contamination. The seeds were sowed in the experiment. Salt solution was prepared to give the concentration levels (50, 100, 200, 300 and 350) m mol/L of NaCl, and distilled water was applied as control treatment. Three replicates of 50 seeds of each species were germinated between two rolled sheets of filter paper with 5 ml of each test solution. The filter paper was replaced every day to prevent accumulation of salts and seeds are incubated at 20 ± 1°C, RH 90% in 12 h day. A seed was considered germinated when 3 radicles emerged and coleoptile elongated to 2 mm. Seed germination were recorded every 24 h for 14 day. Radicle length and shoot length were measured at the end of the experiment. The experimental design was two factors factorial arranged in a completely randomized design (CRD) with three replications and 50 seeds per replicate.

Germination percentage was calculated using this formula:

$$GP = [\text{No. germinated seeds} / \text{Total No. of seeds}] \times 100$$

Germination rate was calculated using this formula:

$$GR = \sum [n / t]$$

n: number of germinated seed

t: germination period

Mean germination time (MGT) was calculated using the method of Cantliffe (1991):

$$MGT = A_1D_1 + A_2D_2 + \dots + A_nD_n / A_1 + A_2 + \dots + A_n$$

A: number of germinated seeds

D: day number during germination period

n: number of last day of recording

Vigor Index was calculated using this formula:

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

RL: radicle length

SL: shoot length

The experimental data was analyzed using SPSS ver. 17 software and treatment means were compared using Duncan's multiple range test (DMRT) at the 5% level of probability and graphs were drawn using Excel 2010 software.

RESULTS AND DISCUSSION

Germination percentage, germination rate and mean germination time

GP, GR and MGT were affected by salinity concentration, species and interaction of these factors (Table 1). Increasing salt concentration decreased GP and GR independent of the species. The highest GP (97%) obtained in the control treatment in *S. ceremont* and the lowest GP observed in 300 and 350 mmol/L of NaCl in *S. cereale* and *S. ceremont* (Fig. 1). In 50 mmol/L, the highest GP (49%) was observed in *S. ceremont*. Hanif and Davies (1998) reported that NaCl can decline germination in *S. cereale* and also in *Triticum aestivum* by increasing salinity. Bojović *et al.* (2010) reported that salt stress decreased germination in several crops such as *Solanum lycopersicum* L., *Brassica oleracea* and *Capiscum annuum*.

By increasing NaCl concentration, germination is delayed and decreased in all cultivars of bread wheat (Akbarimoghaddam *et al.* 2011).

Salt stress decreased GR in all species. The highest germination rate was related to control treatment with 39 mean in *S. ceremont*, while the lowest were observed in 300 and 350 mmol/L (Fig. 2). It could be due to existence of inorganic anion and cation decreased water potential that is because of its solubility in water (Singah *et al.* 1988). We also can say that this reduction in germination rate relies on salinity could be because of its bad effect on physiological processes which are effective on seed germination (Khan *et al.* 2002).

Salt stress increased MGT of *Secale* seeds (Fig. 3). Shahi-Gharahlar *et al.* (2010) noticed that MGT increased by increasing salt concentration in snake melon (*Cucumis melo* var. *flexuosus*). The highest MGT observed in 300 and 350 mmol/L NaCl in *S. cereale* and *S. ceremont* and 2.78 was least days that the seeds of *S. cereale* need to germinate. Tobe *et al.* 2002 reported that negative effect of salinity on seed germination and early growth could be due to the toxic effect of NaCl on seeds, or due to the osmotic effect, that prevents the seeds from imbibition.

Our findings agree with those of Atak *et al.* (2006) in triticale and Asci (2011) in red clover who determined that germination and seedling growth were reduced in saline condition while NaCl affected the germination of seeds. As a comparison, *S. cereale* germinated in a shorter time than the other species while *S. ceremont* needed longer time than the others to germinate.

Table 1 Analysis of variance of the effect of salinity and species on *Secale* L. seed germination and early seedling.

| Source of variance | Mean of square | | | |
|--------------------|---------------------|--------------------|-------------|------------|
| | Radicle length (cm) | Shoot length (cm) | Vigor index | GP (%) |
| salt | 3.30** | 2.73 ** | 34.20** | 694.825 ** |
| species | 26.61 ** | 22.65 ** | 48.92** | 657.05 ** |
| salt × species | 17.91* | 0.84 ^{ns} | 3.16* | 427.37 ** |
| Error | 4.99 | 0.23 | 16.4 | 26.7 |
| CV (%) | 14.64 | 21.98 | 12.58 | 9.22 |

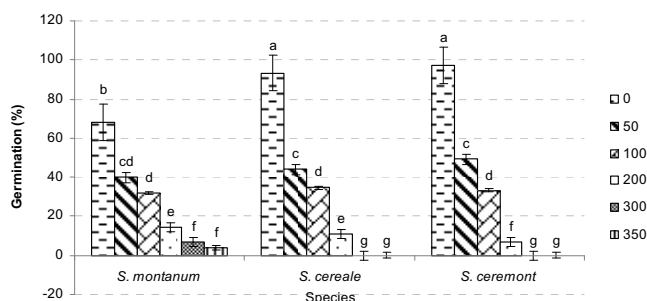


Fig. 1 Effect of salinity (mmol/L NaCl) and species on germination percentage of *Secale* L.

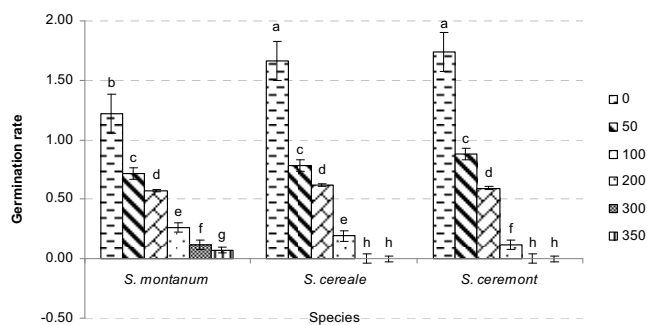


Fig. 2 Effect of salinity (mmol/L NaCl) and species on germination rate of *Secale* L.

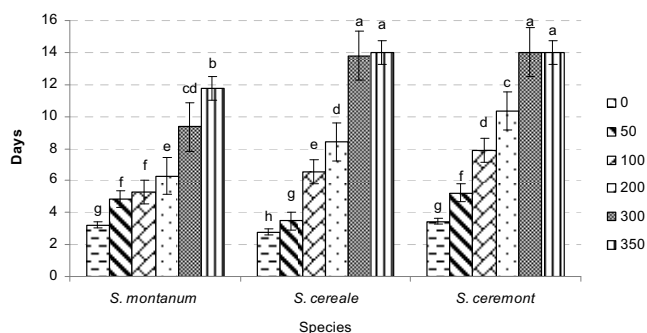


Fig. 3 Effect of salinity (mmol/L NaCl) and species on mean germination time (days) of *Secale* L.

Radicle and shoot length

Salinity and species had significant effects on both radicle and shoot length. Results showed that under non-saline conditions radicle length increased significantly when seeds were pretreated without NaCl. The longest radicle formed in control treatment in *S. montanum* with mean 3.53 cm and the shortest radicle formed in 350 mmol/L in *S. ceremont* with a mean of 0.57 cm (Fig. 4). Our results agree with those of Maghsoudi Moud and Maghsoudi (2008), who showed that *Triticum aestivum*'s radicle and coleoptile length was reduced in saline condition. Also, Jalali *et al.* (2010) reported that increasing salinity (NaCl) levels (from 0 to 150 mmol/L) resulted in a decrease in root length of

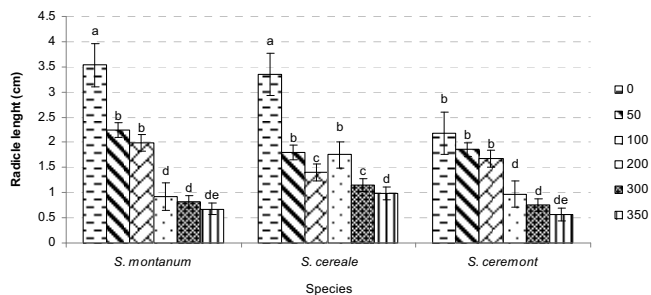


Fig. 4 Effect of salinity (mmol/L NaCl) and species on radicle length of *Secale* L.

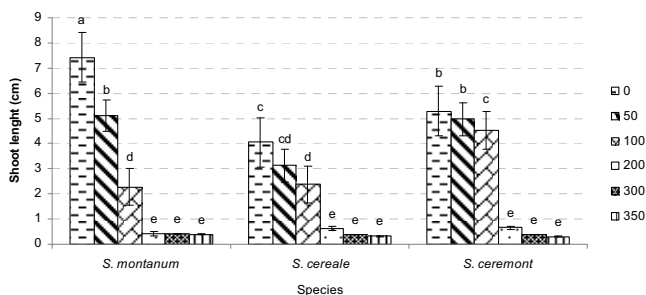


Fig. 5 Effect of salinity (mmol/L NaCl) and species on shoot length of *Secale* L.

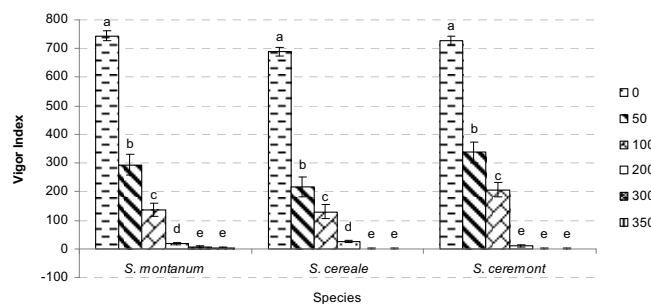


Fig. 6 Effect of salinity (mmol/L NaCl) and species on vigor index of *Secale* L.

Festuca rubra.

Fig. 5 shows that under non-saline condition between species there is significant effect. *S. montanum* had the longest shoot length and *S. cereale* showed the short shoots. **Fig. 5** shows that under saline condition the tallest shoots formed when seeds were sowed in control treatment and shortest shoot obtained in 300 and 350 mmol/L NaCl.

The shoot length of the three *Secale* species differed under the different salinity levels as shown in **Fig. 5**.

Okçu *et al.* (2005) showed that in pea (*Pisum sativum* L.) shoot length was severely influenced by salt and also no shoot length was recorded for some cultivars. Shoot and root lengths are the most important traits for selection under salt stress conditions, because roots are in direct contact with soil and absorb water (Jamil and Rha 2004) and nutrient from soil, and shoot grows until the root can fully support it. Toxic effects of NaCl as well as unbalanced nutrient uptake by seedlings may be responsible for reduction in root and shoot growth under salinity stress (Asci 2011). Salinity which is result of osmotic pressure leads reduction in water absorbance so cell division and differentiation reduce and reduction of plumule and radicle length will be explainable (Bijeh Keshavarzi *et al.* 2011).

Vigor index

Salinity influenced vigor index significantly. Vigor index decreased dramatically in saline treatments. At the high levels of salinity (300 and 350 mmol/L) the VI in all species except *S. montanum* was zero. *S. cereale* was more seriously

affected (**Fig. 6**). Similar results were found by Bijeh Keshavarzi *et al.* (2011) in spinach (*Spinacia oleracea* L.) seedlings and by Janmohammadi *et al.* (2008) in maize seedlings, which were significantly affected under different salt stresses. Seed vigor index is related to the special impact of ions and reduction of environmental water potential in the presence of salinity (Bijeh Keshavarzi *et al.* 2011).

ACKNOWLEDGEMENTS

The authors thank Dr. Jaime A. Teixeira da Silva for improving the figure quality.

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