Effects of salinity and drought stress on germination and seedling growth of *Avena fatua* L. and *Phalaris minor* L.

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Abstract

Avena fatua and *Phalaris minor* are narrow-leaved weeds that pose problems for many crops. In order to study the effects of sodium chloride (NaCl) and polyethylene glycol (PEG) on germination and initial seedling growth of *Avena fatua* and *Phalaris minor*, an experiment was conducted based on a completely randomized design with 4 replications under laboratory conditions during 2011-2012. Eight levels of NaCl: 0 (control), 50, 100, 150, 200, 250, 300, and 350 mM, and 8 PEG concentrations: 0 (control), 15.6, 100.7, 151.4, 190.5, 223.6, 279.3, and 326.2 g/L were used. The findings suggested that in both weed plantlets, increase in NaCl and PEG concentrations resulted in reduced seed germination, and seedling shoot and rootlet length, as compared with the control with no drought stress. In the treatments with high concentrations of NaCl and PEG, seed germination in both weeds approached zero which suggests their sensitivity to salt and drought stress. Overall, it seems that *Avena fatua* was more tolerant to salt and drought stress in comparison with *Phalaris minor.*

Key words: narrow-leaved weed; abiotic stress, NaCl; PEG

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Introduction

Biological study of the weeds has generally been considered an accepted practice in weed management. In fact, weed management programs cannot be properly implemented without an understanding of their biology and ecology (Norris, 2002). In any efficient weed control program, the study of weed biology is necessary from both economic and environmental points of view. This includes studies on morphology, germination, seed dormancy, growth physiology, competition power, and reproducing potential (Garcia-Baudin, 1999).

Generally, weeds pose numerous problems for agriculture and cause considerable damage to crop yield. Wild amaranth for example, produces more than 100000 seeds in each plant. Seeds of many weeds still maintain a portion of their potential to grow after 5.5 years. Therefore, even a small number of uncontrolled weeds are capable of producing enough seeds to trouble the farm for several years.

Many studies have been carried out on the factors involved in germination patterns and the importance of these factors in weed management. Identification of the factors

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affecting seed germination and growth of seedlings is useful to stop invasion of weeds to new regions and take novel measures in weed control (Peters et al., 2000). An understanding of the germination extent and time schedule has remarkable practical application in weed biology. Through determining the variability in germination behavior of different populations of the same weed, it is possible to apply appropriate control methods in different regions. Extensive studies have been reported on the abiotic factors such as light and alternative temperature on seed germination of weeds. Seasonal and daily fluctuations can control germination in weeds or limit it to the region close to soil surface (Pawlak et al., 1990).

Annual weeds because of sexual reproduction and abundant seeds are considered as the most problematic unwanted plants in the world (Holt and Orcutt, 1996). Among annual weeds, wild oat or *Avena fatua* and *Phalaris minor* are the most important weeds belonging to Poaceae family (Medd, 2006).

Wild oat or *Avena fatua* is native to Europe (Sharama, 2008) and is one of the most important weeds in more than 20 main crops in 55 countries. It is certainly the most important grass in wheat and barley farms around the world. It is reported as an important weed in irrigated and dry land cereal farms, lentils and other crops (Medd, 2006). This weed grows on abandoned farms, fallows, roadsides, gardens, and meadows. It grows in various light and heavy as well as alkaline to acid soils even though it prefers well-plowed lime and heavy soils. Wild oat is usually seen in pH 4.5 – 9. The highest population of wild oat is reported in wheat farms with annual precipitation between 375 and 750 mL (Medd, 2006). It is an annual, hexaploid, and T_3 weed with 2n=42 chromosomes. In the fields where *Avena fatua* is farmed, there is a possibility of crossing its species.

In *Phalaris minor*, glum has sharp and folded ends. It is an important weed in autumn crops such as wheat, barley and many vegetables. *Phalaris minor* was not considered an important weed by about 2 decades ago. Continuous application of herbicides to control wild oat brought *Phalaris* into focus as a weed. The origin of *Phalaris minor* is the Mediterranean and its other species include *P. brachystachi* and *P. paradoxa* (Kee et al., 2006).

Avena fatua and *Phalaris minor* are more prevalent in some regions in Iran than before. Considering the relatively insufficient attention of the researchers in the country to the weed biology and regarding the importance of anticipating the future course of action in weed control programs, a clear understanding of the abiotic factors on germination and growth of problematic weeds is an important initial step in biological studies of the weeds and this is the primary aim of the present research.

Materials and Methods

Optimal temperature for seed germination

Well-grown seeds of *Avena fatua* and *Phalaris minor* were obtained from Iranian Research Institute of Plant Protection, Weed Research Department. After removing the seed coat, *Avena fatua* seeds were sterilized in %5 sodium hypochlorite solution for 30-60 seconds. In order to break seeds dormancy, they were put in Petri dishes containing gibberellic acid instead of distilled water, or they were kept at 5 °C for 48 hrs. The Petri dishes were then transferred to a germinator with alternative temperatures 10 °C and 20 °C and 16/8 hr photoperiod. *Phalaris minor* seeds were also put in Petri dishes. The seeds were treated with concentrated sulfuric acid for 3 min followed by sterilization with %5 sodium hypochlorite for 1 min. The Petri dishes were then transferred to the germinator with alternating temperatures of 10 °C and 20 °C and 16/8 hr photoperiod.

Van Hoff relationship (1) and NaCl was used to obtain different osmotic potentials:

(1) $\Psi S = -miRT$

where ΨS is the osmotic potential (bar); m is the solution molarity; i is ionization coefficient; R is the universal constant for gases (0.0832 bar. Lit. and T is the temperature in Kelvin scale. Polyethylene glycol (PEG 6000) was used to obtain various Matric potentials through equation (2):

(2) $\Psi = -(1.18\times10^{-2})\ C - (1.18\times10^{-4})\ C^2 + (2.67\times10^{-4})\ CT + (8.39\times10^{-7})\ C^2 T$

where C is the osmotic potential (bar); Ψ is the polyethylene glycol content ($gL^{-1)}$; and T is the temperature (°C).

Salt stress

7 mL sodium chloride solution with concentrations of 0 (control), 50, 100, 150, 200, 250, 300 and 350 mM were added to the Petri dishes containing seeds of *Avena fatua* and *Phalaris minor*. The dishes were then kept in the germinator under the aforementioned temperature conditions. After 21 days, the effects of various salinity treatments were recorded on seed germination, and length of seedling rootlets and shoots.

Drought stress

In order to investigate the effects of drought stress on germination of *Avena fatua* and *Phalaris minor* seeds, 0 (control), 15.6, 100.7, 151.4, 190.5, 223.6, 279.3, and 326.2 g/L polyethylene glycol were added to the Petri dishes containing their seeds. The dishes were then kept in the germinator for 21 days under the mentioned temperature conditions. Then, the effects of drought stress treatment on seed germination, and length of seedling rootlets and shoots were determined.

Table 1
Analysis of variance of the effect of salinity and drought stress on seed germination of *Avena fatua* and *Phalaris minor*

		Avena fatua	*Phalaris minor*
Source of Variation	D.F.		
Treatment	3	49.90**	8.36**
Error	12	0.45	0.017
C. V. (%)		10.92	4.20

**: significant difference at 0.01 probability

Table 2
Analysis of variance of the effect of salinity and drought stress on seedling growth of *Avena fatua* and *Phalaris minor*

		Avena fatua		*Phalaris minor*	
Sources of Variation	D.F.	Rootlet Length	Shoot Length	Rootlet Length	Shoot Length
Treatment	7	0.17**	0.266**	1.58**	2.70**
Error	16	0.021	0.014	0.043	0.10
C. V. (%)		13.58	10.66	7.90	11.28

**: significant difference at 0.01 probability

Statistical analysis

The experiment was carried out through a completely randomized design with 8 treatments and 4 replications. The treatments included sodium chloride 0, 50, 100, 150, 200, 250, 300, and 350 mM and polyethylene glycol 0, 15.6, 100.7, 151.4, 190.5, 223.6, 279.3, and 326.2 g/L. The mean differences were adjudged using Duncan's Multiple Range Test.

Results

Effects of salinity and drought stress

Analysis of variance of the effects of salinity and drought stress on seed germination of *Avena fatua* and *Phalaris minor* and the length of rootlets and shoots of these plants after 21-day treatment suggested that these treatments made significant changes at p≤01 (Tables 1 and 2).

Interaction of weed species, salinity and drought stress on seed germination

The findings suggested that *Avena fatua* had the highest seed germination (60%) in response to 50 mM sodium chloride which was significantly different in comparison with other salinity treatments except 100 mM sodium chloride and 100.7 gL^{-1} polyethylene glycol. Increase in concentration of sodium chloride solution led to decrease in seed germination. *Avena fatua* seeds did not germinate under 300 and 359 mM sodium chloride treatments. These treatments belonged to the same statistical group as 150, 200, and 250 mM sodium chloride and 223.6, 279.3, and 326.2 gL^{-1} polyethylene glycol.

Avena fatua showed the highest seed germination (82.5%) in response to 15.6 gL^{-1} PEG which was significantly different in comparison with other PEG concentrations. Increase in polyethylene glycol concentration led to a reduction in seed germination and at 326.2 g L^{-1} polyethylene glycol concentration, *Avena fatua* seed germination was zero (Fig. I).

The highest germination in *Phalaris minor* seeds (82.5%) were recorded in response to 50 mM sodium chloride. Increasing sodium chloride concentration resulted in some fluctuations in seed germination of the weeds which necessitates repetition of the experiments to reach a conclusive result. Germination of *Phalaris minor* seeds was zero at 300 mM sodium chloride treatment which belonged to the same statistical group as 200, 250, 300, and 350 mM sodium chloride and 226.7, 279.3, and 326.2 g L^{-1} polyethylene glycol treatments.

Under drought stress, the highest germination in *Phalaris minor* seed (87.5%) was recorded in response to 15.6 gL^{-1} polyethylene glycol which was in the same statistical group as 50 mM sodium chloride and 100.7 gL^{-1} polyethylene glycol. Increase in the concentration of polyethylene glycol reduced seed germination and at 216.2 gL^{-1} PEG, germination percent was zero (Fig. II).

Interaction of weed species, salinity and drought stress on seedling rootlet length

Interaction of effects of salinity and drought stress, and the weed species on seedling rootlet length showed that *Avena fatua* had the longest rootlets (10.8 cm) in 50 mM sodium chloride treatment which was significantly different from other treatments except 151.4 and 190.5 g L^{-1} polyethylene glycol. Increasing the concentration of sodium chloride decreased seedling rootlet length in *Avena fatua* and at 300 and 350 mM NaCl, it was zero.

Under drought stress, *Avena fatua* seedlings had the longest rootlets (9.62 cm) in response to 15.6 gL^{-1} polyethylene glycol. As concentration of polyethylene glycol increased, seedling rootlet length decreased reaching zero in 223.6 gL^{-1} polyethylene glycol (Fig. III).

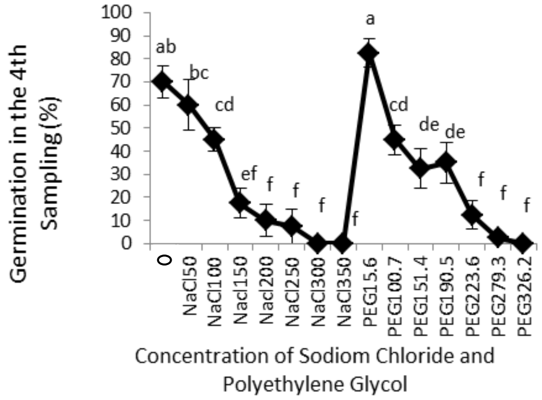

Fig. I. Germination rate of *Avena fatua* L. seeds in response to salinity and drought stress after 21 days

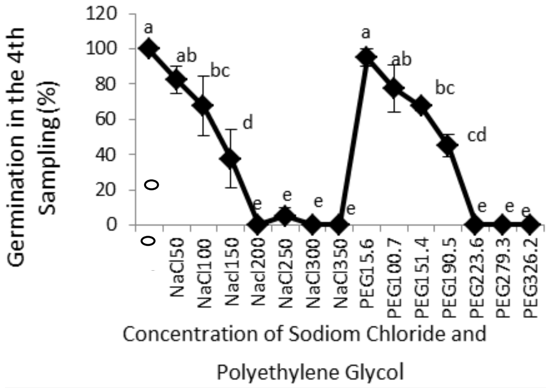

Fig. II. Germination rate of *Phalaris minor* seeds in response to salinity and drought stress after 21 days

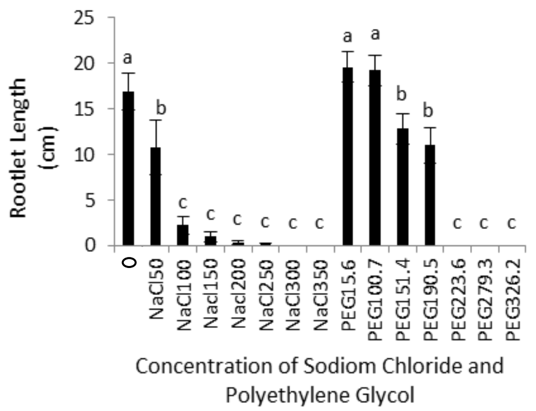

Fig. III. Length of *Avena fatua* L. rootlets under salinity and drought stress

Phalaris minor seedlings had the longest rootlets (2.8 cm) in 50 mM sodium chloride and there was no significant difference between 100.7 and 115.4 gL^{-1} polyethylene glycol. Increase in sodium chloride concentration led to a reduction in seedling rootlet length and at 300 and 350 mM sodium chloride, this parameter was zero. These treatments belonged to the same statistical group as 100, 150, 200, and 250 mM sodium chloride and 223.6, 279.3, and 326.2 gL^{-1} polyethylene glycol.

In drought stress conditions, the longest rootlets for *Phalaris minor* seedlings (4.44 cm) were observed in response to 15.6 gL^{-1} polyethylene glycol. As concentration of polyethylene glycol increased, rootlet length in *Phalaris minor* seedlings decreased reaching zero in 223.6 gL^{-1} polyethylene glycol.

Interaction of weed species, salinity and drought stress on seedling shoot length

Interaction of effects of salinity and drought stress, and weed species on shoot length showed that *Avena fatua* had the longest shoots (9.75 cm) in 50 mM sodium chloride which were significantly different from other treatments except 190.5 gL^{-1} polyethylene glycol. Increasing the concentration of sodium chloride decreased shoot length in *Avena fatua* seedlings and at 300 and 350 mM concentrations, shoot length was zero.

Avena fatua seedlings had the longest shoots (17.05 cm) under drought stress in response to 15.6 gL^{-1} polyethylene glycol which is in the same statistical group as 100.7 and 151.4 gL^{-1} polyethylene glycol. As concentration of polyethylene glycol increased, shoot length in *Avena fatua* seedlings decreased reaching zero in 223.6 g L^{-1} polyethylene glycol (Fig. IV).

Phalaris minor seedlings produced the longest shoots (3.81 cm) under 50 mM sodium chloride. Increase in sodium chloride concentration did not result in a regular pattern in shoot length where some fluctuations were observed in this parameter and there is a need to repeat the experiment to reach conclusive results. At 300 and 350 mM sodium chloride concentrations, shoot length was zero.

In response to drought stress, *Phalaris minor* seedlings produced the longest shoots at 15.6 gL^{-1} polyethylene glycol. As concentration of polyethylene glycol increased, shoot length of the seedlings decreased reaching zero at 223.6 gL^{-1} polyethylene glycol (Fig. VI).

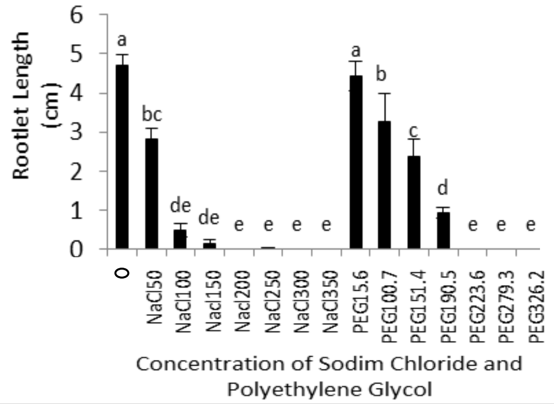

Fig. IV. Length of *Phalaris minor* rootlets under salinity and drought stress

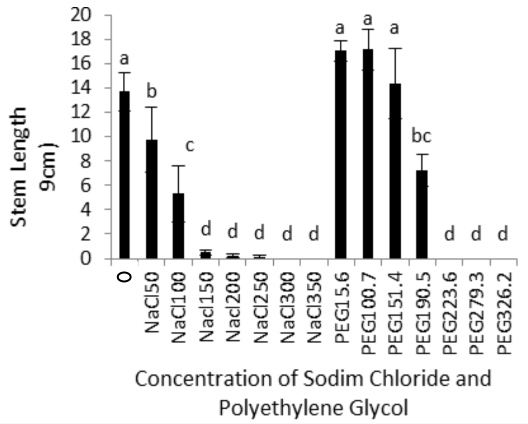

Fig. V. Length of *Avena fatua* L. stems under salinity and drought stress

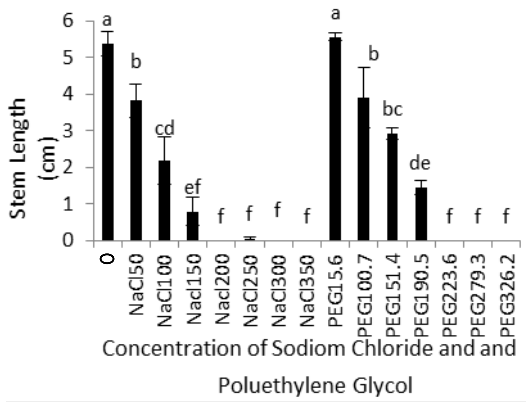

Fig. VI. Length of *Phalaris minor* stems under salinity and drought stress

Discussion

Seed germination, shoot length and rootlet length in the seedlings under study reduced with an increase in sodium chloride and polyethylene glycol concentrations compared with the control. In *Avena fatua* seedlings, seed germination was more resistant to salinity and drought stress compared with shoot and rootlet length. Rootlet length was more sensitive to salinity and drought stresses compared with shoot length. In *Phalaris minor* seedlings, germination and shoot and rootlet growth showed similar responses to salinity and drought stress.

Simpson (2008) after a 4-day treatment of *Avena fatua* seeds with various polyethylene glycol osmotic potentials in light condition suggested that the reduce in the osmotic potential of polyethylene glycol solution (0 – 21 bar) increased secondary dormancy and then reduction of osmotic potential from 75% to 45% of polyethylene glycol 6000 solution reduced the secondary dormancy. This is in agreement with the findings of the present study.

Reduction of osmotic potential is the well-known result of combined salinity and drought stresses. This means that under both these abiotic stresses, the plant loses its internal osmotic potential. This is done through augmentation of mineral ions or synthesis and augmentation of organic substances. While the first reaction is more important under salinity stress, in drought stress conditions the second reaction is of principal importance (Yan et al., 2012).

Salinity stress is an important ecological factor and in fact it is the most serious limiting factor in plant growth (Gulick and Dvorak , 2000). The main challenge for the plant in saline soils is that, since the external osmotic potential is much less than in non-saline soils, high amount of osmotic substances must be augmented to obtain water potential slope which in turn facilitates transfer of water into the plant. On the other hand, increased concentration of ions in cytoplasm will eventually lead to toxic concentrations (Gouia *et al*., 2004). Plants are usually adapted to salinity through reducing their internal osmotic potential in order to absorb water. The mechanism of this osmotic adaptation involves ion absorption and a change in the balance between organic and mineral ions present in the cellular sap.

A study on the effects of drought stress in seven indigenous and exotic weeds in northeast of Australia suggested that germination in indigenous weeds subjected to water shortage significantly reduced and the exotic weeds were more resistant to drought stress (Pérez-Fernández, 2011). Treatment of weed seeds with 50, 100, 150, 200, 250, 300, 350, 400, 450, and 500 mM sodium chloride showed that increase in sodium chloride concentration reversely affected plantlet growth (Yelmas et al., 2006). This same study suggested that root growth in 250 mM concentration of sodium chloride remarkably decreased and at 500 mM concentration the ratio of water to biomass severely reduced in the plants under study (Yelmas et al., 2006). Finally, a study on germination and growth of *Schinopsis quebracho Colorado* seedlings under 0– 300 mM sodium chloride revealed that up to 100 mM concentration of sodium chloride had no effect on seed germination. However, at 200 – 300 mM concentrations the seeds did not germinate (Meloni et al., 2008)

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