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Salt Tolerance at Germination of Two Forage Grasses for Reclamation of Salinity Habitats

Alireza Shahriari[1]

[1] Faculty of Natural Resources, University of Zabol, Zabol, Iran

Correspondence: Alireza Shahriari, Faculty of Natural Resources, University of Zabol, PO box98615-538, Zabol, Iran. Tel: 98-091-5541-7882. E-mail: nimaaryan2002@yahoo.com

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Abstract

Environmental stresses are among the most limiting factors to crop plant productivity. Salinity is one of the most detrimental ones. Establishment of seedlings at early growth stages of crop plants as one of the most important. Determinants of high yield are severely affected by soil salinity. Therefore, high germination rate and vigorous early growth under salty soils is preferred. *Aeluropus lagopoides* and *A. littoralis* are perennial grasses distributed in many saline areas and they are important economic plants used for sand fixation, pastures and other purposes. Seed germination is the critical stage for species survival. Seeds of halophytes usually show optimal germination in freshwater similar to glycophytes, but differ in their ability to germinate at higher salinities. The main objective of this study was to determine seed germination percentages and rates of *Aeluropus lagopoides* and *A. littoralis* when exposed to increased salinity. Mature seeds were collected from natural populations and a factorial complete randomized design of seven salinity levels 0 (control), 75, 150, 225, 300, 375 and 450 mM NaCl were used. Seeds were incubated in light at 25˚C and checked daily for 14 days. Maximum germination occurred in absence of salt, and salinity significantly decreased germination percentages and rates. The highest and the lowest resistance to increased salinity were observed in *Aeluropus littoralis* and *Aeluropus lagopoides* respectively. Thus, *Aeluropus littoralis* is more suitable than *Aeluropus lagopoides* for reclamation of salinity habitats.

Keywords: germination, salinity, grass, Aeluropus lagopoides, Aeluropus littoralis

1. Introduction

Biotic and abiotic stresses affect germination and the growth of plants (Saberi et al., 2011). Saline soils are a major environmental, agricultural and community problem in arid and semi-arid regions, where soil salt content is high and precipitation is insufficient to leach them. In these regions, planting salt-tolerant species, particularly N2-fixing species, is the most useful approach to rehabilitating salt-affected degraded lands (Oba et al., 2001). About 23% of the world's 1.5 × 109 ha of cultivated land is saline and 37% is sodic. Approximately one third of the world's irrigated land is salt-affected due chiefly to unsustainable irrigation practices (Khan & Duke, 2001). Salinity induced inhibition of plant growth may occur due to the effects of high Na^+, Cl^- or SO_4^{2-} by decreasing the uptake of essential elements such as P, K, NO_3 and Ca; ion toxicity or osmotic stress (Zhu, 2002).

Salts can affect seed germination either by restricting the supply of water (osmotic effect) or causing specific injury through ions to the metabolic machinery (ionic effect). Moreover most seeds are located near the soil surface, where salt concentration changes due to continuous evaporation of ground water (Ungar, 1991). However, rainfall can quickly leach salt from the surface and supply water to the seed. Thus, for successful establishment of plants in saline environments, seeds must remain viable in high salinity conditions and germinate when salinity decreases (Khan & Ungar 1997). Halophyte seeds are known to remain viable for extended periods of time during exposure to high salinity and germinate when salinity is reduced (Khan & Ungar, 1998; Berrichi et al., 2010). Salinity and its effects on biomass production have been considered by numerous authors (Mehari et al., 2005; Maghsoudi & Maghsoudi, 2008). Salt tolerance of halophytic grasses varies with the ecotype, species, habitat and other environmental factors (Gulzar et al., 2003b). Plants of grasses like *Urochondra setulosa* can survive salinities of up to 1000 mM NaCl (Gulzar et al., 2003a), while seeds of a number of grasses survive in salinity (550 to 600 mM NaCl) approaching seawater (Hester et al., 2001). Some grasses grow in soil salinities ranging between 300 to 500 mM NaCl (Bell & O'Leary, 2003; Peng et al., 2004), while others cannot survive in a salt concentration above 300 mM NaCl (La Peyre & Row, 2003).

Aeluropus lagopoides and *A. littoralis* (Linn.) Trin. Ex Thw. (Poaceae) are salt-secreting rhizomatous perennial grasses distributed from northern Africa (Morocco to Somalia), Sicily and Cyprus, through the Middle East to Central Asia, Iran, Pakistan and India (Cope, 1982). *Aeluropus lagopoides* and *A. littoralis* are distributed in many sandy and saline areas of deserts in Iran. Species of *Aeluropus* occur in saline habitats and could be used to reclaim salinized agricultural and rangeland. Since both of these grasses are extensively used as fodder in developing countries (Gulzar et al., 2003a), they have great potential for use as forage, silage and for biological reclamation of saline wastelands. They are also used for sand fixation, pastures and other purposes. These grasses are capable of vegetative reproduction through rhizome growth after monsoon rains and also can produce numerous flowers and seeds from April to October (Gulzar & Khan, 2001). They are often found in association with *Cressa cretica* in the inland communities and with *Cyperus arenarius*, *Cressa cretica*, and *Halopyrum mucronatum* in coastal communities located at the backwaters of the Hamoon Wetland in Iran. Indeed, little information is available on the salt tolerance of these two species *of Aeluropus* in Iran. Therefore, the purpose of this investigation was to examine effects of seven concentrations of NaCl on seed germination percentage and speed of *Aeluropus lagopoides* and *A. littoralis***.**

2. **Materials and Methods**

Mature seeds were collected from natural populations in the four natural populations (Iran).

These species include two species *are A. littoralis* and *A. lagopoides* for any two samples from different growing regions were considered, so that *A. littoralis* source of the Qom river and Mighan desert and *A. lagopoides* source of Hoveize and Darkhovin chosen.

Seeds were separated from inflorescence in the laboratory, then cleaned and dry-stored in a refrigerator at 4˚C after surface sterilization with 10% (v/v) sodium hypochlorite. Seeds were germinated in 10 cm diameter Petri dishes on one sheet of Whatman No.1 filter paper, moistened with 5 ml of the test solution. The Petri dishes were sealed with transparent tape. Fifty seeds were used for each treatment with four replicates each. We used a factorial complete randomized design swith even salinity treatments of 0 (control), 75, 150, 225, 300, 375 and 450 mM NaCl. The experiment was conducted in a programmed incubator at 25˚C with a 14:10 hr light/dark cycle each day and at 70% relative humidity.

Germination was recorded on alternate days (every 48 h) for 14 days. Germination percentage and rate were measured, Rate of germination was calculated with a modified Timson's germination velocity index: £G/T, where G is the percentage of seed germinated after 2 d intervals, and T is the total time of germination (Khan & Ungar, 1998).

Statistical analyses were carried out using SPSS version 10. A one-way ANOVA was used to determine the differences among treatment group means for percent germination, rate of germination and recovery percent germination A Duncan's multiple comparison test was also done to determine if significant ($p<0.05$) differences occurred between individual treatments.

It should be noted that the habitat *A. lagopoides* Hoveize of germination was very low (even in control conditions (1/5 percent)). Therefore was excluded from analysis of data and data analysis was done with other species. For ease of analysis and conclusions presented in each habitat was given a code. *A. lagopoides* of habitat for Darkhovin Code 1, Code 2 for *A. littoralis* of Qom river habitat for the desert *A. littoralis* Mighan code 3 was considered.

3. Results

Results of variance analysis the effects of concentrations of NaCl on *A. lagopoides* and *A. littoralis* on properties included germination percentage and germination speed were reported in Table 1.

Table 1. A one-way ANOVA of the effects of salinity species and their interaction on germination of species seeds

Source of variation	Sum of Squares	df	Mean Square	F	P
Effect of salinity	23311.717	6	3885.286	6.526	0.00**
Effect of Species	30728.496	2	15364.382	32.388	0.00**
Effect of interaction salinity and species	66440.634	20	3322.032	77.141	0.00**

** Significant at 0.01 level of probability

A one-way ANOVA indicated significant individual effects (P<0.01) of salinity, species and their interaction on percent and rate of germination of *Aeluropus* seeds. Mean comparison Duncan's multiple indicated significant difference (P<0.01) between species seed germination (Table 2).

Table 2. The effect of species on seed germination

Code	Mean germination	Class
1	4.02	c
2	43.88	a
3	13.33	b

a, b, c: denote significant difference among species at 0.01 level of probability

Results showed that germination percentage of *A. lagopoides* and *A. littoralis* was reduced when NaCl concentrations increased. Maximum germination for code 1 (16.43%), code 2 (90.43%) and code 3 (44%) was related to control treatment (Figure 1). though maximum seed germination percentage was related to control treatment (Figure 1).

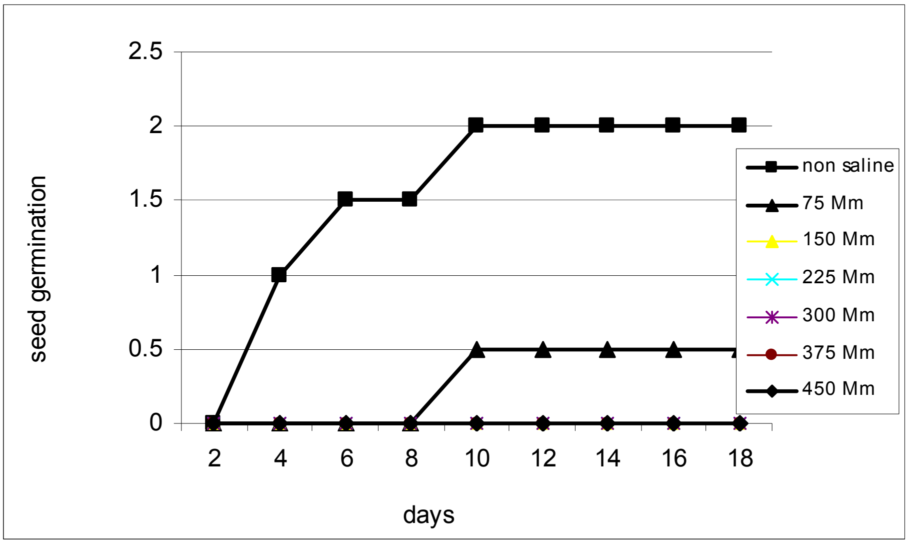
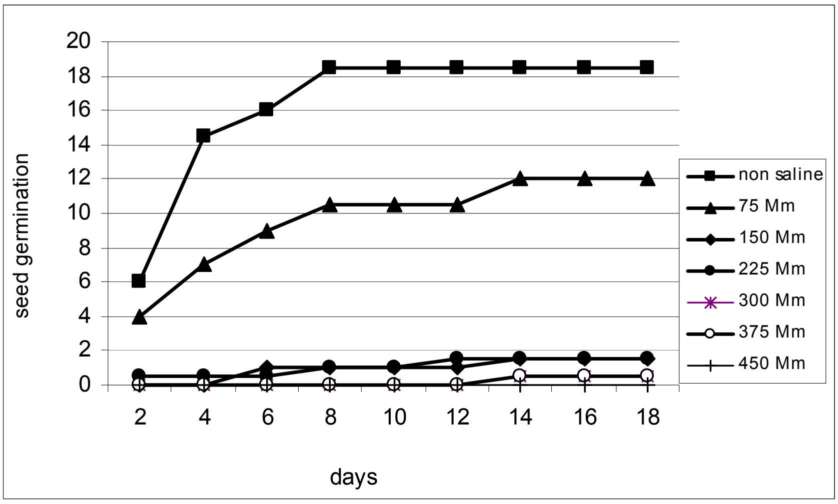
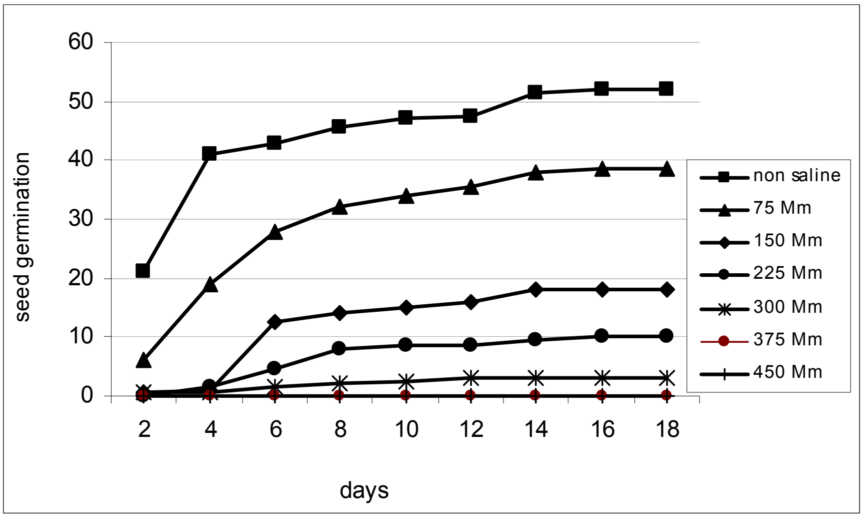


Figure 1. Germination percentage of species seeds in different salinity

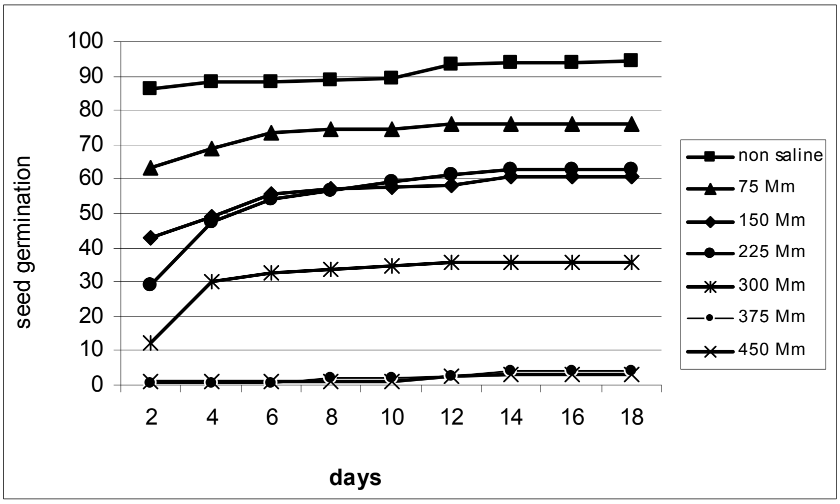
Cumulative mean percent germination of species seeds over time in 0, 75, 150, 225, 300, 375 and 450 mM NaCl in 14 h light: 10 h dark photoperiod and 70% relative humidity (Figures 2, 3, 4).



(a)



(b)



(c)

Figure 2. Mean final percent germination of Aeloropus sp. in various salinity: (a) Code 1 (b) Code 2 (c) Code 3

4. Discussion

Ion ratios could be helpful in categorizing the physiological response of a plant (salt excluding, salt-secreting or salt-diluting) in relation to ion selectivity under increasing substrate salt concentrations (Wang et al., 2002).

Although most halophyte species control accumulation of inorganic ions as the basic mechanism to adjust the osmotic potential so their internal tissues to protect against external salinity, they differ widely in the extent to which they accumulate inorganic ions (Ghassemi-Golzani et al., 2011). Even between species of a single genus, this difference is sometimes of such a high level that efficient salt-tolerant species can be screened within a single genus (Khan et al., 2002). *Aeluropus* species are said to respond differently to salinity.

The test species differed greatly in their response to salinity, as quantified by the percentage germination and the results showed a significant effect of salinity on germination percentage ($P<0.01$). Despite high tolerance of halophytes to salt, maximum germination occurred in the salt free condition, as maximum germination for code 1 (16.43%), code 2 (90.43%) and code 3 (44%) was obtained in non-saline control and it decreased in 450 mM NaCl. Therefore increase in salinity progressively inhibited germination. Gulzar and Khan (2001) reported best germination of halophytic grasses is obtained under nonsaline conditions and their germination decreases with increases in salinity.

Grasses seeds usually are not very tolerant of increased salinity (Khan & Ungar, 2001), and the maximum salt tolerance for germination usually ranges between 250 and 350 mmol/L NaCl (Morgan and Myers, 1989; Gulzar & Khan, 2001). *Spartina alterniflora* is an exception; it can germinate at concentrations higher than 400 mmol/L NaCl (Mooring et al., 1971). It appears from our data that halophytic grasses from coastal areas of Iran are among the highly salt tolerant grasses at germination stage and have the ability to germinate in warm conditions.

The highest and the lowest salt resistance were observed in *Aeluropus littoralis* and *Aeluropus lagopoides* respectively. Recruitment of *Aeluropus* spp. in natural conditions through seed germination appears to take place after monsoon rains. Germination of halophyte seeds in subtropical coastal and inland salt marshes usually occurs after monsoon rains, which causes a reduction in temperature and a lowering of soil salinity (Khan & Gul 1998; Khan & Ungar 1998). Therefore salinity has posed adverse effects on germination of the species. These results are consistent with other studies (Ashraf & McNeilly, 1986; Ashraf et al., 1989) where germination inhibition under saline condition has been reported for grass species.

Since seeds of *A. lagopoides* are highly sensitive to salinity, the species is unable to propagate through seeds in nature unless salinity is greatly reduced. Thus, *Aeluropus littoralis* is more suitable than *Aeluropus lagopoides* for reclamation of salinity habitats.

In summary, our results showed that *A. littoralis* is a highly salt tolerant halophytic grass, and it may be used as a turf grass in the areas where freshwater is not available or to stabilize sand dunes both in the coastal and inland saline deserts of Iran. Gulzar et al. (2003a) indicated that *Aeluropus* species could also be used in increasing forage production in salt affected wastelands because of its high protein content and high salinity tolerance. This grass is used locally as a fodder for livestock and could be useful in coastal sand dune stabilization. It is currently being grown experimentally in the field as a fodder using brackish water irrigation.

5. Conclusion

The in vitro study of the germination of seed Aeluropus plant under saline stress enabled us to conclude that concentrations of NaCl limited the seeds germination. The salt seemed to affect the opening of the plumule rather than germination. These results can important to understanding of salinity mechanisms and founding solutions to prevent of infiltration of these elements to valuable grassland plants. A total this grass is used locally as a fodder for livestock and could be useful in coastal sand dune stabilization. It is currently being grown experimentally in the field as a fodder using brackish water irrigation.

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