Study of Seed Germination Ecology of Rye Grass (*Lolium perenne* L.) and goat grass (*Aegilops cylindrica* Host.)

Mohammad Rezvani*, Noorallah Taghinia and Hasan NikkhahKouchaksaraei

Department of Agronomy and Plant Breeding, Qaemshahr Branch, Islamic Azad University, Qaemshahr, Iran
* Corresponding author, E-mail: m_rezvani52@yahoo.com
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ABSTRACT

In a series of labrotary and green house experiments the effect of salt and osmotic stress, pH, leaching duration and flooding depth and duration on seed germination of goat grass (*Aegilops cylindrica* Host.) and rye grass (*Lolium perenne* L.) was investigated. The experiments were carried out as completely randomized design with three replicates. Results indicated that about 45–55% of freshly harvested goat grass seed were dormant while percentage of rye grass dormant seed was about 20–24. Enhancement of both osmotic and salt stresses significantly reduced seed germination. Both species were fairly tolerant to high water and drought stresses. The pH higher and/or lower than those of 7 significantly reduced germination, while in rye grass germination was limited in pH higher than 7. Long-term flooding for four weeks in all burial depth increased seed germination campare to control treatment. While, short-term flooding and also leaching induced a temporary dormancy in both species. The knowledge obtained from this investigation will increase our information about the germination behavior of goat grass (*Aegilops cylindrica* Host.) and rye grass (*Lolium perenne* L.) in different habitate that could be effective for improving management of the plants in agroecosytems.

Keywords: Osmotic and salt stresses, pH, flooding depth, leaching duration

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INTRODUCTION

Seed germination is the crucial stage of life cycle a plant. Germination of plants seed influences by environmental conditions such as temperature, light, pH, salt and drought stresses (Probert *et al*., 1985). Temperature affects on the capacity and rate of germination. Many weed species germination increased by fluctuating temperatures (Bazzaz, 1979). Chachalis *et al*. (2008) reported higher germination of venice mallow (*Hibiscus trionum*) seeds in fluctuating temterature than the constant ones. Light is an effective environmental factor on germination. Weed seeds that require light for germination normally emerge when scattered to the soil surface and remain dominant in notill or reduced-tillage systems (Chauhan and Johnson, 2010). High seed germination of some weeds occurs over a broad pH range but in the others seed germination is limited by high or low pH. Rezvani and Fani Yazdi (2013) and Fani Yazdi *et al*. (2013) reported germination of black night shade (*Solanum nigrum*) and sheep sorrel (*Rumex acetosella*) in a wide range of pH. Salt and drought stresses influence on seed germination of weeds. There are many reports that show seed germination reduces by the stresses (Chachalis and Reddy, 2000; Chauhan and Johnson, 2010).

Irrigation and flooding are common practices in agricultural ecosystems; seeds in orchards and fields must be able to germinate at low oxygen concentrations (Karimmojeni *et al*., 2011). Therefore, understaning of seed longevity in flooding conditions is important to seed germination in the next crop. Seed longevity declination by flooding can lead to reduction in weed population.

Goat grass (*Aegilops cylindrica* Host.) is an annual and rye grass (*Lolium perenne* L.) is a perennial winter weed belonges to the Poaceae family. These weeds infested winter crops (wheat and canola) and orchards in Qaemshahr, Mazandaran Province, Iran. But, there is a lack of knowledge about seed germination ecology of the weeds in Iran. The objective of the study was to obtain information about the effect of water and salt stress, pH, leaching duration, burial depths and flooding duration on seed germination.

MATERIALS AND METHODS

Seed Collection

Goat grass (*Aegilops cylindrica* Host.) and Rye grass (*Lolium perenne* L.) seeds were collected from plants growing in wheat field and citrus gardens at Qaemshahr (36° 38′ N and 52° 43′ E), Mazandaran Province, Iran on July, 2012. Total monthly rainfall and means of monthly temperature during the mother plants growth in 2011–2012 presented in Figure 1.

Seeds were harvested randomly from more than 1000 plants that had completely ripened and were separated from spike manually and stored in labrotory at 20 ± 5°C until experiments started on November, 2012. The 1000-seed weight, seed length and width were determined by using digital caliper. Seeds were tested for viability with the use of 1% tetrazolium chloride solution before each trial (Peters, 2000).

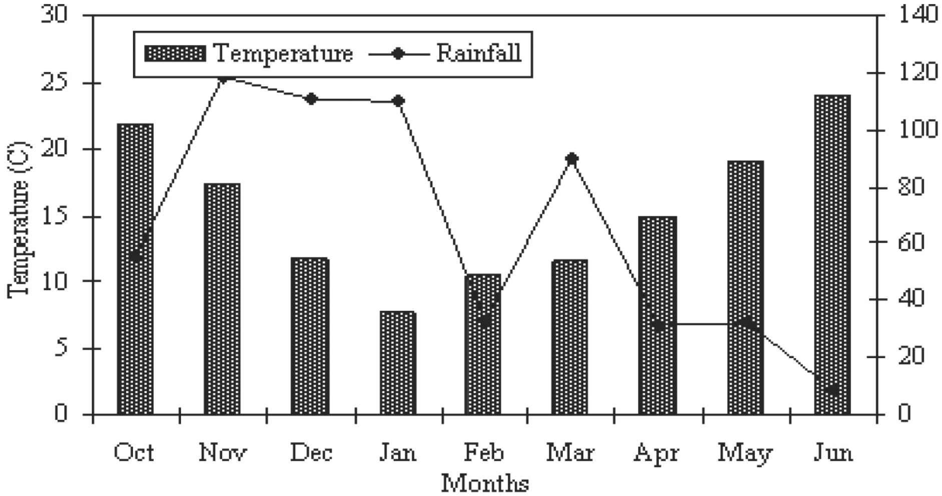

Figure 1 Total monthly rainfall and mean of monthly temperature during the mother plants growth in 2011–2012

Germination Protocol

Seeds were sterilized with 1% sodium hypochloride solution for 1 min and then rinsed 6 times with deionized water. Thirty seeds were placed on a single layer of filter paper in a 9-cm plastic petri dish. The filter paper were moistened with 6 ml of deionized water in flooding duration and leaching duration seed germination test and or treatment solutions in salt and osmotic stresses and pH experiments. Petri dishes were sealed with Parafilm and incubated at temperature 25/15°C (day/night). The photoperiod was set at 12/12 hours (day/night). Fluorescent lamps were used to supply a light intensity of 300 µmol m^{-2} s^{-1}.

The number of germinated seeds was calculated 15 days after the start of tests. The seeds were determined as germinated when produced a sible radicle higher than 1 mm length (Chejara *et al*., 2008).

Effect of Osmotic Stress on Germination

The effect of osmotic stress on seed germination of goat grass and rye grass with water solutions of different osmotic potentials including 0, -0.1, -0.2, -0.4, -0.6, -0.8 and -1 MPa was evaluated. Solutions were prepared by dissolving 0, 4.34, 7.73, 10.16, 12.81, 15.06 and 17.04 g of polyethylene glycol (PEG 6000) in 100 cc of deionized water, respectively. Petri dishes were placed in incubator at a temperature of 25/15°C and photoperiod 12/12 hours

Effect of Salt Stress on Germination

The impact of salt stress on seed germination of goat grass and rye grass was studied by solutions containing 0, 25, 50, 100, 200 and 400 mM NaCl (Merck, Germany). Petri dishes were incubated at a temperature of 25/15°C and photoperiod 12/12 hours.

Effect of pH on Germination

To evaluate the effects of pH on seed germination of goat grass and rye grass, buffered solutions of pH including 3, 5, 7, 9 and 11 were prepared according to Chachalis and Reddy (2000) method. Deionized water (pH 7.2) was used as control treatment. The seeds were incubated at a 12/12 hours photoperiod and 25/15°C temperature.

Effect of Leaching Duration on Germinetion

In two separate experiments, thirty seeds of both goat grass and rye grass were placed in polyethylene bags and imposed to leaching duration treatment by rising with tap water for 12, 24, 48 and 96 hours. After each leaching duration, bags were exhumed and seeds incubated at incubator under conditions described at germination protocol section. Water temperature used for leaching was 9 ± 3°C.

Effect of Burial Depth and Flooding Duration on Seed Longivity

Thirty seeds were placed in polyethylene bags and buried at soil in plastic pots (20 cm by 30 cm) at depths of 0, 5 and 10 cm and flooded for 2 and 4 weeks. Pots were placed outdoors and kept flooded at the durations. After each flooding duration, plastic bags were exhumed from the pots and seeds were rinsed by tap water. In each bag germinated seeds were counted and added to total germination percent. Non-germinated seeds were placed in Petri dishes and incubated at incubator according to conditions described at germination protocol. Germinated seeds were counted after 15 days. Total germination percentage was the sum of germinated seeds at the pots and germinated ones after incubation.

Data Analysis

All experiments were set as a completely randomized design with three replicates. Each experiment was conducted twice and means of two experiments were used for analysis.

Data were subjected to arcsine transformation to improve homogeneity and transformed data were used for statistical analysis. Regression analysis was used to determine the effect of salinity and osmotic stress on seed germination. The pH, leaching duration and burial depth and flooding duration data were subjected to analysis of variance with the use of

SAS software. Means were separated by both standard error bars and LSD test at P = 0.05.

RESULTS AND DISCUSSION

Seed Description and Germination

Thousand seed weight, seed length and width of goat grass were 12.48 g, 7.20 mm and 2.80 mm, respectively. Seeds of goat grass germinated 54.31% in light/dark and 45.36% in darkness regimes. For rye grass thousand seed weight, seed length and width were 1.57 g, 5.68 mm and 1.58 mm, respectively. Initial germination of rye grass seeds in 12/12 hours light/dark regime and complete darkness was 80 and 76%, respectively.

The result of tetrazolium chloride test revealed that 100% seed of goat grass and rye grass were viable. According our results 45–55% of freshly harvested of goat grass showed seed dormancy, while rye grass initial seed dormancy was 20–24% in different light regime.

Osmotic Stress

Linear models including y = -33.906x + 52.99; R^2 = 0.96 and y = -56.078x + 88.376; R^2 = 0.86 with strong correlation was fitted to response of seed germination of goat grass and rye grass to different osmotic potential, respectively. Seed germination of both goat grass and rye grass reduced when increased osmotic stress (Figure 2A and 2B).

In rye grass initial decreasing in germination at concentrations 0 to -0.4 MPa was not remarkable. The maximum reduction in germination was observed in a range of concentration from -0.6 to -0.8 MPa (Figure 2B). At the highest level of osmotic stress (-1 MPa) 26.24% of rye grass seeds were germinated that indicate the ability of rye grass seed in germination at high drought stress condition (Figure 2B). Goat grass seed also gereminated about 15.67% at -1 MPa osmotic stress (Figure 2B). According to our results rye grass seed is more tolerant to high osmotic stress than goat grass. These data also suggest that rye grass and goat grass can tolerate high level of osmotic stress. Drought stress in seed germination stage could be an important factor for germination and establishment of weeds and crops. Reductions in seed germination by osmotic stress have been reported by many researchers in many weeds. Ramirez *et al*. (2010), Rezvani and Fani Yazdi (2013) and Fani Yazdi *et al*. (2013) reported different sensivity of weed species of seed germination to osmotic stress.

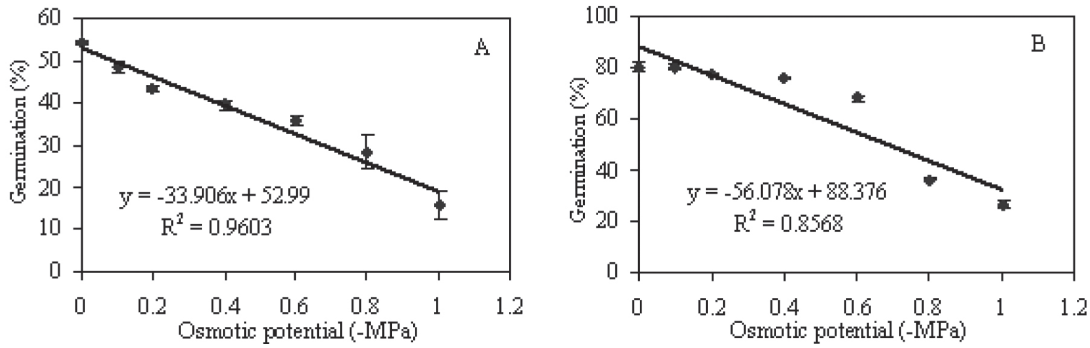

Figure 2 Effect of osmotic stress on seed germination of goat grass (A) and rye grass (B) at 25/15°C (day/night) for 2 weeks (Vertical bars represent standard error)

Salt Stress

Salt stress linearly (y = -0.148x + 56.343; R^2= 0.95) decreased germination of goat grass (Figure 3A). Also, a linear model (y = -0.2185x + 81.03; R^2 = 0.92) described the reaction of rye grass seed germination to salt stress (Figure 3B). Seed germination of both goat grass and rye grass at 400 mM NaCl concentration reached to zero. Germination of goat grass and rye grass were 19.16% and 17.94% at concentration of 200 mM NaCl, respectively (Figure 3A and 3B). Germination of this proportion of seed suggests that both species able to germinate even at salinity levels up to 200 mM. Sensitivity of seed germination to salt stress has also been reported in many weed species. Similarly, increasing the salt stress resulted in declination in germination of Buffalobur (*Solanum rostratum*) (Wei *et al*., 2009), trumpetcreeper (*Campsis radicans*) (Chachalis and Reddy, 2000) and tall morningglory [*Ipomoea purpurea* (L.) Roth.] (Singh *et al*., 2011).

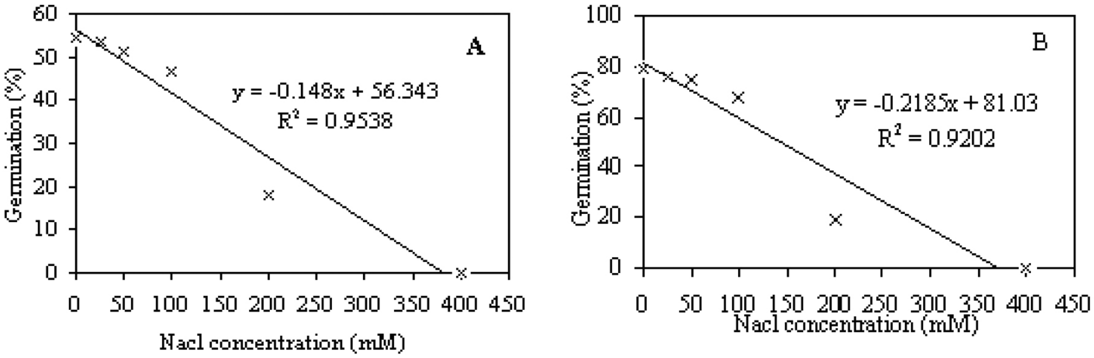

Figure 3 Effect of salt stress on seed germination of goat grass (A) and rye grass (B) at 25/15°C (day/night) for 2 weeks (Vertical bars represent standard error)

pH

The effect of pH on seed germination of both weed was significant (Table 1). Response of goat grass and rye grass seed germination to low pH buffered solution was different. The maximum seed germination for both goat grass and rye grass was observed at pH 7 (Figure 4A and 4B). Seed germination of goat grass reduced at pH 3, 5, 9 and 11 in comparison with pH 7 (Figure 4A). Seed germination of goat grass reduced at pH 3 and 5 while 71.8% to 78.25% of seeds of rye grass were germinated (Figure 4A and 4B). Seed germination of rye grass was minimum at pH 9 and 11 (Figure 4B). Results of Ramirez *et al*. (2012) also indicated that seed germination of common beggars-tick (*Bidens alba*) was significantly impacted by pH of the tested solution. They also showed that germination of common beggars-tick seed inhibited at pH 3, 4, 9 and 10. Rezvani and Fani Yazdi (2013) and Fani Yazdi *et al*. (2013) indicated that the seeds of black nightshade (*Solanum nigrum* L.) and sheep sorrel greatly germinated in a broad range of pH from 4 to 10.

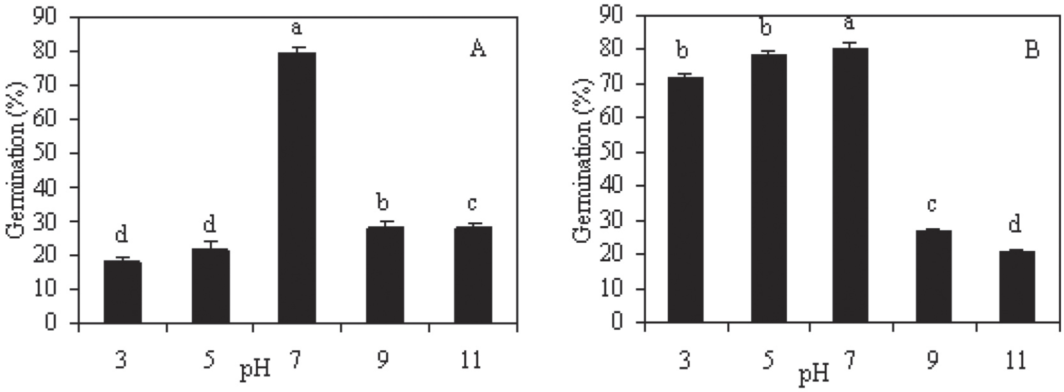

Figure 4 Effect of pH on seed germination of goat grass (A) and rye grass (B) at 25/15°C (day/night) for 2 weeks. Same letters in each column shows non-significant differences (Vertical bars represent standard error)

Leaching Duration

Leaching duration significantly influenced on seed germination of goat grass and rye grass (Table 1). Seed germination of goat grass reduced after 12, 24 and 48 hours leaching compared with control treatment. Goat grass seed germination at 96 hours leaching was similar to the control one (Figure 5A). Leaching duration for 12 and 24 hours reduced seed germination of rye grass in comparison with control (Figure 5B). But, seed at 48 hours was not significantly different comapred with control. Compared with control seed germination of rye grass significantly increased after 96 hours leaching (Figure 5B). Our data suggest a short-term leaching with cold water induces short-term dormancy to seeds of both species. Such short-term dormancy disappeared after leaching for 96 hours.

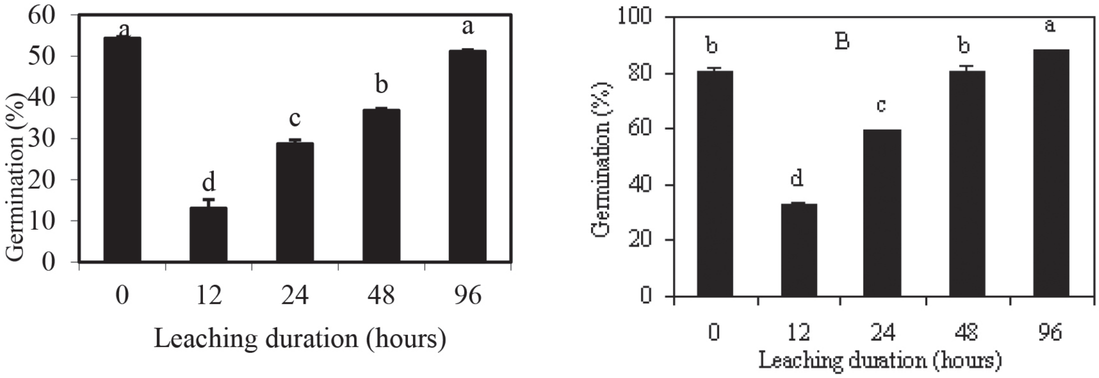

Figure 5 Effect of leaching duration and burial depth on seed germination of goat grass (A) and rye grass (B) at 25/15°C (day/night) for 2 weeks. Same letters in each column shows non-significant differences (Vertical bars represent standard error)

Burial Depth and Flooding Duration

Burial depth and flooding duration influenced seed germination of both goat grass and rye grass (Table 1). In goat grass seed that flooded for two weeks showed lower germination than those of seeds were buried for four weeks. Seed germination of goat grass that buried for four weeks was 100% in all depths (Figure 6A). Increasing of burial depth for seeds that buried for two weeks, caused increased germination percentage of goat grass (Figure 6A). Rye grass seeds that imposed flooding and buried for four weeks showed the maximum germination (100%) that was higher than those of buried seeds for two weeks. After two weeks, also, germination reduced by increasing in burial depth (Figure 6B)

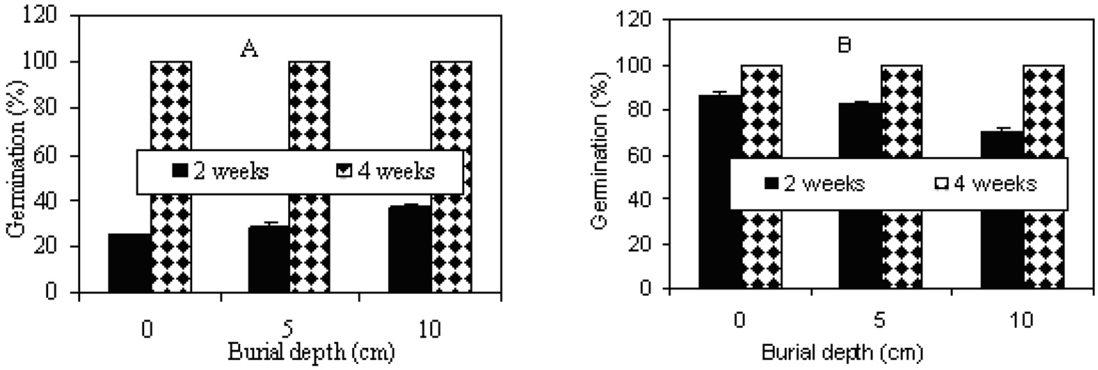

Figure 6 Effect of flooding duration and burial depth on seed germination of goat grass (A) and rye grass (B) at 25/15°C (day/night) for 2 weeks (Vertical bars represent standard error)

An initial reduction in seed germination of gaot grass could be due to inducing temporary dormancy. We found different reportes about the response of seed to flooding. Singh *et al*. (2011) indicated that flooding duration significantly decreased and delayed the emergence of tall morningglory (*Ipomoea purpurea*). Similarly our findings, Baird and Dickens (1991) and Singh and Achhireddy (1984) reported virginia button weed (*Diodia virginiana* L.) and strangler vine [*Morrenia odorata* (Hook. and Arn.) Lindl.] were not sensitive to flooding, respectively.

Table 1 ANOVA for the effect of treatments on seed germination of goat grass and rye grass

Plant species	Treatments	Mean square
Goat grass	pH	822.73*
	Leaching duration	1425.18*
	Burial depth and flooding duration	999.75*
Rye grass	pH	2696.95*
	Leaching duration	2544.58*
	Burial depth and flooding duration	734.58*

*: Significant at $P < 0.05$

CONCLUSION

In north of Iran, following the climate change and global warming the precipitation reduced at winter crops planting date. This reduction is a challenge for seed germination and seedling establishment of winter crops such as wheat, canola and vegetables and weeds. According to our results goat grass and rye grass seed gereminated at -1 MPa osmotic stress suggesting that seed may germinate under high water stress successfully than common winter crops in the region. The seed germination of both weeds was occurred in 200 mM NaCl concentration that suggests goat grass and rye grass are fairly tolerant to high salinity. The response of seed germination of goat grass and rye grass depended on pH. Rye grass germinated in pH range wider than goat grass. Flooding irrigation is a common practice in some cropping systems in Iran; the region that water is not a limitation factor. Also, natural temporary flooding in rainy season is a usual environmental challenge for seeds in soil. Therefore, seeds must be able to remain viable and germinate at low oxygen concentrations.

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