

Effects of Seed Priming on Germination Characteristics of *Bromus* Species under Salt and Drought Conditions

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Abstract It has been estimated that about nine million square kilometers of the world's arid rangelands have been turned into man-made deserts over the past half century. *B. inermis* was introduced as a livestock improvement crop, it has since invaded natural prairies and grasslands, outcompeting native grasses and decreasing biodiversity. The increasing frequency of dry periods in many regions of the world and the problems associated with salinity in irrigated areas frequently result in the consecutive occurrence of drought and salinity on cultivated land. The objective of this study was to determine the effect of seed priming on germination characteristics of *Bromus* species under stressful conditions. For osmopriming treatment, *Bromus* seeds were immersed in -0.6 MPa of PEG solutions at 25°C for 12 hours under dark conditions and seed were soaked for 12h in distilled water for hydropriming treatment. Drought condition was simulated by using PEG6000 according to Kuffman formula. Our results showed that *Bromus* could be categorized as a salt tolerant plant and its more tolerate to salinity than drought stress. Seed priming is a good seed enhancement technique for improving seed germination and faster seed germination of *Bromus* seeds.

Key words: Priming • Germination • Bromus • Salinity • Drought

INTRODUCTION

The increasing frequency of dry periods in many regions of the world and the problems associated with salinity in irrigated areas frequently result in the consecutive occurrence of drought and salinity on cultivated land [1]. It has been estimated that about nine million square kilometers of the world's arid rangelands have been turned into man-made deserts over the past half century [2]. Rangelands are areas unsuitable for cultivation, plant growth is deeply affected by a combination of environmental stresses such as drought, or high salinity. From an agricultural aspect, such stresses are among the most significant factors responsible for substantial and unpredictable losses in crop production. The physiological mechanisms that plants responses to salinity and drought show high similarity, suggesting that both stresses must be perceived by the plant cell as deprivation of water. High salt concentrations (most commonly NaCl) in the soil lead to a decrease in water potential, which affects water availability [3, 4]. Both drought and salinity threaten crop productivity

worldwide. Water deficit or osmotic effects are probably the major physiological mechanisms for growth reduction as both stresses lower the soil water potential.

However, drought and salinity may differentially affect the mineral-nutrient relations in plants. Generally, drought reduces both nutrient uptake by the roots and transport from the roots to the shoots, because of restricted transpiration rates and impaired active transport and membrane permeability [5, 6]. Seed germination and seedling growth are critical life history stages often subject to high mortality rates. Seeds and seedlings may be less stress tolerant than adults or may be exposed to the more extreme environmental fluctuations at or near the soil surface. Increasing salinity generally reduces germination.

Seed priming has been successfully demonstrated to improve germination and emergence in seeds of many crops, particularly seeds of vegetables and small seeded grasses [7, 8]. Among different strategies to cope with salinity issues, seed priming is an easy, low cost and low risk technique and the approach has recently been used to overcome the salinity problem in agricultural lands.

Seed priming with optimal concentrations of cytokinins has been shown to be beneficial to germination, growth and yield of some crop species grown under saline conditions [9, 10]. Giri and Schillinger, [11] reported that wheat seed priming in potassium chloride (KCl), polyethylene glycol (PEG) and water led to enhanced emergence, rate and extent of seedling emergence comparing to checks.

There is little information on the role of seed priming in germination characteristics of *Bromus* species under stress conditions, therefore this study was conducted to evaluate the effect of seed priming on germination performance under salt and drought stress.

MATERIALS AND METHODS

This study was carried out at the Department of Agronomy and Plant Breeding, College of Agriculture and Natural Resources, University of Tehran, Iran. Two *Bromus* species, namely, *Bromus inermis* and *Bromus tomentellus* were used. Germination and early seedling growth (14 days) of the species were studied under distilled water (control) and osmotic potentials of -0.3, -0.6, -0.9 and -1.2 MPa, for NaCl [12] and polyethyleneglycol 6000 (PEG 6000) [13] for simulating salinity and drought stress. Prepared NaCl potentials had the electrical conductivity (EC) values of 6.5, 12.7, 18.4 and 23.5 dSm⁻¹, respectively.

Seed Treatments [b2]: For osmopriming treatment, *Bromus* seeds were immersed in -0.6 MPa of PEG solutions at 25°C for 12 hours under dark conditions and seeds were soaked for 12h in distilled water for hydropriming treatment. The osmopriming concentration and duration were determined in a preliminary experiment (data not shown). Thereafter, the seeds were rinsed with tap water three times and one time with distilled water. The treated seeds were surface-dried and then dried for 24 hours at 25°C.

Germination Tests [b3]: Four replications of 25 seeds were germinated within 90mm Petri dishes and 5ml of salt and drought solutions. Petri dishes and seeds were put into sealed plastic bags to avoid moisture loss. Seeds were allowed to germinate 25±1°C under dark condition for 14 days. Germination was considered to have occurred when radicles were 2mm long. Germination percentage was recorded every 24 h for 14 days. Rate of germination was calculated to assess the speed of germination [14].

$$MGT = \frac{\sum N_i D_i}{N}, RG = \frac{1}{MGT}$$

Where Ni: number of seeds germinated on the day i, Di: Days of germination test. N: total number of seeds.

Root length and shoot length were measured after the 14th day. Vigor index was calculated using the following equation: Vigor index = % germination × seedling length.

Statistical Design [b4]: Two experiments were conducted to investigate the effect of seed priming on each *Bromus* species. The experimental design for each experiment was three factors factorial (3×2×5) arranged in a completely randomized design with four replications and 25 seeds per replicate for each stress. The first factor was stress (salinity and drought), the second treatments (control, hydropriming and osmopriming) and the third was osmotic potential levels (0, -0.3, -0.6, -0.9 and -1.2 MPa). Data for germination percentage were subjected to arcsin transformation before analysis of variance. The differences between the means were compared using least significant difference (LSD) values (P<0.05). Since, similar osmotic potentials for drought and salt stresses were used, performance of *Bromus* seeds were compared under these stress using randomized complete block design. MSTAT C and Minitab 15 were used for data analyses.

RESULTS

B. Tomentellus [b5]: Results showed that there was a significant three-way interaction (p<0.01, df_e=58) for speed of germination, root length, seedling length and mean germination time. For germination percentage two-way interactions was significant. *B. tomentellus* seeds germinated better at drought condition comparing to salt stress and this difference was more obvious at -0.9 and -1.2 Mpa (Fig 1b). Osmoprimed seeds germinated better than hydroprimed and control seeds and this difference was significant but hydropriming and controls had not any significant difference (Fig 1a).

Speed of germination increased with seed priming treatments rather than of controls. Under drought condition, osmopriming exhibited higher speed than other treatment at 0, -0.3 and -0.6 Mpa but hydroprimed seeds germinate faster at -0.9 and -1.2 Mpa. For salinity treatments osmopriming was the best treatment at 0, -0.9 and -1.2 Mpa and only hydropriming exhibited faster germination at -0.3 Mpa (Fig 2).

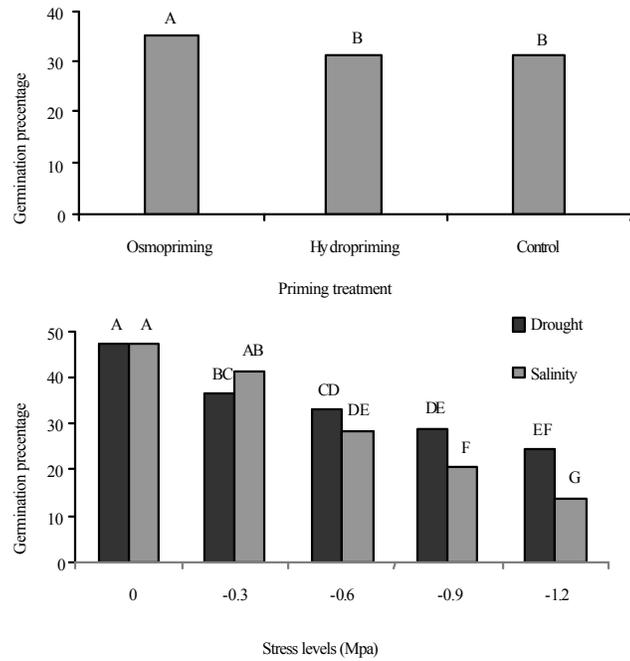


Fig. 1: Germination percentage of *B. tomentellus*: a) effects of seed priming treatments on seed germination, b) germination percentage at different stress conditions

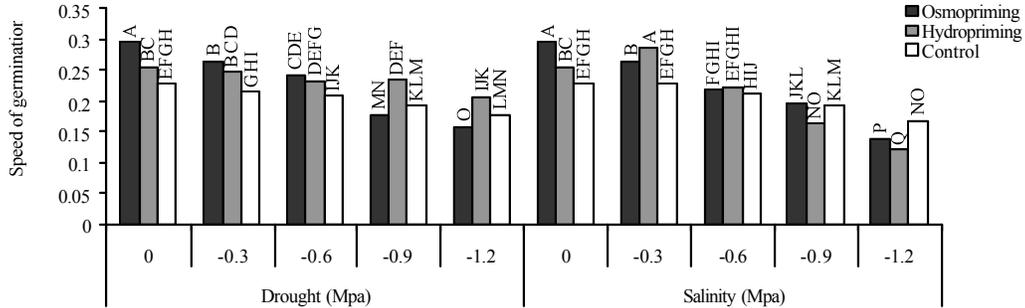


Fig. 2: Effects of seed priming treatments on speed of germination under salinity and drought stresses

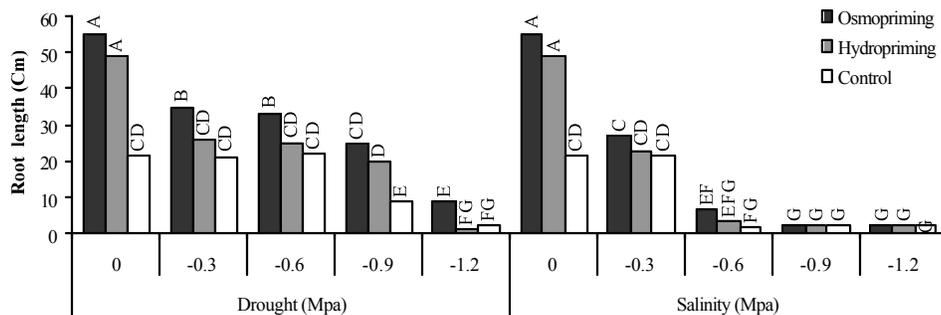


Fig. 3: Effects of seed priming treatments on root length of *B. tomentellus* under salinity and drought stress

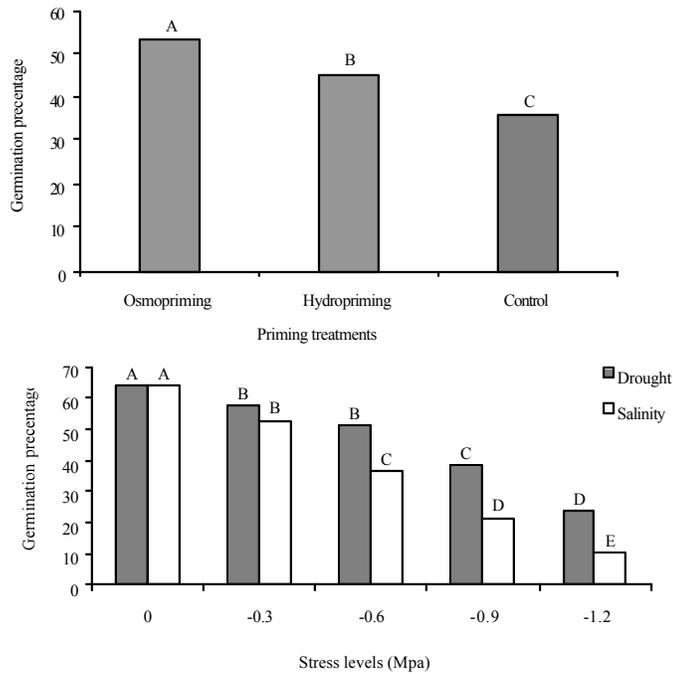


Fig. 4: a) Effects of seed priming treatments on germination percentage of *B. inermis* Effects of different stress conditions on germination percentage of *B. inermis*

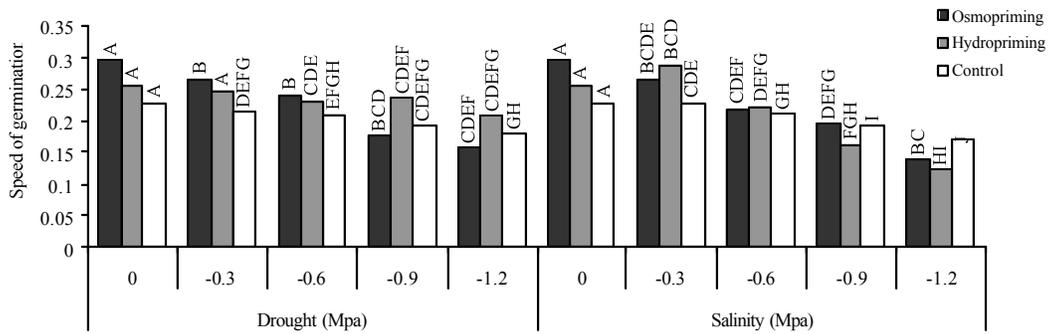


Fig. 5: Effects of seed priming on speed of germination of *B. inermis* at stress conditions *B. inermis* is more sensitive to salinity than drought. No notable root was observed at -0.9 and -1.2 MPa salinity compare to drought same levels (Fig 6)

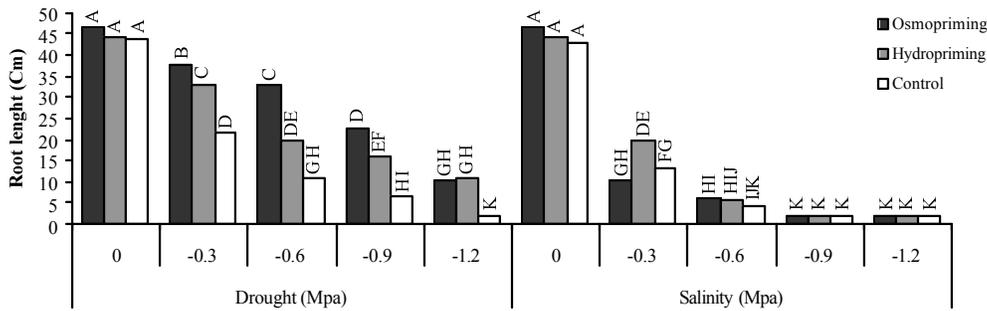


Fig. 6: Effects of seed priming treatments on root length of *B. inermis* under salt and drought conditions.

Root length also decreased by salinity and drought stresses. Osmoprimes had the longest root length at all stress levels. *B.tomentellus* root growth at drought stress is better than salinity stress. This fact was more clear at higher osmotic potentials (Fig 3). No root produced at -0.9 and -1.2 Mpa of salinity stress.

Primed seeds had a higher seedling length than controls. Osmoprimes seed produced longer seedling at higher osmotic potentials of both stress (data are not shown).

Bromus Inermis [b6]: Results showed that there was a significant three-way interaction ($p < 0.01$, $df_c = 58$) for speed of germination, root length, mean germination time and for seedling length ($p < 0.05$, $df_c = 58$). For germination percentage two-way interactions were significant. Osmoprimes seeds germination percentage was higher than hydroprimed and controls (Fig 4 a). Seed germination of *B. inermis* was obviously higher at drought stress levels than salinity (Fig 4 b).

Speed of germination decreased due to increasing stress levels. For this species we could not find any pattern. Seeds germinate faster at drought conditions comparing to salinity and primed seeds took shorter time to germinate comparing to controls (Fig 5).

B. inermis is more sensitive to salinity than drought. No notable root was observed at -0.9 and -1.2 MPa salinity compare to drought same levels (Fig 6).

In the drought conditions osmopriming was the best treatment than hydropriming and controls but at salinity conditions hydroprimed seeds produced longer roots than others especially at -0.3 Mpa salinity.

DISCUSSION

According to our results *Bromus* is more tolerant to drought than salinity stress. Germination characteristics are much higher in drought conditions. With comparing primed and non-primed seeds it is clear that seed priming could enhance seed germination in stress conditions and by the way osmopriming is more suitable to hydropriming. It is suggested that side effects of salinity is because of its toxicity effects than osmotic. This result is on the basis of the same osmotic potentials of drought and salinity stress levels. One of the most general types of stress experienced by plants is water-limitation, which becomes particularly pronounced during periods of drought. The well known definition of drought which accepted by various researchers includes a reduction in plant growth due to a historical decrease in average precipitation

amounts [15]. Osmotic solutions are used to impose water stress reproducibly under *in vitro* conditions [16]. Polyethylene glycol molecules with a $M \geq 6000$ (PEG 6000) are inert, non-ionic and virtually impermeable chains that have frequently been used to induce water stress and maintain a uniform water potential throughout the experimental period [17, 18]. Molecules of PEG 6000 are small enough to influence the osmotic potential, but large enough to not be absorbed by plants [19]. Because PEG does not enter the apoplast, water is withdrawn from the cell and the cell wall. Therefore, PEG solutions mimic dry soil more closely than solutions of low osmotic, which infiltrate the cell wall with solutes [20]. Maiti *et al.* [21] also reported that osmotic seed priming of maize caryopses in copper sulphate, zinc sulphate, manganese sulphate, or boric acid induced high levels of seed germination. Fotia, *et al.* [22] reported that Osmotic seed priming of maize caryopses resulted in more homogenous and faster seed germination as compared to the control.

Finally according to our results *Bromus* is a drought tolerant plant and could be use in arid and semi arid environments for its soil establishing or dedesertification and also it could be used as a good forage plant for such regions. *B. inermis* is more tolerant against stress than *B. tomentellus* and it is suggested that further studies on this species might have interesting results. Kaya *et al.* [23] reported that seed priming could enhance sunflower seed germination under the stress conditions. Duke [24, 25] reported that best adapted to regions with moderate rainfall and moderate cool summer temperatures. Suited to silt or clay soils, deep loams, but also does well on light sandy soils, on well-drained soils and less drought resistant than crested wheatgrass, but does not tolerate temperature extremes. One of the greatest challenges in restoration ecology is to sow a seed type or cultivar that has the capacity to produce abundant biomass and cover in a short period of time [26].

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