

UDK 633.31:581.142

GERMINATION PERCENTAGE AND RECOVERY OF *LOLIUM PERENNE* L. AND *BROMUS TOMENTELLUS* BOISS. (POACEAE, LILIOPSIDA) SEEDS AT SEVERAL OSMOTIC POTENTIAL LEVELS OF ISO-OSMOTIC SOLUTIONS

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Поступила в редакцию 24.01.13 г.

Germination Percentage and Recovery of *Lolium perenne* L. and *Bromus tomentellus* Boiss. (Poaceae, Liliopsida) Seeds at Several Osmotic Potential Levels of Iso-Osmotic Solutions. – Tilaki Dianati Ghasem Ali, Gholami Farzaneh, Bezdi Kamal Ghasemi, and Behtari Behzad. – Germination is a most salt-sensitive plant growth stage and severely inhibited with increasing salinity in both glycophytes and halophytes. In the present study, the effect of three levels of a salt (NaCl) and polyethylene glycol 6000 (PEG) on the germination of *Lolium perenne* L. and *Bromus tomentellus* Boiss. seeds was studied. The object was to reveal factors responsible for seed germination due to salt toxicity or the osmotic effect. The electrical conductivity (EC) values of NaCl solutions were 0, 5, 7, and 9 dSm⁻¹. PEG 6000 induced drought conditions at the same osmotic potential of 0, -2, -3, and -4 bar. Our results showed that NaCl and PEG treatments had significant ($p = 0.05$) effect on the germination percentage and recovery of seed germination. In *L. perenne*, a lower germination percentage (65.2%) was obtained from PEG compared with NaCl at an equivalent water potential in each treatment but in *B. tomentellus*, seed germination was better in PEG than in NaCl. When non-germinated seeds under various NaCl and PEG treatments were transferred to distilled water, they were recovered significantly, indicating little ionic and osmotic effect of salinity on viability. Germination inhibition, therefore, appears to be either osmotic or ionic, depending on the species. A similar recovery response was noted when seeds were transferred from a PEG solution to water. PEG had no toxic effect, since the seeds germinated after removing the PEG stress.

Key words: Polyethylene Glycol 6000, NaCl, Salinity, Stress, Ionic and Osmotic Effect.

Всхожесть и восстановление семян *Lolium perenne* L. и *Bromus tomentellus* Boiss. (Poaceae, Liliopsida) при различных уровнях осмотического потенциала изоосмотических растворов. – Тилаки Дианати Хасем Али, Холами Фарзане, Безди Камаль Хасеми, Бехтари Бехзад. – Прорастание является одной из наиболее чувствительных к солям стадий роста растений и сильно ингибируется с увеличением солёности у гликофитов и галофитов. В данной работе изучали влияние трёх уровней соли (NaCl) и полиэтиленгликоля 6000 (ПЭГ) на всхожесть семян *Lolium perenne* L. и *Bromus tomentellus* Boiss. Цель состояла в выявлении факторов, ответственных за нарушение прорастания (токсичность соли или осмотический эффект). Электропроводность растворов NaCl составляла 0, 5, 7 и 9 дСм⁻¹. Засушливость имитировали введением ПЭГ 6000 при сохранении осмотического водного потенциала 0, -2, -3 и -4 бар. Результаты показали, что обработка NaCl и ПЭГ значимо ($P = 0.05$) влияет на прорастание и восстановление всхожести семян. Меньшая доля прорастания семян *L. perenne* (65.2%) наблюдалась при воздействии ПЭГ по сравнению с NaCl при равном вод-

ном потенциале при каждой обработке, но для *Bromus tomentellus* Boiss. прорастание семян было выше при воздействии ПЭГ, нежели NaCl. Непроросшие при обработке NaCl и ПЭГ семена после переноса в дистиллированную воду восстанавливали всхожесть, что указывает на слабое влияние ионного и осмотического эффекта солёности на прорастание семян и их жизнеспособность. Ингибирование всхожести, таким образом, может иметь осмотическую или ионную природу в зависимости от вида растения. Сходный уровень восстановления наблюдался при переносе семян из раствора ПЭГ в воду. ПЭГ не обладает токсическим влиянием, и семена прорастают после прекращения его воздействия.

Ключевые слова: полиэтиленгликоль 6000, NaCl, солёность, стресс, ионное и осмотическое воздействие.

INTRODUCTION

Saline soils are widespread in arid and semiarid regions of the world. Salinity is one of the main problems that negatively affects soil fertility and limits plant production (Richards, 1954). The factors affecting plant growth under salinity can be divided into three groups, namely: i) water stress, ii) ion toxicity, and iii) problems in nutrient uptake and translocation to green plants' parts, and, as a result, disorders in cells due to disruption of ionic balances such as in the case of K^+ and Ca^{++} . Under salt stress, physiological drought may play an important role by limiting water uptake from the soil. On the other hand, excessive salt uptake by plants disrupts cellular functions and damages their physiological processes such as photosynthesis and respiration (Leopold, Willing, 1984). However, salt stress affects germination percentage, germination rate, and seedling growth in different ways depending on plant species. High NaCl concentrations induce dormancy in the seeds of many halophyte species (Debez et al., 2004), while the seeds of glycophyte species lose their viability under similar conditions. A plant's ability to tolerate salinity stress varies with the stage of growth in its life cycle (Khan, 2002). Germination is a most salt-sensitive plant growth stage and severely inhibited with increasing salinity in both glycophytes and halophytes (Sosa et al., 2005). Salts can affect seed germination by either restricting the supply of water (an osmotic effect) or causing specific injury through their ions to the metabolic machinery (an ionic effect). Soil salinity may affect the germination of seeds either by creating an external osmotic potential to the seed preventing water uptake, or through the toxic effects of Na^+ and Cl^- ions on the seed germination (Khajeh-Hosseini et al., 2003). Salt and osmotic stresses are responsible for inhibition or delayed seed germination and seedling establishment (Almansouri et al., 2001). Under these stresses, there is a decrease in the water uptake during imbibitions and, furthermore, salt stress may cause excessive uptake of ions (Murillo-Amador et al., 2002).

Lolium perenne L. (perennial ryegrass) is a cool-season perennial bunchgrass native in Europe, Middle Asia, and North Africa. *Lolium perenne* L. plays an important role in forage/livestock systems. Its high palatability and digestibility make this species highly valued for dairy and sheep forage systems. *Lolium perenne* L. is adapted to medium textured soils with a pH between 5.1 and 8.4. It requires a minimum of 18 to 25 inches of precipitation annually, at least half of which should be received as rain.

Bromus tomentellus Boiss. (Brome grass) is a densely growing bunchgrass reaching a height of 20 – 70 cm. It is used for pasture and for erosion control and grows in spring and summer. In northern Africa and western Asia, it can be found at elevations of 1,000 – 3,400 m (USDA, 1950).

The aim of the present study was to determine factors responsible for failures of germination percentage and germination recovery of *Lolium perenne* L. and *Bromus tomentellus* Boiss. seeds under saline conditions due to an osmotic barrier or the toxic effect of NaCl by comparison of seed germination under a range of osmotic potentials causing by NaCl and PEG.

MATERIALS AND METHODS

Germination conditions. The present study was carried out at Faculty of Natural Resources, Tarbiat Modares University, Noor, Iran. The seed materials of *Lolium perenne* L. and *Bromus tomentellus* Boiss. were obtained from the Seed Gene Bank of Natural Source of Iran, which is commonly cultivated in Iran.

Seeds were separated from inflorescence in the laboratory and surface disinfected by ozone treatment for 30 min (Duan et al., 2004). The seeds were germinated in Petri dishes (10 cm) containing two layers of Whatman 42 filter paper moistened initially with 10 ml of distilled water (0 bar as control) and three different solutions of NaCl or polyethylene glycol (PEG 6000) (Michel, Kaufmann, 1973) under osmotic potentials of -2, -3, and -4 bar. The papers were being replaced every 2 days to prevent accumulation of salts (Rehman et al., 1996). The NaCl solutions had the electrical conductivity (EC) values of 0, 5, 7, and 9 dSm⁻¹, respectively. After 7 days, the germination of species was studied after different treatments.

Germination tests. The experiment was carried out within 15 – 25°C under 8 h darkness / 16 h lights (38u mol m⁻²s⁻¹) for 7 days. Germination was considered to have occurred when the radicles were 2 mm long (Hardegree, Van Vactor, 2000). Germination percentage was recorded every 24 h for 7 days. Non-germinated seeds were transferred to distilled water to determine the germination recovery. The recovery percent was calculated using the following index (Ajmal Khan, Gulzar, 2003):

$$\% \text{Recovery} = \frac{a - b}{c - b} \times 100,$$

where *a* is the total number of germinated seeds after having been transferred to distilled water, *b* the total number of germinated seeds in the saline solution, and *c* the total number of seeds. High recovery germination percentages would indicate that previous seed germination was inhibited by an osmotic effect, whereas low recovery germination would indicate specific ion toxicity (Khan, 2002).

Experimental design. We employed a three-factor factorial (2×2×4) experiment arranged in a completely randomized design; with three replications and 50 seeds per replicate. The first factor was the nature of species (*Lolium perenne* L. or *Bromus tomentellus* Boiss.), the second was the iso-osmotic agent (NaCl or PEG), and the third was the osmotic potential level (0, -2, -3 or -4 bar). Data were subjected to analysis of variance followed by Duncan's multiple range test to determine significant differences among the mean values at the probability level of 0.05 or 0.01. Statistical analysis was done with SPSS version 11.0 for Windows statistical software package.

RESULTS AND DISCUSSION

Effects of iso-osmotic agents on germination percentage

In the present study, a significant three-way interaction (species, iso-osmotic agents (NaCl and PEG) and osmotic potential levels) was found ($P < 0.01$) for all investigated characters. Table 1 shows the mean of seed germination percentage of *L. perenne* and *B. tomentellus* using different osmotic potential levels of iso-osmotic solutions (NaCl and PEG). The -4 bar solution was highly effective in reducing the germination percentage of both species (*L. perenne* and *B. tomentellus*). Generally, seed germination decreased as the osmotic potential levels of NaCl and PEG increased. The highest and lowest germination percentage was observed in *B. tomentellus* by the 0 bar (control) osmotic potential and by the -4 bar osmotic potential of NaCl, respectively (Table 1).

Table 1

Effects of the osmotic potential level on the germination percentage of *Lolium perenne* L. and *Bromus tomentellus* Boiss. under osmotic stresses of PEG and NaCl (with EC: 0, 5, 7, 9)

Osmotic potential (bar)	<i>Lolium perenne</i>		<i>Bromus tomentellus</i>	
	NaCl	PEG	NaCl	PEG
0	86.19±1.3 abc*		90.1±2.1 a	
-2	84±1.3 abcd	83±1.6 abcd	82±2.9 bcd	83±2.4abcd
-3	79.9±1.7 cd	77.1±2.3 d	64.2±4.5 e	76.6±3.1 d
-4	75.4±2.1 d	65.2±3.9 e	45.3±4 f	52.2±4.4 f

* Means (±SE) followed by the same letter are not significantly different at $P < 0.01$.

These results confirm the findings of Misra & Dwivedi, 2004; they reported that an increased osmotic potential level of salinity led to a reduced germination percentage of greengram cultivars. Seed germination of *L. perenne* was always better in NaCl than in PEG at an equivalent water potential in line with the earlier observation made for soybean by Khajeh-Hosseini et al. (2003); Demir et al. (2006) in sunflower (*Helianthus annuus* L.), and Dianati et al. (2009) in alfalfa. This may be due to the uptake of Na^+ and Cl^- ions by the seeds, maintaining a water potential gradient allowing water uptake during seed germination. The NaCl-treated seeds compared to the PEG-treated seeds were allowed to imbibe water for a longer time and through the first stage of germination without protrusion of the radicle. These results are in agreement with Murillo-Amador et al. (2002) in cowpea, Demir & Van De Venter (1999) in watermelon; they affirmed that drought or salinity may influence germination by decreasing the water uptake. Moreover, salinity perturbs plant hormone balance (Khan, Rizvi, 1994) and reduces the utilization of seed reserves (Ahmad, Bano, 1992).

The iso-osmotic solutions of NaCl and PEG in *B. tomentellus* have similar effects on seed germination at a similar osmotic potential, except -3 bar; at this osmotic potential level (-3 bar), seeds germination was better in PEG than in NaCl (Fig. 1). In general, the ionic toxicity of the salt (NaCl) stress treatment causes more damage to plant cells than that in PEG drought stress conditions, and plays a major role in membrane injury, organelle damage, and pigment degradation prior to cell death, which is well documented in many plant species such as sugarcane (Errabii et al., 2007), *Centaurea rugosina*

(Radi et al., 2005, 2006), *Fraxinus angustifolia* (Tonon et al., 2004), durum wheat (Lutts et al., 2004).

The high accumulation of Na^+ and Cl^- can be related to the greater sensitivity of the crop compared with other important grains (Ashraf, McNeilly, 1989). According to Ashraf & Wahid (2000) findings, the adverse effect of NaCl on seed germination

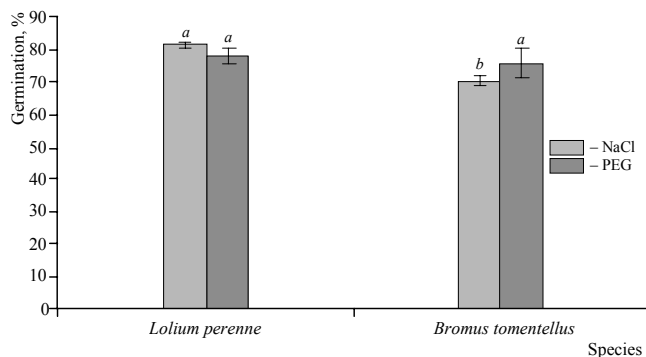


Fig. 1. Germination percentage in *Lolium perenne* L. and *Bromus tomentellus* Boiss. with osmotic solutions of NaCl and PEG

in maize seedlings is partly due to impairment in breakdown of seed lipids so as to supply soluble sugars to the respiratory metabolism of the growing embryo. Salt tolerance in glycophytes is associated with the ability to limit uptake and/or transport of saline ions (mainly Na^+ and Cl^-) from the root zone to the aerial parts (Greenway, Munns, 1980). A lower uptake of toxic mineral elements and a higher uptake of beneficial mineral elements are generally associated with greater salt tolerance of most species (Shannon, Grieve, 1999). Na^+ is the main toxic ion in salinized soils. Low Na^+ and high K^+ in the cytoplasm are essential for the maintenance of a number of enzymatic processes (Munns, Tester, 2008). Na^+ enters plant cells through the high-affinity K^+ transporter (HKT) and through non-selective cation channels (Zhu, 2003). The similarity in size of the hydrated ionic of Na^+ and K^+ makes them difficult to discriminate between, and this is the basis of Na^+ toxicity (Blumwald, 2000). Under salt stress, Na^+ competes with K^+ for uptaking into roots (Munns, Tester, 2008). Ionic imbalance in plants is caused mainly by the influx of excess Na^+ (Munns, Tester, 2008; Yang et al., 2007).

Effects of iso-osmotic agents on germination recovery

Table 2 shows the mean of seed germination recovery of *L. perenne* and *B. tomentellus* using different osmotic potential levels of the iso-osmotic solutions (NaCl and PEG).

Table 2

Effects of the osmotic potential level on the recovery percentage of *Lolium perenne* L. and *Bromus tomentellus* Boiss. under osmotic stresses of PEG and NaCl (with EC: 0, 5, 7, 9)

Osmotic potential (bar)	<i>Lolium perenne</i>		<i>Bromus tomentellus</i>	
	NaCl	PEG	NaCl	PEG
0	—			
-2	11.7±5 de	8±3.3 e	32±6.4 ab	26±5.9 bc
-3	10.7±2.6 de	8.8±2.2 e	35.8±5 ab	24.2±4.1 bcd
-4	15.7±3.8 cde	14.1±2.4 cde	41.8±3.6 ab	24.1±4.9 bcd

* Means (±SE) followed by the same letter are not significantly different at $P < 0.01$.

GERMINATION PERCENTAGE AND RECOVERY

When seeds were transferred to distilled water 7 days after their salinity treatments, the seed recovery percentage increased with increasing of the osmotic potential of PEG and NaCl for both species. The germination recovery of both species was significantly

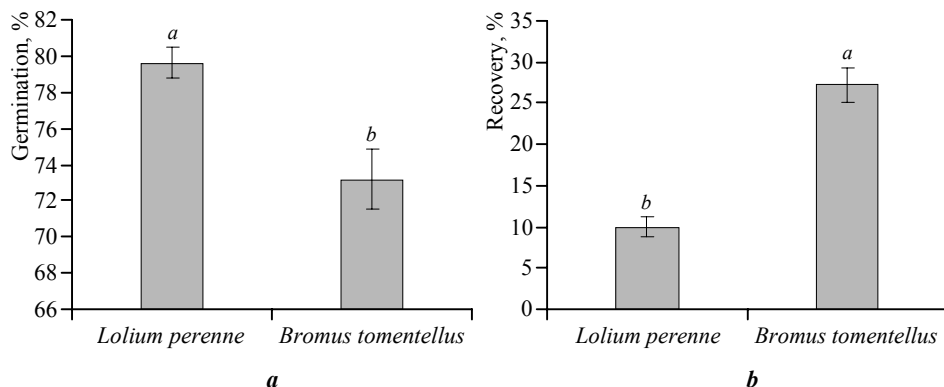


Fig. 2. Seed germination percentage (a) and germination recovery (b) of *Lolium perenne* L. and *Bromus tomentellus* Boiss. Values with the same superscript letter are not significantly different at $P < 0.01$ according to Duncan's multiple comparison range tests

increased in response to the highest dose of NaCl (-4 bar); a high recovery percentage was recorded (41.8). There was a significant difference between the species in their recovery percentage. *B. tomentellus* had the highest recovery percentage compared to *L. perenne* (Fig. 2, b).

There was a non-significant difference between the iso-osmotic solutions at all the osmotic potential levels (Fig. 3). The present study revealed that PEG had no toxic effect, because seeds germinated after removing PEG stress. Mehra et al. (2003) and Michel (1983) indicated that PEG molecules do not enter to the seed and Khajeh-Hosseini et al. (2003) found that there was no toxicity of PEG.

Under salt stress, Na^+ and Cl^- may be taken up by the seeds and toxic effect of NaCl might appear.

Strong salinity stress in our study did not devitalize seed germination ability; it only inhibited the germination temporarily and the viability was maintained. It is because

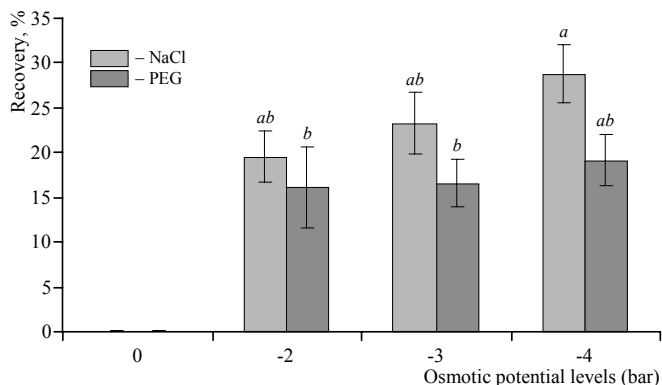


Fig. 3. Recovery of seed germination in *Lolium perenne* L. and *Bromus tomentellus* Boiss. at several osmotic potential levels of NaCl and PEG solutions. Values with the same superscript letter are not significantly different at $P < 0.01$ according to Duncan's multiple comparison range tests

seed germination was recovered when the stress was removed. Seeds of some species when pre-treated with salinity stresses showed a priming effect of salinity on germination, while others showed no effect of salinity on germination and recovered immediately after removing salinity stress was removed. Another species failed to germinate when exposed to high salinity stress (Khan, Ungar, 1996). Under hyper-saline conditions, seed survival may be an appropriate criterion for success rather than germinability, since recovery germination does occur in the seeds of *B. tomentellus*, *L. perenne*, and other species when hyper-saline conditions are alleviated (Redondo et al., 2004; Song et al., 2005; Tlig et al., 2008). Yuying et al. (1999) reported that after removing the stress, the seed germination and early seedling growth were higher than those of the untreated seeds were.

CONCLUSIONS

Salinity affects the germination process in *Lolium perenne* and *Bromus tomentellus* seeds. However, the evaluated selections have different levels of tolerance to iso-osmotic solutions. The components of salinity (osmotic and ionic) affect the evaluated two species differently. *Lolium perenne* was more affected by the osmotic effect, while *Bromus tomentellus* was more affected by the ionic effect. When seeds were transferred to a non-saline medium after 7 days of their exposure to salinity, there was a substantial recovery of germination. However, seed germination under natural conditions is more complicated and influenced by many factors such as salinity, drought, light, and temperature. Future studies would focus on the interactive effects of these factors and on the understanding of the ecophysiological strategies of plants for survival under natural environmental conditions. *Lolium perenne* and *Bromus tomentellus* are important plants for forage production in Iran which could be used to improve the quality of degraded saline land (with moderate salinity) as well as a high protein diet for animals.

The authors wish to thank the Tarbiat Modares University for financial support of their study.

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