SEED INVIGORATION TECHNIQUES TO IMPROVE GERMINATION AND EARLY GROWTH OF INBRED LINE OF MAIZE UNDER SALINITY AND DROUGHT STRESS

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Summary. Poor seed germination and crop stand are major problems in saline areas. However, seed vigour enhancement treatments might be able to alleviate the negative effects of salinity. The impact of pre-sowing treatments (control, osmopriming at -1.2 MPa of urea for 96 h and hydropriming for 36 h) on germination and early growth of maize (*Zea mays* L.) inbred line (Mo17) was evaluated under four levels of osmotic potential (0.0, -0.4, -0.8 and -1.2 MPa) induced by NaCl and polyethylene glycol (PEG-6000), as salinity and drought stress, respectively. Germination and early growth were affected by both stresses, while at the same osmotic potentials the depressive effect of PEG was more severe than NaCl. Hydropriming significantly improved germination and seedling growth presented as final germination percentage, germination index, seedling vigour index and length of seedling under both stress and non-stress conditions. Hydropriming could alleviate the effects of salinity and drought stress on germination and seedling early growth. This study indicated that hydropriming could be suitable seed invigoration treatment under saline and drought-prone environments.

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Key words: Maize inbred line, germination, invigoration, priming, salinity stress.
Abbreviations**:** PEG - polyethylene glycol; EC - electronic conductivity; SVI - Seedling vigour index.

INTRODUCTION

Among various environmental stresses, soil salinity has become a critical problem worldwide due to its dramatic effects on plant physiology and performance. Over 400 Mha across the world are affected by salinity that is about 25 % of the world's total area (including 15 % of Iran's lands) (Ghassemi *et al.,* 1995). A major concern of agriculture in Iran could thus be salinity which is a serious threat to the sustainability of crop or seed production in many parts of the country.

Salinity delays the onset, reduces the rate and increases the dispersion of germination events, resulting in reduced plant growth and final crop yield (Ashraf and Foolad, 2005). Seeds are particularly vulnerable to stress encountered between sowing and seedling establishment while plant salt tolerance usually increases with plant ontogeny. Soil salinity may affect the germination of seeds either by creating osmotic potential external to the seeds preventing water uptake or through the toxic effects of Na^+ and Cl^- ions on germinating seed (Khajeh-Hosseini *et al.,* 2003).

Maize is a protandrous plant (anthesis is sooner than silking), hence to achieve the synchronization of silking and anthesis stages of parental lines, delayed sowing dates have been suggested for male lines. In this situation accelerated germination and uniform emergence are essential. Any unfavorable environmental factor such as salinity during germination may interfere with this synchronism. It seems that one way to achieve this requirement could be the use of pre-sowing seed treatments.

Seed priming was defined as pre-sowing treatments in water or in an osmotic solution that allows seed to imbibe water to proceed to the first stage of germination, but prevents radicle protrusion through the seed coat. The most important priming treatments are osmopriming and hydropriming. Osmopriming refers to soaking seed in solutions of sugars, polyethylene glycol (PEG), glycerol, sorbitol (Ashraf and Foolad, 2005) or fertilizers such as urea (Al-Mudaris and Jutzi, 1999), followed by drying the seed before sowing. Hydropriming involved soaking of seed in water before sowing. Previous work (Afzal, 2005; Afzal *et al.,* 2005; Ashraf and Rauf, 2001; Basra *et al.,* 2006; Roy and srivastava, 2000) suggested that the adverse and depressive effects of salinity and water stress on germination can be alleviated by various seed priming treatments. Although the effects of priming treatments on germination of some seed crops has been studied, but relatively little information is available on the invigorating of maize inbred lines seed under salt stress.

The aim of the study was to evaluate whether priming with water and osmotic solution (urea) results in enhancement of seed vigour in maize inbred line (Mo17) under a range of osmotic potentials due to NaCl and PEG, respectively. Further to realize whether responsible factors for failure of maize seed germination under saline condition is an osmotic blockade or is due to toxic effects of NaCl.

MATERIALS AND METHODS

Seeds of inbred line maize MoI7 (as pollen parent) were used for this study. Seeds were obtained from Seed and Plant Improvement Institute, Karaj. The study was conducted in a seed laboratory of the Department of Seed Sciences and Technology, University of Tehran, Karaj.

Seed treatments consisted of T1: control (untreated seeds), T2: soaking in distilled water for 36 h (hydropriming). T3: soaking in -1.2 MPa solution of urea for 96 h (osmopriming). Both priming treatments were conducted at 15 °C in the dark.

Germination and early seedling growth were studied using distilled water (control) and under osmotic potentials of -0.4, -0.8 and -1.2 MPa for NaCl and polyethylene glycol (PEG 6000), respectively. NaCl concentrations had the electrical conductivity (EC) values of 3.7, 12.3, 17.4 and 21.8 dSm^{-1}, respectively. Referred osmotic potential of NaCl solution (-0.4, -0.8 and -1.2 MPa) were prepared by using of 5.25, 10.5 and 15.75 grams of NaCl per liter. Osmotic solution of PEG were prepared by using of 161, 241 and 302 gram of PEG per liter, respectively for -0.4, -0.8 and -1.2 MPa.

Three replications of 50 seeds were germinated in 12 cm diameter glass Petri dishes at 25±1 °C in a dark growth chamber with 45 % relativity humidity. 10 ml osmotic solution was added to each Petri dish and a seed scored germinated when root length reached 2 mm. Germinating seed were counted daily, and terminated when no further germination occurred.

Seedling vigour index (SVI) was calculated following modified formula of Abdul-Baki and Anderson (1973):

$$SVI = [seedling\ length\ (cm) \times germination\ percentage]$$

The germination index (GI) which expressed as speed of germination was calculated as described in the Association of Official Seed Analysts (AOSA, 1983).

Mean shoot and root lengths at the end of germination were measured per replication. Dry weights of seedlings were taken with the help of an electric balance after drying each replication at 70 °C in the oven to get the constant weight (Afzal *et al.,* 2005).

For comparison of control (untreated seeds) in stress and normal conditions the reduction of germination (RPG) was calculated according to the formula of Madidi *et al*, (2004).

The experimental design was three factors factorial (2×3×4) arranged in a completely randomized design (CRD) with three replications. First factor was solution (NaCl and PEG), the second seed treatments (control, hydropriming and osmopriming) and third was osmotic potential levels (0, -0.4, -0.8, -1.2 MPa). For statistical analysis, the data of germinating percentage were transformed to $\arcsin\sqrt{X/100}$. Data were subjected to analysis of variance (ANOVA) procedures (SAS Institute Inc., 1988), and LSD test was applied at 5 % probability level to compare the differences among treatment means.

RESULTS AND DISCUSSION

Analysis of variance showed that there is a significant three way interaction (seed treatment × solution× osmotic potential) for germination percentage (Table 1). Germination percentage showed the significant reduction with

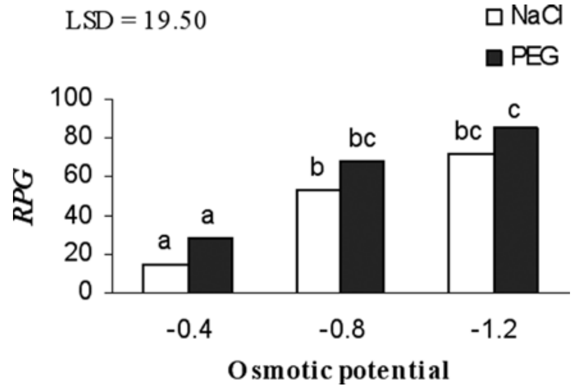

Fig. 1. Interaction effect of solution (NaCl or PEG) and osmotic potential on reduction percentage of germination of maize inbred line (Mo17). Figures not sharing same letters differ significantly at p=0.05, (RPG at each osmotic potential is average of all seed treatments).

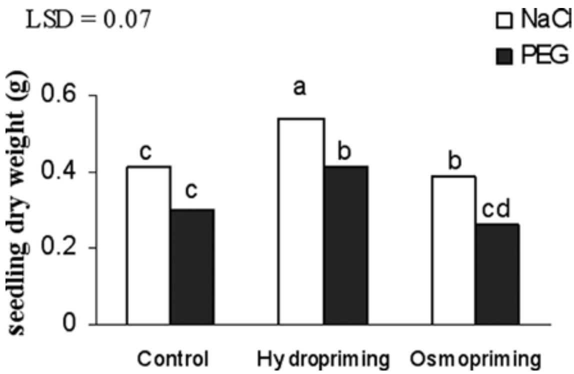

Fig. 2. Influence of different seed treatments on seedling dry weight of maize inbred line seeds (Mo17) under drought (PEG solution) or salt stress (NaCl solution). The bars with different alphabets are statistically different at p=0.05.

decrease in osmotic potential, in both solutions and all seed treatments. Hydroprimed seeds achieved maximum germination especially during the higher osmotic potentials (i.e. 0 and -0.4 MPa). Minimum germination was recorded at untreated seeds (control) followed by osmopriming (Table 2). At both seed treatments greater reduction in germination percentage due to PEG compared to NaCl was recorded (Figure 1).

A significant interaction of seed treatment × solution was found for seedling dry weight. Maximum seedling dry weight was attained from hydroprimed seeds under saline condition, while osmopriming could not improve this character under all condition as compared to control (figure 2).

There was a significant interaction of seed treatment × osmotic potential

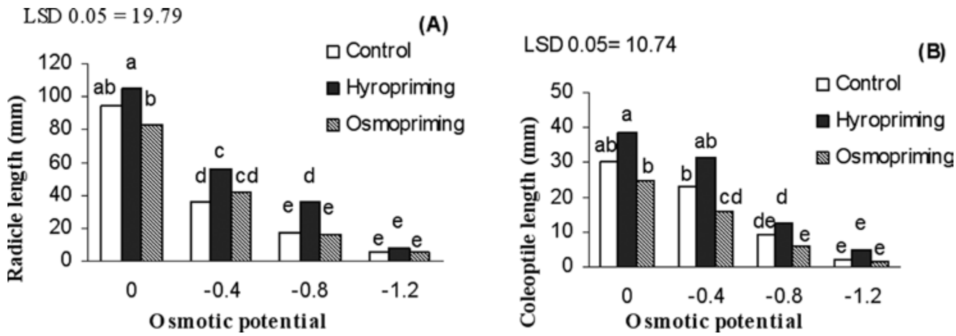

Fig. 3. Effect of different seed treatment (control, soaking in water and urea) on (A) radicle length, (B) coleoptile length averaged from Mo17 under different levels of osmotic potential during germination test. The vertical bars with different alphabets are statistically different (at p=0.05) indicating interactive effect of seed treatments and osmotic potential.

on both coleoptile and radicale length (Figure 3A, B). The radicle and coleoptile length of seeds that were subjected to hydropriming significantly differed from those subjeced to osmopriming and control, especially in high osmotic potentials (i.e. 0 and -0.4 MPa).

Interaction of seed treatment and osmotic potential for germination index (GI) showed that under high osmotic potentials (i.e. 0 and -0.4 MPa), hydroprimed seeds had higher GI as compared to osmoprimed or untreated seeds (Figure 4).

Interaction effect of seed treatment and osmotic potential significantly

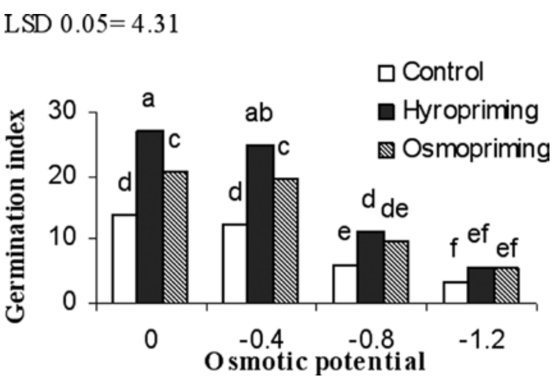

Fig. 4. Influence of different seed treatments on germination index of maize inbred line seeds (Mo17) under different osmotic potential levels. The bars with different alphabets are statistically different at p=0.05. (Germination index of each osmotic potential is averaged from NaCl and PEG solution).

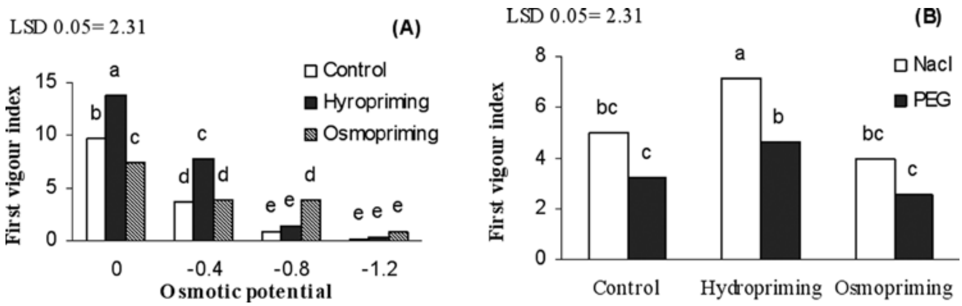

Fig. 5. Effect of different seed treatments on seedling vigour index of maize inbred line seeds (Mo17), (A) during different osmotic potentials (B) under drought (PEG solution) or salt stress (NaCl solution). The bars with different alphabets are statistically different at p=0.05.

affected the seedling vigour index (SVI). Hydropriming significantly increased SVI, mainly at high osmotic potentials. Averagely SVI of hydroprimed seeds was 56 % higher than untreated seeds (Figure 5A). Interaction of solution (NaCl or PEG) and seed treatment was significant for SVI. In the similar way hydropriming could improve this parameter while maximum value was recorded from the saline conditions (Figure 5B).

Present study showed that both salinity and drought stress affected germination adversely while the effects of drought stress were more severe than salinity stress. Compared to the control both seed treatments showed enhanced performance under stress conditions. Hydropriming technique compared with osmopriming clearly improved seed germination and seedling early growth under both stress and non-stress conditions. Hydroprimed seeds could achieve earlier and more uniform germination, or by higher GI and longer and heavier seedlings.

The findings of present study are in agreement with the results of Kaya *et al.* (2006) and Basra *et al.* (2006) who reported the hydroprimed seeds of sunflower and wheat could germinate faster and produced longer seedling under salinity stress, compared with untreated seeds.

Although some earlier studies referred that osmopriming can contribute to improve germination rate and seedling emergence in different plant species by increasing the expression of aquaporins (Gao *et al.,* 1999), enhancement of ATP_{ase} activity, RNA and acid phosphathase synthesis (Fu *et al.,* 1988),

Table 1. Factorial analysis of the seed priming effect on germination and seedling growth of maize inbred line (Mo17) under different levels of osmotic potential induced by NaCl and PEG.

Factorial analysis		Germination percentage		Seedling dry weight		Coleoptile length		Radicle length		GI		SVI	
Source of varience	**Df**	**F**	**P**	**F**	**P**	**F**	**P**	**F**	**P**	**F**	**P**	**F**	**P**
Seed priming (a)	2	4.16	<0.05	36.82	<0.01	6.03	<0.05	4.93	<0.01	61.72	<0.01	38.19	<0.01
Solution (b)	1	12.64	<0.01	4.79	<0.1	3.28	<0.1	19.58	<0.01	3.63	<0.05	62.11	<0.01
Osmotic potential (c)	3	34.32	<0.01	48.94	<0.01	39.24	<0.01	54.65	<0.01	17.84	<0.01	92.76	<0.01
Interaction a×b	2	1.53	0.289	26.03	<0.01	1.73	0.342	1.64	0.202	2.13	<0.1	5.34	<0.01
Interaction a×c	6	2.05	<0.1	2.52	<0.1	4.12	<0.01	2.46	<0.05	8.59	<0.01	2.19	<0.1
Interaction b×c	3	3.19	<0.05	0.84	0.227	2.09	0.124	1.37	0.324	0.94	0.261	3.16	<0.05
Interaction a× b×c	6	2.49	<0.05	1.16	0.164	0.481	0.673	0.68	0.723	0.644	0.897	0.928	0.552

Table 2. Germination percentage of maize inbred line seeds (Mo17) treated with urea (osmopriming), hydropriming and control (untreated) at water induced by NaCl and PEG.

MPa	Seed treatments					
	Control		Hydropriming		Osmopriming	
	NaCl	PEG	NaCl	PEG	NaCl	PEG
0	91.8	93.8	95.6	93.8	90.2	88.7
-0.4	67.6	53.0	83.7	74.0	74.4	60.6
-0.8	32.9	23.4	49.2	40.7	34.5	29.7
-1.2	21.7	13.5	27.4	18.9	26.3	14.5

LSD (Int) = 12.49 (96 d.f.).

also by increase of amylases, proteases or lipases activity (Ashraf and Foolad, 2005). Results of the present study showed that osmopriming with urea compared to hydropriming can not be recommended as suitable invigoration treatment under both stress and non-stress conditions. It may be due to toxic effect of urea or might be because of long period of priming or low osmotic potential (lower than critical potential). However, the superiority of hydropriming on germination could be due to soaking time effects rather than urea treatment. Hydroprimed seeds imbibed water for a longer time, compared to osmoprimed seeds, and went through the first stage of germination without protrusion of radicle.

Seeds germinated better in NaCl than PEG at the equivalent water potential, possibly due to the uptake of Na^+ and Cl^- ions by the seed, maintaining a water potential gradient allowing water uptake during seed germination. With no toxicity effect of PEG reported (Khajeh-Hosseini *et al.*, 2003), the lower germination percentage obtained from PEG compared with NaCl suggests that adverse effects of PEG on germination were due to osmotic effect rather than specific ion accumulation.

Our results showed significant improvement in germination and early growth of inbred line of maize (Mo17) due to hydropriming treatment. Soaking seeds for 36 h resulted in invigorate of germination under salinity and drought stress as well as normal conditions. Finding of present study also revealed that at equivalent osmotic potentials drought stress induced by PEG had more drastic inhibitory effects on germination. Thus, it is concluded that under salinity stress the osmotic effect is rather important than toxic effect in loss of seed germination. Our results suggest that hydropriming could be as suitable, cheap and easy seed invigoration treatment for inbred lines of maize, especially when germination is affected by salinity and drought stress.

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