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DESERT 16 (2011) 61-68

# The effect of hydropriming and halopriming on germination and early growth stage of wheat (*Triticum aestivum* L.)

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Received: 14 March 2010; Received in revised form: 17 April 2010; Accepted: 2 May 2010

## Abstract

In order to study of hydropriming and halopriming on germination and early growth stage of wheat (*Triticum aestivum* L.) an experiment was carried out in laboratory of the Department of AgroNomy and Plant breeding, Shahrood University of Technology. Seed treatments consisted of T1: control (untreated seeds), T2: soaking in distilled water for 18 h (hydropriming). T3: soaking in -1.2 MPa solution of $CaSO_4$ for 36 h (halopriming). 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. Results showed that Hydroprimed seeds achieved maximum germination seedling dry weight, especially during the higher osmotic potentials. Minimum germination was recorded at untreated seeds (control) followed by halopriming. Under high osmotic potentials, hydroprimed seeds had higher GI (germination index) as compared to haloprimed or untreated seeds. Interaction effect of seed treatment and osmotic potential significantly affected the seedling vigour index (SVI).

*Keywords:* Wheat; Hydropriming; Halopriming; Germination

## 1. Introduction

Soil salinity is one of the great concerns in arid and semi-arid regions in the world. According to the studies %7 of the world lands is saline and %3 is high saline, because of low precipitation, high evaporation and irrigation by saline waters, soil salinity is getting increased (Teimouri *et al*, 2009). The most important problems for economic crops production in arid regions are high concentration of ions especially NaCl either in soil or in irrigation water (Moeinrad, 2008). Salinity is one of the major and increasing problems in irrigated agricultural systems in Iran, particularly in wheat grown areas. Ghassemi *et al*. (1995) estimated about 14% of irrigated land to be badly affected by salinity in Pakistan. The adverse effects of high concentration of salts for plants are due to the osmotic retention of water and to specific ionic effects on the protoplasm. Water is osmotically held in salt solutions, so as the concentration of salt increased water becomes less and less accessible to the plant. Poor germination and seedling establishment are the results of soil salinity. It is an enormous problem adversely affecting growth and development of crop plants and results in to low agricultural production (Garg & Gupta, 1997). Salt and osmotic stresses are responsible for both inhibition or delayed seed germination and seedling establishment (Almansouri *et al*. 2001). Under these stresses there is a decrease in water uptake during imbibitions and furthermore salt stress may cause excessive uptake of ions (Murillo-Amador *et al*. 2002). Seed priming has been successfully demonstrated to improve germination and emergence in seeds of many crops, particularly

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seeds of vegetables and small seeded grasses (Bradford, 1986). Dharmalingam and Basu (1990) reported beneficial effect of a hydration-dehydration seed treatment on germination of sunflower.

Wheat is grown on all type of soils and is classified as a moderate, salt tolerant crop (Mass & Hoffman, 1977). Yield losses on salt affected soils of Iran average about 64%. 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 halopriming and hydropriming. Halopriming is a pre-sowing soaking of seeds in salt solutions, which enhances germination and seedling emergence uniformly under adverse environmental conditions. Hydropriming involved soaking of seed in water before sowing. Previous work (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 wheat seed under salt stress.

The aim of the study was to evaluate whether priming with water and salt solution ($CaSO_4$) results in enhancement of seed vigour in wheat (cv. Pishtaz) under a range of osmotic potentials due to NaCl and PEG, respectively. Further to realize whether responsible factors for failure of wheat seed germination under saline condition is an osmotic blockade or is due to toxic effects of NaCl.

## 2. Material and methods

Seeds of wheat (*Triticum aestivum* L.) cv. Pishtaz were used for this study. The study was conducted in laboratory of the Department of Agronomy and Plant breeding, Shahrood University of Technology. Seed treatments consisted of T1: control (untreated seeds), T2: soaking in distilled water for 18 h (hydropriming). T3: soaking in -1.2 MPa solution of $CaSO_4$ for 36 h (halopriming). Both priming treatments were conducted at 20 °C in the dark separately and re dried up to original weight with forced air under shade following Basra *et al*. (2005) procedure. 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). 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 was prepared by using of 161, 241 and 302 gram of PEG per liter for -0.4, -0.8 and -1.2 MPa respectively. 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 % relative humidity. 10 ml of each osmotic solution was added to each Petri dish and a seed scored germinated when radicle length reached 2 mm or more. Germinating seed were counted daily, and terminated when no further germination occurred. Germination percentage (GP) was calculated following formula:

$GP = [\text{Total seeds germinated (when no further germination occurred)}/\text{Total number of seeds}] \times 100$

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

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

The germination index (GI) which expressed as speed of germination was calculated as described in Scott *et al*. (1984:)

$GI = \sum (TiNi)/S$

Where *Ti* is the number of days after sowing, *Ni* is the number of seeds germinated on *i*th day, and *S* is the total number of seeds used. 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 percentage of germination (RPG) was calculated according to the formula of Madidi *et al*, (2004). The experimental design was three factors factorial (2×3×4) based on completely randomized design (CRD) with three replications. First factor was solution (NaCl and PEG), the second factor was seed treatments (control, hydropriming and halopriming) 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 $X$ /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.

## 3. Results and discussion

Analysis of variance showed that there is a significant three way interaction (seed treatment × solution× osmotic potential) for germination percentage (Table1). Germination percentage showed the significant reduction with decrease in osmotic potential, in both solutions and all seed treatments (Table2). 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 halopriming (Table 2). At both seed treatments greater reduction in germination percentage due to PEG compared to NaCl was recorded (Figure 1). Abbasdokht *et al.* (2004) reported that high quality of germination in crops will result high yield. A significant interaction of seed priming × solution was found for seedling dry weight (Table1). Maximum seedling dry weight was attained from hydroprimed seeds due to NaCl condition, while halopriming could not improve this character under all condition as compared to control (Fig. 2).

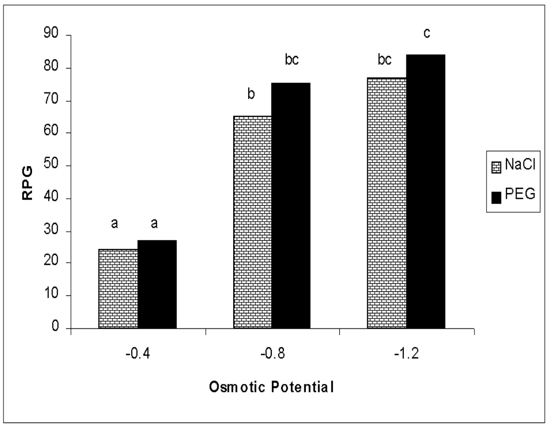

Fig. 1. Interaction effect of solution (NaCl or PEG) and osmotic potential on reduction percentage of germination (RPG) of wheat (cv. Pishtaz). Figures not sharing same letters differ significantly at P=0.05, (RPG at each osmotic potential is average of all seed treatments)

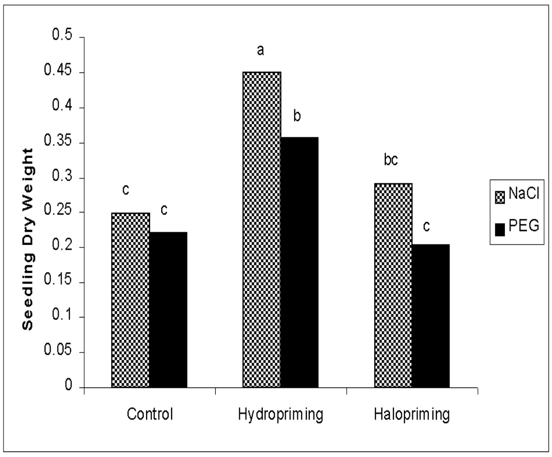

Fig. 2. Influence of different seed priming treatments on seedling dry weight (gr) of wheat (cv. Pishtaz) under drought (PEG solution) or salt stress (NaCl solution)

Neamatollahi *et al*. (2009) reported that hydropriming increased seedling dry weight under saline condition. There was a significant interaction of seed treatment × osmotic potential on both root length and shoot length (table 1). The root and shoot length of seeds that were subjected to hydropriming significantly differed from those subjected to halopriming and control, especially in high osmotic potentials (i.e. 0 and -0.4 MPa). The root and shoot length decreased by increasing in osmotic potential in both NaCl and CaSo4 but shoot length was affected severely in comparison to root length (Fig.3A, B). Interaction of seed treatment and osmotic potential for germination index (GI) showed that under 0 and -0.4 MPa, hydroprimed seeds had higher GI as compared to haloprimed or untreated seeds (Fig.4). Interaction effect of seed treatment and osmotic potential significantly affected the seedling vigour index (SVI). Hydropriming significantly increased SVI, mainly at high osmotic potentials (Fig.5a).

Table 1. Factorial analysis of the seed priming effect on germination and seedling growth of wheat (cv. Pishtaz) under different levels of osmotic potential induced by NaCl and PEG.

| Source of variance | df | Germination percentage | Seedling dry weight | Shoot length | Root length | Germination index | seedling vigour index |
|---|---|---|---|---|---|---|---|
| Priming (a) | 2 | $5.2^{*}$ | $25.12^{**}$ | $5.02^{*}$ | $5.33^{**}$ | $58.1^{**}$ | $45.12^{**}$ |
| Solution (b) | 1 | $13.11^{**}$ | $2.17^{ns}$ | $4.12^{ns}$ | $22.11^{**}$ | $4.17^{*}$ | $54.38^{**}$ |
| Osmotic potential (c) | 3 | $46.65^{**}$ | $33.23^{**}$ | $42.13^{**}$ | $47.44^{**}$ | $22.63^{**}$ | $86.27^{**}$ |
| Interaction a × b | 2 | $2.23^{ns}$ | $18.11^{**}$ | $2.15^{ns}$ | $1.05^{ns}$ | $3.15^{ns}$ | $7.11^{**}$ |
| Interaction a × c | 6 | $3.21^{ns}$ | $1.05^{ns}$ | $3.63^{**}$ | $3.02^{*}$ | $10.61^{**}$ | $1.43^{ns}$ |
| Interaction b × c | 3 | $4.17^{*}$ | $0.12^{ns}$ | $2.12^{ns}$ | $2.17^{ns}$ | $1.09^{ns}$ | $4.18^{*}$ |
| Interaction a × b × c | 6 | $3.35^{*}$ | $0.8^{ns}$ | $0.95^{ns}$ | $1.01^{ns}$ | $1.63^{ns}$ | $1.11^{ns}$ |

*P < 0.05; **P < 0.01; Ns: not significant

Table 2. Germination percentage of wheat seeds (cv. Pishtaz) primed with $CaSO_4$ (halopriming), hydropriming and control (untreated) at water induced by NaCl and PEG

| MPa | Seed treatments | | | | | |
|---|---|---|---|---|---|---|
| | Control | | Hydropriming | | Halopriming | |
| | NaCl | PEG | NaCl | PEG | NaCl | PEG |
| 0 | 90.11 | 91.16 | 95.13 | 96.13 | 92.11 | 93.23 |
| -0.4 | 74.37 | 81.13 | 79.12 | 80.78 | 73.17 | 65.18 |
| -0.8 | 41.4 | 35.63 | 48.13 | 50.14 | 27.15 | 30.11 |
| -1.2 | 30.7 | 27.15 | 34.72 | 33.16 | 23.13 | 17.18 |

LSD=10.33

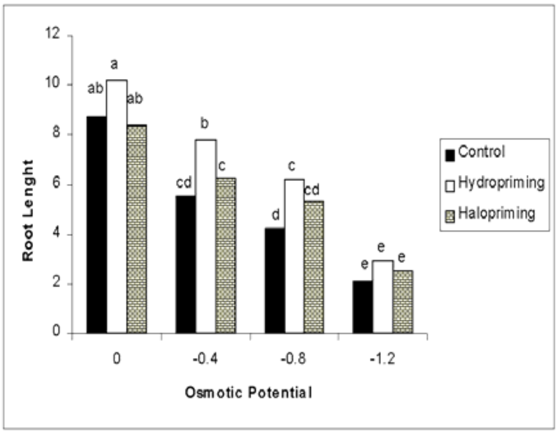

Fig. 3(a). Effect of different seed priming treatments (control, hydropriming and halopriming) on root length (cm), under different levels of osmotic potential during germination test. The vertical bars with different alphabets are statistically different (at p=0.05) indicating interactive effect of seed priming treatments and osmotic potential

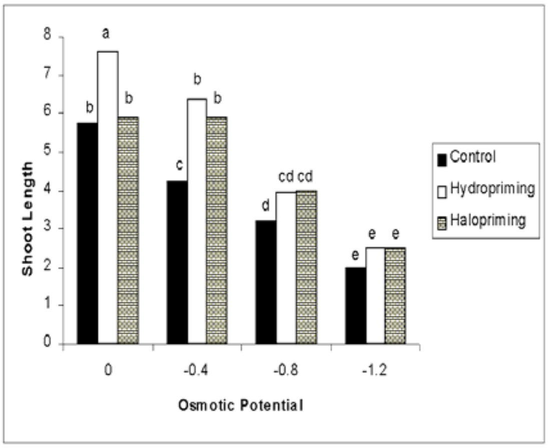

Fig. 3(b). Effect of different seed priming treatments (control, hydropriming and halopriming) on shoot length (cm) averaged from wheat (cv. Pishtaz) under different levels of osmotic potential during germination test. The vertical bars with different alphabets are statistically different (at p=0.05) indicating interactive effect of seed priming treatments and osmotic potential

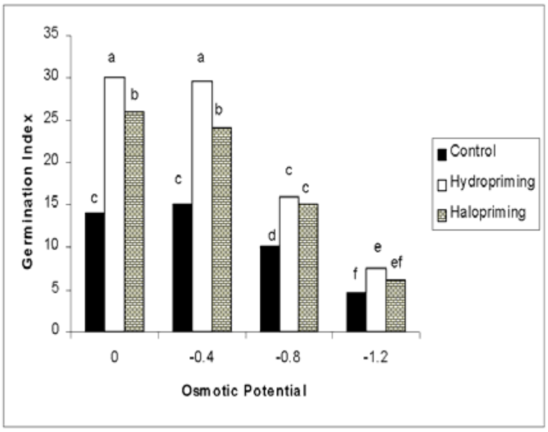

Fig. 4. Influence of different seed priming treatments on germination index of wheat (cv. Pishtaz) under different osmotic potential levels. The bars with different alphabets are statistically different at P= 0.05. Germination index of each osmotic potential is averaged from NaCl and PEG solution)

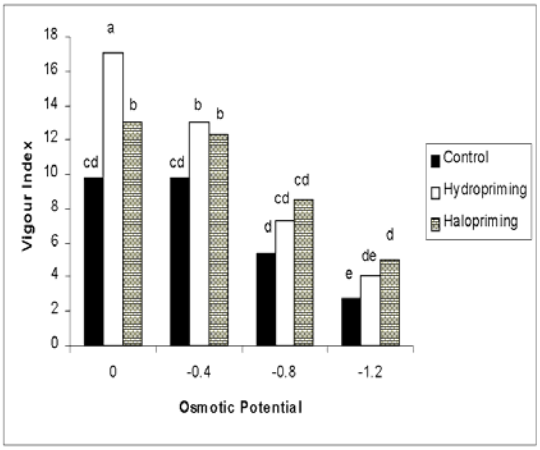

Fig. 5(a). Effect of different seed priming treatments on seedling vigour index of wheat (cv. Pishtaz), during different osmotic potentials. The bars with different alphabets are statistically different at p=0.05

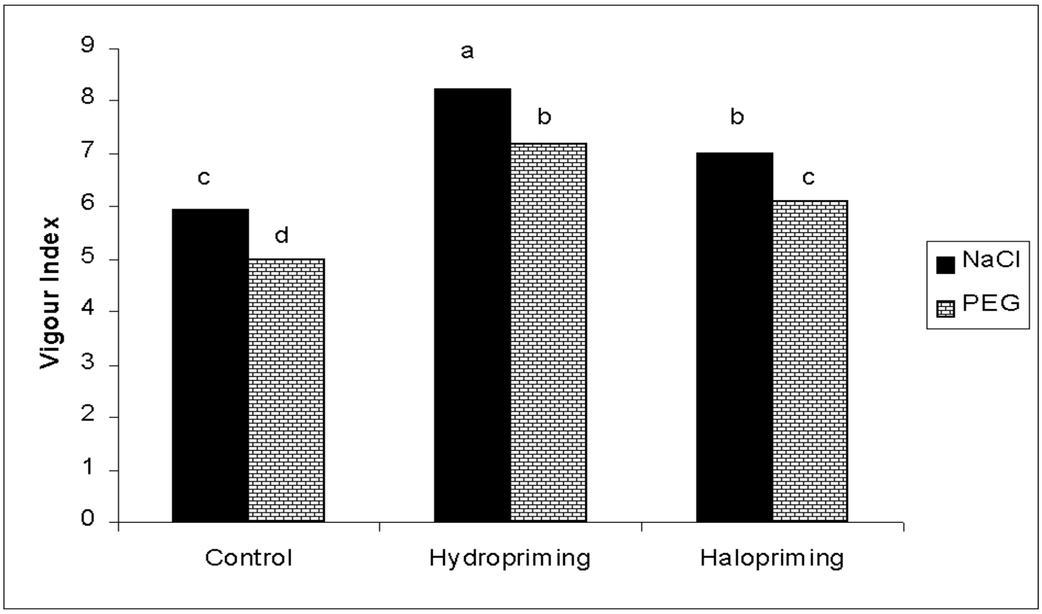

Fig. 5(b). Effect of different seed priming treatments on seedling vigour index of wheat (cv. Pishtaz), under drought (PEG solution) or salt stress (NaCl solution). The bars with different alphabets are statistically different at p=0.05

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 NaCl 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 halopriming 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. Caseiro *et al* (2004) found that hydro priming was the most effective method for improving seed germination of onion, especially when the seeds were hydrated for 96 h compared to 48 h. 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 halopriming 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 ATPase activity, RNA and acid phosphathase synthesis (Fu *et al.,* 1988), also by increase of amylases, protease or lipases activity (Ashraf and Foolad, 2005).

Results of this study showed that halopriming with $CaSO_4$ compared to hydropriming can not be recommended as suitable treatment under both stress and non-stress conditions. It may be due to toxic effect of $CaSO_4$ 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 $CaSO_4$ treatment. Hydroprimed seeds imbibed water for a longer time, compared to haloprimed seeds, and went through the first stage of germination without protrusion of root. 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 wheat (cv. Pishtaz) 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 wheat, especially when germination is affected by salinity and drought stress. The results of priming among species, varieties, and seedlots have been variable (Heydecker, 1977). Because of this variability in response, Bradford (1986) has suggested that treatment conditions must be optimized for each seedlot. However, maximum priming can be achieved in a particular seedlot through various combinations of temperature, water potential and treatment duration.

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