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ORIGINAL ARTICLE

Seed Germination, Seedling Growth and Enzyme Activity of Wheat Seed Primed under Drought and Different Temperature Conditions

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The study aimed was to determine the effects of drought stress (0, -4, -8, -12 bar) and osmo-priming (-15 bar PEG 6000 for 15 at 24 h) on seed germination, seedling growth and enzyme activity at different temperatures were assessed in the laboratory for wheat. Results showed that the highest germination percentage (GP) (94.33%), normal seedling percentage (NSP) (92%), germination index (GI) (44.85) and seedling length (11.03 cm) were attained from osmo-priming in control conditions. Therefore, seed priming with PEG 6000 significantly ($p \leq 0.01$) increased germination characteristics as compared to the unprimed seeds under drought stress. Also, osmo-priming increased catalase (CAT) and ascorbate peroxidase (APX) as compared to the unprimed.

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Drought and salinity are widespread problems around the world (Soltani *et al*., 2006). Therefore Davidson and Chevalier (1987) and Owen (1972) reported that seed germination and seedling growth of wheat (*Triticum aestivum* L.), like other crops, were negatively affected by drought stress. Parera and Cantliffe (1994) reported that rapid and uniform field emergence is an essential prerequisite to reach the yield potential, quality and ultimately profit in annual crops.

Seed characteristics are usually essential process in seedling establishment and plant development to obtain seedling numbers those results in higher seed crop (Almansouri *et al*., 2001; Murungu *et al*., 2003). Also, Patade *et al*. (2011) reported that seed germination and establishment are the most sensitive stages to salinity stress. Abiotic stresses maybe delay seed germination and reduce the rate (Patade *et al*., 2011; Rouhi *et al*., 2011; Ansari and Sharif-Zadeh, 2012; Ansari *et al*., 2012; Ansari *et al*., 2013). Ashraf and Foolad (2005) showed that seed priming is one of the methods that can be taken to counteract the adverse effects of abiotic stresses. Seed priming techniques have been used to increase germination, improve germination uniformity, improve seedling establishment and stimulate vegetative growth in more field crops (Ansari and Sharif-Zadeh, 2012; Ansari *et al*., 2012;

Patade *et al*., 2011; Foti *et al*., 2008) under stressed conditions. Also, other researchers reported that, the priming strategies enhanced activities of free radical scavenging enzymes such as CAT and APX (Ansari and Sharif-Zadeh, 2012; Rouhi *et al*., 2011; Ansari *et al*., 2013). In addition, the priming increased the germination percentage and activities of glyoxylate cycle enzymes in Persian Silk Tree (*Albizia julibrissin* Durazz) (Sadghi *et al*, 2011). Therefore, the study aimed was to determine the effects of drought stress (0, -4, -8, -12 bar) and osmo-priming (-11 bar PEG 6000 for 15 at 24 h) on seed germination, seedling growth and enzyme activity at different temperatures were assessed in the laboratory for wheat.

MATERIALS AND METHODS

The study was conducted in the seed laboratory of Natural Resources Faculty, University of Tehran, Iran.

Drought stress at osmotic potentials of 0 (as control), - 4, -8 and -12 bar were adjusted using PEG 6000 before the start of the experiment.

Seeds of were pretreated with PEG 6000. For halo-priming, seeds were exposure in -15 bar concentrations PEG 6000 for 24 h at 15±1°C. Wheat seeds were exposure in 20 cm glass petri dishes containing 15 ml solution. The imbibed seeds were then washed three times with tap water and dried on filter paper at 15±1°C for 24 h (Ansari and Sharif-Zadeh, 2012; Ansari *et al*., 2013).

This experiment was carried out in 4 levels of dorught stress (0, -4, -8, -12 bar) under different temperature incubations (15, 20 and 25 °C). For the germination tests, three replicates of 50 seeds were surface sterilized and imbibed on two layers of blotter paper in 9-cm-diameter Petri dishes at 15, 20 and 25 ˚C incubation under different drought stress. Germinated seeds were recorded every 24 h for 8 days. After test time expiration, some germination indexes were evaluated such as: Germination percentage, normal seedling percentage, germination Index and seedling length.

All extraction procedures were carried out at 4 °C. The seed samples, weighting about 0.3 gr, were homogenized with 3 ml of tris (PH 7.8), followed by centrifugation of 20000 g for 20 min. The supernatants were used for determination of enzyme activity. The supernatants were used for determination of enzyme activity. Catalase (CAT, EC 1.11.1.6) activity was determined spectrophotometric ally following H_2O_2 consumption at 240 nm (Chiu *et al*., 1995). Ascorbate peroxidase (APX, EC 1.11.1.7) activity was determined according to the procedures of Johnson and Cunningham (1972). The activities of APX and CAT were expressed per mg protein, and one unit represented 1 µmol of substrate undergoing reaction per mg protein per min.

All data were analyzed statistically by analysis of variance using SAS Software. Data for germination and normal germination percentages were subjected to arcsine transformation before analysis of variance was carried out with SAS software. Mean comparisons were performed using an ANOVA protected least significant difference (Duncan) ($P < 0.01$) test.

RESULTS AND DISCUSSION

Analyze of variance for osmo-priming showed that Temperature × Treatment × Drought stress interaction was significantly ($P < 0.01$) for all traits under drought stress (Table 1). Also, our results showed that the highest germination percentage (GP) (94.33%), normal seedling percentage (NSP) (92%), germination index (GI) (44.85) and seedling length (11.03 cm) were attained from osmo-priming in control conditions at 20 °C (Fig 1-4).

In agreement with the results, earlier reports (Ansari *et al*., 2012; Patade *et al*., 2011; Ansari *et al*., 2013), have shown negative affect stress conditions on germination characteristics. The results of our study suggested that osmo-priming cause improvement in the seed characteristics as compared to the unprimed. Cumulative germination showed that the highest GP at 15 °C, 20 °C and 25 °C was attained from seed primed under control condition (Fig 6-8).

In agreement with the results, earlier reports (Ansari *et al*., 2012; Rouhi *et al*., 2011; Patade *et al*., 2011; Ansari *et al*., 2013) positive effects of priming in relation to seed performance, germination percentage and seedling indices. In the present investigation osmo-priming increased catalase and ascorbat peroxidase as compared to the unprimed seed (Fig 5). Seed priming highly increased APX and CAT activities in mountain rye (Ansari *et al*., 2012; Ansari *et al*., 2012. Also, Rouhi *et al*. (2012) showed that antioxidant enzyme activities (superoxide dismutase, catalase, and peroxidase) in treated seeds of Berseem clover (*Trifolium alexandrinum* L.) were significantly increased compared to those in control group. Oxidative stress blocks growth and development by decreasing cell division, therefore protection from oxidative stress is critical for seed germination. Recent studies show that the presence of several antioxidative and hydrolytic enzymes in dry cereal grains, and activities raised considerably after the start of seed imbibition (Chang *et al*., 2000; Demeke *et al*., 2001). Recently ansari and Sharif-Zadeh (2012) in Mountain rye and Rouhi *et al*. (2012) in Berseem clover (*Trifolium alexandrinum* L.) showed that antioxidant enzyme activities (superoxide dismutase, catalase, and peroxidase) in treated seeds of were significantly increased compared to those in control group.

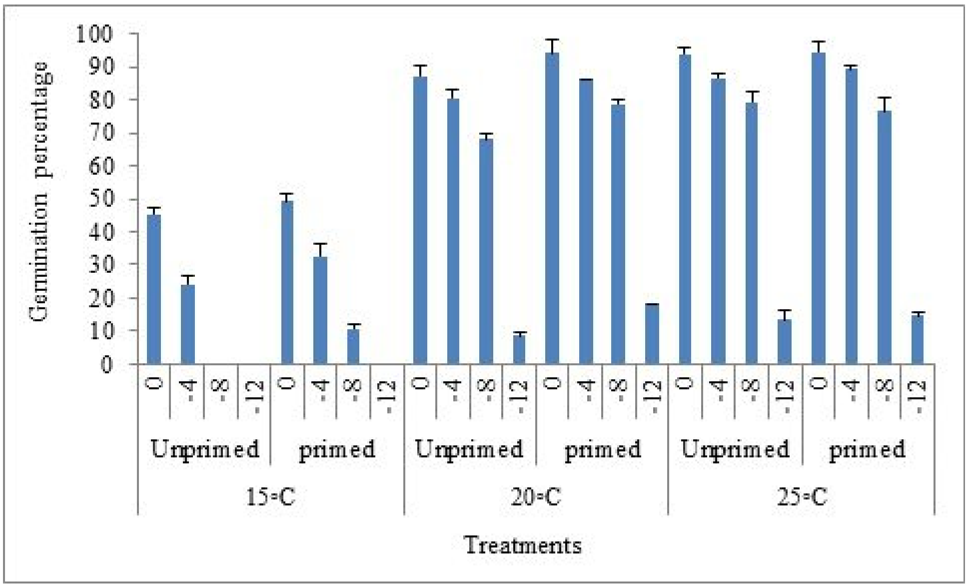

Figure 1. Effect of Temperature × Treatment × drought stress interaction on germination percentage.

Table 1. Variance analysis of studied traits in wheat under drought stress.

S.O.V	df	GP	NSP	GI	LS
Temperature (A)	2	9643.77**	13367.86**	5202.23**	54.32**
Treatment (B)	1	344.34**	1505.07**	24.99**	6.16**
Salinity stress (C)	3	8537.25**	9980.74**	3586.81**	55.35**
A*B	2	70.05**	331.22**	3.62ns	0.41**
A*C	6	296.58**	1799.99**	498.8**	11.01**
B*C	3	24.25ns	341.08**	1.48ns	1.41*
A*B*C	6	46.11**	552.23**	3.15**	0.81**
Error	-	12.01	4.31	1.22	0.04
CV	-	9.44	8.18	6.17	13.11

*and ** indicate significant difference at 5% and 1% probability level respectively.

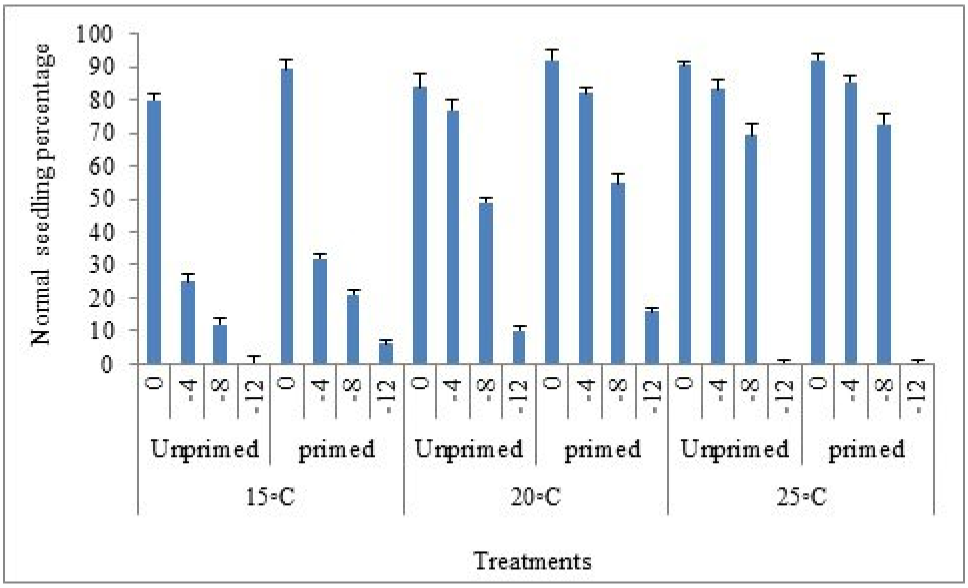

Figure 2. Effect of Temperature × Treatment × drought stress interaction on normal seedling percentage.

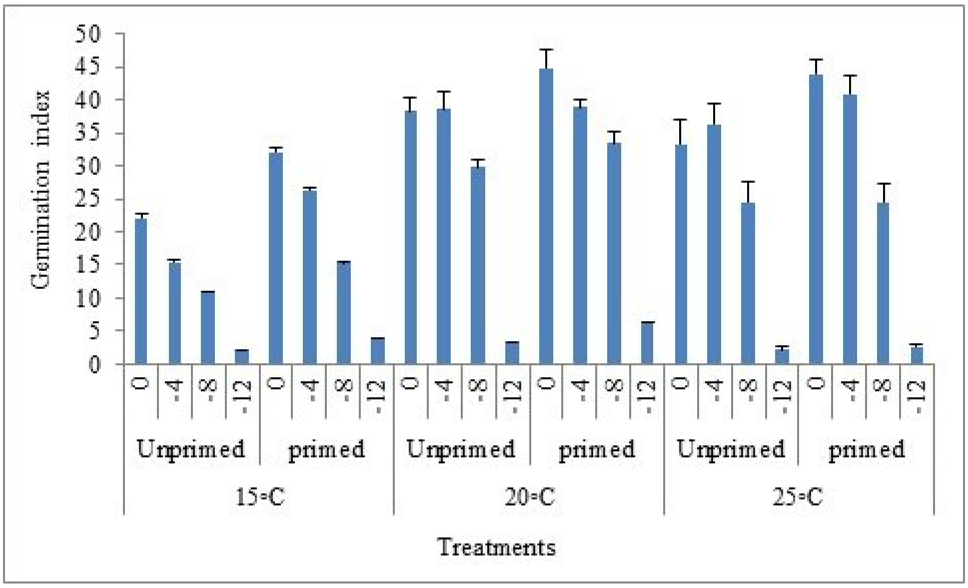

Figure 3. Effect of Temperature × Treatment × drought stress interaction on germination index.

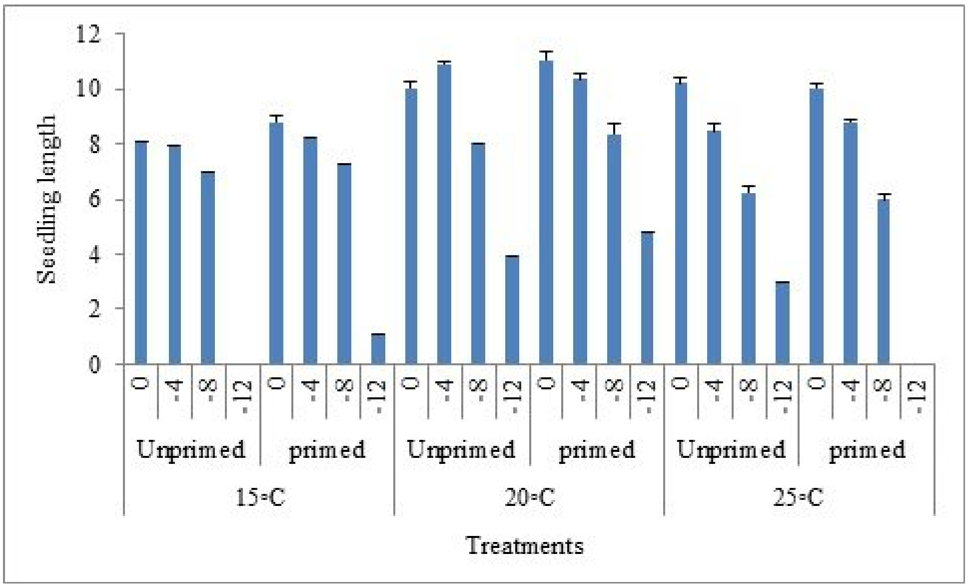

Figure 4. Effect of Temperature × Treatment × drought stress interaction on seedling length.

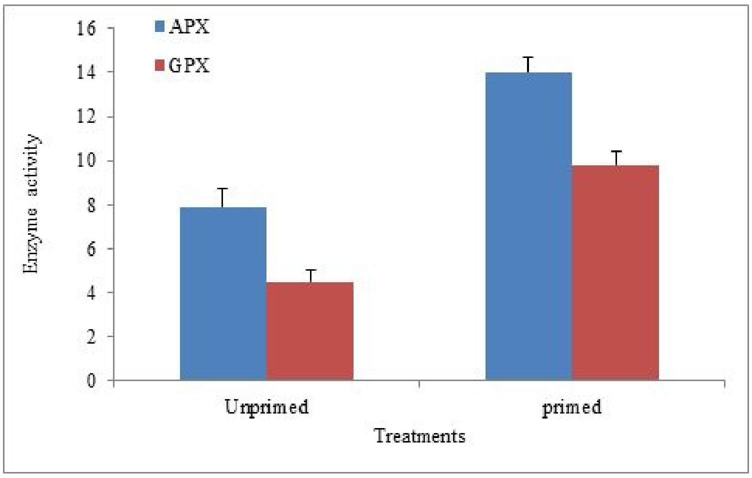

Figure 5. Effect of osmo-priming on enzyme activity.

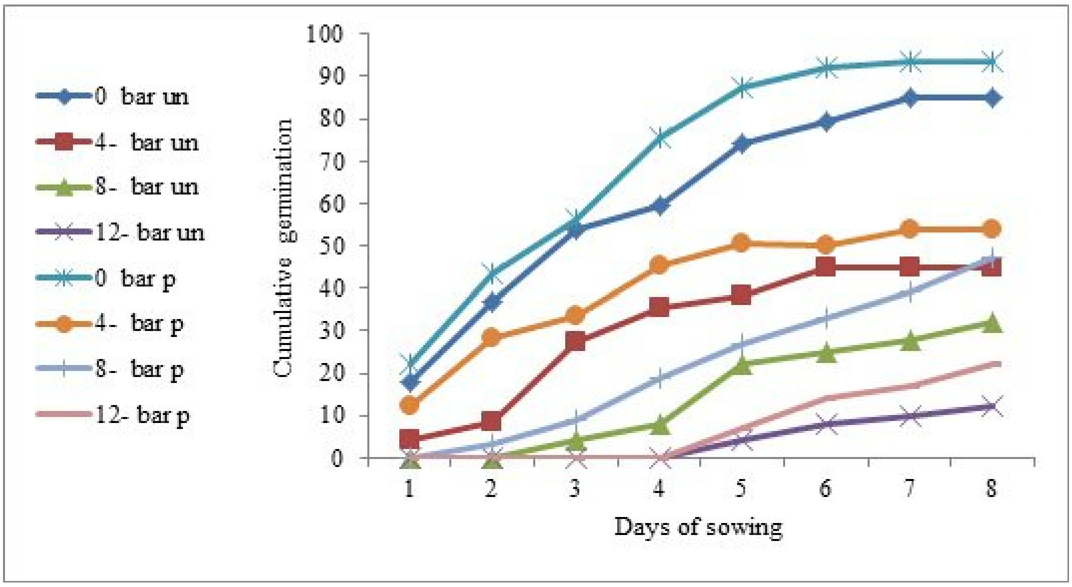

Figure 6. Cumulative seed germination of pre-sowing treated and control of wheat seeds germinated at 15 °C. (un: unprimed, p: primed).

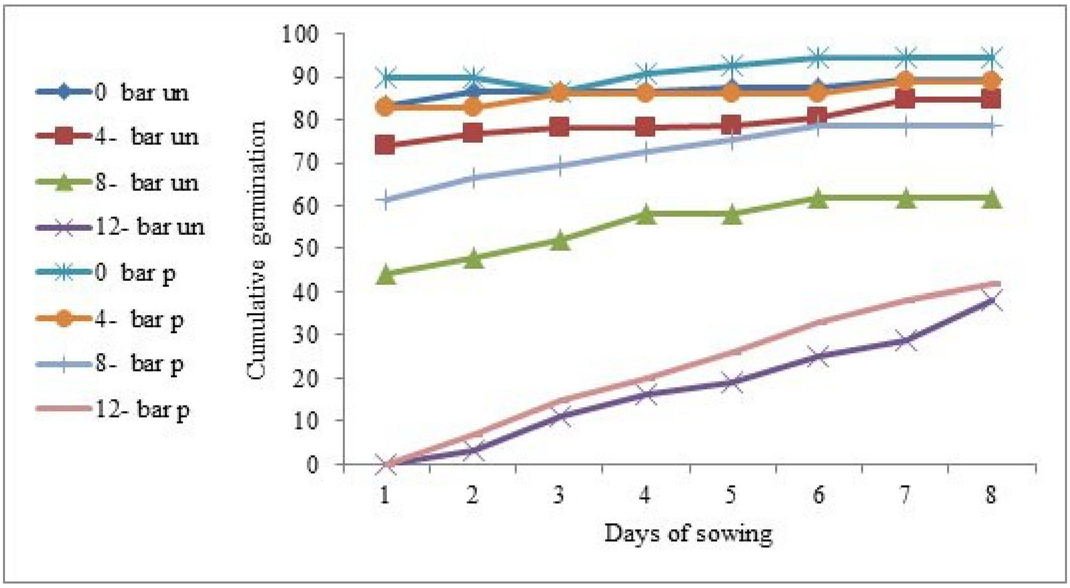

Figure 7. Cumulative seed germination of pre-sowing treated and control of wheat seeds germinated at 20 °C. (un: unprimed, p: primed).

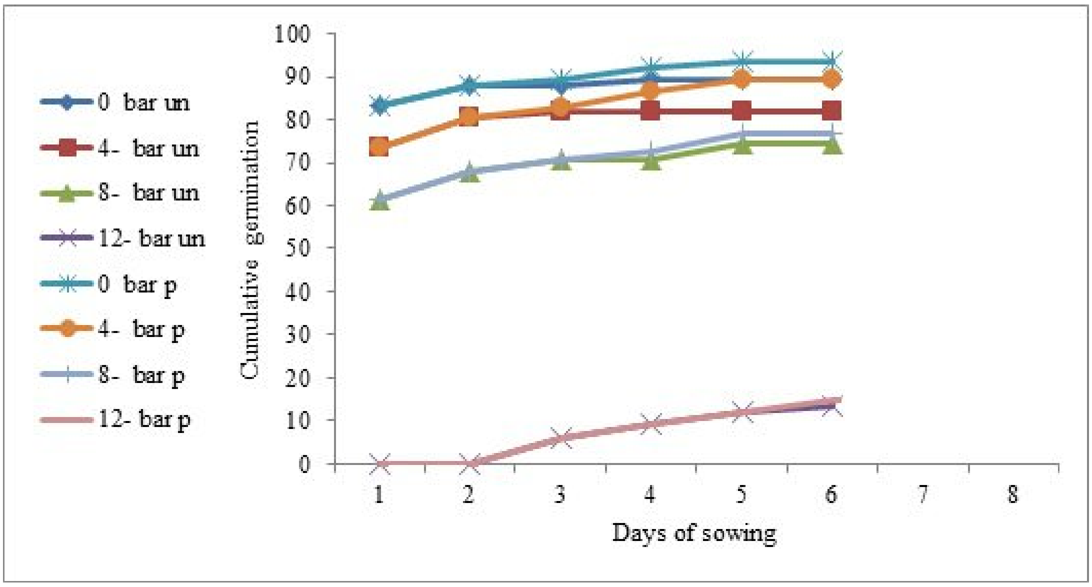

Figure 8. Cumulative seed germination of pre-sowing treated and control of wheat seeds germinated at 25 °C. (un: unprimed, p: primed).

CONCLUSION

The highest germination characteristics were attained from osmo- priming under control condition in each temperature. Osmo-priming increased germination characteristics as compared to the unprimed seeds. Also, priming increased CAT and APX. therefore can be said that improvement this germination characteristics of primed seeds could be results of increasing the antioxidant profile of treated seeds.

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