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ORIGINAL ARTICLE

Effect of Osmo-priming on Germination and Enzyme Activity in Barley (*Hordeum vulgare* L.) Seeds under Drought Stress Conditions

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Seed priming was used in barley to increase seed germination and tolerance on stress exposure. Barley seeds were treated with PEG (Polyethylene 6000 mw). After 7 days our results showed that, seed priming treatments significantly ($p \leq 0.01$) affected germination percentage, normality seedling percentage, and germination index. Seed priming with PEG increased germination characteristics as the compared to the unprimed. Also, priming increased catalase as compared to the unprimed seeds. Therefore, the highest germination characteristics and catalase activity were attained from priming with PEG.

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Key words: Osmo-priming, Seed germination, Drought stress, Enzyme activity, Hordeum vulgare L.

Barley is one of the most widely grown crops in arid and semiarid regions of the world. The seeds of barley show a delayed or reduced germination when the water potential of surrounding medium decreases. Stress conditions are widespread problem around the world.

Germination 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). Seed germination and establishment are the most sensitive stages to abiotic stress in more crop species (Patade *et al.*

2011, Redmann, 1974). Abiotic stress maybe delay seed germination and reduce the rate (Patade *et al.*, 2011. Rouhi *et al.*, 2011. Ansari *et al.*, 2012). Patade *et al.* (2009) and Ashraf and Foolad (2005) reported that seed priming is one of the methods that can be taken to counteract the adverse effects of abiotic stress (Patade *et al.*, 2009. Ashraf and Foolad., 2005). The purpose of seed priming is to a partially hydrated with water, or various chemical solutions the seeds to a point where germination processes are begun but not completed (Asfrac and Foolad., 2005), followed by drying of seeds to the original moisture level. Seed priming techniques

have been used to increase germination, improve germination uniformity, improve seedling establishment and stimulate vegetative growth in more field crops (Iqbal and Ashraf, 2007; Kaya *et al.*, 2006; Kaur *et al.*, 2002; Patade *et al.*, 2011; Saglam, *et al.*, 2011; Sadeghi *et al.*, 2011), under stressed conditions. Also, the priming strategies enhanced activities of free radical scavenging enzymes such as CAT and SOD (Ansari *et al.*, 2012). Therefore, the study aimed was to determine the effect of osmo-priming treatment on germination characteristics and enzyme activity of barley, under drought stress.

MATERIALS AND METHODS

The study was conducted in the Faculty member, Agricultural and natural resources reasearch center of Yazd,Iran.

Drought stress at osmotic potentials of 0 (as control), -4, -8, -12 and -16 bar were adjusted using PEG 6000 before the start of the experiment.

Seeds of were pretreated with PEG 6000. For osmo-priming, seeds were exposure in -8 bar concentrations PEG for 24 h at $15 \pm 1^\circ\text{C}$. Barley 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 $15 \pm 1^\circ\text{C}$ for 24 h (Ansari and Sharif-Zadeh, 2012).

After test time expiration, some germination indexes were evaluated such as: germination percentage, normality seedling percentage, and germination index.

All extraction procedures were carried out at 4°C (Primed and unprimed). 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 (Ansari and Sharif-Zadeh, 2012). 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 (Bailly *et al.*, 1996). The activities of 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

According to our results of variance analysis, effect of priming treatments on GP, GI, NSP and MTG, under droght stress conditions were significant ($P < 0.01$) (Table 1).

Osmo-priming increased germination characteristics as compared unprimed seeds under drought stress conditions (Fig. 1, 2, 3 and 4).

Our results showed that germination and seedling growth were significantly improved in barley seeds primed with PEG as compared to the unprimed seeds. The highest germination percentage was attained from osmo-priming in control conditions but was no significantly with unprimed seeds (Fig. 1). Priming with PEG, also improved the GI as compared to the unprimed seeds under drought stress (Fig. 2). The highest NSP was attained from treatment of PEG in control conditions (Fig. 3). The highest MTG was attained from unprimed seeds under drought stress conditions (Fig. 4).

Seed germination and seedling growth are critical stages in the life cycle of a plant, especially under adverse abiotic stresses. Seed priming is one of the methods that can be taken to counteract the adverse effects of abiotic stress (Patade *et al.*, 2009. Ashraf and Foolad., 2005). The results are in agreement with the earlier study Ansari *et al.* (2012) who reported the significant reduction in the germination as well as growth of Rye. Also, earlier reports (Patade *et al.*, 2011; Ansari *et al.*, 2012; Rouhi *et al.*, 2011) have shown positive effect of priming in relation to seed performance, germination percentage and seedling indices. Therefore, our results showed that in across drought stress levels priming increased germination indexes as compared to the unprimed seeds.

Enzyme activity was significantly improved in barley seeds primed as compared to the unprimed (Fig. 5). CAT significantly improved in barley seeds primed with PEG as compared to the unprimed. Also APX significantly improved in barley seeds

primed with PEG as compared to the unprimed (Figure 5).

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, Morohashi 2002, Reichheld *et al.* 1999). Increasing CAT could significantly increase seed tolerance to environmental conditions (Ansari *et al.*, 2012). Recently Moosavi *et al.* (2009) in Amaranth genotypes 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. The priming strategies enhanced activities of free radical scavenging enzymes such as CAT and SOD (Rouhi *et al.*, 2012).

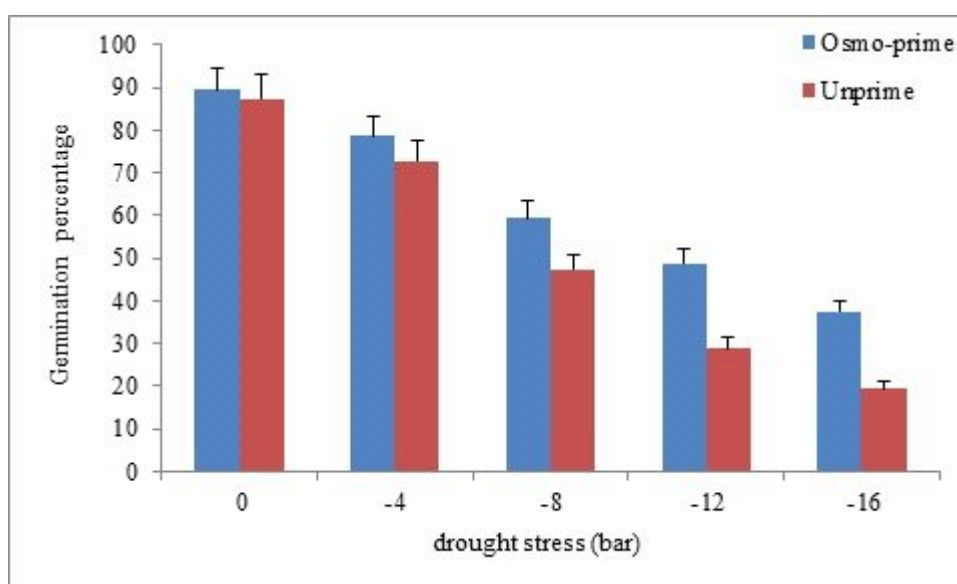


Figure 1. Effect of priming on germination percentage of barley seeds under drought stress.

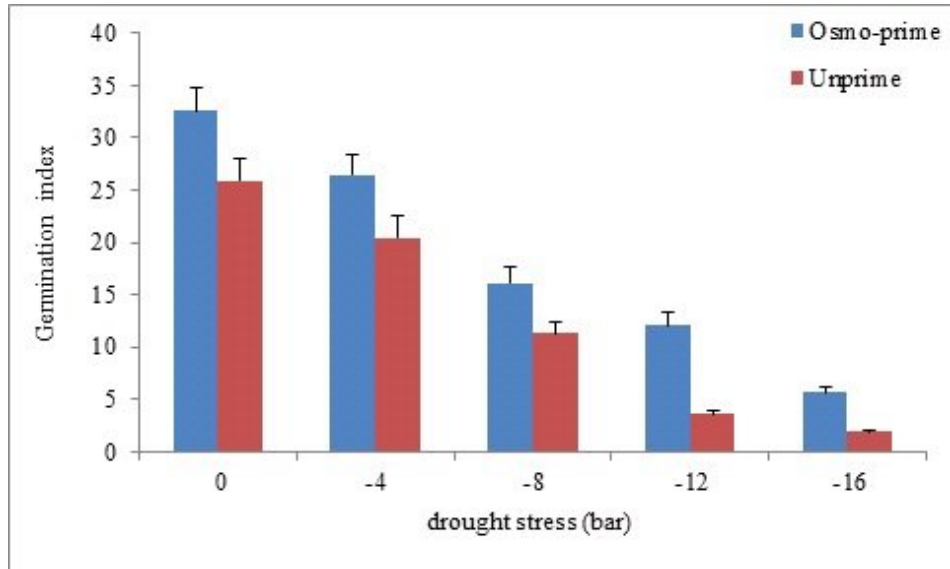


Figure 2. Effect of priming on germination index of barley seeds under drought stress.

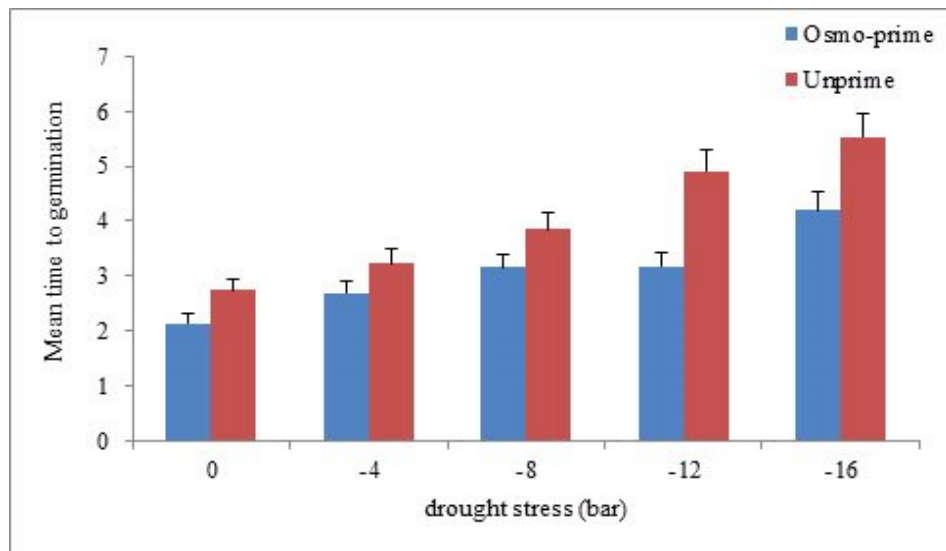


Figure 3. Effect of priming on mean time to germination of barley seeds under drought stress.

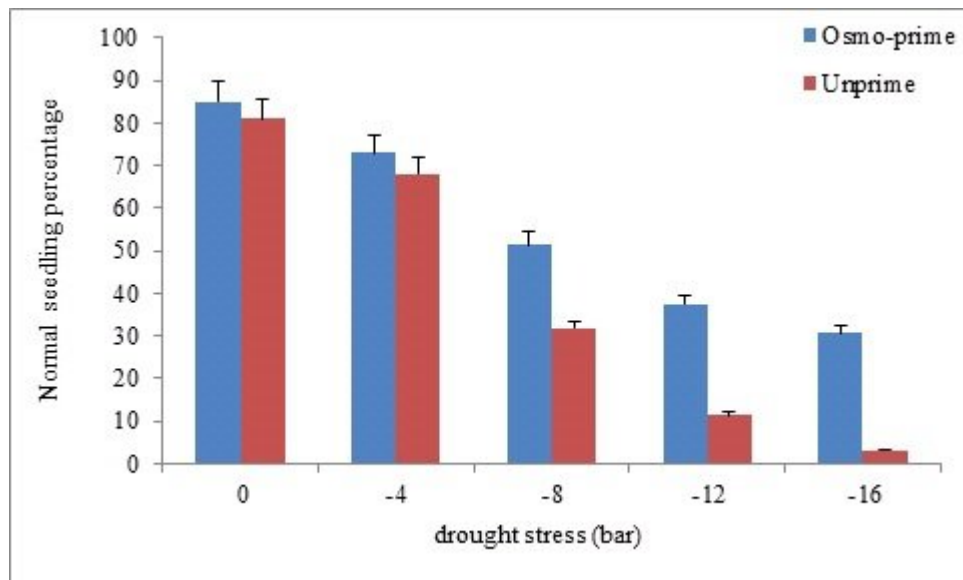


Figure 4. Effect of priming on normal seedling percentage of barley seeds under drought stress.

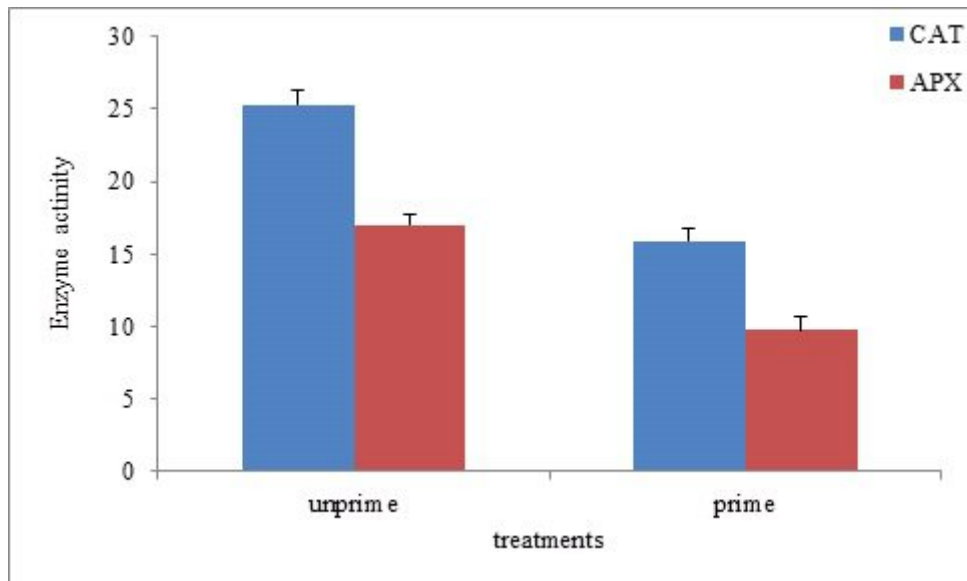


Figure 5. Effect of priming on enzyme activity.

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

S.O.V	df	Germination percentage	Germination index	mean time to germination	Normal seedling percentage
Treatment (T)	1	1009.2**	263.41**	7.25**	2033.63**
Stress (S)	4	3752.2**	676.7**	5.35**	4953.05**
T*S	4	88.2**	4.8**	0.42**	192.55**
Error	20	7.33	0.6	0.029	3.66
CV %	-	4.76	4.97	4.84	4.05

CONCLUSIONS

Osmo-priming improved germination percentage, germination index, normality seedling percentage, and reduced means time to germination, in barley seeds under stressed conditions. 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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