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ORIGINAL ARTICLE

Osmo and hydro priming improvement germination characteristics and enzyme activity of Mountain Rye (*Secale montanum*) seeds under drought stress

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Impacts of various concentrations of polyetylenglycol 6000 (PEG 6000) (0, -9, -11, -13 and -15 bar) and hydro priming on Mountain Rye (*secale montanum*) germination characteristic and enzyme activity were evaluated under drought stress in the seed laboratory of Natural Resources Faculty, University of Tehran, Karaj, Iran. Analyze of variance for hydro priming showed that temperature × time of priming interaction was significantly for germination percentage (GP), normal seedling percentage (NSP), coefficient of velocity of germination (CVG), seedling vigor index (SVI), coefficient of allometry (AC) and seedling length (SL) under drought stress and for osmo priming showed that Concentration of PEG × Temperature × Time of priming interaction was significantly for all traits under drought stress. Results of interaction effects for hydro priming showed that the highest GP (53%) and NSP (23.5%) were attained from hydro priming for 16h at 15°C and the highest CVG (0.21) and AC (0.49) were attained from hydro priming for 8h at 10°C, also hydro priming for 8h at 15°C increased SL (3.15) as compared to the unprimed. Osmo priming with concentration of -15 bar PEG for 24h at 15°C increased GP (80.5 %), GI (17.9), NSP (45 %), SVI (257.85) and SL (5.73 cm) and decreased MTG as compared to the unprimed and other treatments of osmo priming. The highest CVG was attained from concentration of -9 bar PEG for 24h at 10°C. the highest AC was attained from concentration of -9 bar PEG for 12h at 15°C. Also osmo and hydro priming increased catalase (CAT) and ascorbate peroxidase (APX) as compared to the unprimed.

Key words: Osmo priming, Secale montanum, Germination characteristics, enzyme activity, drought stress.

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Abiotic stresses are widespread problems around the world. Germination characteristics negatively affected by abiotic stresses in more crops (Davidson and Chevalier, 1987; Okçu *et al.,* 2005; Ansari et al., 2012). Seed germination is the most sensitive stage to abiotic stress (Patade et al., 2011; Redmann, 1974; Khajeh- Hossaini et al., 2003). Koster and Leopold, (1988) reported that drought stress is a critical environmental factor that restricts seed germination and seedling establishment. Drought stress could be detrimental or even lethal to the germinating seeds, especially if it occurred when the seeds were hydrated beyond critical moisture content (Chen et al., 2010). Seed priming can be taken to counteract the adverse effects of abiotic stress (Patade *et al.,* 2009. Ashraf and Foolad, 2005). Also seed priming techniques have been used to increase germination, improve germination uniformity in more field crops under stressed conditions (Iqbal and Ashraf, 2007; Kaya *et al.,* 2006; Patade *et al.,* 2011). Osmo priming can contribute to improve seedling emergence in different plant species by increasing the expression of aquaporin's (Gao et al., 1999), improvement of ATPase activity, RNA and acid phosphatase synthesis (Fu et al., 1998), also by improve of amylases, lipases and protease synthesis (Ashraf and Foolad 2005). The priming strategies enhanced activities of free radical scavenging enzymes such as CAT and SOD (Rouhi et al., 2012). The mountain rye (*Secale montanum*.) is a native wild species in southern Europe, Morocco, Iran and Iraq (De Bustos & Jouve 2002). The value of *S. montanum* as a pasture crop has been tested successfully in the United States (Robert et al. 1988), Australia and New Zealand (Oram 1996). Montanum rye (*Secale montane*) is an important plant in world that has more feed uses. The study aimed was to determine the effect of osmo and hydro prming on germination seedling growth and enzyme activity of mountain rye (*secale montanum*), under drought stress.

MATERIALS AND METHODS

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

Seed priming: Seeds of Rye were treated with water (Hydro priming) and polyethylene glycol 6000 (Osmo priming). For osmo priming treatments, concentrations of -9, -11, -13 and -15 bar PEG 6000 were used. The seeds were imbibed in distilled water in hydro priming treatments. The seeds without any treatment were termed as unprimed. Treated seeds with PEG and water were kept in darkness in an incubator at 10 and 15±1°C. Time of priming for hydro priming was 8 and 16 h and for osmo priming was 12 and 24 h. After priming, samples of seeds were removed and rinsed three times in distilled water and then dried at 15±1°C to back the original moisture level.

Seed germination and seedling growth: Seeds of primed and unprimed (control) Mountain Rye (*secale montanum*) were placed in critical stress (-14 bar), in dark condition for 7 days at 20±1°C. Germination was considered to have occurred when the radiceles were 2 mm long. Fifty seeds per dish were used for each treatment. Germinated seeds were recorded every 24 h for 7 days. After test time expiration, some germination indexes were evaluated such as: GP, NSP, GI, MTG, CVG, SVI, AC and SL.

Antioxidant enzymes assay: After the results of hydro and osmo priming effect on investigated traits and determined elite treatment (16h at 10◦C for hydro priming and -15 bar PEG for 24h at 15◦C for osmo priming) for *secale montanum*, determination of antioxidant enzyme activity was performed. 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 (Bailly et al., 1996). 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.

Statistical analysis: The study was conducted in the seed laboratory of Natural Resources Faculty, University of Tehran, Karaj, Iran, Using a randomized complete design with 4 replications. 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

Hydro priming

Analysis of variance for hydro priming showed that main effects of temperature on all factors measured significant expect for AC and SL (Tab 1), but main effects of time on all factors measured was significant (Tab 1). Analysis of variance showed that there is a significant interaction effects (Temprature × time of priming) for all traits expect for GI, SVI and MGT (Tab 1).

For time of priming the highest GI (8.26) was attained with 16h priming and the highest SVI (36.96) and MTG (5.74) were attained with 8h priming (Tab 2). For temperature of priming the highest GI (8.39), SVI (50.66) and MTG (5.4) were archived at 10°C (Tab 2). Results of interaction effects showed that highest GP (53%) and NSP (23.5%) were attained from hydro priming for 16h at 15°C (Table 3). The highest CVG (0.21) and AC (0.49) were attained from hydro priming for 8h at 10°C. Hydro priming for 8h at 15°C increased SL as compared to the unprimed and other treatments of hydro priming (Table 3).

Osmo priming

Analyze of variance for osmo priming showed that Concentration of PEG × Temperature × Time of priming interaction was significantly for all traits under drought stress (Table 4). Osmo priming like of hydro priming increased germination characteristics as compared to the unprimed (Table 5). Osmo priming with concentration of -15 bar PEG for 24h at 15°C increased GP , GI, NSP, SVI and SL and decreased MTG as compared to the unprimed and other treatments of osmo priming (Table 5). The highest CVG was attained from concentration of -9 bar PEG for 24h at 10°C (Table 5). The highest AC was attained from concentration of -9 bar PEG for 12h at 15°C (Table 5).

Antioxidant enzyme activity

Enzyme activity was significantly improved in *secale montanum* seeds primed with as compared to the unprimed (Figure 1). CAT significantly improved in *secale montanum* seeds primed with Osmo priming as compared to the unprimed and hydro priming. Also APX significantly improved in *secale montanum* seeds primed with Osmo priming as compared to the unprimed but no significantly difference with hydro priming (Figure 1).

Table 1 Variance analysis of studied traits in *Secale montanum* under drought stress

S.O.V	DF	GP	GI	NSP	SVI	CVG	MGT	AC	SL
Temperature	1	184.25**	23.81**	578.61**	3106.18**	0.0013**	0.71**	0.0035ns	0.0057ns
Time of priming	1	254.8**	19.03**	60.56**	1517.65**	0.0072**	4.85**	0.09**	7.29**
Temperature × Time of priming	1	14.51*	0.0365ns	34.57*	17.1ns	0.0004*	0.14ns	0.033**	0.58**
Error	12	1.62	0.18	4.52	70.48	0.00009	0.058	0.0027	0.1
CV	-	3.18	5.94	10.54	19.18	4.96	4.65	11.75	14.68

*, ** and ns, indicate significant difference at 5%, 1% probability level, and no significantly respectively.

Table 2 Main effects time of priming and Temperature of priming for germination index (GI), seedling vigour index (SVI) and means time to germination (MTG) under Critical potential with hydro priming treatments.

treatment		GI	SVI
Time of priming	8h	6.08b	36.96a
	16h	8.26a	25.35b
Temperature of priming	10°C	8.39a	50.66a
	15°C	5.95b	14.51b

Table 3 Interaction effects (temperature × time of priming) on germination percentage (GP), normality seedling percentage (NSP), coefficient of velocity of germination(CVG), coefficient of allometry (AC) and seedling length (SL) under Critical potential with hydro priming treatments.

Treatment					
Temperature	Time	GP	NSP	CVG	AC
10°C	8h	43b	16.5b	0.21a	0.49a
	16h	53a	23.5a	0.2ab	0.48a
15°C	8h	42b	6.5c	0.18bc	0.43ab
	16h	34c	7c	0.17bc	0.31b
Un		30.5c	0d	0.22c	0c

Table 4 - Variance analysis of studied traits in *Secale montanum* under drought stress

S.O.V	DF	GP	GI	NSP	SVI	CVG	MGT	AC	SL
Temperature (B)	1	240.25**	11.73**	12.88ns	318.71ns	0.00015ns	0.015ns	1.23**	0.05ns
Time of priming (C)	1	82.3**	34.11**	10.24ns	946.54ns	0.004*	0.4**	0.39*	0.71ns
Concentration of PEG (A)	3	15.34ns	1.45*	20.18*	2865.2ns	0.0005ns	0.03ns	0.27*	1.2ns
A*B	1	44.02*	0.52ns	16.15ns	623.78ns	0.003*	0.06ns	0.08ns	0.02ns
A*C	3	44.63**	6.7**	33.56**	3190.48ns	0.0003ns	0.05ns	0.7**	1.21ns
B*C	3	59.364**	14.51**	155.63**	9537.25**	0.0034**	0.39**	0.143ns	4.03**
A*B*C	3	85.9**	2.03*	23.13*	7476.5**	0.002*	0.15*	0.32**	4.84**
Error	-	10.36	0.51	6.73	1190.74	0.0006	0.04	0.07	0.55
CV	-	5.52	4.97	8.33	32	6.84	7.35	14.85	21.8

*, ** and ns, indicate significant difference at 5%, 1% probability level, and no significantly respectively.

germination percentage (GP), germination index (GI), normality seedling, percentage (NSP), seedling vigour index (SVI), coefficient of velocity of germination(CVG), means time to germination (MTG), coefficient of allometry (AC) and seedling length (SL)

Table 5 Interaction effects (temprature × time of priming) on germination percentage (GP), germination index (GI), normality seedling percentage (NSP), seedling vigour index (SVI), coefficient of velocity of germination(CVG), means time to germination (MTG), coefficient of allometry (AC) and seedling length (SL) under Critical potential with hydro priming treatments.

Treatment		traits							
Temperature	PEG	GP	GI	NSP	SVI	CVG	MGT	AC	SL
10°C	-9	71.5^{abcdef}	14.6^{bc}	33^{b}	100.65^{b}	0.35^{bcd}	2.87^{bcde}	1.97^{abc}	3.05^{b}
	-11	72.5^{abcde}	14.4^{bc}	32^{b}	101.12^{b}	0.354^{abcd}	2.8^{bcde}	1.99^{abc}	3.16^{b}
	-13	69^{bcdef}	12.3^{de}	27^{bc}	99.36^{b}	0.36^{abcd}	2.76^{bcde}	1.75^{bc}	3.68^{b}
	-15	60.5^{f}	11.6^{e}	26^{bc}	84.24^{b}	0.32^{d}	3.07^{b}	1.67^{cd}	3.24^{b}
	-9	65.5^{ef}	14.2^{c}	24^{bc}	74.4^{b}	0.4^{a}	2.47^{ef}	1.58^{cd}	3.1^{b}
	-11	65^{ef}	13.3^{cd}	26.5^{bc}	99.64^{b}	0.353^{abcd}	2.84^{bcde}	2.06^{abc}	3.76^{b}
	-13	79^{abc}	15.9^{b}	31^{b}	115.63^{b}	0.36^{abcd}	2.76^{bcde}	1.62^{cd}	3.73^{b}
	-15	68.5^{cdef}	14.7^{bc}	31^{b}	100.75^{b}	0.39^{ab}	2.54^{def}	1.21^{d}	3.25^{b}
15°C	-9	67.5^{def}	14.1^{c}	31^{b}	117.8^{b}	0.38^{abcd}	2.63^{cdef}	2.49^{a}	3.8^{b}
	-11	77^{abcd}	13.7^{cd}	30^{b}	99.9^{b}	0.356^{abcd}	2.8^{bcde}	1.86^{bc}	3.33^{b}
	-13	69.5^{bcdef}	13.8^{c}	28.5^{bc}	104.595^{b}	0.36^{abcd}	2.73^{bcde}	1.96^{abc}	3.67^{b}
	-15	74.5^{abcde}	14^{c}	25.5^{bc}	61.965^{b}	0.33^{cd}	2.99^{bc}	1.89^{bc}	2.43^{b}
	-9	79.5^{ab}	14.6^{bc}	26^{bc}	62.14^{b}	0.34^{bcd}	2.92^{bcd}	2.1^{abc}	2.39^{b}
	-11	72^{abcde}	14.1^{c}	19.5^{c}	53.04^{b}	0.36^{abcd}	2.7^{bcde}	1.73^{cd}	2.72^{b}
	-13	79.5^{ab}	15.7^{b}	30.5^{b}	102.48^{b}	0.359^{abcd}	2.78^{bcde}	1.62^{cd}	3.36^{b}
	-15	80.5^{a}	17.9^{a}	45^{a}	257.85^{a}	0.38^{abc}	2.26^{f}	2.41^{ab}	5.73^{a}
Un		30.5^{g}	4.6^{f}	0^{d}	0^{c}	0.22^{e}	6.1^{a}	0^{d}	0^{c}

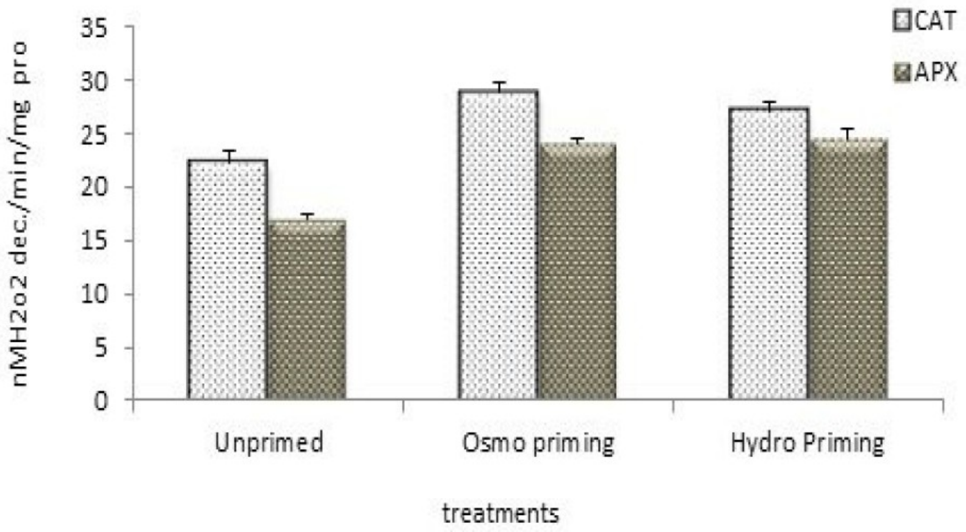

Figure 1. Effect of priming on activity of enzymes

DISCUSSION

In the present investigation, drought stress affected on GP, GI, NSP, SVI, CVG, MTG, AC and SL in *Secale montanum*. In agreement with the results, earlier reports (Ansari et al., 2012; Okçu *et al.,* 2005), have shown negative affect drought stress on germination characteristics. The results of our study suggested that hydro and osmo priming cause improvement in the seed characteristics as compared to the unprimed. In agreement with the results, earlier reports (Ansari et al., 2012; Ashraf and Foolad 2005) positive effects of priming in relation to seed performance, germination percentage and seedling indices. Priming can improve germination of many crops species, particularly under adverse conditions (Kaur et al., 2002; Ansari et al., 2012). Also, Rouhi et al. (2011) and Amini (2011) showed that hydro and osmo priming increased germination percentages, germination index and seed vigor, while decreased mean germination time, the time to get 50% germination and energy of germination. Hydro and osmo priming reduced MTG as compared to the unprimed in our study. Gharib and Hegazi (2010) for Bean (*Phaseolus vulgaris* L.) and Rouhi et al. (2011) for four grass species showed that priming reduced means time to germination**.** In the present investigation hydro and osmo priming increased catalase, ascorbat peroxidase and total protein as compared to the unprimed seed. Moosavi et al. (2009) reported that seed priming highly increased POD and PPO activities in Amaranth genotypes. 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. Osmo priming can be contribute to improve seedling emergence in different plant species by increasing the expression of aquaporins (Gao et al., 1999), improvement of ATPase activity, RNA and acid posphathase synthesis (Fu et al., 1998), also bye improve of amylases, lipases and protease synthesis (Ashraf and Foolad, 2005). 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, Morohashi 2002, Reichheld et al. 1999). Higher germination percentage and also higher germination rate of osmo primed seeds could be results of increasing the antioxidant profile of treated seeds. This well elucidates that POX should play a more significant role than CAT in detoxifying the produced H_2O_2 since the activity of POX increased, in contrast to that of CAT (Dey et al., 2007). It is well documented that CAT is less efficient than POX in scavenging H_2O_2 because of its low substrate affinity. Higher POX could increase cell division so it could play as a key factor in seedling growth (Erdal and Dumlupinar., 2010), therefore increasing CAT could significantly increase seed tolerance to environmental conditions. 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.

CONCLUSIONS

Osmo and hydro priming increased germination characteristics as compared to the unprimed. 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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