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ORIGINAL ARTICLE

Effect of Hormone Priming on Germination Characteristics and Enzyme Activity of Mountain Rye (*Secale montanum*) Seeds under Drought Stress Conditions

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Seed priming techniques have been used to increase germination, improve germination uniformity and seedling establishment under stressed conditions. Seed priming was used in Rye Mountain (*Secale montanum*) to increase seed germination and tolerance on stress exposure. Rye seeds were treated with various priming agents for different time and temperatures. The effect of priming was assessed on germination characteristics on subsequent exposure to drought (PEG -14 bar) stress for 7 days. Seed priming treatments significantly ($p \leq 0.01$) affected, Germination percentage (GP), normality seedling percentage (NSP), germination Index (GI), germination uniformity (GU), means time to germination (MTG), coefficient of velocity of germination (CVG), seedling vigour index (SVI) and coefficient of allometry(AC). Seed priming with gibberelic acid (GA) and salicylic acid (SA) ($p \leq 0.01$) increased germination characteristics as the compared to the unprimed. Seed priming with concentrations 25, 50 ppm of GA for 15 h at 10 °C and 25 ppm of SA for 12 h at 10 °C, may be considered as optimal treatment for priming of *Secale montanum* seeds in drought stress conditions. Also priming increased catalase (CAT) and ascorbate peroxidase (APX) as compared to the unprimed.

Key words: Hormone priming, Seed germination, Drought stress, Enzyme activity, Secale montanum.

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Key words: Hormone priming, Seed germination, Drought stress, Enzyme activity, Secale montanum.

The perennial 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.

Seed germination and rapid germination are usually essential processes in seedling establishment and plant development to obtain seedling numbers those results in higher seed crop (Almansouri *et al*., 2001; Murungu *et al*., 2003). In more crop species, seed germination and establishment are the most sensitive stages to abiotic stress (Patade *et al* 2011; Redmann, 1974). Abiotic stress such as: drought stress, salt stress and cold stress maybe delay seed germination and reduce the rate (Patade *et al*., 2011; Rouhi *et al*., 2011; Ansari *et al*., 2012). 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 like polyethylene glycol (osmo priming) or salts like $CaCl_2$, $CaSO_4$ and NaCl (Halopriming) the seeds to a point where germination processes are begun but not completed (Asfraf and Foolad, 2005), followed by drying of seeds to the original moisture level (McDonald, 2000). Seed priming techniques have been used to increase germination, improve germination uniformity, improve seedling establishment and stimulate vegetative growth in more field crops such as: wheat (Iqbal and Ashraf, 2007), sunflower seeds (Kaya *et al*., 2006), Chickpea (Kaur *et al.*, 2002), Capsicum (Patade *et al.*, 2011), lenti (Saglam *et al.*, 2011) and soybean (Sadeghi *et al.*, 2011), under stressed conditions. Also similarity indicated, in maize (Foti *et al.*, 2008), cucumber (Ghasemi-Golezani and Esmaeilpour, 2008). Priming has also been shown to induce nuclear DNA synthesis in the radicle tip cells in several plant species including: tomato (Liu *et al*., 1997), pepper (*Capsicum annuum*) (Lanteri *et al*., 1993), maize (*Zea mays*) (Garcia *et al*. 1995), and leek (Ashraf and Bray, 1993; Clark and James, 1991). Also, the priming strategies enhanced activities of free radical scavenging enzymes such as CAT and SOD (Ansari *et al*., 2012). In addition, the hormone-priming increased the germination percentage and activities of glyoxylate cycle enzymes in Persian Silk Tree (*Albizia julibrissin Durazz*) (Sedghi *et al.*, 2011). Although effect of seed priming in other field crops are documented, no reports are available on potential of various seed priming treatments and responses of the Mountain Rye seeds on subsequent exposure to drought stress. Therefore, the study aimed was to determine the effect of different priming treatments on germination seedling growth of Mountain Rye (*Secale montanum*), under drought stress and to find the best combination and time regime in different materials priming.

MATERIALS AND METHODS

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

Determine the critical drought potential: Drought tolerance of Mountain Rye (*Secale Montanum*) seeds was tested by using solutions of 0, -2, -4, -6, -8, -10, -12, -14 and -16 bar Polyethylene glycol (PEG-6000) concentrations. Four replicates of 50 seeds for each treatment were used. Rye seeds were germinated in 10 cm glass Petri dishes containing 6 ml PEG6000 solution in a germinator at 20±1°C, in dark condition for 7 days. Germinated seeds were recorded every 24 h for 7 days. After test time expiration, for Determine the critical drought potential, some germination indexes were evaluated such as: germination percentage (GP) and normal seedling percentage (NSP).

Seed priming: Seeds of were pretreated with salicylic acid and gibberellic acid. In hormone priming treatments, seeds were exposure in 0, 25, 50, 75 and 100 ppm concentrations salicylic acid and gibberellic acid for 12 and 15 h at 10±1°C and 12 h at 15±1°C. Rye 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.

Seed germination test: Seeds of primed and unprimed, rye mountain (*Secale montanum*) for drought tolerance were placed in 10 cm glass petri dishes containing 6 ml, critical potential in a germinator at 20±1°C for 7 days. Four replicates of 50 seeds for each treatment were used. Seeds were considered germinated when a 2 mm length radicle protruded through the seed coat. Germinated seeds were recorded every 24 h for 7 days. After test time expiration, some germination indexes were evaluated such as: Germination percentage (GP), normal seedling percentage (NSP), germination Index (GI), germination uniformity (GU), means time to germination (MTG), coefficient of velocity of germination (CVG), seedling vigor index (SVI) and coefficient of allometry (AC).

Antioxidant enzymes assay: After the results of priming effects on investigated traits and determined elite treatment (25 ppm for 12h at 10◦C for GA and SA) 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 (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). 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: 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

Critical drought tolerance test: The ability of seed drought tolerance was indicated by the germination percentage and normality seedling percentage, which showed the critical drought concentration for rye seeds. Seeds germination of Rye was significantly affected ($P<0.01$) by PEG concentrations (Figure 1). The highest germination percentage and normality seedling percentage was found in distilled water, followed by -2 and -4 bar PEG. The highest PEG concentrations (-14 and -16 bar PEG) showed substantial reduction in seed germination (Figure 1) and showed substantial reduction in normality seedling percentage (%) (Figure 1). The differences between the variants were significant and mathematically proven ($P<0.01$). However, none of the treatments had a statistically significant effect on seed germination and normality seedling until -6 bar PEG, whereas –14 and -16 bar treatments significantly reduced the germination and normality seedling (Figure 1). Suffered a drastic decrease at -10 bar PEG. Rye seeds were more sensitivity in a higher concentration of -10bar.

The effect of different treatments seed priming on germination characteristics and enzyme activity in response to drought stress:

Priming with gibberellic acid: Results showed germination percentage (GP) (Figure 2) and germination index (GI), were significantly improved in *Secale Montanum* seeds primed with GA as compared to the unprimed. The highest Germination percentage (GP), germination index (GI), normality seedling percentage (NSP) and seedling vigour index (SVI), were attained from treatment 25 ppm of GA at 10°C for 12 h (Table 1). The minimum germination uniformity (GU) was attained from 0 ppm at 10°C for 15 h (Table 1). Coefficient of velocity of germination (CVG), was significantly improved in *Secale montanum* seeds primed with treatments, 0 ppm at 10°C for 12 and 15 h, 0 ppm at 15°C for 12 h, 25 ppm at 10°C for 12 h and 100 ppm at 10°C for 12 h (Table 1). The minimum Means time to germination (MTG) was attained from treatment, 50 ppm at 10°C for 12 h and the highest coefficient of allometry (AC) was attained from treatment, 0 ppm at 10°C for 15 h and 25 ppm at 15°C for 12 h (Table 1).

Priming with salicylic acid: Results for SA showed germination percentage (GP) (Fig 3) and germination index (GI), was significantly improved in seeds primed with concentration 25 ppm SA at 10°C for 12 h, normality seedling percentage (NSP) was significantly improved in seeds primed with concentrations 25 ppm SA at 10°C for 12 h as compared to the unprimed and other concentrations SA (Table 2). The highest seedling vigour index (SVI) and coefficient of velocity of germination (CVG) were attained from treatment, 75 ppm SA at 10°C for 12 h.. Germination uniformity (GU) was no significantly difference in *Secale montanum* seeds primed with all treatments SA as compared to the unprimed (Table 2). The highest and minimum means time to germination (MTG) was attained respectively from 50 ppm SA at 10°C for 15 h and 75 ppm SA at 10°C for 12 h (Table 2). The best treatment for Coefficient of allometry (AC) attained from treatments 0 ppm, at 10°C for 12, 15 h and 50 ppm SA at 15°C for 12 h (Table 2).

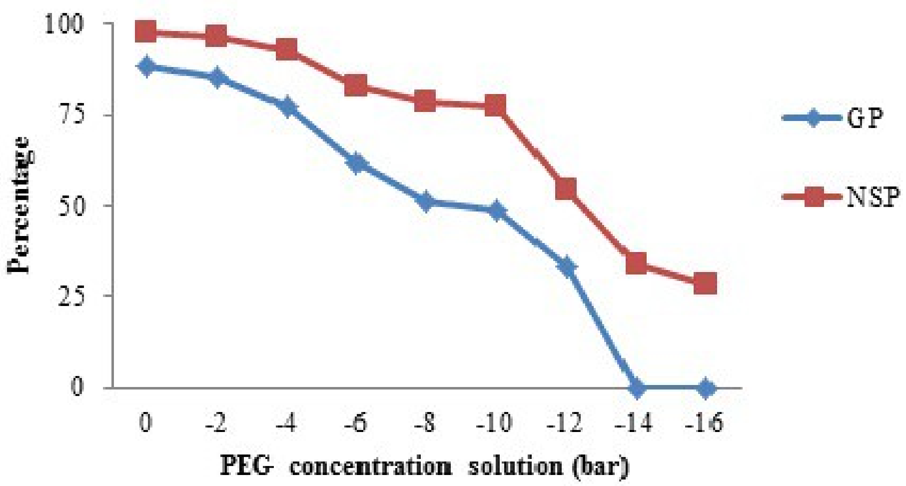

Figure 1: Changes of the germination percentage (GP) and Normality seedling percentage (NSP) of Rye mountain (*Secale montanum*), seeds under different PEG concentration solution.

Table 1. Effect of priming with gibberellic acid on germination percentage (GP), normality seedling percentage (NSP), germination Index (GI), germination uniformity (GU), means time to germination (MTG), coefficient of velocity of germination (CVG), seedling vigour index (SVI) and Coefficient of allometry (AC), under Critical potential.

Treatments	GI	NSP	SVI	GU	CVG	MTG	AC
0ppm- 10°C- 12 h	7.63^{ab}	14.5^{bc}	26.7^{ed}	0.3^{cde}	4.65^{ab}	0.25^{cd}	0.51^{abc}
0ppm- 10°C- 15h	7.65^{abc}	13.5^{cd}	19.33^{h}	0.23^{e}	4.59^{ab}	0.23^{d}	0.58^{a}
0ppm- 15°C- 12 h	4.93^{c}	10^{ef}	20.10^{f}	0.27^{de}	4.51^{abcd}	0.22^{d}	0.51^{abc}
25ppm- 10°C- 12 h	8.96^{a}	18^{ab}	34.51^{c}	0.32^{bcde}	4.69^{ab}	0.24^{cd}	0.41^{abcd}
25ppm- 10°C- 15h	6.75^{b}	10^{ef}	20.95^{e}	0.31^{bcde}	3.50^{efg}	0.27^{bcd}	0.37^{abcd}
25ppm- 15°C-12 h	7.48^{ab}	6.5^{g}	10.02^{g}	0.26^{de}	3.58^{efg}	0.23^{cd}	0.58^{a}
50ppm- 10°C- 12 h	8.87^{a}	13^{cde}	30.91^{c}	0.47^{a}	2.83^{g}	0.34^{a}	0.31^{bcd}
50ppm- 10°C- 15h	8.5^{a}	20^{a}	54.2^{a}	0.33^{bcde}	4.40^{abcd}	0.26^{bcd}	0.28^{d}
50ppm- 15°C- 12 h	6.7^{b}	10.5^{de}	21.70^{e}	0.31^{bcde}	3.84^{cde}	0.26^{bcd}	0.43^{abcd}
75ppm- 10°C- 12 h	9.16^{a}	15^{bc}	31.86^{c}	0.33^{bcde}	3.49^{efg}	0.28^{bcd}	0.24^{d}
75ppm- 10°C- 15h	7.53^{ab}	14^{bcd}	35.51^{b}	0.36^{bcd}	3.77^{de}	0.29^{bc}	0.44^{abcd}
75ppm- 15°C- 12 h	7.97^{ab}	7.5^{fg}	13.99^{f}	0.28^{cde}	3.72^{def}	0.25^{cd}	0.36^{abcd}
100ppm- 10°C- 12 h	8.9^{a}	15^{bc}	30.6^{cd}	0.38^{abc}	3.00^{fg}	0.31^{ab}	0.38^{abcd}
100ppm- 10°C- 15h	8.79^{a}	13.5^{cd}	22.49^{de}	0.31^{bcde}	3.76^{de}	0.28^{bcd}	0.46^{abcd}
100ppm- 15°C- 12 h	7.68^{ab}	14.5^{bcd}	25.58^{e}	0.29^{cde}	4.90^{a}	0.24^{cd}	0.51^{abc}
Unprimed	4.65^{c}	0^{h}	0^{i}	0.39^{ab}	4.07^{bcde}	0.26^{bcd}	0.00^{e}

Table 2. Effect of priming with salicylic acid acid on germination percentage (GP), normality seedling percentage (NSP), germination Index (GI), germination uniformity (GU), means time to germination (MTG), coefficient of velocity of germination (CVG), seedling vigour index (SVI) and Coefficient of allometry (AC), under Critical potential.

Treatments	GI	NSP	SVI	GU	CVG	MTG	AC
0ppm- 10°C- 12 h	7.63^{cd}	14.5^{b}	26.69^{c}	4.65^{a}	0.25^{bcd}	4.04^{abcd}	0.51^{a}
0ppm- 10°C- 15h	7.65^{cd}	13.5^{b}	19.33^{f}	4.59^{a}	0.23^{cd}	4.41^{abc}	0.58^{a}
0ppm- 15°C- 12 h	4.90^{fg}	10^{cd}	20.10^{f}	4.51^{a}	0.22^{cd}	4.51^{ab}	0.51^{ab}
25ppm- 10°C- 12 h	9.83^{ab}	20^{a}	37.42^{ab}	4.90^{a}	0.23^{bcd}	4.28^{abcd}	0.46^{abcd}
25ppm- 10°C- 15h	6.71^{de}	12.5^{bc}	24.78^{c}	4.42^{a}	0.21^{d}	4.66^{ab}	0.41^{bcde}
25ppm- 15°C-12 h	4.41^{g}	7^{efg}	14.23^{d}	3.94a	0.23^{bcd}	4.30^{abcd}	0.35^{cde}
50ppm- 10°C- 12 h	7.70^{cd}	11.5^{bc}	27.90^{b}	4.60^{a}	0.25^{bcd}	4.03^{abcd}	0.34^{de}
50ppm- 10°C- 15h	6.54^{de}	8.5^{de}	13.95^{d}	4.89^{a}	0.21^{d}	4.76^{a}	0.45^{abc}
50ppm- 15°C- 12 h	4.53^{g}	5.5^{f}	7.55^{e}	4.48^{a}	0.22^{cd}	4.50^{ab}	0.58^{a}
75ppm- 10°C- 12 h	10.14^{a}	19.5^{a}	41.44^{a}	4.10^{a}	0.31^{a}	3.28^{e}	0.38^{bcde}
75ppm- 10°C- 15h	5.91^{ef}	4.5^{f}	10.78^{d}	4.22^{a}	0.28^{abc}	3.66^{cde}	0.28^{e}
75ppm- 15°C- 12 h	6.52^{de}	9de	13.96^{d}	3.94a	0.25^{bcd}	4.02^{abcd}	0.47^{abc}
100ppm- 10°C- 12 h	8.87^{bc}	13.5^{b}	25.38^{c}	4.52^{aa}	0.28^{ab}	3.61^{de}	0.41^{bcde}
100ppm- 10°C- 15h	6.47^{de}	5^{f}	11.09^{d}	4.10^{a}	0.28^{abc}	3.68^{cde}	0.33^{de}
100ppm- 15°C- 12 h	5.59^{efg}	5^{f}	10.41^{d}	4.51^{a}	0.23^{bcd}	4.31^{abcd}	0.32^{e}
Unprimed	4.65^{gf}	0^{g}	0^{g}	4.07^{a}	0.26a^{bcd}	3.91^{bcde}	0.00^{f}

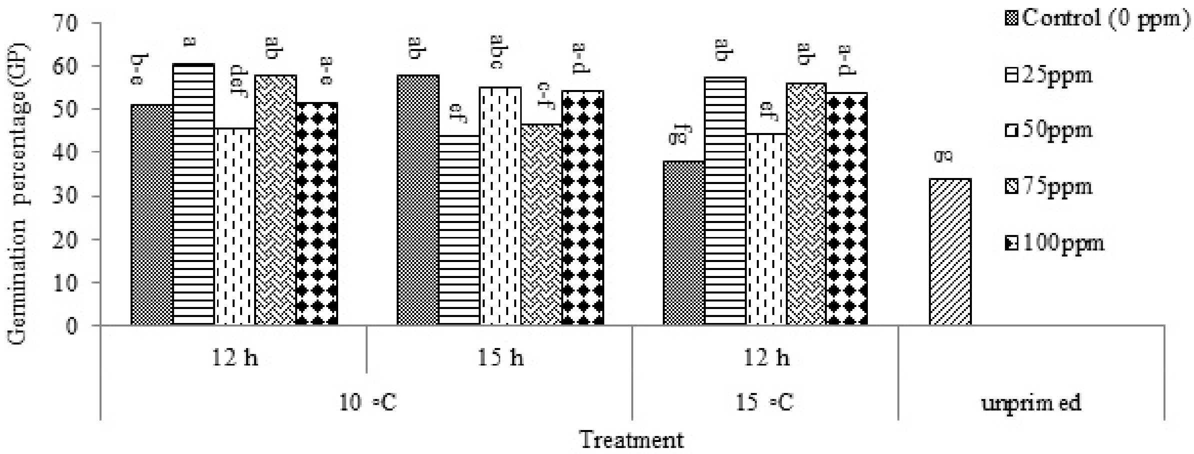

Figure 2. Effect of priming with gibberellic acid on germination percentage (GP) under Critical potential.

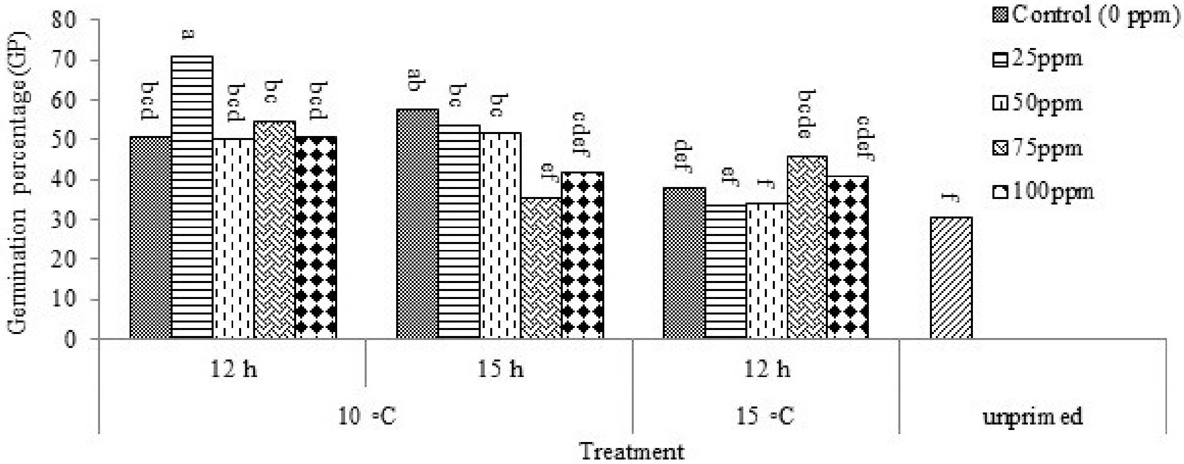

Figure 3. Effect of priming with salicylic acid on germination percentage (GP) under Critical potential.

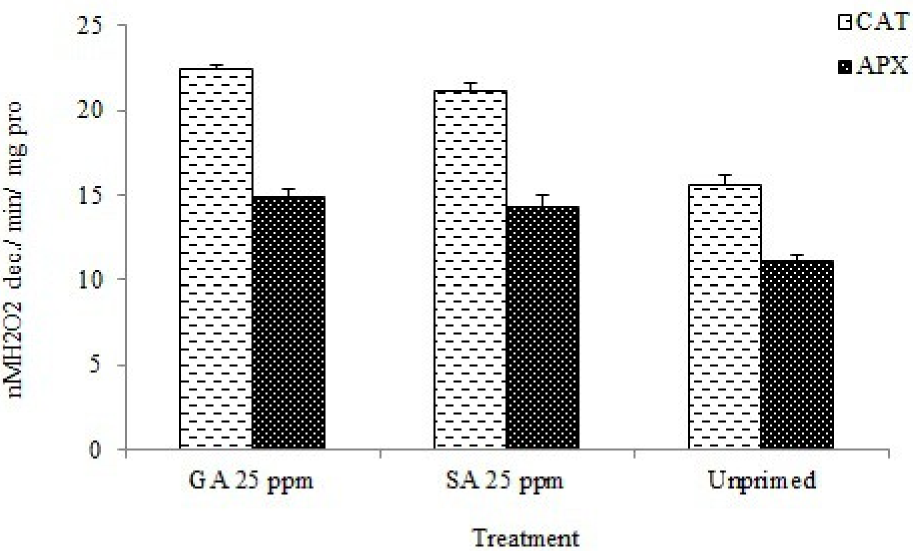

Figure 4. Effect of priming on activity catalase (CAT) and ascorbate peroxidase (APX)

Antioxidant enzyme activity: Enzyme activity was significantly improved in *Secale montanum* seeds primed with as compared to the unprimed (Figure 4). CAT significantly improved in *Secale montanum* seeds primed with GA and SA as compared to the unprimed. Also APX significantly improved in *Secale montanum* seeds primed with GA and SA as compared to the unprimed (Figure 4).

Thus, seed priming with concentration 25, 50 ppm of GA for 15 h at 10 °C and concentration 25 ppm of SA for 12 h at 10 °C, maybe considered as optimal treatment for priming of *Secale montanom* seeds in drought stress.

DISCUSSION

In the present investigation, drought stress affected Germination percentage (GP), normality seedling percentage (NSP), germination Index (GI), germination uniformity (GU), means time to germination (MTG), coefficient of velocity of germination(CVG), seedling vigour index (SVI) and Coefficient of allometry (AC) in *Secale montanum*. The -6 bar PEG or higher concentration reduced the germination characteristics significantly. 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. As the PEG concentration of -14 and -16 bar inhibited completely the normality seedling percentage therefore, -14 bar PEG was used to study the potential critical in *Secale montanum*. 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). In the present study, effect of various seed priming agents was assessed on germination and seedling growth, under control conditions, various seed priming, significantly increased germination index (GI) (data no shown). In agreement with the results, 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. However, the priming treatments in the present investigation like GA and SA with appropriate concentration, temperature and time, improved germination characteristics. Seed priming with all prime materials in this experiment improved GP, NSP, GI, GU, SVI and other traits than unprimed expect some treatments for some traits. GA increase the synthesis of hydrolytic enzymes at aleuron layer and by the activity of these enzymes, storage compounds convert to transferable and transfer to emberyo (Sedghi *et al*., 2008). GA treatments may improve rapid and uniform seedling germination-emergence and plant development in nurseries and in greenhouses (Tzortzakis, 2009). Also, SA increased germination percentage and other germination indices as compared to unprimed. The promotive effect of SA on shoot and root parameters has been reported in wheat and barley (Singh and Usha, 2003; Hayat *et al*., 2005). Salicylic acid changes the balance of hormones in plants, increased IAA and ABA and prevented the reduction of Cytokinins under no stress conditions (Shakirova *et al*., 2003). Improved germination and seedling growth were recorded in wheat, when the seeds were subjected to pre-sowing seed-soaking treatment in salicylic acid (Shakirva; 2007). Treat of wheat seeds to ascorbic acid decreased bad effective's drought stress on fresh and dry weight of radical and plumule (Hamad and Hamada, 2001). Afzal *et al*. (2006) reported that ascorbate and salicylic acid caused reduction of effectives salinity stress on the germination and seedling growth of wheat. It was identified that salicylic acid regulate cell division, cell extension and death (Zhang *et al*., 2002). In the present investigation priming increased catalase and ascorbat peroxidase as compared to the unprimed. Rouhi *et al*. (2012) and Ansari *et al*. (2012) showed that antioxidant enzyme activities in treated seeds of Berseem clover (*Trifolium alexandrinum* L.) increased as compared to the unprimed. Priming 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). Thus Can Said, effect of treatments priming on germination and seedling growth at concentrate, time of priming, temperature for priming and plant species.

CONCLUSIONS

Seed priming treatments improved germination percentage (GP), normality seedling percentage (NSP) and seedling vigour index (SVI), and reduced germination uniformity (GU), means time to germination (MTG), coefficient of velocity of germination (CVG) in *Secale montanum* 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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