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# OSMO AND HYDRO PRIMING MEDIATED GERMINATION IMPROVEMENT UNDER COLD STRESS CONDITIONS IN MOUNTAIN RYE *(SECALE MONTANUM)*

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**ABSTRACT.** 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 (Cold stress). Rye seeds were treated with water (Hydro priming) and polyetelyn glycol 6000 (Osmo priming). The effect of priming was assessed on germination characteristics under cold stress (at 3°C) for 7 days. Analyze of variance for hydro priming showed that temperature × time of priming interaction was significantly (P < 0.01) for all traits under cold stress and for osmo priming showed that Concentration of PEG × Temperature × Time of priming interaction was significantly (P < 0.01) for all traits under cold stress except seedling dry weight (SDW). For hydro priming the highest germination percentage (GP) was attained from hydro priming at 15±1°C for 16 h (70%), whereas the highest normal seedling percentage (NSP) (22.5%), germination index (GI) (8.57), seedling vigor index (SVI) (38.9) and seedling length (SL) (1.7 cm) were in the hydro priming at 10±1°C for 16 h. For osmo priming the highest germination percentage (GP) (80 and 80.5%), normal seedling percentage (NSP) (48 and 45%), seedling vigor index (SVI) (101.26 and 85.25) and seedling length (SL) (2.11 and 1.89 cm) observed in the osmo priming with concentrations of -9 and -15 bar PEG at 15±1°C for 24 h.

**Key words:** Priming treatments; *Secale montanum;* Germination; Seedling growth; Cold stress.

## INTRODUCTION

*Secale montanum* is an important plant in world that has more feed uses. De Bustos and Jouve (2002) reported than perennial mountain rye (*Secale montanum*) is a native wild species in southern Europe, Morocco, Iran and Iraq. The value of *S. montanum* as a pasture crop has been tested

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successfully in the United States (Robert *et al.,* 1988), Australia and New Zealand (Oram, 1996).

Primary stages 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). Seed germination is the most sensitive stage to cold, drought and salinity stress, in more crops (Patade *et al.,* 2011; Redmann 1974; Murrillo-Amador *et al.,* 2002; Khajeh-Hosseini *et al.,* 2003; Ghassemi-Golezani *et al.,* 2008). Strategies for improving the growth and development of crop species have been investigated for many years. In more field crops seed priming techniques have been used to improved germination characteristicsis (Iqbal and Ashraf, 2007; Kaya *et al.,* 2006; Kaur *et al.,* 2002; Casenave and Toselli 2007; Patade *et al.,* 2011). During priming, seeds are partially hydrated so that pre-germination metabolic activities proceed, while radicle protrusion is prevented, then are dried back to the primary moisture level (McDonald, 2000). Various priming treatments have been employed to increase the rate and uniformity of seed germination (Bradford, 1986). Common priming techniques include osmo priming (soaking seeds in osmotic solutions such as polyethylene glycol), halo priming (soaking seeds in salt solutions), hydro priming (soaking seeds in water), hormone priming (soaking seeds in hormone solutions) and other various chemical solutions. Priming contributes to significant improvement in seed germination and seedling growth in different plant species (Singh and Usha, 2003; Sadeghi *et al.,* 2011; Hayat *et al*., 2005; El-Tayeb, 2005; Tzortzakis, 2009; Hamad and Hamada, 2001; Patade *et al.,* 2011), also Gharib and Hegazi (2010) showed that priming improved germination percentage, germination rate and seedling criteria, compared with control seeds under optimal and low temperature stress conditions.

Although effects 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 cold stress. Therefore this research was conducted to investigate the effects of hydro priming and osmo priming on germination characteristics of *Secale montanum* seeds under cold stress.

## MATERIALS AND METHODS

### 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. Rye seeds were exposure in 20 cm glass petri dishes that containing 15 ml solution. 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 low temperature (3±1°C) in germinator, in dark condition for seven days. 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 seven days. After test time expiration, some germination indexes correlating to seed vigor were evaluated such as: germination percentage (GP), normality seedling percentage (NSP), germination index (GI), means time to germination (MTG), coefficient of velocity of germination (CVG), seedling vigor index (SVI), seedling dry weight (SDW), seedling length (SL).

### Statistical analysis

The study was conducted in the seed laboratory of Natural Resources Faculty, University of Tehran, Karaj, Iran. Data of germination percentage were subjected to data transformation (arcsine) before the statistical analysis in order to unify the variance of the data. Data of experiment were subjected to factorial analysis. All data were analyzed statistically by analysis of variance using MSTAT-C and Microsoft Excel software. Mean comparisons were performed using an ANOVA protected least significant difference (Duncan) ($P < 0.01$) test.

## RESULTS

### Hydro priming

Analyze of variance for hydro priming showed that Temperature × Time of priming interaction was significantly ($P < 0.01$) for all traits under cold stress (*Tabel 1*).

Hydro priming increased germination percentage (GP), normal seedling percentage (NSP), germination index (GI), means time to germination (MTG), coefficient of velocity of germination (CVG), seedling vigor index (SVI), seedling dry weight (SDW) and seedling length (SL) as compared to the unprimed (*Fig. 1*). The highest germination percentage (GP) (*Fig. 1a*) was attained from hydro priming at 15±1°C for 16 h (70%), whereas the highest normal seedling percentage (NSP) (22.5%) (*Fig. 1a*), germination index (GI) (8.57) (*Fig. 1b*), seedling vigor index (SVI) (38.9) (*Fig. 1c*) and seedling length (SL) (*Fig. 1d*) (1.7 cm) were in the hydro priming at 10±1°C for 16 h. Seedling dry weight (SDW) was significantly increased with hydro priming at 15±1°C for 12 and 16 h (1.25 mg) as compared to the unprimed and hydro priming at 10±1°C (*Fig. 1e*). The minimum means time to germination (MTG) (4.26) was attained from hydro priming at 15±1°C for 16 h (*Fig. 1f*). Hydro priming at 15±1°C for 16 h increased coefficient of velocity of germination (CVG) (0.045) (*Fig. 1g*) as compared to the unprimed and other treatments.

**Table 1 - Variance analysis of studied traits in *Secale montanum* under cold stress for hydro priming**

| S.O.V | DF | GP | GI | SDW | SL | NSP | SVI | CVG | MGT |
|---|---|---|---|---|---|---|---|---|---|
| Temperature | 1 | 141.26$^{ns}$ | 1.36$^{ns}$ | 5.40$^{**}$ | 0.00008995$^{ns}$ | 28.24$^{*}$ | 98.00$^{ns}$ | 0.00029901$^{**}$ | 1.3803$^{**}$ |
| Time of priming | 1 | 548.86$^{**}$ | 60.67$^{**}$ | 8.28$^{ns}$ | 0.0063304$^{ns}$ | 81.03$^{**}$ | 279.81$^{*}$ | 0.00087286$^{**}$ | 4.0423$^{**}$ |
| Temperature × Time | 1 | 423.81$^{**}$ | 3.92$^{**}$ | 2.69$^{**}$ | 0.3600476$^{**}$ | 97.10$^{**}$ | 836.69$^{**}$ | 0.00005695$^{**}$ | 0.5067$^{**}$ |
| Error | 12 | 38.65 | 0.32 | 2.56 | 0.0122212 | 4.85 | 33.21 | 0.00000265 | 0.0368 |
| CV | | 12.76 | 9.71 | 15.76 | 7.19685 | 9.92 | 16.65 | 3.75490500 | 3.6618 |

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

**Table 2 - Variance analysis of studied traits in *Secale montanum* under cold stress for osmo priming**

| S.O.V | DF | GP | GI | SDW | SL | NSP | SVI | CVG | MGT |
|---|---|---|---|---|---|---|---|---|---|
| Concentration of PEG (A) | 3 | 695.73$^{**}$ | 72.98$^{**}$ | 2.174$^{*}$ | 0.6951064$^{**}$ | 260.7$^{**}$ | 3118$^{**}$ | 0.00228848$^{*}$ | 0.1426$^{*}$ |
| Temperature (B) | 1 | 65.632$^{*}$ | 11.3$^{**}$ | 2.918$^{*}$ | 0.0754532$^{*}$ | 12.58$^{ns}$ | 243$^{ns}$ | 0.00000694$^{ns}$ | 0.0021$^{ns}$ |
| Time of priming (C) | 1 | 0.6644$^{ns}$ | 0.846$^{ns}$ | 5.478$^{ns}$ | 0.1006079$^{**}$ | 5.176$^{ns}$ | 206.5$^{ns}$ | 0.00094562$^{ns}$ | 0.0648$^{ns}$ |
| A*B | 3 | 25.134$^{ns}$ | 1.368$^{*}$ | 1.546$^{ns}$ | 0.028448$^{ns}$ | 6.99$^{ns}$ | 80.3$^{ns}$ | 0.0010737$^{ns}$ | 0.0715$^{ns}$ |
| A*C | 3 | 206.07$^{**}$ | 6.83$^{**}$ | 1.506$^{ns}$ | 0.0135152$^{ns}$ | 24.09$^{ns}$ | 85.7$^{ns}$ | 0.0018327$^{*}$ | 0.1123$^{ns}$ |
| B*C | 1 | 59.364$^{*}$ | 0.007$^{ns}$ | 7.305$^{ns}$ | 0.3878954$^{**}$ | 7.071$^{ns}$ | 894.7$^{**}$ | 0.0006183$^{ns}$ | 0.0137$^{ns}$ |
| A*B*C | 3 | 34.451$^{*}$ | 2.412$^{**}$ | 3.874$^{ns}$ | 0.094986$^{**}$ | 111.3$^{**}$ | 888.2$^{**}$ | 0.00275772$^{**}$ | 0.1579$^{*}$ |
| Error | | 14.307 | 0.467 | 6.342 | 0.0115746 | 8.828 | 61.84 | 0.00061502 | 0.0416 |
| CV | | 6.6057 | 9.099 | 12.09 | 7.098222 | 9.042 | 15.5 | 6.843052 | 7.3406 |

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

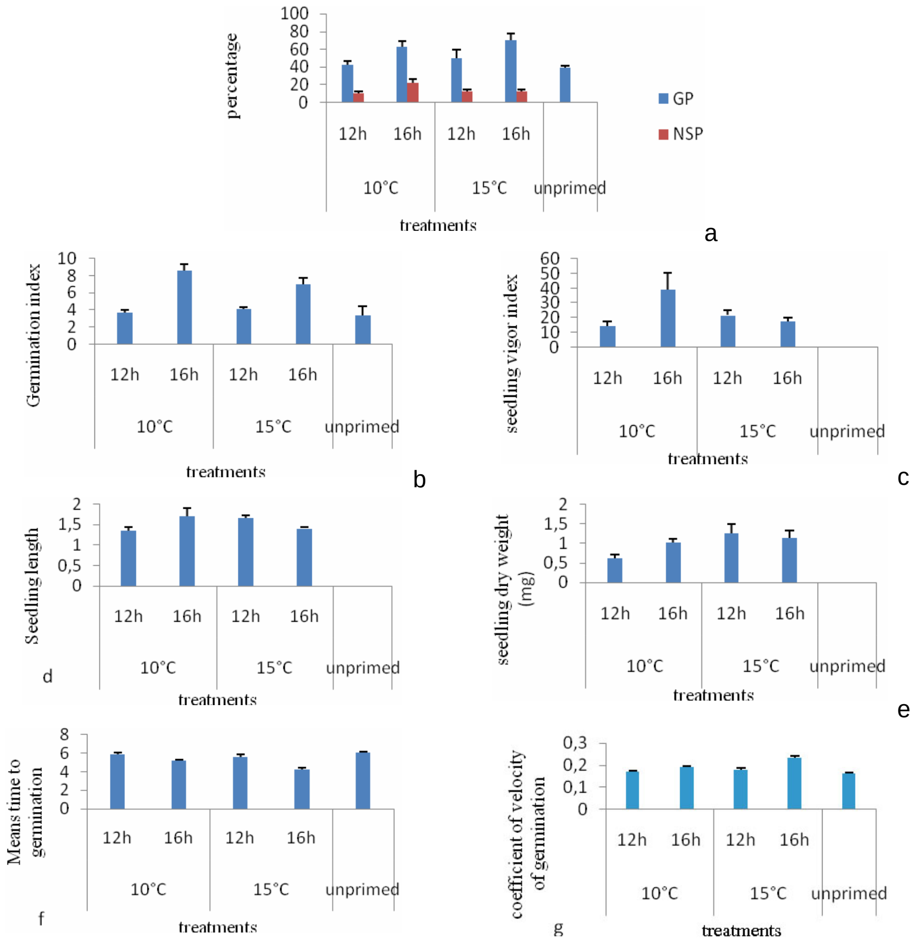


**Figure 1 - Effect of hydro priming on germination percentage (GP) (a), normale seedling percentage (NSP) (a), germination index (GI) (b), means time to germination (MTG) (f), coefficient of velocity of germination (g), seedling vigor index (SVI) (c), seedling dry weight (SDW) (e), Seedling length (SL) (d) in *Secale montanum* under cold stress. Vertical bars show SD.**

## Osmo priming

Analyze of variance for osmo priming showed that Concentration of PEG × Temperature × Time of priming interaction was significantly (P < 0.01) for all traits under low temperature except seedling dry weight (SDW) (*Table 2*).

Osmo priming like of hydro priming increased germination percentage (GP), normal seedling percentage (NSP), germination index (GI), means time to germination (MTG), coefficient of velocity of germination (CVG), seedling vigor index (SVI), seedling length (SL) as compared to the unprimed (*Fig. 1*).

The highest germination percentage (GP) (80 and 80.5%), normal seedling percentage (NSP) (48 and 45%), seedling vigor index (SVI) (101.26 and 85.25) and seedling length (SL) (2.11 and 1.89 cm) observed in the osmo priming with concentrations of -9 and -15 bar PEG at 15±1°C for 24 h (*Fig. 2a, b, c*).

Osmo priming at 15±1°C for 24 h increased germination index (GI) as compared to the unprimed and other treatments (*Fig. 3a*). Osmo priming reduced means time to germination (MTG) as compared to the unprimed (*Fig. 3b*). The minimum coefficient of velocity of germination (CVG) was in the unprimed (*Fig. 3c*).

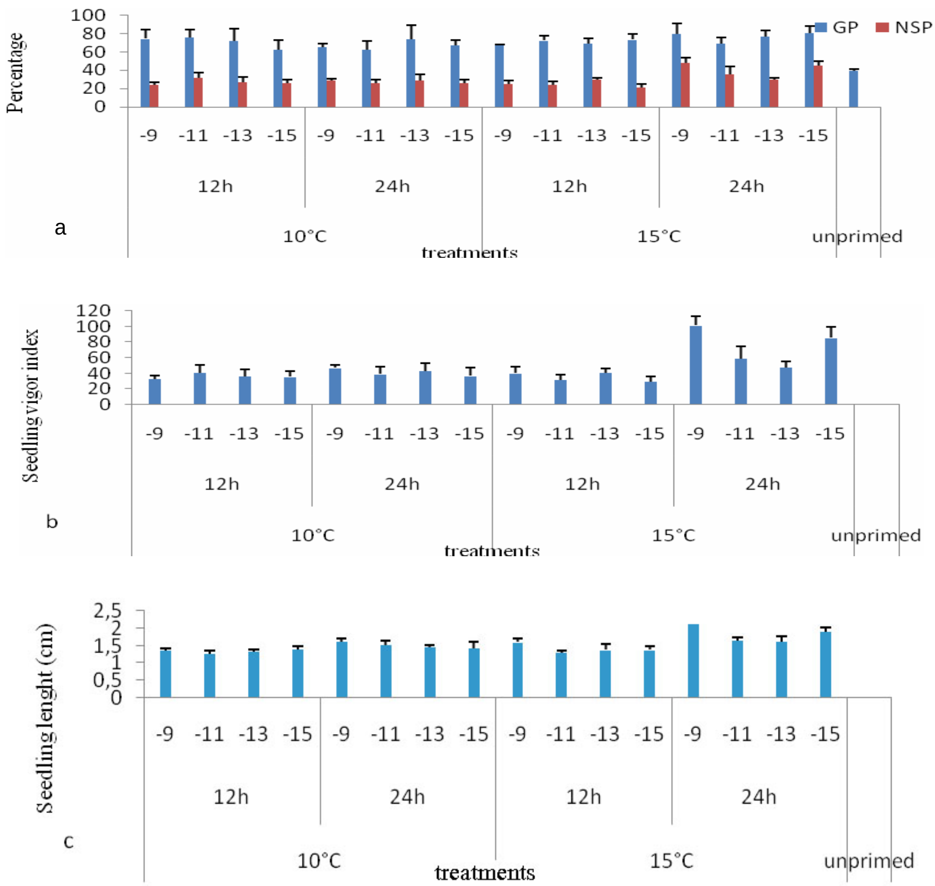


**Figure 2 - Effect of osmo priming on germination percentage (GP) (a), normal seedling percentage (NSP) (a), seedling vigor index (SVI) (b) and seedling length (SL) (c), in *Secale montanum* under cold stress. Vertical bars show SD**.

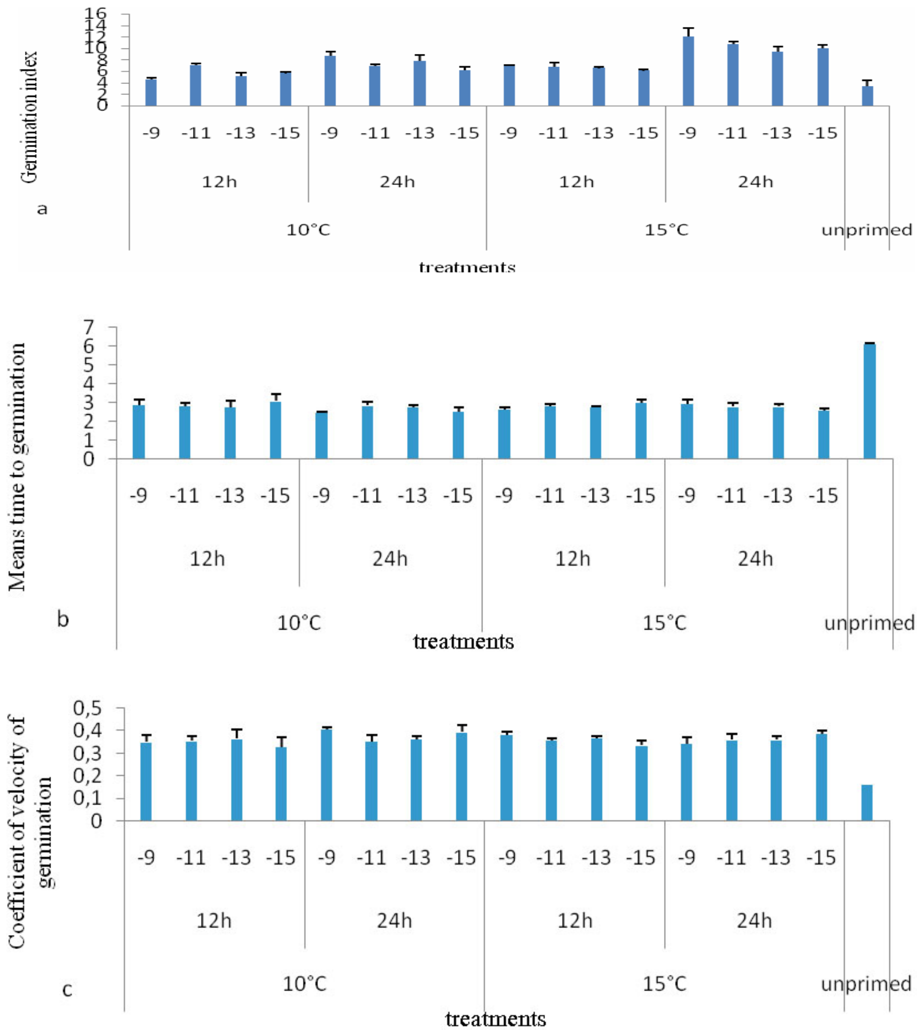


**Figure 3 - Effect of osmo priming on germination index (GI) (a), means time to germination (MTG) (b) and coefficient of velocity of germination (c) in *Secale montanum* under cold stress. Vertical bars show SD.**

## DISCUSSION

In the present investigation, cold stress affected on germination percentage (GP), normal seedling percentage (NSP), germination index (GI), means time to germination (MTG), coefficient of velocity of germination (CVG), seedling vigor index (SVI), seedling dry weight (SDW), seedling length (SL) as compared to the unprimed in *Secale montanum*. In agreement with the results, earlier reports (Patade *et al.*, 2011; Gharib & Hegazi, 2010; Tobeh & Jamaati-e-Somarin, 2012), have shown negative affect cold stress on germination characteristics. Results showed that priming treatments cause improvement in the seed characteristics. In agreement with the results, earlier reports (Sadeghian & Yavari, 2004; Chen *et al.,* 2005; Kaya *et al.,* 2006) 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 such as low temperature (Zheng *et al.,* 1994; Hardegree and Van Vactor, 2000; Patade *et al.,* 2011). Also, Sadeghi *et al.* (2011) and Ganji Arjenaki *et al*. (2011) showed that 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.

In our study priming reduced means time to germination (MTG) as compared to the unprimed. 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**.** Osmo priming can 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). The promotive effect of priming on germination parameters has been reported in more crops under abiotic stress conditions (Patade *et al.,* 2011; Gharib and Hegazi, 2010; Sadeghi *et al.,* 2011; Ganji Arjenaki *et al.,* 2011, El-Tayeb, 2005).

## CONCLUSIONS

Seed priming treatments improved germination percentage (GP), normal seedling percentage (NSP), germination index (GI), means time to germination (MTG), coefficient of velocity of germination (CVG), seedling vigor index (SVI), seedling dry weight (SDW), seedling length (SL) as compared to the unprimed in *Secale montanum* under cold stress. Among the treatments, seed priming with PEG (Osmo priming) was more effective in cold stress than the hydro priming. Thus it is conclude that under cold stress, hydro priming at 15±1°C for 24 h and osmo priming with solutions -9 and -15 bar at 15±1°C for 24 h could be a suitable strategy for cold regions.

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