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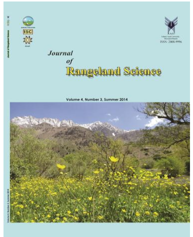

Full Length Article:

Comparative Effects of Hydropriming and Halopriming on Germination Performance of *Secale montanum* Guss. under Salinity Stress

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Abstract. Salinity stress is one of the most important abiotic stress factors limiting the plants' growth. Seed priming is known as a method which improves the germination performance in seeds in stress conditions. The objective of this study was to compare the effectiveness of Hydropriming and Halopriming on germination performance of *Secale montanum* while growing under the NaCl-salinity stress. Hydropriming and Halopriming were used in the study to prime seeds. For the Halopriming treatments, concentrations of 0, 125, 250 and 500 mM of sodium chloride were prepared. This experiment was carried out in four levels of salinity stress. A significant tow-way interaction (priming × stress) was found for all characteristics. Germination percent in Halopriming showed a significant decrease with the increases in duration and concentration of treatments. Higher root and shoot length values of *S. mountanum* were observed in Hydropriming treatment but as compared to the control, it was not statistically significant. *S. montanum* appears to be moderately salt-tolerant. The results of this study indicated that Halopriming had a negative effect on the germination and growth of *S. montanum*. The disadvantage may be due to the varied plant species, imbibing time and seed moisture. On the other hand, these effects are probably due to an excessive increase and accumulation of Na^+ and Cl^- ions to the seed tissue. From the present study, it can be concluded that seeds of *S. montanum* are sensitive to priming technique and priming of this species needs to use the other priming methods.

Key words: Hydropriming, Halopriming, Imbibing, Salinity stress, *Secale montanum*

Introduction

The genus *Secale* belongs to the family Poaceae. This genus includes perennial or annual and herbaceous species. The main distribution of *Secale* is in south Asia, turkey, Lebanon, Syria, Iran, Iraq and Afghanistan (Sencer and Hawkes, 1980). *Secale montanum* is one of the most important forage crops in Iran. One of the major problems in forage cultivation in Iran is salinity. The seeds are sown in seedbeds for the reclamation or foraging purposes having inadequate moisture because of the lack of rainfall at germination stage. Another major restriction to seed germination is soil salinity. Soil salinity affects a large part of the regions in Iran that approximately covers 12.5 % of the total area of the country (Akhani and Ghorbanli, 1993). Salinity causes a decrease in water uptake during imbibitions stage and this stress may cause excessive absorption of ions such as Na^+ and Cl^- (Murillo-Amador *et al.*, 2002). Plants are of three ways of adaptations to salinity: osmotic stress tolerance, Na^+ or $Cl^–$ exclusion, and the tolerance of tissue to the accumulated Na^+ or $Cl^–$ (Rady *et al.*, 2013). Seed priming is known as a method which improves the germination performance and emergence in seeds of many crops and small seeded grasses in stress conditions (Heydecker and Coolbaer, 1977; Afzal *et al.*, 2008). A wide variety of priming treatments has been used to enhance seed germination. Halopriming as a pre-sowing seed in NaCl solution has been reported to increase the germination performance in many plant species under salinity stress conditions (Sivritepe *et al.*, 2003; Smith and Cobb, 1991). Actually, Halopriming is one of the techniques for coping with salinity. Hydropriming is the simplest method to hydrating seeds. Hydropriming is included in soaking the seed in distiller water and re-drying before the complete conducting of germination. Peng *et al.* (2013) mentioned that Hydropriming treatment is an effective strategy to improve seed germination and plant growth of *Festuca sinensis*. Generally, all priming techniques allow some of the metabolic processes necessary for germination to occur without germination (Rahimi, 2013). Therefore, the main objective of this study was to compare the effects of Hydropriming and Halopriming on germination performance of *S. montanum* while growing under the NaCl-salinity stress.

Materials and Methods

Seed origin

This study was carried out in the seed technology laboratory of the Faculty of Natural Resources, Tarbiat Modares University (TMU) of Iran. The origin of the seeds was Esfahan province (32°39′27.72″ N, 51°40′ 9.12″ E, 1590 m.a.s.l) in Iran. The seeds were stored in paper bags at constant temperature of 4 °C in darkness.

Seed priming

Hydropriming was used according to the method described by Taylor *et al.* (1998). Seeds of *S. montanum* were separately placed in nylon-net bags and immersed in sterile distilled water at a temperature of 20 °C and hydrated for 24 h. For the Halopriming treatments, concentrations of 125, 250 and 500 mM of sodium chloride were prepared. Then, seeds were immersed in NaCl solution at 20 °C for 24 h in the dark. Seeds without the treatment served as a control group.

After treatment, the seeds were rinsed with tape water for three minutes and slightly hand-dried. While still damping, the seeds were sprayed with Thiram fungicide at a rate of 0.65 mL kg^{-1} of seeds (Giri and Schillinger, 2003). Then, seeds were shade-dried until the moisture level comes back to the original content at room temperature. Hydropriming and Halopiming treated seeds were equilibrated at room temperature for 2 days.

Salt stress treatments and germination test

This experiment was carried out in four levels (0, 100, 200 and 300 mM) of salinity stress. The fifty primed and unprimed seeds were placed on two layers of blotter paper watered with 5 ml of different levels of NaCl solution in 9-cm-diameter Petri dishes with three replications. Germination tests were conducted in a germinator maintained at 15– 25 ºC for a 8 h period of darkness and a 16 h one of light with a light intensity of 38 $\mu molm^{-2}s^{-1}$ provided by cool-white fluorescent lamps (ISTA, 1985). The germination was monitored every day while the seeds were counted when they exhibited radicle extensions of ≥ 2 mm (Hardegree and Van Vactor, 2000). The germination percent was calculated according to the total number of seeds germinated. The Germination Rate (GR) was calculated using the following formula (Panwar and Bhardwaj, 2005) (Equation 1).

$$GR = \sum_{i=1}^{n} \left[\frac{n}{t} \right] \qquad \text{(Equation 1)}$$

Where

GR= Germination Rate

n= number of newly germinated seeds at time t

t = number of days since sowing

Vigor Index (VI) of the seedlings was calculated according to the following formula (Abdul-Baki and Anderson, 1973) (Equation 2).

$$VI = (RL + SL) \times GP \qquad \text{(Equation 2)}$$

Where

RL= Root Length (cm)

SL= Shoot Length (cm)

GP= Germination Percent

The seedlings were weighed to record the fresh mass. After the growth, root and shoot lengths of ten seedlings were measured. The seedlings were then placed in an oven at 80 °C for 24 h. These dried seedlings were weighed to record the seedling dry mass. Analysis of variance of the data was computed using the MSTATC Program (Michigan State University). LSD test at a 5% level of probability was used to evaluate the differences among the means.

Results

A significant two-way interaction (priming and stress) was found for all characteristics. So, these main effects will not be interpreted.

The comparison of means indicates that the germination rate was significantly affected by Halopriming and Hydropriming (Table 1). The maximum value of germination rate was recorded in the control treatment in 0 stress level (16.20) which was not statistically significant as compared to the Hydropriming (14.66). Germination rate in Halopriming and Hydropriming treatments drastically declined with the increases of stress and concentration of Halopriming solution. The germination rate was decreased with the increase in duration of Halopriming in all concentration levels.

Table 1. Germination rate (days) of *Secale montanum* treated with Hydropriming, Halopriming for 24 and 72 h in four levels of NaCl (0, 125, 250 and 500 mM) and control (untreated) at different stress levels of NaCl

Stress Levels (mM)	Seed Priming							
	Control	Hydropriming	Halopriming					
		24 h	24 h			72 h		
		-	125mM	250 mM	500 mM	125mM	250 mM	500mM
0	16.20aA*	14.66aA	12.68bA	11.53bA	8.04cA	11.98bA	9.52cA	6.27dA
100	10.16aB	6.72cB	9.52abB	8.74bB	5.72cB	9.85abB	8.54bB	4.21dB
200	7.58abC	5.39bB	8.43aB	6.49bC	4.13cC	6.21bC	5.87bC	3.51cB
300	5.78aD	3.72bC	5.00aC	3.59bD	3.05bC	3.36bD	2.00cD	2.70cB

*a, b, c, d: Means with different lower case letter in the same row differ significantly (P < 0.05)

*A, B, C: Means with different capital letter in the same column differ significantly (P < 0.05)

$LSD_{0.05}$ = 1.55 (64 d.f.)

A higher germination percent was observed in the control in 0 stress level which was significantly compared to all priming treatments (P> 0.05) (Table 2). Hydropriming had statistically higher values of germination percent than the Halopriming. Germination percent in Halopriming showed a significant decrease with the increase in duration and concentration of treatments. So, a lower germination percent was recorded in Halopriming 500 mM for 72 h in 300 mM stress level. In this trait, a significant decrease was observed with the increase in stress level.

Table 2. Germination percent of *Secale montanum* treated with Hydropriming, Halopriming in 24 and 72 h
in four levels of NaCl (0, 125, 250 and 500 mM) and control (untreated) at different stress levels of NaCl

Stress Levels (mM)	Seed Priming							
	Control	Hydropriming	Halopriming					
		24 h	24 h			72 h		
		-	125 mM	250 mM	500 mM	125 mM	250 mM	500 mM
0	92.67aA*	68.67bA	61.33bA	42.67cA	20.67dA	36.00cA	16.00dA	7.33eA
100	48.67aB	35.33bB	43.33aB	22.00cB	9.33dB	26.67cB	10.67dA	4.33eA
200	34.00aC	17.33bC	14.0bC	15. 0bB	6.67cB	13.33bcC	8.33cAB	6.00cA
300	15.33aD	8.33abD	5.33bD	5.00bC	2.00cB	9.33abC	6.67bB	3.67cA

*a, b, c, d: Means with different lower case letter in the same row differ significantly ($P < 0.05$)
*A, B, C: Means with different capital letter in the same column differ significantly ($P < 0.05$)
$LSD_{0.05}$ = 8.01 (64 d.f.)

Higher values of shoot length were recorded in Hydropriming treatment (Table 3) but as compared to the control, it was not statistically significant. In general, a decreasing trend of shoot length was observed with the increasing levels of salinity stress. The minimum and statistically significant shoot length rates as compared to the control were observed in Halopriming 500 mM for 72 h at 300 mM stress level.

Table 3. Shoot length (cm) of *Secale montanum* seedlings treated with Hydropriming, Halopriming for 24 and 72 h at four levels of NaCl (0, 125, 250 and 500 mM) and control (untreated) at different stress levels of NaCl

Stress Levels(mM)	Seed Priming							
	Control	Hydropriming	Halopriming					
		24 h	24 h			72 h		
		-	125 mM	250 mM	500 mM	125 mM	250 mM	500 mM
0	7.42aA	7.48aA	6.79abA	6.19bA	5.62bcA	5.34cA	4.97cA	2.00dA
100	2.22abB	2.28abB	2.50aB	2.34abB	2.13abB	2.73aB	2.10abB	1.74bB
200	0.42aC	0.42aC	0.30aC	0.29aC	0.23aC	0.27aC	0.12aC	0.17aC
300	0.63aC	0.39aC	0.26aC	0.20aC	0.72aC	0.17aC	0.14aC	0.04bC

*a, b, c, d: Means with different lower case letter in the same row differ significantly ($P < 0.05$)
*A, B, C: Means with different capital letter in the same column differ significantly ($P < 0.05$)
$LSD_{0.05}$ = 0.79 (64 d.f.)

Similar to shoot length, the higher values of root length of *Secale mountanum* were observed in Hydropriming treatment (Table 4) but as compared to the control, it was not statistically significant. Also, a decreasing trend of root length was observed with the increasing levels of salinity stress.

Table 4. Root length (cm) of *Secale montanum* seedlings treated with Hydropriming, Halopriming for 24 and 72 h in four levels of NaCl (0, 125, 250 and 500 mM) and control (untreated) at different stress levels of NaCl

Stress Levels(mM)	Seed Priming							
	Control	Hydropriming	Halopriming					
		24 h	24 h			72 h		
		-	125 mM	250 mM	500 mM	125 mM	250 mM	500 mM
0	3.53aA	3.57aA	3.20abA	2.97bA	2.82bA	2.87bA	2.34bA	2.41bA
100	2.00aB	2.34aB	1.60bB	1.51bB	1.45bB	1.32bB	1.16bB	0.80cB
200	0.92abC	1.2aC	0.69bC	0.51bC	0.35bcC	0.59bC	0.34bcC	0.17cC
300	0.82aC	0.94aC	0.43bC	0.39bC	0.17bcC	0.30bC	0.25bcC	0.10cC

*a, b, c, d: Means with different lower case letter in the same row differ significantly ($P < 0.05$)
*A, B, C: Means with different capital letter in the same column differ significantly ($P < 0.05$)
$LSD_{0.05} = 0.38$ (64 d.f.)

The maximum value of vigor index was recorded by the control in 0 mM stress level which (Table 5) was statistically significant as compared to the other treatments, and the minimum value of vigor index was recorded for Halopriming 500 mM for 72 h in 300 mM salinity stress. In all treatments, vigor index was decreased by increasing the stress level.

Table 5. Vigor index of *Secale montanum* treated with Hydropriming, Halopriming for 24 and 72 h at four levels of NaCl (0, 125, 250 and 500 mM) and control (untreated) at different stress levels of NaCl

Stress Levels(mM)	Seed Priming							
	Control	Hydropriming	Halopriming					
		24 h	24 h			72 h		
		-	125 mM	250 mM	500 mM	125 mM	250 mM	500 mM
0	1014.17aA	756.00bA	609.33cA	389.03dA	174.66fA	293.52eA	110.2gA	35.7hA
100	199.89aB	145.27bB	168.23abB	90.03cB	34.34dB	91.37cB	35.22dB	9.24eA
200	45.89aC	17.68aC	15.04aC	10.60C	3.47aC	10.38aC	2.23aC	1.25aA
300	19.05aC	8.43aC	5.00aC	3.17aC	0.68aC	3.54aC	2.10aC	0.47aA

*a, b, c, d: Means with different lower case letter in the same row differ significantly ($P < 0.05$)
*A, B, C: Means with different capital letter in the same column differ significantly ($P < 0.05$)
$LSD_{0.05} = 47.13$ (64 d.f.)

Discussion and Conclusion

Germination and the early seedling development are the growth phases which are the most sensitive stages to the stresses (De Souza *et al.*, 2013; Rahimi, 2013). Salinity stress is one of the most important abiotic stress factors limiting the plants' growth. The results showed that *Secale montanum* can germinate and early grow well up to 300 mM of salinity stress. Therefore, this species appears to be moderately salt-tolerant. Koocheki and Zarif ketabi (1996) found that *Secale cereale* is more resistant to salinity stress as compared to *Dactylis glomerata*, *Bromus tomentellus*, *Agropyron elongatum*, *Agropyron desertorum* and *Agropyron cristatum* species. Generally, salt stress inhibited the growth of shoots, roots and germination performance of *Secale montanum*. This is consistent with the study done by Hanif and Davies (1998) which showed that increasing the salinity stress reduced the germination and growth parameters of *Secale cereale* seeds; so in this study, a higher decline in the germination and growth parameters was observed at the highest salt concentration (300 mM). According to Demir and Mavi (2008), the negative effects on germination may be due to poor hydration and differences in the mobilization of stored reserves and structural organization of proteins. Hydropiming and Halopriming have proved to be a successful strategy to reduce the adverse effects of salt stress and improve the seed germination performance in grass, vegetables and field crops (Kaya *et al.*, 2006; Guzman and Olave, 2004; Afzal *et al.*, 2004;

Sivritepe *et al.*, 2003; Li *et al.*, 2011; Dianati *et al.*, 2010). Nonetheless, diverse population of native grass seed genetically uses this method with great complexity. Therefore, there are critical points on its use (Di Girolamo and Barbanti, 2012) regarding that it is not always a successful method about all species. The results of this study indicated that Halopriming and Hydropriming had negative effects on the germination and growth of *Secale montanum*. Evenari (1964) found that pre-sowing treatments of seed did not increase grain yield of *Sorghum* and even had negative impacts. The disadvantage may be due to the varied plant species (Murungu *et al*., 2004), imbibing time and seed moisture. Drying rate also influences the effects of priming (Madakadze *et al.*, 2000). Our results are similar to the findings of Ghassemi-Golezani and Esmaeilpour (2008) who reported that Halopriming does not affect, or has little effects on *Cucumis sativus* seed. Interpretation of reduction in the growth parameters of *Secale montanum* can be difficult because of many influential factors which occur during the halopriming and hydropriming presses. It can be argued that these negative effects arise from high seed imbibition during priming that can be over swelling endosperm and restrict the radicle protrusion. The positive effects of seed priming on seed invigoration critically depend on the priming duration (Ashraf *et al*., 2005). On the other hand, these effects are probably due to an excessive increase and accumulation of Na^+ and Cl^- ions to the seed tissue duration of 24 an 72 h of Halopriming treatment which causes the inhibitory effects on the activities of germination process. In Halopriming duration treatment, Na+ and Cl− may be taken up by the seed and toxic effects of NaCl might appear. Our findings at high salinity concentration of Halopriming media (500mM) showed that a decrease in the germination performance is more than the other concentrations. Therefore, the present study revealed that NaCl priming or Haloprming had toxic effects on *S. montanum* seeds. Also, Hydropriming technique had no significant effects on the germination performance and growth parameters of *S. montanum* under salinity and non-salinity stresses. From the present study, it can be concluded that seeds of *S. montanum* are sensitive to the priming technique and priming of this species needs further study. In general, the other seed priming methods should be compared to select the optimal priming conditions in later studies.

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بررسی مقایسه تاثیر هیدروپرایمینگ و هالوپرایمینگ بر عملکرد جوانه‌زنی گونه چاوارکوهی (*Secale montanum* Guss.) تحت تنش شوری

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چکیده. تنش شوری یکی از مهمترین عوامل غیر زنده محدود کننده رشد گیاهان است. پرایمینگ یکی از روش‌های شناخته شده است که باعث بهبود عملکرد جوانه‌زنی بذور در شرایط تنش می‌شود. هدف این مطالعه بررسی اثر بخشی هیدروپرایمینگ و هالوپرایمینگ بر عملکرد جوانه‌زنی گونه چاودار کوهی تحت تنش شوری بود. هیدروپرایمینگ و هالوپرایمینگ برای مطالعه روی بذور پرایم شده مورد استفاده قرار گرفتند. برای تیمار هالوپرایمینگ غلظت‌های ۱۲۵، ۲۵۰ و۵۰۰ میلی‌مول کلرید سدیم آماده شد. این آزمایش در چهار سطح تنش شوری انجام شد. اثرات متقابل دو جانبه (پرایمینگ × تنش) برای همه شاخص‌ها مشخص شد. درصد جوانه‌زنی در هالوپرایمینگ با افزایش مدت زمان و غلظت تیمار، کاهش معنی‌داری را نشان داد. افزایش بیشتر طول ریشه‌چه و ساقه‌چه در تیمار هیدروپرایمینگ مشاهده شد اما در مقایسه با شاهد از نظر آماری معنی‌دار نبود. چاودار کوهی به نظر می‌رسد که مقاومت متوسطی به شوری دارد. نتایج این مطالعه نشان داد که هالوپرایمینگ بر جوانه‌زنی و رشد چاودار کوهی تاثیر منفی دارند که اشکال عمده آن ناشی از نوع واریته گیاهی و مدت زمان جذب آب و رطوبت بذر می‌باشد. از سوی دیگر این اثر احتمالاً به دلیل افزایش و تجمع بیش از حد یون‌های کلر و سدیم در بافت بذر می‌باشد. از این مطالعه می‌توان نتیجه گرفت که بذور چاودار کوهی به تکنیک پرایمینگ حساس هستند و پرایمینگ در این گونه نیاز به مطالعه روش‌های دیگر دارد.

کلمات کلیدی: هیدروپرایمینگ، هالوپرایمینگ، جذب، تنش شوری، ***Secale montanum***