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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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Received on: 13/04/2014 Accepted on: 05/07/2014

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*

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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 maior 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 complete conducting of before the 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 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 9cm-diameter Petri dishes with three replications. Germination tests were conducted in a germinator maintained at 15-25 °C for a 8 h period of darkness and a 16 h one of light with a light intensity of 38 µmolm⁻²s⁻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).

$$GR = \sum_{i=1}^{n} \left\lceil \frac{n}{t} \right\rceil$$
 (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).

$$VI = (RL + SL) \times GP$$
 (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 Halopriming affected by 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 Hydropriming (14.66). Germination rate 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 Halopriming duration of 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	_ Seed Priming								
		Hydropriming	Halopriming						
(mM)	Control	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 $_{0.05} = 1.55 (64 \text{ d.f.})$

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 Primin	g								
	_	Hydropriming	Haloprim	Halopriming						
	Control	24 h	4 h 24 h							
	Control		- 125 mM	250	500 mM	125 mM	250 mM	500		
		-	123 IIIVI	mM	JOO HIIVI	123 IIIVI		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)

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	Seed Priming								
		Hydropriming	Haloprim	ing					
Levels(mM)		24 h	24 h	24 h			72 h		
	Control		125	250	500	125	250	500	
		-	mM	mM	mM	mM	mM	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)

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.

^{*}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.)

^{*}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.)

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

G.	Seed Priming									
Stress Levels(mM)		Hydropriming	Halopriming							
	Control	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)

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									
		Hydropriming	Halopriming	Halopriming						
	Control	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)

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 observed at the highest 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 improve the seed germination performance in grass, vegetables and field crops (Kaya et al., 2006; Guzman and Olave, 2004; Afzal et al., 2004;

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

LSD $_{0.05} = 0.38 (64 \text{ d.f.})$

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

LSD $_{0.05} = 47.13 (64 \text{ d.f.})$

Sivritepe *et al.*, 2003; Li *et al.*, 2011; Dianati et al., 2010). Nonetheless, diverse population of native grass 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 Halopriming indicated that 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 the effects of priming influences (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 be difficult because can 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 needs further study. 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 چاودارکوهی (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 می شود. هدف این مطالعه بررسی اثر بخشی هیدروپرایمینگ و هالوپرایمینگ برای مطالعه روی بذور پرایم شده مورد استفاده قرار تنش شوری بود. هیدروپرایمینگ غلظتهای ۲۵۰، ۲۵۰ و ۵۰۰ میلیمول کلرید سدیم آماده شد. این گرفتند. برای تیمار هالوپرایمینگ غلظتهای ۲۵۰، ۲۵۰ و ۵۰۰ 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 می رسد 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 می باشد. از سوی دیگر این اثر احتمالاً به دلیل افزایش و تجمع بیش از حد یونهای کلر و سدیم در بافت بذر می باشد. از این مطالعه می توان نتیجه گرفت که بذور چاودار کوهی به تکنیک پرایمینگ حساس هستند می برایمینگ در این گونه نیاز به مطالعه روشهای دیگر دارد.

كلمات كليدى: هيدروپرايمينگ، هالوپرايمينگ، جذب، تنش شورى، Secale montanum

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