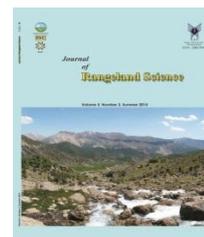


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**Research and Full Length Article:**

## **Growth Responses of *Secale cereale* and *S. ceremont* to Priming Treatments under Salinity Stress**

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Received on: 13/12/2014

Accepted on: 03/05/2015

**Abstract.** Soil salinity affects a large part of the region in Iran. Salinity covers 12.5% of total area of the country. Priming of seeds in sodium chloride and water (hydro-priming) has been reported to be a simple technique for enhancing the seedling establishment and germination performance in the seeds of many crops and small seeded grasses under the stress conditions. Therefore, the aim of this research was to study the effects of halo- and hydro-priming on *Secale cereale* and *S. ceremont* growth response under NaCl induced salinity stress conditions. Seeds of *S. cereale* and *S. ceremont* were immersed in sterile distilled water (hydro-priming). For the halo-priming treatments, concentrations of 125, 250 and 500 mm of sodium chloride were prepared. This experiment was carried out in three levels (0, 100 and 200 mm) of salinity stress. The means comparison indicates that the seedling traits of both species are significantly affected by both halopriming and hydropriming. In both species of *Secale*, all the traits were decreased significantly by increasing salt stress levels so that the most reduction of all the traits was observed at the highest level of salinity stress concentration as compared to the control. Increasing NaCl concentration in halopriming treatment solution significantly reduced all the traits in both species so that the most reduction of all traits was observed at the highest level of salinity stress concentration as compared to the control. *S. cereale* and *S. ceremont* appear to be poor salt-tolerant. Halo-priming in the *S. ceremont* with the concentrations of 125 and 250 mmol for 24h at the highest level of salt stress showed the alleviation of detrimental effects of salinity. Halo-priming somewhat in the *S. ceremont* was suggested in the areas with high salinity to increase the efficiency of species.

**Key words:** Halopriming, Hydropriming, Imbibing, Salt-tolerant, *Secale cereale*, *Secale ceremont*

## Introduction

*Secale* spp. belongs to the family Poaceae and Triticeae tribe. It includes perennial and annual species. The main distribution of the genus *Secale* spp. is in south Asia, Turkey, Lebanon, Syria, Iran, Iraq and Afghanistan (Sencer and Hawkes, 1980). Rye (*Secale cereale*) is the annual or biennial cool season grass. *S. cereale* has an extensive fibrous root system that may go as deep as 1.5 m. Rye culms are slender, erect and mostly glabrous (Heuzé *et al.*, 2013). *S. cereale* is widely cultivated in northern Europe and North America. Rye is mainly used for its grain but it is also a valuable fodder (Kelley and Mutch, 2011). *S. ceremont* is an annual species used as forage.

One of the major problems in forage cultivation in Iran is salinity. The seeds are sown in seedbeds for the reclamation or foraging purpose having inadequate moisture due to the lack of rainfall at germination stage. Another major restriction to seed germination is soil salinity. Soil salinity affects a large part of the region in Iran. Salinity covers 12.5% of the total area of the country (Akhani and Ghorbanli, 1993).

Priming of seeds in osmoticums such as mannitol, polyethylene glycol and sodium chloride (osmo-priming) and in water (hydro-priming) has been reported to be an economical, simple and safe technique for increasing the capacity of seeds for the osmotic adjustment and enhancing the seedling establishment and germination performance and emergence in the seeds of many crops and small seeded grasses under stress conditions (Kaur *et al.*, 2005; Heydecker and Coolbaer, 1977; Afzal *et al.*, 2008). A wide variety of priming treatments has been used to enhance the seed germination. Halo-priming as 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, holo-

priming is one of the techniques for coping with salinity. Hydro-priming is the simplest method to hydrate the seeds. Hydro-priming is associated with soaking the seed in distilled water and re-drying it before completing the germination. Peng *et al.* (2013) mentioned that Hydropriming treatment is an effective strategy to improve seed germination and plant growth of *Festuca sinensis*. Although the priming of seeds had been reported to result in better seedling growth under salinity stress conditions in various grasses, little is known about annual *Secale* induced by seed priming. Therefore, the aim of this research is to study the effects of halo- and hydroprimed *S. cereale* and *S. ceremont* growth response under NaCl induced salinity stress conditions.

## Materials and Methods

This study was carried out in Seed Technology Laboratory of Natural Resources, Tarbiat Modares University (TMU) of Iran. The origin of seeds was Esfahan province, Iran. The seeds were stored in paper bags at constant temperature of 4°C in darkness.

Hydro-priming was used according to the method described by Taylor *et al.* (1998). Seeds of both species were separately placed in nylon-net bags and immersed in sterile distilled water at temperature of 20°C and allowed to hydrate for 24 h. For the halo-priming treatments, concentrations of 125, 250 and 500 mM of sodium chloride were prepared. Then, seeds were immersed in NaCl solutions at 20°C for 24 h in darkness. Seeds without the treatment served as a control group.

After treatment, the seeds were rinsed with tap water for three minutes and lightly hand-dried. While still damp, the seeds were sprayed with Thiram fungicide at a rate of 0.65 ml kg<sup>-1</sup> of seed (Giri and Schillinger, 2003). Then, seeds were shade-dried until the moisture level came back to the original content at room

temperature. Hydro-priming and halo-priming treated seeds were equilibrated at room temperature for two days.

This experiment was carried out in three levels (0, 100 and 200 mM) of salinity stress. Fifty primed and unprimed seeds were with three replications placed on two layers of blotter paper watered with 5 ml of different levels of NaCl solution in 9-cm-diameter Petri dishes. Germination tests were conducted in a germinator maintained at 15-25°C for a period of 8 hours of darkness and 16 hours of light with the light intensity of  $38 \mu \text{molm}^{-2}\text{s}^{-1}$  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  $>2 \text{ mm}$  (Hardegree and Van Vactor, 2000).

The germination percent was calculated according to total number of germinated seeds. The germination rate was calculated using the following formula (Panwer and Bhardwaj, 2005) (Equation 1).

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

Where

n = number of newly germinated seeds at time t

t=number of days since sowing

The vigor index (VI) of seedlings was calculated according to the following formula (Abdul-Baki and Anderson, 1973) (Equation 2):

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

Where:

RL = root length (cm),

SL= shoot length (cm),

GP= germination percent

The seedlings were weighed to record the fresh mass.

After growth, root and shoot lengths of ten seedlings were measured. The seedlings were then placed in an oven run at 80°C for 24 h. These dried seedlings were weighed to record the seedling dry

mass. Analysis of variance of data was conducted using the MSTAT-C Program (Michigan State University). The Duncan's Multiple Range post hoc test at 5% probability level was used to test the differences among treatments.

## Results

The results of analysis of variance (ANOVA) showed significant effects of priming and salinity stress on parameters of germination in *Secale cereale* and *S. cereale*.

### Effects of Priming

a) *S. cereale*: Means comparison indicates that the seed germination traits of *S. cereale* were significantly affected by both halopriming and hydropriming (Table 1).

The maximum germination percent was recorded in control at 0 stress level (52%) which was significantly higher than hydro-priming (37%) and all the other priming treatments. Seed hydro-priming significantly increased the germination rate as compared to halo-priming treatment (Table 1) but it had no significant differences with control. Based on the results in *S. cereale*, higher values of fresh weight, vigor index and shoot and root length were observed in control, but for the root length and fresh weight, there were no significant differences between hydro-priming and control. Increasing NaCl concentration in halo-priming treatment solution significantly reduced all the traits in *S. cereale* (Table 1).

**Table 1.** Effects of priming with Hydro-priming and Halo-priming (24 h in four solutions of NaCl (125, 250 and 500 mM) and control (untreated) on germination percent, germination rate, root and shoot lengths and fresh weight (g) of *Secale cereale*

Treatments	Germination %	Germination Rate	Vigor Index	Root Length (cm)	Shoot Length (cm)	Fresh Weight (g)
Halopriming 125 mmol-24h	25.44 c*	9.65 a	124.53 c	1.88 ab	2.07 b	49.59 b
Halopriming 250 mmol-24h	9.78 d	8.20 b	53.22 d	1.46 bc	1.93 b	53.67 b
Halopriming 500 mmol-24h	8.67 d	6.89 c	26.26 d	1.07 c	1.69 b	48.38 b
Hydropriming 24h	37.00 b	10.33 a	225.77 b	2.13 a	2.17 b	84.00 a
Control	52.00 a	9.77 a	306.24 a	2.17 a	3.31 a	94.42 a

\*Values in columns marked with the same letter are insignificantly different on significance level  $\alpha = 0.05$

b) *S. ceremont*: Results of *S. ceremont* showed that the patterns of response to priming treatments were the same as *S. cereale*. In all but one case (root length), the highest values were observed in control (Table 2). Root length was more

developed in halo-priming as 125 mmol-24h (1.27 cm) than non-primed ones (1.61 cm). The minimum values for all the traits were observed in the halo-priming of 500 mM for 24 h (Table 2).

**Table 2.** Effects of priming with Hydropriming and Halopriming (24 h in four solutions of NaCl (125, 250 and 500 mM) and control (untreated) on germination percent, germination rate, root and shoot lengths and fresh weight (g) of *Secale ceremont*

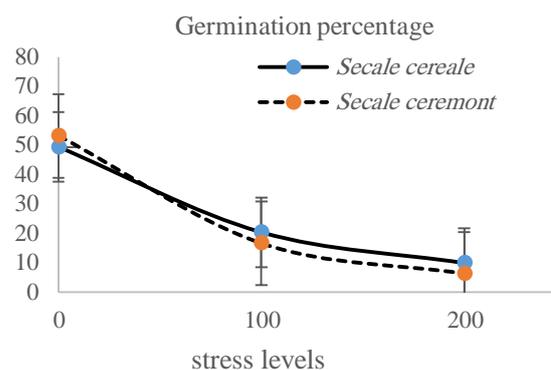
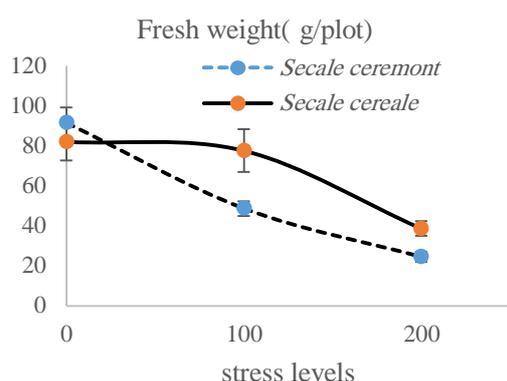
Treatments	Germination %	Germination Rate	Vigor Index	Root Length (cm)	Shoot Length (cm)	Fresh Weight (g)
Halopriming 125mmol-24h	23.44 b*	8.18 b	129.42 b	1.27 a	2.05 b	54.83 bc
Halopriming 250 mmol-24h	18.67 c	6.72 c	67.18 c	0.80 b	1.48 c	45.32 cd
Halopriming 500mmol-24h	18.11 c	5.10 d	41.03 c	0.40 b	1.15 c	35.21 d
Hydropriming 24h	20.56 bc	9.18 b	125.35 b	1.61 a	2.20 b	60.62 b
Control	46.22 a	10.61 a	284.75 a	1.61 a	2.82 a	78.40 a

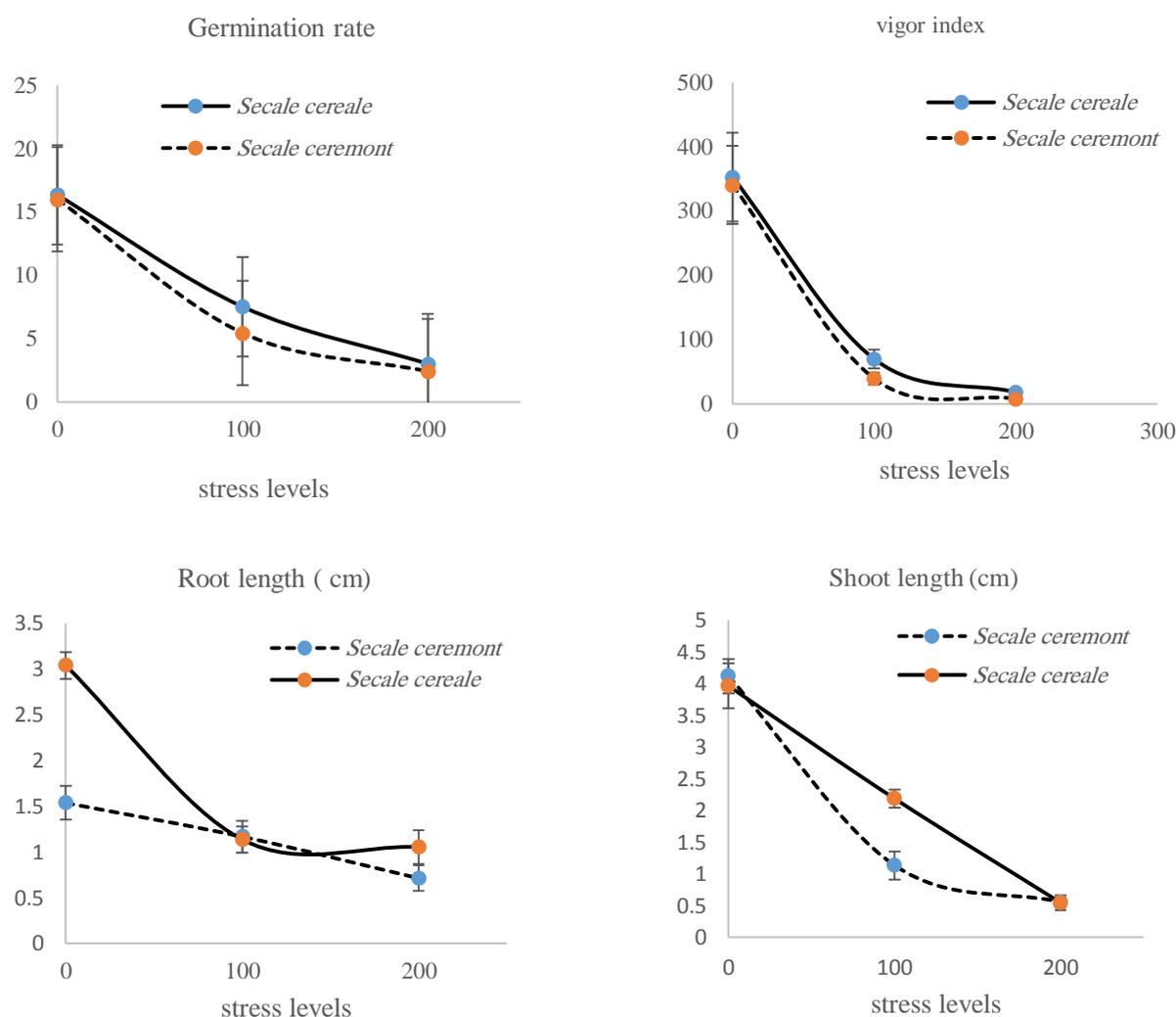
\*Values in columns marked with the same letter are insignificantly different on significance level  $\alpha = 0.05$

### Relationships between salinity levels and seed germination traits

Effects of salt treatments on shoot and root length, fresh weight (g), germination percent, germination rate, vigor index of *S. ceremont* and *S. cereal* are shown in Fig. 1. In both species of *Secale*, all the

traits decreased significantly by increasing salt stress levels so that the most reduction of all the traits was observed at the highest level of salinity stress concentration as compared to the control.





**Fig. 1.** Effects of salinity stress under 0, 100 and 200 Mm NaCl on germination percent, germination rate, root and shoot lengths and fresh weight (g) of *Secale ceremont* and *S. cereale*

### Salinity by priming interaction effects

*S. ceremont*: Means comparison of interaction effects of *S. ceremont* indicates that the germination percent was negatively affected by priming treatment (Table 3) so that the maximum value of germination percent was recorded in control at 0 stress level which was statistically significant as compared to all the priming treatments. Germination percent drastically declined with the increase of stress levels and concentration of haloprimering solution. Almost a similar response of germination percent was observed in all the traits. The highest shoot length was observed in

control at 0 stress level while no significant difference regarding shoot length was observed between control and Hydro-priming of 24h and halo-priming 125 mmol for 24h at stress level of 0. The result showed that for root length, no significant difference was observed between haloprimering 125 mmol for 24h at stress level of 0 and hydro-priming for 24h at stress level of 0.

**Table 3.** Interaction effects of priming with Hydropriming and Halopriming (24 h) in four solutions of NaCl(125, 250 and 500 mM) and control (untreated) under salinity stress 0, 100 and 200 Mm on germination percent, germination rate, vigor index and fresh weight (g) of *Secale ceremont*

Treatments	Stress Level (mmol NaCl)	Germination %	Germination Rate	Vigor Index	Fresh weight (g)	Shoot Length (cm)	Root Length (cm)
Halopriming 125 mmol-24h	0	56.00 b	15.28 c	353.17 b	87.17 c	46.0 a	1.60 ab
	100	38.00 c	12.44 d	169.77 c	74.33 cd	1.3d ef	1.44 a-d
	200	28.00 d	8.19 e	105.56 d	58.40 def	1.2d ef	0.98 b-e
Halopriming 250 mmol-24h	0	44.67 c	19.56 b	332.58 b	108.00 b	3.35 f	1.13 b-e
	100	10.67 ef	6.34 ef	29.35 h	53.40 d-g	0.71 ef	0.67 de
	200	14.67 e	5.37 fg	25.37 h	42.13 f-i	0.37 f	0.58 de
Halopriming 500 mmol-24h	0	16.67 e	4.24 fgh	12.05 h	32.47 f-j	2.92 bc	0.59 de
	100	11.33 ef	5.38 fg	34.86 h	47.50 e-h	0.29 bc	0.37 e
	200	3.67 f	2.92 ghi	5.75 h	23.93 ij	0.25 f	0.25 e
Hydropriming 24h	0	3.33 f	2.35 hi	6.40 h	19.50 ij	4.43 a	2.18 a
	100	9.67 ef	2.88 ghi	5.49 h	14.77 i	1.51 de	1.68 ab
	200	5.67 f	2.59 hi	8.61 h	26.37 hij	0.65 ef	0.98 b-e
Control	0	99.33 a	24.54 a	742.74 a	129.37 a	5.30 a	2.18 a
	100	30.00 d	5.91 ef	96.55 d	68.00 cde	2.00 cd	1.68 ab
	200	9.33 ef	1.37 i	14.97 h	37.83 e-j	0.36 f	0.78 de

Means followed by the same letter in a column do not differ significantly at  $p \leq 0.05$

Trend of responses to priming treatments in *S. cereale* was similar to *S. ceremont* so that in all the cases of priming, higher values were observed in control in 0 stress level (Table 4).

Traits in halo-priming showed a significant decrease with the increased concentration of treatments. So, lower values of traits were recorded in halopriming 500 mM for 24 h.

**Table 4.** Interaction effects of priming with Hydropriming and Halopriming (24 h) in four solutions of NaCl (125, 250 and 500 mM) and control (untreated) under salinity stress 0, 100 and 200 mmol on germination percent, germination rate and vigor index of *Secale cereale*

Treatments	Stress level (mmol NaCl)	Germination %	Germination Rate	Vigor Index	Shoot Length (cm)	Root Length (cm)
Halopriming 125 mmol-24h	0	41.33 c	17.37 b	271.57 c	3.67 b	3.03 a
	100	19.00 cd	15.02 c	139.84 d	2.11cde	1.32cde
	200	11.33 ef	11.86 c	61.79 ef	0.43 fg	1.31cde
Halopriming 250 mmol-24h	0	77.33 b	18.08 ab	566.59 b	3.77 b	2.98 a
	100	22.67 d	8.48 ef	79.59 def	1.82def	0.92 de
	200	6.00 f	6.24 g	16.89 ef	0.22 g	0.49 ef
Halopriming 500 mmol-24h	0	10.00 f	6.09 g	15.46 ef	3.29 bc	2.50 ab
	100	24.00 d	9.65 e	89.35 de	1.63 def	0.64 def
	200	12.33 ef	3.09 h	22.44 ef	0.15 g	0.09 f
Hydropriming 24h	0	4.33 f	3.34 h	2.93 f	3.50 bc	3.35 a
	100	4.67 f	2.71 h	1.53 f	2.4bcde	1.41 cd
	200	9.67 f	3.25 h	21.37 ef	0.62 fg	1.64 c
Control	0	98.00 a	19.45 a	725.45 a	5.60 a	3.35 a
	100	39.33 c	7.17 fg	149.19 d	3.00 bcd	1.41 cd
	200	18.67 cd	2.70 h	44.08 f	1.32 efg	1.75 bc

Means followed by the same letter in a column do not differ significantly at  $p \leq 0.05$

### Discussion and Conclusion

The results of investigation indicate that NaCl salinity caused growth inhibition in *Secale ceremont* and *S. cereale* due to the decreases in germination parameters and

fresh weight seedling. These effects of salinity on *S. ceremont* and *S. cereale* supported the finding of Hanif and Davies (1998) which showed that the increased salinity stress reduced the

germination percent and growth parameters of *S. cereale* seeds. Severity of salt damage has been found to be dependent on the species, the exposing period to salinity, the salinity level and growth stage of plant (Botia et al., 1998; Carvajal et al., 1998; Del Amor et al., 1999). In this study, the strongest decline in germination and growth parameters was observed in the highest salt concentration (200mM). 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. Our results showed that seeds of *S. ceremont* and *S. cereale* germinated at low concentrations of NaCl (100 mM) while at higher concentrations, (200) seeds of these species were inhibited and decreased significantly. Therefore, *S. ceremont* and *S. cereale* appear to be poor salt-tolerant. In the present study, we did not detect that halo-priming diminished the inhibiting effects of salinity stress on germination and growth response in *S. cereale* as it has been shown in some of species such as melon (Sivritepe et al., 2003) and *Distichlis spicata* (Sargeant et al., 2006). In some parameters, especially *S. ceremont*, halo-priming with concentrations of 125 and 250 mmol for 24h at the highest levels of salt stress (200) showed the alleviation of detrimental effects of salinity. In other words, halo-priming of seeds of *S. ceremont* could decrease the amount of reduction in germination percent, germination rate, vigor index, fresh weight and shoot and root length of seedlings as compared to the seedlings raised from non-primed (controls) seeds. Therefore, this treatment, especially in *S. ceremont* can be used in the areas with high salinity to increase the efficiency of species. These results are in agreement with the works done by Jisha and Puthur (2014) on *Vigna radiata* (L.) Wilczek who reported that halo-priming with

NaCl increased the stress tolerance potential in three *V. radiata* (L.)

In contrast to our findings about *S. ceremont*, hydro-priming and halo-priming had proved to be successful strategies to reduce the adverse effects of salt stress and improve the seed germination performance in grass, vegetable 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, genetically diverse population of native grass seed makes use of this method with great complexity. Therefore, there are critical points on its use (Di Girolamo and Barbanti, 2012) and it is not always a successful method for all the species. Results demonstrated that growth response of *S. cereale* was not affected by halo-priming and hydro-priming treatments. Our results were similar to the findings of Ghassemi-Golezani and Esmailpour (2008) who reported that halo-priming has no or little effects on *Cucumis sativus* seeds. Evenari (1964) found that pre-sowing treatments of seeds did not increase the grain yield of sorghum and even had a negative affect. The disadvantage may be due to the varied plant species (Murungu et al., 2004), imbibing time and seed moisture. Drying rate also influences the priming (Madakadze et al., 2000). Interpretation of reduction in growth parameters of *S. cereale* can be difficult because of many influential factors which occur during halo-priming and hydro-priming presses. It can be argued that these negative effects arise from high seed imbibition during priming that can swell endosperm considerably and restrict the radicle protrusion. The positive effects of seed priming on seed invigoration are critically dependent on priming duration (Ashraf et al., 2005). On the other hand, these effects are probably due to an excessive increase and accumulation of Na<sup>+</sup> and Cl<sup>-</sup> ions to the seed tissue 24 an 72 h duration of halo-priming treatment

which causes the inhibitory effects on the activities of germination process. However, halo-priming treatment in *S. ceremont* showed that it somewhat can be useful.

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## پاسخ رشد گونه‌های *Secale cereale* و *Secale cereale* به تیمارهای پرایمینگ تحت تنش شوری

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تاری دریافت: ۱۳۹۳/۰۹/۲۲

تاریخ پذیرش: ۱۳۹۴/۰۲/۱۳

**چکیده.** شوری خاک قسمت اعظمی از ایران را تحت تاثیر قرار داده است. شوری حدود ۱۲/۵ درصد کشور را در بر گرفته است. گزارش شده است که پرایمینگ بذر با استفاده از کلرید سدیم و آب (هیدروپرایمینگ) به عنوان تکنیک ساده ای برای افزایش استقرار گیاهچه و عملکرد جوانه‌زنی در برخی از گیاهان زراعی و گراس‌های کوچک بذر تحت شرایط استرس به کار می‌رود. بنابراین هدف از این تحقیق مطالعه بررسی تاثیرات هالوپرایمینگ و هیدروپرایمینگ روی پاسخ رشد دو گونه *Secale cereale* و *S. cereale* تحت القای استرس شوری با استفاده از کلرید سدیم بود. بذر دو گونه به منظور انجام تیمار هیدروپرایمینگ در آب مقطر فرو برده شد. برای تیمارهای هالوپرایمینگ محلولهای ۱۲۵، ۲۵۰ و ۵۰۰ میلی مول از کلرید سدیم تهیه شد. این آزمایش در سه سطح شوری (صفر، ۱۰۰ و ۲۰۰ میلی مول) انجام شد. مقایسه میانگین‌ها نشان داد که خصوصیات گیاهچه در هر دو گونه به‌طور معنی‌داری تحت تاثیر تیمارهای هالوپرایمینگ و هیدروپرایمینگ قرار گرفت. در هر دو گونه چاودار تمامی صفات، کاهش معنی‌داری با افزایش سطح تنش شوری نشان داد به طوری که بیشترین کاهش در تمامی صفات در بالاترین سطح غلظت تنش شوری نسبت به شاهد مشاهده شد. افزایش در غلظت کلرید سدیم در محلول‌های هالوپرایمینگ‌ها به‌طور معنی‌داری سبب کاهش خصوصیات رشد هر دو گونه شد. به طوری که بیشترین کاهش در خصوصیات رشد در بالاترین غلظت محلول هالوپرایمینگ نسبت به تیمار شاهد مشاهده شد. به نظر می‌رسد دو گونه *S. cereale* و *S. cereale* مقاومت کمی به شوری دارند. هالوپرایمینگ در گونه *S. cereale* با غلظت ۱۲۵ و ۲۵۰ میلی مول به مدت ۲۴ ساعت در بالاترین سطح تنش شوری اثرات مخرب شوری را کمتر کرد. نتایج نشان داد که هالوپرایمینگ تا حدودی می‌تواند برای افزایش کارایی در گونه *S. cereale* در مناطق با شوری بالا مفید باشد.

**کلمات کلیدی:** هالوپرایمینگ، هیدروپرایمینگ، جذب، تحمل شوری، *Secale cereale*، *Secale cereale*

*ceremont*