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**Full Length Article:**

## Effects of Salinity and Drought Stresses on Seed Germination and Seedling Growth of Desert Wheatgrass *Agropyron desertorum*

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**Abstract.** Considering forage grass species, germination is one of the important stages concerning the growing stage that is often influenced by environmental stresses, particularly salinity and drought stresses. Thus, this study aims to assess the effects of salinity and drought stresses on germination characteristics and seedling growth of desert wheatgrass, *Agropyron desertorum* (Fisch. ex Link) Schult. in laboratory conditions. Two separate experiments were conducted using completely randomized design with four replicates. Six salinity treatments (control, 50, 100, 150, 200 and 300 mM NaCl) and six drought treatments (control, -0.2, -0.4, -0.6, -0.8 and -1 MPa polyethylene glycol) were utilized in four replicates. Germination percentage and rate were calculated while computing the germinated seeds every day. Growth parameters (rootlet, shoot and seedling length), allometric index and seed vigor have been accordingly given. For each experiment, one-way ANOVA was used to determine significant effects of treatments and mean comparison was done by the means of Duncan method using SPSS software. Results showed that salinity and drought stresses had the significant effects on germination and growth indices of *Agropyron desertorum* so that germination rate and percentage, root length, shoot length, seedling length and seed vigor indices were decreased by increasing the concentrations of salinity and drought treatments. Also, seed germination and growth were observed only until -0.4 MPa drought level so that germination at  $\varphi_s = -0.6$  MP was completely stopped.

**Key words:** Salinity and drought stresses, Germination, Vigor index, *Agropyron desertorum*

## Introduction

Unsuitable amount and intensity of environmental factors that cause some damages in the plant (directly or indirectly) and create some limitations in growth and other vital processes for the plants can be called environmental stress (Fowden *et al.*, 1993). Germination stage is one of the most sensitive stages to salinity and drought stresses and if the plant is able to tolerate stresses in this stage, it can pass the later growth stages (Gholami *et al.*, 2010).

Most of areas in Iran are located on the arid belt of the world and have the arid and semi-arid climate and almost 12.5% of the country areas may be comprised of salty lands (Azarnivand and Zare Chahouki, 2010). Since a large part of Iran's rangelands has salty and alkaline soils, soil salinity constitutes one of the inhibiting factors for fodder production so that high levels of salinity in germination stages can be one of the obstacles for rangeland species to spread their regenerations (Manchanda and Garg, 2008). In addition to the reduction of free potentials of water, salinity has the adverse effects on seed germination via toxic effects of Na<sup>+</sup> and Cl<sup>-</sup> ions (Greenwood and Macfarlen, 2009).

Drought stress is directly or indirectly one of the most important stresses affecting other stresses (Tamartash *et al.*, 2010). Plants' resistance to drought stress will resist to the other physical stresses in the environment. Drought stress occurs when water gained by the plant is lower than the lost water (Kafi and Keshmiri, 2011). It can be due to excessive losses of water or decreased absorption or both of them. Decreased osmotic and total water potentials accompanied by the loosed turgidity, closed stomata and reduced growth are the special signs of water stress and where the stress is very intense, it can result in extreme decreased photosynthesis, impaired physiological processes, impaired growth and eventually, death of plant (Singh and

Patel, 1996). Numerous studies have been carried out about environmental stresses. Gholami *et al.* (2010) and Ghaderi *et al.* (2011) assessed the germination indices on *Vicia monantha* and on *Vicia villosa* L. under salinity and drought stresses. They found significant effects of both stresses on the decrease of seed characteristics. Also, they noted that germination of this species was continued until salinity of 300 mM and drought level of 0.2 MPa; in the salinity of 400 mM and drought level of 0.4 MPa, germination was stopped. Zia and Khan (2004) on *Limonium stocksii*, Mehrabi *et al.* (2011) on *Brassica napus* and Zirehzadeh *et al.* (2010) on *Thymus vulgaris* reached the same conclusion.

Akhavan Armaki *et al.* (2012) in the assessment of drought stress effects on germination indices of three rangeland species from *Bromus* genus showed that by increasing drought level, all germination and growth traits of seedling decreased significantly and among assessed species, *Bromus tomentellus* showed the appropriate germination in the investigated stress potentials.

Considering the lack of exact understanding of the responses of rangelands species to salinity and drought stresses, it is necessary to evaluate other grass species resistant to salinity and drought for the establishment of vegetation cover in the arid and semi-arid areas by a better knowledge.

Desert wheatgrass, namely *Agropyron desertorum* (Fisch. ex Link) Schult is a cool season grass belongs to Poaceae family and is of good adaptability in the vegetative growth stage in the arid climates. Appropriate fodder and seed yield, good palatability for livestock, soil conservation and ability of growing in most of soils indicate that this species is suitable for the restoration and rehabilitation of rangelands in the arid and semi-arid regions of the country (Azarnivand and Chahooki, 2010). Thus, considering its importance for above-

mentioned reasons in this investigation, we assess the effects of two environmental stresses (salinity and drought) on the germination and seedling growth traits to evaluate the ability of this species as a resistant plant.

### Materials and Methods

For conducting this investigation, *Agropyron desertorum* seeds were collected from the rangelands of Kerchiskai village, Jiroft city, Kerman province, Iran. Seeds were transferred to the laboratory and were disinfected with 10% of sodium hypochlorite for 10 minutes and after rinsing with the distilled water three times, they were placed in 0.001 of Benomyl solution for 20 minutes to disinfect completely. Then, seeds were rinsed with the distilled water. To eliminate the effects of other factors influencing the experiments, all laboratory instruments including Petri dishes, filter paper and forceps were sterilized in an autoclave at 120 °C for 30 min.

To assess the effects of salinity and drought stresses, two separate experiments were conducted by the help of completely randomized design with four replications and 6 treatments. Sodium chloride (NaCl) and polyethylene glycol (PEG 6000) were used to exert drought and salinity stresses, respectively to simulate the stress conditions. For salinity stress, 6 different potential levels including (control 0), 50 and 150, 200 and 300 mM NaCl and for drought stress, 6 different potential levels including (control 0), -0.2, -0.4, -0.6, -0.8 and -1 MPa were applied. For calculating PEG required for the experiment, Kaufman and Eckard formula (1971) was used as bellow (Equation 1):

$$\varphi_s = -(1.18 \times 10^{-2})C - (1.8 \times 10^{-4})C^2 + (2.67 \times 10^{-4})CT + (8.39 \times 10^{-7})C^2T$$

(Equation 1)

Where

C= required PEG (grams per kilogram of water),

T= environment temperature which is supposed to be 25°C,

φs= osmotic pressure (MPa)

To avoid the evaporation of the solution and constant concentration, Petri dishes were placed in nylon bags during the experiment; then, they were maintained in a germinator with 26° C for 16 h photoperiod and 8 h darkness with the relative humidity of 70%. Germinated seeds were counted every day till 25<sup>th</sup> day. From 21st day to 25th day, no germinated seeds were observed. Seedling root and shoot length and seedling length were measured in the last day of experiment. Germination Percentage (GP) was calculated from the final number of germinated seeds divided by the number of sown seeds and Germination Rate (GR) (Equation 2) according to Maguire (1962).

$$GR = \sum_{i=1}^n S_i / D_i$$

(Equation 2)

Where

GR= germination rate,

S<sub>i</sub> =number of germinated seeds in each counting,

D<sub>i</sub> = number of days till counting and

n = number of counting times (days)

At the end of experiments, allometry index (root/shoot length ratio) and seed vigor index (germination percentage × mean seedling length (mm))/100) were calculated (Abdul baki and Anderson, 1973). Since some of data sets did not follow a normal distribution, these data sets were normalized for the analysis. Thus, before any statistical analysis, logarithmic transformation was done to normalize the germination percentage. For normalizing other indices,  $Y = \sqrt{X+0.5}$  transformation was performed. To determine the significance of data obtained from treatments, one-way ANOVA was conducted using SPSS software. The given means were tested by the means of Duncan method.

## Results

### Analysis of variance

Results of one-way ANOVA showed significant effects of different salinity and drought treatments on the germination

and seed growth indices of *Agropyron desertorum* species for all the traits except Alomtric index for salinity stress (Table 1).

**Table 1.** Analysis of variance of effects of salinity and drought stresses on seed traits of *Agropyron desertorum* species

Variables Name	Salinity Stress		Drought Stress	
	F	P-value	F	P-value
Germination percentage	42.14**	0.001	68.03**	0.001
Germination rate	41.20**	0.001	47.63**	0.001
Root length	36.53**	0.001	30.01**	0.001
Shoot length	17.22**	0.001	59.7**	0.001
Seedling length	38.89**	0.001	75.26**	0.001
Seed vigor index	45.01**	0.001	84.48**	0.001
Alomtric index	1.12	0.32	18.09**	0.001

\*\* Significant at 1% probability level

### Means of traits comparisons

#### a) Germination percentage

Results of mean comparisons showed that germination percentage of *Agropyron desertorum* seeds decreased with the increase in salinity level from the control solution to 300 mM NaCl; however, there were no significant differences between control and 50 mM treatments, 100 and 150 mM treatments and 200 and 300 mM treatments (Fig. 1).

In a drought experiment, germination percentage decreased as the drought value was increased and maximum germination percentage was observed in control treatment. By increasing drought intensity, germination percentage was reduced from 100% in control treatment to 9% in -0.4 MPa one (Fig. 1). No germination was observed in a treatment greater than -0.4 MPa.

#### b) Germination rate

Germination rate was significantly reduced by increasing the NaCl concentration. There were no significant differences between control and 50 mM treatments, 100 and 150 mM treatments and 200 and 300 mM treatments; however, the highest and lowest germination rates were found in control and 300 mM treatments, respectively (Fig. 2).

In the drought experiment, there were significant differences in germination rate between different levels of treatments so that the highest germination rate was observed for control treatment. Germination rate was significantly decreased by the increase of polyethylene glycol concentration (Fig. 2).

#### c) Root length

As it can be observed in Figure 3, different salinity and drought levels had significant effects on root length. Root length was decreased by increasing NaCl concentration so that the highest and lowest root length rates were observed in control and 300 mM treatments, respectively (Fig. 3).

In the drought experiment, root length was decreased by increasing the drought level. There were no significant differences between -0.2 and -0.4 MPa and the highest root length was found in control treatment (Fig. 3).

#### d) Shoot length

Results of means' comparisons at different salinity levels showed that shoot length decreased by the increase of NaCl so that the highest shoot length was found in control treatment. Although 50 mM NaCl treatment was not significantly different from 100 mM treatment, there was a significant difference between 50 mM and control treatments (Fig. 4).

In case of polyethylene glycol, it should be noted that there were significant differences between control, -0.2 and -0.4 MPa treatments ( $P < 0.01$ ) (Fig. 4).

**e) Seedling length**

Different salinity concentrations had significant influences on the seedling length so that seedling length was generally decreased by increasing NaCl concentration. Significant differences were not observed between 150, 200 and 300 mM treatments in case of salinity effects but there were significant differences between the mentioned treatments and control and 50 and 100 mM treatments (Fig. 5). Statistically, there was a significant difference between 50 and 100 mM treatments at the confidence level of 99%.

In the drought experiment, significant differences were found between different drought levels in case of seedling length so that the highest seedling length was found in control treatment; in other words, seedling length was significantly

decreased by increasing polyethylene glycol concentration (Fig. 5).

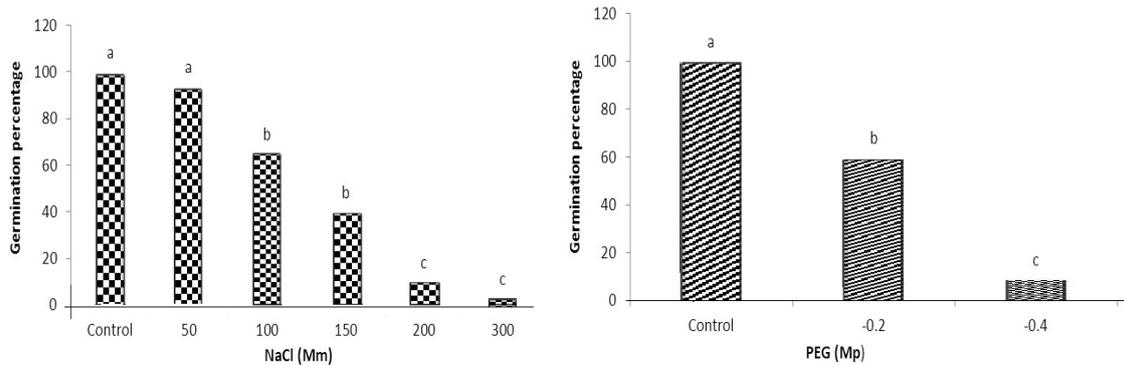
**f) Seed vigor index**

Seed vigor index decreased by the increase of salinity and drought levels. In salinity stress, treatments with different concentrations of NaCl were significantly different so that seed vigor index was reduced by increasing salinity (Fig. 6).

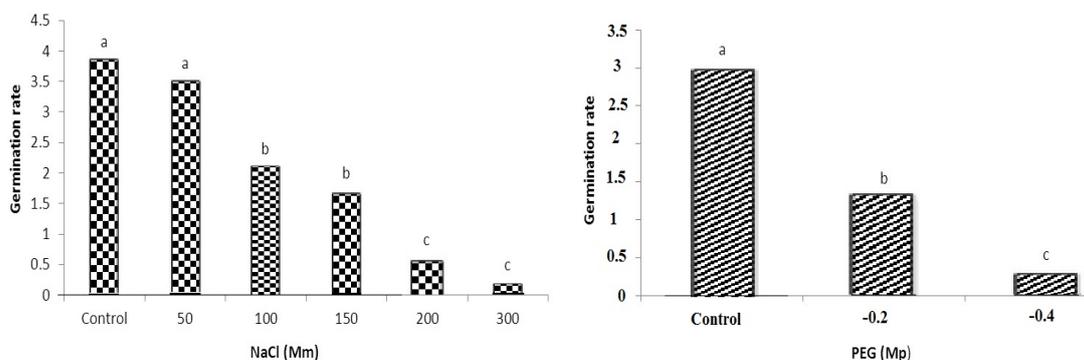
In the drought experiment, three control, -0.2 and -0.4 MPa treatments were significantly different and the highest vigor index was found in control treatment (Fig. 6).

**g) Allometry index**

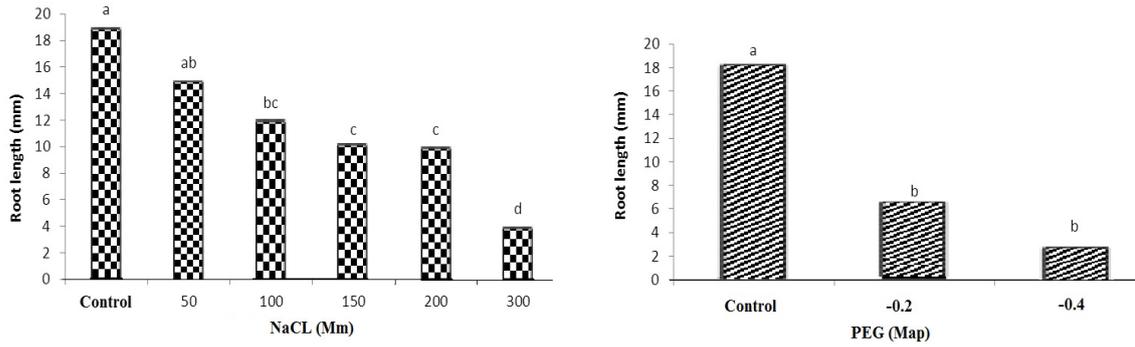
Salinity treatment did not show significant differences in case of allometry index (root/shoot length ratio) and drought treatment had significant effects on allometry index. Comparisons of mean allometry index in different drought treatments showed that though control and -0.4 MPa treatments were not significantly different, these two treatments were significantly different from -0.2 treatment (Fig. 7).



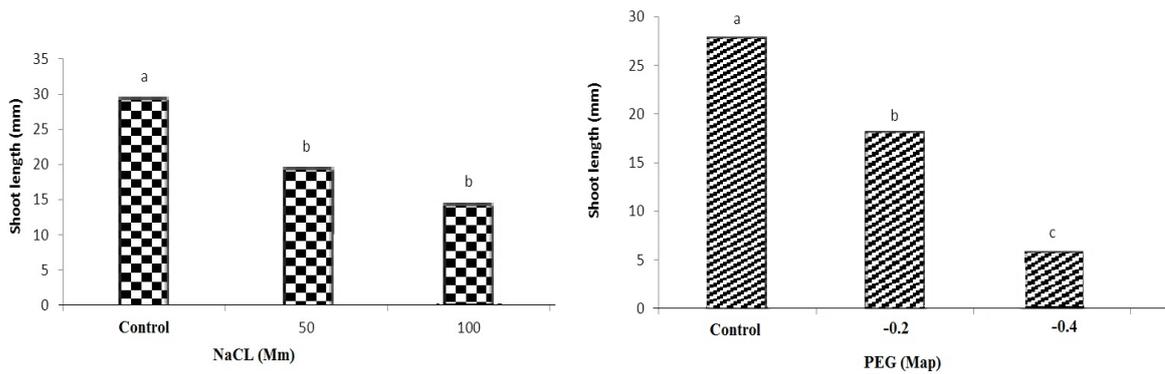
**Fig. 1.** Means of effects of salinity and drought stresses on germination% of *Agropyron desertorum*



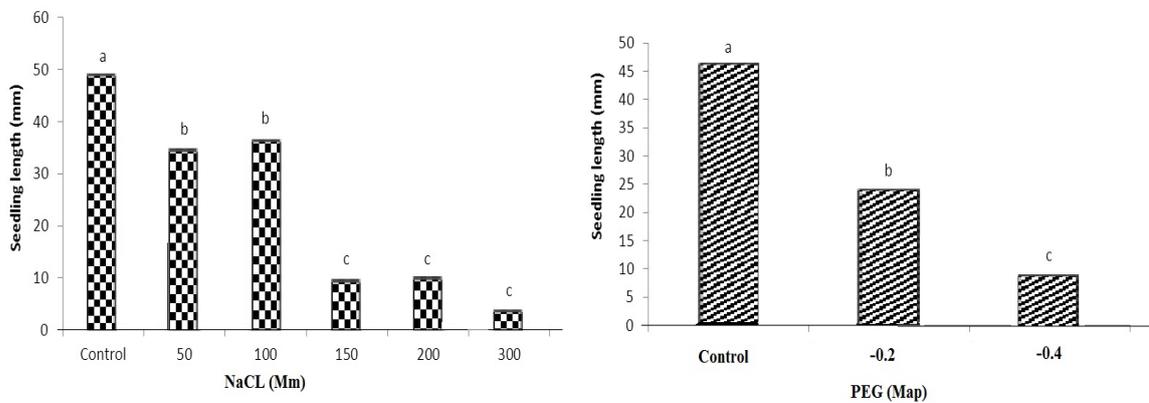
**Fig. 2.** Means of effects of salinity and drought stresses on germination rate of *Agropyron desertorum*



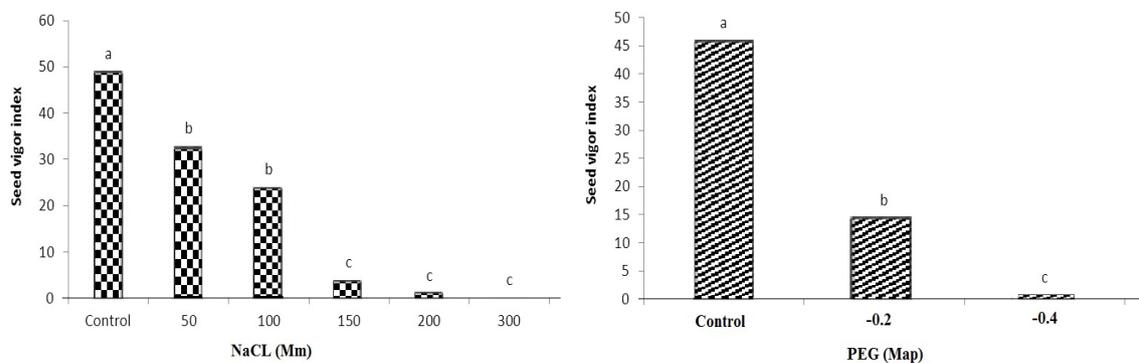
**Fig. 3.** Means of effects of salinity and drought stresses on root length of *Agropyron desertorum*



**Fig. 4.** Means of effects of salinity and drought stresses on shoot length of *Agropyron desertorum*



**Fig. 5.** Means of effects of salinity and drought stresses on seedling length of *Agropyron desertorum*



**Fig. 6.** Means of effects of salinity and drought stresses on seed vigor index of *Agropyron desertorum*

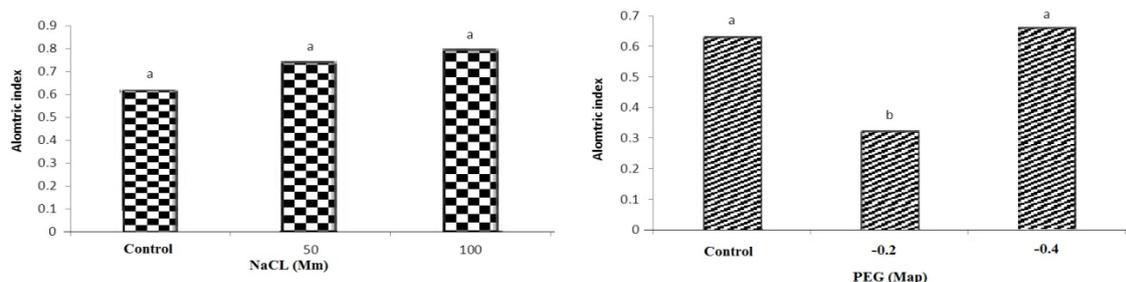


Fig. 7. Means of effects of salinity and drought stresses on Allometry index of *Agropyron desertorum*

### Discussion and Conclusion

Based on results obtained by this investigation, it can be noted that salinity and drought stresses have influenced germination indices of *Agropyron desertorum*. In other words, high concentrations of NaCl and polyethylene glycol created inappropriate media for germination and seedling growth of *Agropyron desertorum* seeds so that germination traits were decreased by increasing salinity and drought; these results are consistent with those presented by the studies done by Tamartash *et al.* (2010), Akhavan-Armaki *et al.* (2012), Zirehzadeh *et al.* (2010), Mehrabi *et al.* (2011) and Gharedi *et al.* (2011) on other range and crop species. Germination percentage and rate were reduced by the increase of salinity since osmotic pressure of solution increases by increasing salinity. This leads to the ionic imbalance that had a negative effect on the seeds' crucial interactions and prevents seed germination (Khan and Ungar, 2001). Also, drought stress causes the decrease in seedling germination and seedling growth that can be due to the direct influence of slower breakdown of endosperm materials in cotyledons accompanied by the slower transfer of materials broken down to seedling. Also, drought stress affects germination by limiting water absorption by seed, by transferring seed reserves or by directly influencing on organic structure and protein synthesis of embryo. Decreased germination percentage and rate under the effects of drought stress have been reported by the studied donr by Gholami

*et al.* (2010) on *Vicia monantha* species and Kafi and Keshmiri (2011) on *Cuminum cyminum* species. Root and shoot length which may be the important traits in primary establishment of seedling decreased significantly under drought stress. In this investigation, root and shoot length were decreased that could be explained by turgescence pressure limitation or the accumulation of dry materials in root storage tissues that is consistent with the study of Sharma *et al.* (2004) about the decrease in seedling length through the reduced tissue water in seedling under the effects of increased salinity levels. Since under high levels of salinity and drought stresses, germination percentage decreased in addition to seedling length, seed vigor index was decreased. Nonetheless, in stages after germination, the tolerance of seedlings to salinity stress depends on the ability of them to store toxic  $\text{Na}^+$  and  $\text{Cl}^-$  ions in their vacuoles if the cell metabolism is not affected (Wahid *et al.*, 1997). Results obtained from this investigation showed that different salinity concentrations had significant effects on root and shoot length, allometry index and seed vigor and these indices were reduced by increasing salt concentration. In the arid regions, seeds having a proper germination percentage and growth speed for root and shoot can be established better and represent higher yields. Since present investigation has been conducted in laboratory conditions, it is necessary to assess the germination and establishment of this species in filed conditions so that by comparing the results obtained from

two situations, the results of current study are rationally used. It is worthwhile to note that by doing such researches, the species resistant to salinity and drought stresses can be introduced to the arid and semi-arid regions and they can be used to establish the rangeland vegetation tolerant to salinity and drought. Results of present study have indicated that *Agropyron desertorum* could tolerate salinity stress better than drought stress. This plant could germinate well in the area where precipitation could change soil moisture up to threshold of -0.2 MPa osmotic potentials and 100 mM salinity during the sowing season. It seems that the established plants probably could grow in higher stress conditions such as -0.4 MPa drought and 200 mM salinity. Therefore, *Agropyron desertorum* as a cool season forage grass is not recommended for the rehabilitation of pastures with sever drought and salinity conditions.

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## بررسی اثرات تنش خشکی و شوری بر خصوصیات جوانه‌زنی و رشد گیاهچه در گونه *Agropyron desertorum*

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**چکیده.** مرحله جوانه‌زنی گیاهان یکی از مراحل مهم در طول دوره رشدی آنها است که اغلب تحت تأثیر تنش‌های محیطی بویژه شوری و خشکی قرار می‌گیرد. هدف از این تحقیق بررسی اثرات شوری و خشکی بر جوانه‌زنی و رشد گیاهچه در گونه *Agropyron desertorum* در شرایط آزمایشگاهی می‌باشد. بدین منظور برای بررسی تأثیر این عوامل محیطی بر شاخص‌های جوانه‌زنی و رشد بذور این گونه، دو آزمایش در قالب طرح کاملاً تصادفی با شش تیمار شوری (شاهد، ۵۰، ۱۰۰، ۱۵۰، ۲۰۰ و ۳۰۰ میلی‌مولار کلرید سدیم) و شش تیمار خشکی (شاهد، ۰/۲، ۰/۴، ۰/۶، ۰/۸، ۱ و ۱- مگاپاسکال پلی‌اتیلن گلیکول) در چهار تکرار انجام شد. با بررسی میزان بذور جوانه‌زده در هر روز، درصد و سرعت جوانه‌زنی محاسبه و میزان رشد با اندازه‌گیری طول ریشه‌چه، ساقه‌چه و گیاهچه و نیز ضریب آلومتری و بنیه بذر تعیین گردید. جهت تعیین معنی‌داری داده‌های حاصل از تیمارها، از تجزیه واریانس یکطرفه استفاده شد و میزان اثر عوامل مورد بررسی بر تیمارها با مقایسه میانگین‌ها به روش دانکن در نرم‌افزار SPSS انجام شد. نتایج این پژوهش بیانگر این است که تنش‌های شوری و خشکی بر شاخص‌های مختلف جوانه‌زنی و رشد گیاهچه گونه *Agropyron desertorum* اثر معنی‌داری دارند و با افزایش غلظت تیمارهای مختلف شوری و خشکی، درصد جوانه‌زنی، سرعت جوانه‌زنی، طول ریشه‌چه، طول ساقه‌چه، طول گیاهچه و بنیه بذر کاهش می‌یابند. همچنین جوانه‌زنی و رشد بذور تا سطح خشکی ۰/۴- مگاپاسکال مشاهده شده و از سطح خشکی ۰/۶- مگاپاسکال جوانه‌زنی بصورت کامل متوقف شد.

**کلمات کلیدی:** تنش‌های شوری و خشکی، جوانه‌زنی، بنیه بذر، *Agropyron desertorum*