



## RESEARCH PAPER

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## Influence of salicylic acid pretreatment on germination and seedling growth of wheat (*Triticum aestivum* L.) cultivars under salt stress

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### Abstract

Salicylic acid enhances salt tolerance in wheat seedlings and resistance to water deficit. The experiment was applied to determine the effect of salicylic acid pretreatment on wheat germination and seedling growth under salt stress. Traits such as seed germination, root length, shoot length, seedling fresh weight, germination rate and seed vigor index were evaluated. The data obtained in accordance factorial experiment with three replications in a completely randomized design were analyzed using MSTAT-C statistical software. Analysis of variance of the effects of salicylic acid and salinity was significant for all traits, but the cultivars for the characters germination percentage, germination rate and seed vigor index was highly significant and variety effect for other traits were not statistically significant. Comparison of means for main effects showed that the cultivars are different in traits such as, germination, vigor index and germination rate. With increasing salinity, decrease germination percentage, germination rate, and seedling root length and shoot fresh weight and seed vigor. Consumption of salicylic acid in the without consumption increased the root and shoot length in the normal conditions. But there stresses plants Concentration 0/5 mM salicylic acid in 80 mM NaCl treatment not decreased the shoot length and root length and has been Increased in the seedling fresh weight trait. Chamran cultivar response to higher concentrations of SA was more appropriate in terms of salinity.

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## Introduction

Wheat is the major crop in Iran. In Iran, 6.2 million hectares are under wheat cultivation, of which 33% is irrigated and 67% is rain fed (Fateh *et al.*, 2012). One of the main problems is salinity in semiarid regions of the world (Bai and Sui, 2006). Salinity is a limiting factor for plant growth because it causes nutritional limitations by reducing phosphorus, potassium, and calcium nitrate to increase intracellular ion concentration and drought stress. Salinity and drought affect the plants in a similar way (Ashraf and Ali, 2008). The osmotic potential of saline soils is low and high concentrations of salts can damage the plant. Osmotic potential in saline soils is low and high concentrations of salts can damage the plant. Salt stress causes the accumulation of reactive oxygen species in cells and damage to membrane lipids, proteins and nucleic acids are antioxidants that neutralize the free radicals that are present in plants can be the most ascorbic acid mentioned (Zhang and Kirkham, 1996). Kaya and Day (2008) compared the germination and seedling growth of wheat under salinity compete with weeds as they are traits that salinity reduced germination and emergence time increasing salinity, but increased in. Dai *et al.* (2009) the effect of salinity on germination rate and root and shoot growth of many crops showed Salinity tolerance during germination in many species, is a reliable test. Between species and varieties, there are differences in the response to salt stress, quite a lot of research done on the germination of various crops reflects the fact that with increasing salinity, root length, shoot and seedling dry weight decreased significantly compared to the control. Increased antioxidant activity has been reported under salinity stress tolerance in wheat cultivars (Sairam *et al.*, 1998). Rahman *et al.* (2008) effects of NaCl salinity on wheat as received, with increasing salt concentration, decreased root length, shoot dry weight occurs.

Many molecules such as salicylic acid (SA) and polyamines have been suggested as signal transducers and messengers who may have profound effects on plant growth and development (Sarin and Naranayan, 1968). Salicylic acid plays an essential role in the

regulation of various physiological processes such as growth, plant development, absorption, germination, photosynthesis and plays (Huang *et al.*, 2008).

Salicylic acid is a phenol compound of plant hormones produced by cells of roots and Different shapes in the air, leaf and root cells around there and having antioxidant activity is involved in the regulation of plant physiological processes. El Tayeb (2005) expressed, salicylic acid production, role in regulating various physiological processes such as growth, plant development, absorption, germination, photosynthesis and plays. Salicylic acid has a protective role in plants are under environmental stress The Senaranta *et al.*, 2002) reported that acetylsalicylic acid (salicylic acid derivative similar) effects of drought and salinity decreases in wheat. Also Shakirova and Sahabutdinova (2003) expressed the use of SA was increased wheat yield.

The aim of this study was to evaluate the effect of salicylic acid pre-treatment on salt tolerance on germination and seedling growth of wheat genotypes.

## Materials and methods

### *Plant materials*

In order to study the effect of salicylic acid on germination and seedling growth of Wheat genotypes under different levels of salinity stress, an experiment was conducted in the Research Laboratory of Islamic Azad University of Shoushtar, Iran in 2012. The experimental design was factorial based on completely randomized design (CRD) with three replications. The treatments have been used including of Treatments of salicylic acid (0, 0/5 and 1 mM), genotypes of bread wheat (CV-Chamran and Sardari) and four levels of salinity stress was induced by NaCl (0, 80, 160 and 240 mM). The wheat seed disinfected with sodium hypo chloride for 5 min and 96% ethanol for 30 seconds, rinsing well with distilled water. Then seeds of two genotypes were soaked in solutions with concentrations (0 (distilled water), 5/0, and 1 mM) of salicylic acid for 24 hour, separately, Then seeds were transferred to sterile Petri dishes containing 30 seeds on filter paper. It also was used a solution of NaCl salinity concentrations and was applied distilled water (control) at a rate of 10 ml per petri dish.

Then placed in Petri dishes and the door were closed with parafilm and were in germinator at 22° C temperature. Germinated seeds were counted every 12 hours and 10 days. The germination tests were calculated for each petridish. Since the second day of experiment, the number of germinated seeds was counted every day (Standard germination is when the root tip was approximately 2 mm). After the final day of testing (the 10th day), germination percentage (GP), germination rate (GR), seed vigor index (VI), root length (RL), shoot length (SL) and fresh weight (FW) of seedling was calculated using the following formulas:

The germination percentage was obtained from the ratio of the number of seeds germinated after 5 days to the total number of seeds (Gharineh *et al.*, 2004).

We use the following formula to calculate the percentage of germination (Nicols and Heydecker, 1968):

$$\{S/T\} \times 100$$

S: The number of germinated seeds.

T: Total number of seeds.

and we use the following formula to calculate the germination rate (Maguire, 1962):

$$R = \frac{\sum n}{\sum dn}$$

R: germination rate

$\sum n$ : The number of germinations during dth day

$\sum dn$ : The number of days, since the beginning of germination

(VI): Were calculated by the method of Abdul-Baki and Anderson (1970), respectively:

$$VI = \frac{GP \times MSH}{100}$$

Root length and shoot length of the ruler mm measure will help. In order to open the curvature and length of seedling root and shoot length shall be measured from end-to-junction seed. Seedling fresh weight, root length and shoot length was measured as the average of five plant.

### Statistical analysis

For statistical analysis the data of germinating percentage were transformed to arcsine  $\frac{\sqrt{x}}{100}$ .

Analysis of variance was performed using PROC ANOVA of SAS (1997). Each treatment was analyzed in three replications. The comparison of the means was done by Duncan test at a probability level of 5 percent.

## Results and discussion

### The percentage of germination

The results of analysis of variance showed (Table 1) that salt stress had significantly (at 1% probability level) effect on the percentage of germination and so SA in the various treatments caused to significant differences (at the level 1%). These results were observed by other researcher such as, Rajasekaran *et al.*, (2002) and Munns (2002). The germination percent decreased with increasing in NaCl concentration (Fig. 1). This reduced in Sardari variety was more than Chamran variety. This reduced in the Sardari variety was over 40% compared to control. Pretreatment of seeds with SA under salt stress caused an increase in germination percentage in Chamran variety compared to Sardari (Fig 1). The highest percentage of germination Sardari was obtained in treatments without stress and also treatments with 1 mM SA and without stress and for Chamran variety highest percentage of germination was obtained in treatment 160 mM stress. The lowest percentage of germination was also seen in severe stress (Table 2). Seed pretreatment with SA in severe salt stress increased germination in Chamran variety compared to Sardari (Fig 1). The germination percentage increasing of wheat in the salt and treatment with SA was observed in the research of Dolatabadian *et al.*, (2009).

The interaction between salinity and SA was significant on germination percentage (Table 1), so that 0/5 mmol SA increased it under stress and no-stress conditions but the two SA concentrations did not exhibit any differences under control treatment,

while at 80 mM salinity in Chamran variety increase in germination percentage was higher with 0.5 mmol SA than Sardari variety (Fig. 1).

In total, it was shown that the SA-treated wheat seeds had little reduce germination but it was higher in Sardari variety. The studies of Torabian (2010) on alfalfa, Shakirova *et al.*, (2003) on wheat and El-Tayeb (2005) on barley gave the same results respecting SA pretreated seeds which shows the increase in salinity resistance by the application of SA.

#### *Germination rate*

The results of analysis of variance showed (Table 1) that with increasing salt stress decreased (at 1% probability level) germination rate. The study of germination rate, found that the germination of Chamran genotype in high salinity conditions has a higher germination rate (Fig. 2). Steep decline in germination rate and germination percentage was similar for both genotypes. De and Kar (1994) reported that the absorption of water by seeds is impaired or slowly be absorbed into the seed germination metabolic activities will be carried out slowly and therefore the time required rooting out the seed germination increases and the germination rate decreases.

According to Fig 2 with increased salt concentration decreased germination rate, so in compared to severe stress and control treatments in Sardari variety was seen 40% reduction in germination rate and so for Chamran variety was 34%. Pretreatment of seeds with salicylic acid on this genotypes could not compensate damages caused by salinity and enhances the germination rate little (Fig 2). The highest germination rate was in treatments without stress in Sardari and 80 mM salinity in Chamran variety. Seed priming with SA and also the lowest germination rate was observed in the treatment of severe stress.

Seeds need to have enough water for critical activities and the start of germination. If water absorption is impaired or proceeds slowly activities inside the seed

will be too slow and increases duration exiting the radicle of seeds and the expression decreases germination rate and uniformity. Homayoun *et al.*, (2011) and Torabian (2010) reached similar conclusions about the wheat plant.

#### *Root and shoot length*

The results of analysis of variance showed that salinity and SA significantly affected root and shoot length (Table 1). Analysis of variance showed that the effect of salinity in root and shoot length were significantly and this traits reacted the different levels of salinity and salicylic acid, but no statistical difference was observed between the cultivars studied. Interactions effects are not significant, indicating that the behavior of cultivars against salinity and salicylic acid were similar for the two traits. In this study, increasing salinity levels, shoot length and root have shown a statistically significant decrease. This is corresponded with results reported researchers at several plants.

Kaya and Dey (2008) In a study done on sunflower seed germination expressed With increasing salinity, root length, shoot and seedling dry weight were significantly decreased compared with controls, but which cannot be said to be operating properly, a more important role has in the inhibition of seed germination under salt stress conditions.

Salicylic acid intake compared with non-smoking treatments increased root length and shoot it without stress. But there stresses plants 0/5 mM Concentration of SA only affects on shoot length and increases its, at 80 mM NaCl treatments (Fig. 3). Also, use 5/0 mM salicylic acid, increased root length Chamran variety in stress treatments compared to the use not salicylic acid (Fig. 4). Mechanism of salicylic acid increases in root and shoot growth of some plants is not well understood. But Shakirova *et al.*, (2003) showed that the probability of salicylic acid to regulate elongation and cell division, along with other substances such as auxin. However Fariduddin *et al.*, (2003) stated salicylic acid prevents by oxidation of auxin.

### Fresh weight of seedling

The results of the analysis of variance showed a significant effect of salicylic acid pretreatment on seedling fresh weight (Table 1). Fresh weights decreased significantly with exposure to NaCl salinity and reduction was severe at 160 mM of NaCl treatment without SA. Increase in seedling fresh weight of salicylic acid is affected by Dolatabadian *et al.*, (2009) were reported in wheat.

Effect of cultivar for the fresh weight of seedlings was not statistically significant, in the sense that the cultivars under salt stress condition, there was no difference in seedling fresh weight. But the traits and the interaction between salinity and cultivars for salinity effects were significant. This result indicates that the salinity difference between the varieties is different.

The mean fresh weight (Table 2) showed that there was no significant difference between the varieties. According to the varieties results at lower salinity (80 mM) seedling fresh weight compared with the control (distilled water) had a smaller reduction. Exogenously treated 0.5 mM SA increased fresh weights in severe at 80 mM saline and non-saline conditions (Fig 5). Compare to control level; SA without NaCl was the highest fresh weight group.

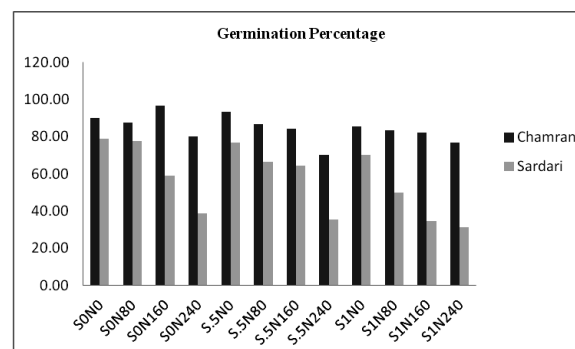
### Seed vigor index (VI)

Results of Analysis of Variance for traits vigor index showed that there were significant differences between cultivars of these parameters (Table 1), therefore concluded that salt stress in wheat varieties with different seed vigor physiological, biochemical activities, and can even affect tolerance mechanisms.

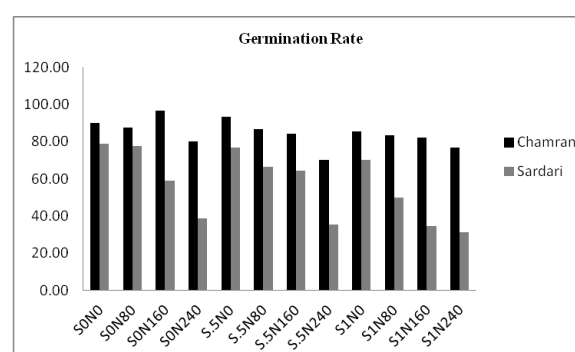
Also, various levels of salinity and salicylic acid effect were not significant (Table 1).

In both varieties of seed vigor decreased with increasing salinity levels (Fig. 6). This is according reports by other researchers. Such Khodarahmpour (2011) and Mensuh *et al.* (2006) that had been stated vigor reduced by salinity stress. Salicylic acid intake

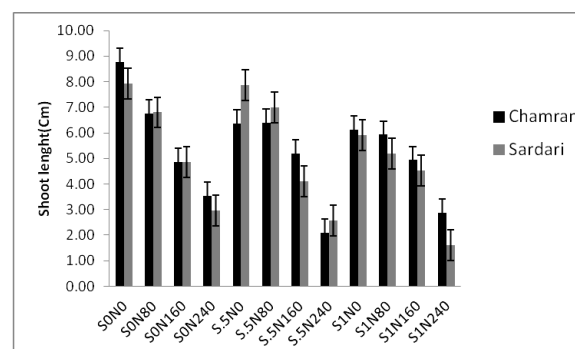
compared to not consumption treatments increased root length and shoot be without stress.



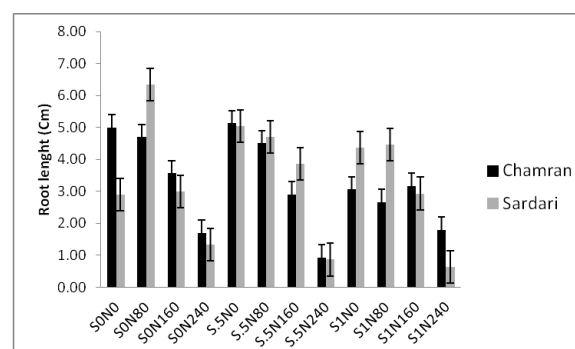
**Fig. 1.** Germination percentage of salicylic acid pretreatment and salinity levels on wheat cultivars.



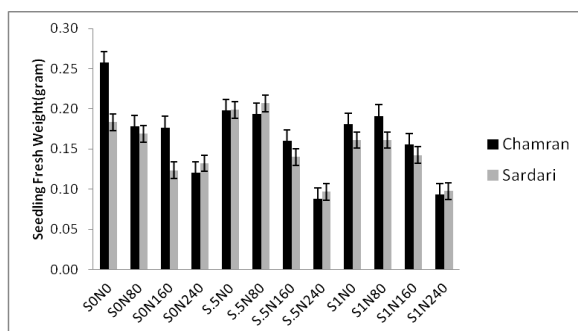
**Fig. 2.** Germination rate of salicylic acid pretreatment and salinity levels on wheat cultivars.



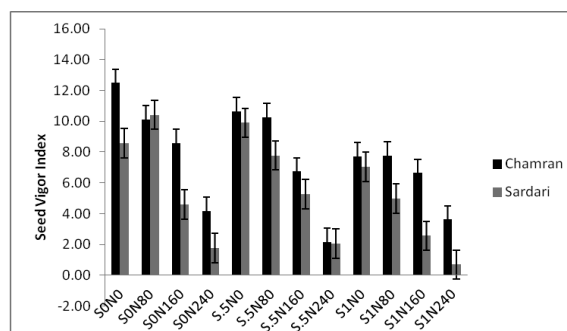
**Fig. 3.** mean of shoot length of salicylic acid pretreatment and salinity levels on wheat cultivars.



**Fig. 4.** mean of root length of salicylic acid pretreatment and salinity levels on wheat cultivars.



**Fig. 5.** mean of seedling fresh weight of salicylic acid pretreatment and salinity levels on wheat cultivars.



**Fig. 6.** mean of seed vigor of salicylic acid pretreatment and salinity levels on wheat cultivars .

**Table 1.** Variance analysis the effect of salicylic acid on wheat germination under different salinity stress levels.

SOV	DF	GP	GR	SFW	LR	LS	VI
SA	2	0.199**	19.13**	0.04*	0.37*	0.36*	1.45**
Variety	1	2.24**	195.9**	0.02ns	0.003 ns	0.11 ns	4.27
SA× Variety	2	0.028**	4.59*	0.0023 ns	0.06 ns	0.05	0.44
Salt	3	0.35**	32.8**	0.03**	3.94*	4.74**	9.28**
Salt × SA	6	0.029 ns	1.16 ns	0.0016 ns	0.14 ns	0.087	0.12
Variety × Salt	3	0.12*	3.58**	0.029*	0/43 ns	0.026	0.24
Salt×Variety ×SA	6	0.013 ns	0.65 ns	0.0015 ns	0/16 ns	0.079	0.25*
Error	48	0.033	1.5	0.001	0/15	0.14	0.21
CV (%)		17/5	19.6	19	12.4	16.7	19.3

\*\*, \* and ns: significant at the 1%, 5% probability levels and non significant respectively

Salt: NaCl, SA: Salicylic acid, CV: Coefficient variation

GP: Germination percent, GR: Germination rate, LR: length root, LS: Length soot, SFW: Seedling fresh weight

**Table 2.** Mean comparisons the effect of salicylic acid on wheat germination under salt stress levels.

VI	LS (Cm)	LR (Cm)	SFW (gram)	GR (in 24 hours)	GP (%)	Treatment
						Variety
2.67 a	2.26 a	1.74 a	0.16 a	7.89 a	1.21 a	Chamran
2.17 b	2.18 a	1.73 a	0.15 a	4.59 b	0.86b	Sardari
						SA (mMol)
2.63 a	2.34 a	1.89 a	0.17 a	7.19 a	1.13 a	0
2.43 a	2.09 b	1.7 b	0.16 a	6.12 b	1.04 ab	0.5
2.15 b	2.03 b	1.63 b	0.14 b	5.4 b	0.95 b	1
						NaCl (mMol)
3.03 a	2.66 a	2.09 a	0.2 a	7.57 a	1.17 a	0
2.87 a	2.55 a	2.3 a	0.18 a	7.02 a	1.1 ab	80
2.33 b	2.14 b	1.7 b	0.15 b	5.83 b	1.04 b	160
1.43 c	1.53 c	1.06 c	0.1 c	4.54 c	0.84 c	240

Means followed by similar letters in each column are not significantly different at the 5% level of probability according to duncan test.



## Conclusions

In all the traits in normal conditions (before application of distilled water) was Chamran superior Sardari variety. In the early stages of plant growth, resulting in better plant establishment and faster growth in other phases is improved. It is concluded that with increasing salinity, decrease germination percentage, germination rate, root length, shoot length and seedling fresh weight and seed vigor.

Chamran response to higher concentrations of salicylic acid was more suitable on salt stress conditions. The response characteristics of different varieties and applied to various levels salicylic acid to determine the threshold level is need for further studies.

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