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# Effect of salicylic acid on alleviation of salt stress on growth and some physiological traits of wheat

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## Abstract

To evaluate the effect of salt stress and salicylic acid application on growth and yield component traits of wheat, an experiment was conducted in factorial based on RCBD design with 3 replications in research farm (green house condition) of University of Tehran (Karaj-Iran) during 2010-11. Salt stress factor including three levels (control, salt stress with Nacl 4 ds/m and Nacl 8 ds/m) and acid salicylic (application and none application). The experiment was carried out on two variety of wheat, separately. The results indicated that maximum height was achieved in control × SA none application treatment and minimum height was achieved in Nacl8 ds/m × SA none application treatment. Also SA application increased number of grain in spike. SA application alleviated destructive effect of salt stress. The results indicated that interaction effect of salt stress × SA had significant effect ($p \leq 0.01$) on Tabasi variety but had not significant effect on Arvand variety on total chlorophyll and relative water content traits. It can be concluded that foliar application of wheat cultivar plants with salicylic acid stimulate the growth of wheat plants via the enhancement of the biosynthesis of photosynthetic pigments; improved relative water content, decreasing of organic solutes (proline) and thus salicylic acid treatment improved wheat growth especially on Tabasi variety.

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## Introduction

Wheat (*Triticum aestivum* L.) is the major cereal crop of Iran, which is grown all over the country. Per hectare yield of wheat is far below than its yield potential, which may be due to different reasons such as drought, nutrient managements, and salinity. The restriction of wheat growth and productivity due to salinity is especially acute in arid and semiarid regions of Iran.

Salinity is a major environmental stress that adversely affects plant growth and metabolism. Salinity impact on plants in two main ways: osmotic stress and ion toxicity (Munns, 2005). Osmotic stress is caused by ions (mainly $Na^+$ and $Cl^-$) in the soil solution decreasing the availability of water to roots. Ion toxicity occur when plant roots take up $Na^+$ and/or $Cl^-$ and these ions accumulated to detrimental levels in leaves. Ion imbalances and nutrient deficiency, particularly for $K^+$ nutrition, can be also occur (Tejera *et al.* 2007).

Under these conditions, plants have adopted mechanisms to attenuate and/or tolerate osmotic and ionic stresses. These mechanisms include osmotic adjustment by accumulation of compatible inorganic as well as organic solutes, the production of stress proteins (Sairam and Tyagi, 2004). Proline accumulation in salt stressed plants is a primary defense response to maintain the osmotic pressure in a cell. The role of proline in cell osmotic adjustment, membrane stabilization and detoxification of injurious ions in plants exposed to salt stress (Ashraf and Foolad, 2007).

Salicylic acid (SA) is accumulated in the plant tissues under the impact of unfavorable abiotic factors, contributing to the increase of plants resistance to salinization (Ding *et al.* 2002; Kang and Saltveit, 2002). In addition, SA-induced increase in the resistance of wheat seedlings to salinity (Shakirova and Bezrukova, 1997). Thus the detrimental effects of high salts on the early growth of wheat seedlings may be alleviated by treating seeds with the proper concentration of a suitable hormone (Darra *et al.* 1973).

Gutierrez Coronado *et al.* (1998) observed significant effect of SA on soybean increases in shoots growth, root growth and plant height. Khodary (2004) reported that SA increased the fresh and dry weight of shoot and roots of salt stressed maize plants.

Under salinity stress photosynthetic pigments greatly decreased. Dela-Rosa and Maiti (1995) observed that chlorophyll biosynthesis decreased salt conditions of sorghum plants. El-Tayeb (2005) found that Chl a, b and carotenoids decreased significantly in NaCl treated plants in comparison to controls of barley plants.

Stogonov *et al.* (1970) suggested that the chlorophyll content of salt stressed plants depend on the biological processes and development stages of the plant and also on the type and concentration of the salt. Keeping in view the importance of wheat and salinity, the aim of present study was to identify the effects of salicylic acid (SA) on salt conditions of wheat plants. Under salt stress, plants have to cope with water stress imposed by the low external water potential, and with ion toxicity due to accumulation inside the plant (Munns, 2005). The knowledge of alterations in physiological processes mediated by NaCl and SA may provide a basis to enhance the productivity of wheat plants in areas adversely affected by salt stress.

Therefore, the present investigation was undertaken to study the impact of spraying salicylic acid on some morphological criteria, yield as well as some biochemical constituents of wheat (*Triticum aestivum* L.) to improve growth, yield, grain quality and nutritional value.

## Material and methods

*Location and experimental design*

This research was conducted at factorial experiment based on randomized complete block design with three replications in greenhouse of Department of Agronomy and Crop Breeding, Tehran University, Karaj, Iran in 2012. The experimental site is located in 35: 56 northern latitude and 50: 58 eastern longitude with 1112.5 m height from the sea level. Two varieties of Wheat seeds (cv. Tabasi, sensitive to salt stress and Arvand, tolerant to salt stress) were planted in pots (20 cm diameter and 30 cm tall) filled with a 1:2:3 mixtures of soil, sand and manure.

*Soil analysis*

Soil analysis of the experimental pots is presented in Table 1.

**Table 1.** Physical and chemical soil analysis.

| Texture | Total N (mg/kg) | P (mg/kg) | K (mg/kg) | pH | EC |
|---|---|---|---|---|---|
| loam | 160 | 14.2 | 151 | 7.2 | 2 dS/m |

*Crop management*

Plants were thinned to five at the 3 to 4 leaf stage. The plants were kept in a glasshouse at a day/night temperature of 25/13 oC and a day length of 16 h. The plants were subjected to three different salinity (EC) levels of irrigation water, namely 2 dS/m as the control, 4 dS/m as the first level, and 8 dS/m as the second level with (1.00 mM SA) and without spray of salicylic acid. Salt stress was carried out by adding NaCl to irrigation water. Plants were subjected to salinity stress at the 3 to 4 leaf stage. Salicylic acid spraying were applied twice once a week after salinity stress and then at flowering stage.

Plant height, spike length, grain number, grain weight, total plant weight, total chlorophyll content, chlorophyll a and chlorophyll b, leaf relative water content were assessed in this study.

*Relative water content*

Flag leaf was harvested at soft dough stage and immediately transferred to the laboratory. First, in small piece of flag leaf, relative water content was calculated using the method described by Merah, (2001):

RWC (%) = (fresh weight - dry weight) / (saturated weight - dry weight) × 100

*Chlorophyll determining*

Total chlorophyll as well as chlorophyll a and b concentrations were calculated according to Arnon (1986).

*Proline content*

Proline was extracted according to the procedure of Irigoyen *et al.* (1992), using 0.3 g of sample and 6 ml of extraction medium. Proline was quantified by spectrophotometry at 515 nm by means of a colorimetric reaction with ninhydrin (Irigoyen *et al.*, 1992). The reaction mixture contained 1.5 ml of 25% (w: v) ninhydrin, 1.5 ml acetic acid and 0.5 ml of the extract. Samples were incubated for 1 h in a boiling water bath, and thereafter the reaction was stopped on ice. The reaction was mixed with 2 ml toluene, vigorously agitated and finally the upper organic phase was extracted to measure the absorbance. For the calculation of proline concentration, a standard curve was prepared with L-proline.

*Statistical analyses*

Analysis of variance was performed for all traits by SAS (9.1) software. Means were separated by application of Duncan's test when the F test proved significant at P = 0.05.

**Results**

Analysis of variance revealed that interaction between the salinity and SA was significant ($p \leq 0.01$) for plant height and spike length, in Tabasi variety whereas for Weight of seed per spike in Arvand variety (Table 2). Comparisons of the mean value of salinity × SA interaction were showed that the highest and lowest rates of plant height were recorded as 30.51 cm in 2 dS/m salinity × without SA application treatment and 17.95 cm in 8 dS/m salinity × without SA application treatment, respectively in Tabasi variety (Table 3).

**Table 2.** Mean square of salicylic acid and Nacl salt on the physiological and growth characters of two varieties of wheat.

| Source | df | Total chlorophyll | Chl a | Chl b | Plant height | Spike length | Proline content | Relative water content | No. seed per spike | Weight of seed per spike | Total weight of plant |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | M S | | | | | |
| | | | | | | Var.1 (Tabasi) | | | | | |
| Replication | 3 | 0.00016 | 0.0000442 | 0.0000867 | 4.4107 | 0.0517 | 0.6830 | 13.101 | 0.1527 | 0.0031 | 0.2606 |
| NaCl | 2 | 0.0193$^{**}$ | 0.01094$^{**}$ | 0.001197$^{**}$ | 51.55$^{**}$ | 2.1217$^{**}$ | 26.3093$^{**}$ | 39.413$^{*}$ | 4.291$^{**}$ | 0.0674$^{**}$ | 3.632$^{**}$ |
| SA | 1 | 0.0839$^{**}$ | 0.08066$^{**}$ | 0.000223$^{**}$ | 283.722$^{**}$ | 0.1552$^{**}$ | 24.391$^{**}$ | 125.287$^{**}$ | 3.375$^{**}$ | 0.0388$^{**}$ | 5.639$^{**}$ |
| NaCl × SA | 2 | 0.00259$^{**}$ | 0.00202$^{**}$ | 0.000044 | 42.182$^{**}$ | 4.384$^{**}$ | 12.205$^{**}$ | 27.440$^{**}$ | 0.375$^{ns}$ | 0.0893$^{ns}$ | 0.230$^{ns}$ |
| Error | 15 | 0.00178 | 0.001815 | 0.000086 | 5.9007 | 0.27202 | 0.9426 | 9.959 | 0.1861 | 0.00177 | 0.0887 |
| | | | | | | Var.2 (Arvand) | | | | | |
| Replication | 3 | 0.00239 | 0.00219 | 0.00147 | 2.3406 | 1.0929 | 2.5065$^{*}$ | 3.211 | 0.944 | 0.0245 | 0.1031 |
| NaCl | 2 | 0.0169$^{**}$ | 0.01983$^{**}$ | 0.000219$^{ns}$ | 29.0030$^{**}$ | 2.6567$^{*}$ | 54.7$^{**}$ | 11.268$^{*}$ | 93.791$^{**}$ | 0.4788$^{**}$ | 1.3083$^{**}$ |
| SA | 1 | 0.0336$^{**}$ | 0.02487$^{**}$ | 0.000670$^{ns}$ | 114.712$^{**}$ | 0.0410$^{ns}$ | 20.164$^{**}$ | 31.483$^{**}$ | 10.666$^{*}$ | 0.0319$^{ns}$ | 0.4108$^{ns}$ |
| NaCl × SA | 2 | 0.000287$^{ns}$ | 0.0001578$^{ns}$ | 0.000772$^{ns}$ | 1.3261$^{ns}$ | 0.6753$^{ns}$ | 4.0003$^{**}$ | 1.349$^{ns}$ | 0.541$^{ns}$ | 0.05436$^{*}$ | 0.127$^{ns}$ |
| Error | 15 | 0.000822 | 0.00268 | 0.00141 | 3.782 | 0.5991 | 0.6150 | 2.745 | 1.977 | 0.01173 | 0.127 |

ns ‹*and **: non-significant, significant differences at 1% and at 5% probability levels

**Table 3.** Mean comparisons of on the physiological and growth characters of Tabasi variety.

| Treatments | Total chlorophyll (mg g⁻¹FW) | Chl a (mg g⁻¹FW) | Chl b (mg g⁻¹FW) | Plant height (cm) | Spike Length (cm) | Proline Content (mg g⁻¹FW) | Relative Water Content (%) | No. seed per spike | Weight of seed per spike (gr) | Total weight of plant (gr) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Salt stress | | | | | |
| NaCl 0 | 0.6266a | 0.4286a | 0.1979a | 26.597a | 3.170b | 5.87b | 75.08a | 2.625a | 0.153b | 3.825a |
| NaCl 4 | 0.5253b | 0.4053b | 0.1819b | 28.42a | 4.14a | 6.71b | 71.32b | 1.500b | 0.288a | 2.898b |
| NaCl 8 | 0.5301c | 0.3562c | 0.1739b | 23.408b | 3.94a | 9.43a | 71.13b | 1.250b | 0.113b | 2.515c |
| | | | | | Salicylic acid | | | | | |
| SA (-) | 0.5230b | 0.3387b | 0.1842a | 29.58a | 3.83a | 8.34a | 70.22b | 1.416b | 0.145b | 2.595d |
| SA (+) | 0.6450a | 0.4547a | 0.1903a | 22.70b | 3.67a | 6.33b | 74.79a | 2.166a | 0.225a | 3.564a |
| | | | | | Salt stress × Salicylic acid | | | | | |
| NaClo × SA(-) | 0.555c | 0.362d | 0.192ab | 30.51a | 3.74bc | 6.22b | 74.91a | 2.00a | 0.160b | 3.172b |
| NaClo × SA(+) | 0.697a | 0.494a | 0.203a | 22.68b | 2.600d | 5.52b | 75.21a | 3.25a | 0.146bc | 4.478a |
| NaCl4 × SA(-) | 0.523d | 0.336e | 0.186bc | 29.36a | 3.275cd | 7.04b | 68.05b | 1.25cd | 0.127bc | 2.585c |
| NaCl4 × SA(+) | 0.667b | 0.473b | 0.193ab | 27.48a | 4.917a | 6.38b | 74.58a | 1.75bc | 0.450a | 3.312b |
| NaCl8 × SA(-) | 0.489e | 0.316f | 0.173c | 28.86a | 4.385ab | 11.783a | 67.69b | 1.00d | 0.147bc | 2.027d |
| NaCl8 × SA(+) | 0.570c | 0.395c | 0.174c | 17.95c | 3.500c | 7.084b | 57.74a | 1.500bcd | 0.080c | 3.002bc |

Means with the same letter in each column are not significantly different at 5% probability level

The highest and lowest rates of spike length were recorded as 4.14 cm in 4 dS/m salinity treatment and 3.17 cm in 2 dS/m salinity treatment, respectively in Tabasi variety (Table 3). Comparisons of the mean value of salinity × SA interaction were showed that the highest and lowest rates of spike length were recorded as 4.917 cm in 4 dS/m salinity × SA spray treatment and 2.60 cm in 2 dS/m salinity × SA spray treatment, respectively in Tabasi variety (Table 3). The highest and lowest number of grains per spike was observed at 2 dS/m salinity (2.625) and 8 dS/m salinity (1.25), respectively. Spray of SA was increased the number of grains per spike. The highest and lowest grains weight was observed at 4 dS/m salinity (0.288 gr) and 8 dS/m salinity (0.113 gr), respectively in Tabasi variety (Table 3).

The highest and lowest plant total weight was observed at 2 dS/m salinity (3.825 gr) and 8 dS/m salinity (2.51 gr) respectively in Tabasi variety (Table 3). Comparisons of the mean value of salinity × SA interaction were showed that the highest and lowest rates of Weight of seed per spike were recorded as 1.013 gr in 0 dS/m salinity × with SA application treatment and 0.535 gr in 8 dS/m salinity × without SA application treatment, respectively in Arvand variety (Table 4).

There was significant ($p \leq 0.01$) difference among salinity treatments with reference to total chlorophyll content in two variety. In addition, there was significant ($p \leq 0.01$) difference among SA treatments with reference to total chlorophyll content in two variety. Interaction between the salinity and SA was also significant in Tabasi variety but had no significant effect on Arvand variety (Table 2).

**Table 4.** Mean comparisons of on the physiological and growth characters of Arvand variety.

| Treatments | Total chlorophyll (mg $g^{-1}$FW) | Chl a (mg $g^{-1}$FW) | Chl b (mg $g^{-1}$FW) | Plant height (cm) | Spike Length (cm) | Proline Content (mg $g^{-1}$FW) | Relative Water Content (%) | No. seed per spike | Weight of seed per spike (gr) | Total weight of plant (gr) |
|---|---|---|---|---|---|---|---|---|---|---|
| Salt stress | | | | | | | | | | |
| NaCl 0 | 0.723a | 0.515a | 0.207a | 40.63a | 7.806a | 5.712c | 80.36a | 15.62a | 1.046a | 4.79a |
| NaCl 4 | 0.695a | 0.493a | 0.202a | 39.19a | 7.645a | 7.489b | 79.26ab | 13.25b | 0.592b | 4.47a |
| NaCl 8 | 0.633b | 0.420b | 0.213a | 36.85b | 6.737b | 10.860c | 77.99b | 8.87c | 0.662b | 3.99b |
| Salicylic acid | | | | | | | | | | |
| SA (-) | 0.646b | 0.444b | 0.202a | 36.71b | 7.355a | 8.937a | 78.06b | 11.91b | 0.730a | 4.292a |
| SA (+) | 0.721a | 0.508a | 0.213a | 41.08a | 7.437a | 7.104b | 80.35a | 13.25a | 0.803a | 4.553a |
| Salt stress × Salicylic acid | | | | | | | | | | |
| NaCl0 × SA(-) | 0.690bc | 0.478ab | 0.2120a | 38.35b | 8.100a | 6.130cd | 79.11ab | 15.25a | 1.08a | 4.596ab |
| NaCl0 × SA(+) | 0.756a | 0.552a | 0.2035a | 42.92a | 7.513ab | 5.29d | 81.60a | 16.00a | 1.013a | 5.001a |
| NaCl4 × SA(-) | 0.660c | 0.462bc | 0.197a | 36.65bc | 7.455ab | 8.09b | 78.57bc | 12.50b | 0.577c | 4.270bc |
| NaCl4 × SA(+) | 0.731ab | 0.523ab | 0.207a | 41.73a | 7.835a | 6.88c | 79.96ab | 14.00ab | 0.607c | 4.68ab |
| NaCl8 × SA(-) | 0.589d | 0.391c | 0.197a | 35.12c | 6.51b | 12.58a | 76.49c | 8.00c | 0.535c | 4.01c |
| NaCl8 × SA(+) | 0.677c | 0.449bc | 0.228a | 38.60b | 6.965ab | 9.134b | 79.48ab | 9.75c | 0.790b | 3.98c |

Means with the same letter in each column are not significantly different at 5% probability level

There was significant ($p \leq 0.01$) difference among salinity treatments with reference to chlorophyll a in both variety. In addition, there was significant ($p \leq 0.01$) difference among SA treatments with reference to chlorophyll a in both variety. Interaction between the salinity and SA was also significant on Tabasi variety but no significant on Arvand variety. Chlorophyll b was significantly ($p \leq 0.01$) affected by salinity treatments in Tabasi variety but had no significant effect on Arvand variety. The interaction between salinity and SA was not significant on both of varieties (Table 2).

RWC is a useful measure of the physiological water status of plants (Gonzalez & Gonzalez-Vilar, 2001). Analysis of variance revealed that salinity and SA treatments had a significant effect on RWC at level of 5 and 1 percent, respectively in two varieties similarly. Interaction between the salinity and SA was significant in Tabasi but no significant on Arvand variety.

Also, salinity and SA treatments and interaction of them had a significant effect on proline content ($p \leq 0.01$). Proline contents remained decreased with SA application. Salicylic acid treatments decreased proline concentrations in plants. However, proline augmented as NaCl doses increased. The higher values (9.43 in Tabasi var. and 10.86 in arvand var.) were detected in control plants (without SA) exposed to 8 dS/m NaCl.

## Discussion

SA application increased height of plants. Guteirrez Coronado *et al.* (1998) were observed the significant effect of SA on soybean increases in root growth and plant height. Under unfavorable abiotic factors, SA is accumulate in plant tissue and resulting in resistance to salinity (Ding *et al.*, 2002; Saltveit, 2000). Khodary (2004) reported that SA increased the fresh and dry weight of shoot and roots of salt stressed maize plants. Thus, from the results of this study can be concluded that the use of SA as a recognized endogenous signal molecule, which is mainly discussed in plant resistance against salinity stress, can be responsible for alleviating salinity stress and improve plant growth. In this respect, many investigators found that low concentrations of salicylic acid enhanced growth of wheat plants (Shakirova *et al.* 2003; Iqbal and Ashraf, 2006). Foliar application of salicylic acid significantly increased yield and its components of maize (Abdel-Wahed *et al.* 2006) and wheat plants (Iqbal and Ashraf, 2006).

Dela-Rosa and Maiti (1995) found an inhibition in chlorophyll biosynthesis in sorghum plants because of salt stress. Salt stress decreased chlorophyll content as compared to the non-saline conditions. Similarly, the adverse effect of salt stress on chlorophyll content of strawberry has been shown by Kaya *et al.* (2002). Furthermore, salt stress inhibits the chlorophyll content in leaves of many crops (Parida and Das, 2005). In barley plants, El-Tayeb (2005) reported that chlorophyll a, b and carotenoids decreased significantly in NaCl treated plants in comparison to controls. Chlorophyll a, b and carotenoids increased significantly in NaCl treated plants in comparison to controls of bean plants (Turkyilmaz *et al.* 2005). Our results are in agreement with Moharekar *et al.* (2003) for wheat, Yildirim *et al.* (2008) for cucumber.

The results showed that SA spraying was improved chlorophyll content and RWC. Parida and Das, (2005) reported that the relative water content, water potential and osmotic potential of plants become more negative with an increase in salinity. This study showed that SA treatments induced an increase in RWC of the salt stressed plants as compared to the control plants on two varieties especially in Tabasi variety (Table 3, 4). Increases in RWC of wheat plants treated with SA were also reported for other crops grown under salt stress including barley (El-Tayeb, 2005), tomato (Tari *et al.* 2002; Szepsi *et al.* 2005) and cucumber (Yildirim *et al.* 2008). This phenomenon may be attributed to the fact that foliar SA application can increase the leaf diffusive resistance and lower transpiration rates.

Plants with the combined treatment (SA×NaCl) showed contradictory results between salt doses regarding organic solute content for which we have no explanation at this time. In addition, the concentration of proline did not change decreased, with SA treatments. Regarding the variation of proline levels, experiments of Shakirova *et al.* (2003) showed a decrease in proline content in wheat seedlings after 24 h of SA treatment, similar to that detected in common bean nodules subjected to ABA application (Khadri *et al.* 2006). On the other hand, the content of proline progressively increased in leaves with increased salt doses in the growth medium.

In conclusion, our results indicate that SA helps wheat plants to better cope with salinity. Based on these findings, the SA treatments may ameliorate the negative effect of salinity. From the preceding results and discussion, it can be concluded that foliar application of wheat cultivar plants with salicylic acid stimulate the growth of wheat plants via the enhancement of the biosynthesis of photosynthetic pigments; improved relative water content, decreasing of organic solutes (proline) and thus salicylic acid treatment improved wheat growth especially on Tabasi variety.

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