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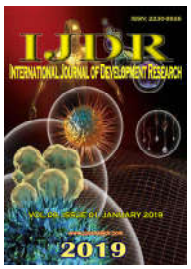

ORIGINAL RESEARCH ARTICLE OPEN ACCESS

SALICYLIC ACID APPLICATION ALLEVIATES THE SALT STRESS EFFECTS IN WHEAT

***[1]Morad Mohammadi, [2]Mohammad Reza Chaichi and [3]Sara Safikhani**

[1]Department of Plant Science, West TN Research & Education Center, Jackson, TN, 38301, USA
[2]Department of Plant Science, Huntley College of Agriculture, California State Polytechnic University, Pomona, California, U.S.A
[3]Department of Agroecology, Shahid Beheshti University, Environmental Sciences Research Institute, Tehran, Iran

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ABSTRACT

The alleviative effect of salicylic acid (SA) on growth and physiological parameters of two wheat verities under salt stress was studied at greenhouse condition in 2016. The experimental treatments were arranged as factorial based on a RCBD with 4 replications. Salinity stress comprised of three levels of control, 4 and 8 dS/m and the salicylic acid treatments were control (without salicylic acid) and 1 mM salicylic acid application. The experiment was carried out on two varieties of wheat (Tabasi, salinity sensitive and Arvand, salinity tolerant). The results indicated that salinity stress especially in 8 dS/m had inhibitory effect on plant height, spike length, grain number per plant, grain weight per plant, chlorophyll content, and relative water content. Salinity stress enhanced the proline content in wheat varieties, especially in sensitive variety. Foliar application of SA mitigated the adverse salinity impacts on the growth characteristics, chlorophyll, proline, and relative water content. SA spraying increased plant height by 24.3% and 7%, spike length by 20.1% and 6.6%, and relative water content by 5.2% and 2% in Tabasi and Arvand varieties, respectively.Maximum total chlorophyll content (0.82 mgg^{-1} FW) was achieved in Arvand variety in control treatment while sprayed by SA. Based on the results of this study, spraying SA on sensitive wheat varieties will warrant much better growth and development under salinity stress.

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INTRODUCTION

Salinity is one of the most important abiotic stresses which reducing the yield in a wide variety of crops all over the world (Shrivastava *et al*., 2015).This problem is very prevalent in arid and semiarid regions and more than 30% of the world (Shrivastava *et al*., 2015). salinity adversely affects plant growth either through osmotic inhibition of water uptake by roots or specific ion effects. Salt stress has toxic effects on plants and leads to metabolic changes like loss of chloroplast activity, decreased photosynthetic and increased photorespiration rate which leads increasing of reactive oxygen species generation (Chawla *et al*., 2013; Abd-Elgawad *et al*., 2016). Salicylic acid (SA) is a common phenolic compound that can act as a plant growth regulator affecting various processes from growth, development, and interaction with other organisms to environmental stress responses (Raskin, 1992). SA is an important signaling molecule, which is involved in local and systemic acquired resistance (SAR) induced by various pathogens and awide range of abiotic stresses (Gao *et al*., 2015). Exogenously applied SA may be used as a priming or hardening compound to enhance the resistance of plants to biotic and abiotic stresses (Khan *et al*., 2014). Application of SA at a suitable concentration could be a powerful tool for the reduction of stress sensitivity. This is done by its hardening effect, which contributes to the maintenance of a dynamic homeostasis of metabolic pathways. Salicylic acid can increase the concentration of abscisic acid (ABA) in plant tissues, furthermore, both SA and ABA may mutually inhibit each other's signaling(Khan *et al*., 2015).SA at high concentration proved to be a potent inhibitor of ethylene biosynthesis(Kepczyriska and Zielinska, 2011), thus

Corresponding author: **Morad Mohammadi**
Department of Plant Science, West TN Research & Education Center, Jackson, TN, 38301, USA

it may control plant growth and development via modulation of ethylene production. Research reportsindicated that priming of tomato plants with SA mitigated the salt stress injury by increasing the activities of enzymatic and non-enzymatic antioxidant mechanisms and osmotic adjustment (Hasanuzzaman *et al*., 2014). Positive effects of SA application werereported on Rice(Jiniand Joseph 2017), Wheat (Wang and Zhang 2017; Mohammadi *et al*., 2013), Mung bean (Ghassemi-Golezani and Lotfi 2015), *Phaseolusvulgaris* L. (Rady *et al*., 2015), *Pisumsativum* (Singh *et al*., 2016), Onion (Semida *et al*., 2016), and *Vignaradiate* (Khan *et al*., 2014; Khan *et al*., 2015)under salinity stress. The present research was designed to evaluate the role of SA in mitigating $MgCl_2$salinity stress on wheat varieties in greenhouse condition. Growth and biochemical parameters were measured to analyze the alleviating effect SA under salt stress condition.

MATERIALS AND METHODS

Experimental design

Two separate pot experiments with a factorial arrangement ofSalicylic Acid and saline irrigation water treatments were conducted using a randomized complete block design (RCBD) with four replications at University of Tehran, Karaj, Iran in 2016.In the first experiment, the treatment effects were studied on Tabasi (sensitive to salt stress), and in the second one the same treatments were applied on Arvand cultivar (tolerant to salt stress). The salinity factor ($MgCl_2$ levels) comprised of three levels (0, 4 and 8dS/m), and the Salicylic acid (SA) was applied at two levels (with/without). Magnesium dichloride was purchased from Merck Millipore Company. The experimental site was located in 35˚ 56′ northern latitude and 50˚ 58′ eastern longitude with 1112.5 m above the sea level.

Crop management

Seeds of two wheat varieties (Tabasi and Arvand) were planted in pots (20 cm diameter and 30 cm height) filled with a 1:2:3 mixtures of soil, sand, and manure (pH: 7.2, EC: 2 dS/cm, total nitrogen: 160 mg/kg, phosphorous: 14.2 mg/kg, potassium 151 mg/kg).Plants were maintained under natural light with a day/night temperature of approximate 25/20˚C, 50% relative humidity during the period of the experiment and a day length of 16 h. Plants in each pot were thinned to five at the 3 to 4-leaf stage. The plants were subjected to three different salinity (EC) levels of irrigation water, 0(control), 4 and 8 dS/m. The control water treatment was distilled water. Salicylic Acid (SA) with 1 mM density was applied twice (one week after salinity stress commencement, and flowering stage) during growing period. Salinity treatment was carried out by adding $MgCl_2$ to irrigation water. Plants were subjected to salinity stress at the 3 to 4-leaf growth stage.

Studied traits

Plant height, spike length, grain number, grain weight per plant, total chlorophyll content, chlorophyll a and chlorophyll b, leaf relative water content were assessed in these studies. Total chlorophyll, as well as chlorophyll a and b concentrations, were calculated according to Arnon (1986). One gram of fresh leaves was taken in the middle of flowering time and ground with 10 ml of 80% acetone. It was then centrifuged at 5000 rpm for 5 minutes. The supernatant was transferred and the procedure was repeated till the residue becomes colorless. The absorbance of the solution was read at 645 nm and 663 nm against the solvent (acetone) blank using a UV-160A UV-vis recording spectrometer (Shimadzu UV 180). Finally, the concentrations of chlorophyll a, chlorophyll b and total chlorophyll were calculated using the following equation:

Chlorophyll a: 12.7(A663) – 2.69(A645)
Chlorophyll b: 22.9(A645) – 4.68(A663)
Total Chlorophyll: 20.2(A645) + 8.02(A663)

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. Flag leaf was harvested at soft dough stage and immediately transferred to the laboratory and relative water content was measured using the method described by Merah (2001):

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

Statistical Analysis

Data were subjected to analysis of variance (ANOVA) to compare the effects of salt stress treatments and SAapplication ontwowheat variety (susceptible and to lerantvariety seperately). 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 $P \leq 0.05$. Excel software was used to draw figures.

RESULTS AND DISCUSSION

Salinity and SA application significantly affected plant height in both wheat varieties. Reduction in plant height under salt stress was observed in both wheat varieties (Table 1). Both salinity levels of 4 and 8 ds/m significantly reduced plant height in Tabasi (salt sensitive) variety compared to control. However, in Arvand (salinity tolerant) variety the moderate salinity level (4 ds/m) did not significantly reduced plant height compared to control (Table 2). This result was consistent with other research works (Radi*et al*., 2013).Salinity inhibits plant growth and metabolism for two reasons: first due to water deficit and second due to salt specific or ion excess effects (Munns *et al*., 2006). The SA application increased plant height by 24.3 % and 7 % in Tabasi (sensitive) and Arvand (tolerant) varieties, respectively (Table 2). Some earlier reports have shown that exogenous SA has obtained particular attention because of inducing protective effects on plants under salinity, and the effects of cytotoxicity induced by salt stress can be ameliorated by the exogenous application of SA (Simaei *et al*., 2011; 2012). The longest spike in Tabasi and Arvand verities (4.89 cm and 7.80 cm, respectively)were observed in control condition while the smallest spike (3.71 cm and 6.54 cm, respectively) were observed on 8 dS/m salinity level.

Table 1. Mean square of salicylic acid and MgCl2 salt on the physiological and growth characters of two varieties of wheat

SOV	df	Plant height	Spike Length	Grain number per plant	grain weight per plant	Ch a	Ch b	Total Ch	Proline Content	Relative Water Content
					Tabasi					
Replication	3	5.2051	0.7284	0.152	0.0051	0.0008	0.0004	0.0019	0.1161	3.5150
$MgCl_2$	2	67.632 **	2.793 **	15.291 **	0.0379 **	0.0139 **	0.0121 *	0.0298 **	12.0429 **	39.1350 *
SA	1	224.257**	3.720 **	7.041	0.1066 **	0.1274 **	0.0069	0.0748 **	1.686 **	79.6760 **
$MgCl_2$×SA	2	4.443	0.3284	0.291	0.1420 **	0.0003 **	0.0041	0.0023	0.2884	6.4000
Error	15	6.506	0.2317	1.552	0.00278	0.0016	0.0028	0.0012	0.1873	8.1870
CV	-	9.04	11.16	35.18	14.71	9.90	24.46	5.491	5.18	3.97
					Arvand					
Replication	3	3.0250	0.0246	0.152	0.0145	0.00006	0.0004	0.0001	0.6147	1.3449
$MgCl_2$	2	83.0493 **	3.2682 **	68.7916 **	0.1763 **	0.0066 **	0.0024**	0.0100 **	6.2768 **	18.0355 **
SA	1	38.7350 **	1.2776 **	2.0416 *	0.0748 **	0.0146 **	0.0026	0.0293 **	2.9516 *	15.7853 *
MgCl2×SA	2	10.6518	0.2476	0.2916	0.0107	0.0043 **	0.0013	0.0010 *	0.0483	4.3113
Error	15	6.2515	0.1452	0.3527	0.0071	0.0005	0.0005	0.0002	0.3986	2.0218
CV	-	6.69	5.27	4.49	9.76	4.42	11.75	1.99	8.92	1.78

*and **: significant differences at 5% and at 1% probability levels

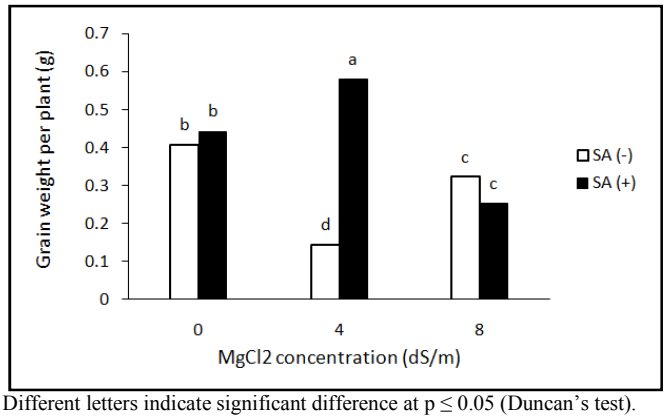
Table 2. Mean comparisons of on the physiological and growth characters two varieties of wheat

Treatments	Plant height (cm)	Spike Length (cm)	Grain number per plant	grain weight per plant	Chl a (mgg-1 FW)	Chl b (mgg-1 FW)	Total Chl (mgg-1 FW)	Proline Content (mgg-1 FW)	Relative Water Content (%)
					Tabasi				
Salt stress									
MgCl2 0	31.56 a	4.89 a	5.00 a	0.43 a	0.44 a	0.25 a	0.70 a	6.95 b	74.50 a
MgCl2 4	26.59 b	4.34 b	3.37 b	0.36 b	0.41 a	0.18 b	0.59 b	8.85 a	70.95 b
MgCl2 8	26.46 b	3.71 c	2.25 b	0.29 c	0.36 b	0.23 ab	0.59 b	9.35 a	70.44 b
Salicylic acid									
SA (-)	25.15 b	3.92 b	3.00 b	0.29 b	0.33 b	0.23 a	0.57 b	8.61 a	70.14 b
SA (+)	31.26 a	4.71 a	4.08 a	0.42 a	0.48 a	0.20 a	0.68 a	8.08 b	73.79 a
					Arvand				
Salt stress									
MgCl2 0	39.19 a	7.80 a	15.75 a	1.03 a	0.57 a	0.21 ab	0.78 a	6.16 c	81.11 a
MgCl2 4	39.18 a	7.32 b	13.87 b	0.80 b	0.53 b	0.22 a	0.75 b	7.14 b	78.97 b
MgCl2 8	33.61 b	6.54 c	10.00 c	0.76 b	0.52 b	0.19 b	0.70 c	7.93 a	78.21 b
Salicylic acid									
SA (-)	36.05 b	6.99 b	12.91 b	0.84 b	0.51 b	0.19 a	0.71 b	7.43 a	78.62 b
SA (+)	38.59 a	7.45 a	13.50 a	0.92 a	0.56 a	0.22 a	0.78 a	6.72 b	80.24 a

Different letters indicate significant difference at $p \leq 0.05$ (Duncan's test).

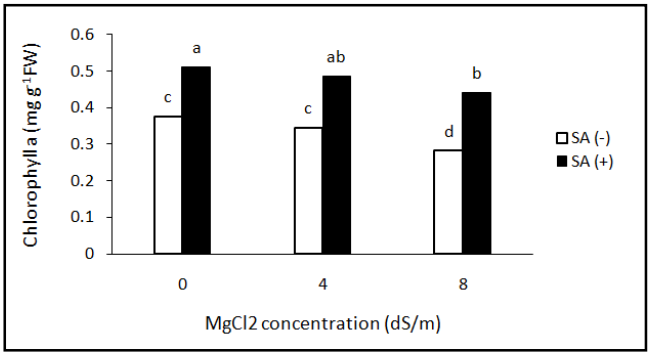
In both sensitive (Tabasi) and tolerant (Arvand) varieties spike length followed a decreasing trend as the salinity level increased. SAapplication on leaves improved spike length in both varieties (20.15% and 6.58%, respectively). This result indicated that sensitive variety (Tabasi) better responded to SA application compared to tolerant variety (Table 2).Tolerance to salt stress by exogenous application of SA has been reported by Jayakannan *et al*.,(2015). SA can alleviate the adverse effect of high salinity by decreasing K^+ leakage from root tissues and by enhancing the H^+-ATPase activity (Jayakannan *et al*., 2013).This process provides a driving force for Na^+/H^+ exchanger at the plasma membrane and leads to reduced sodium accumulation in the cytosol (Shi *et al*., 2000). Increasing salinity levels had destructive effects on grain number per plant in both of varieties. The grain number in Tabasi (salinity sensitive) variety was significantly reduced by both moderate and sever salinity treatments compared to control (32.6% and 55%, respectively). While there was no significant difference between the two salinity levels (Table 2). The grain number per plant in Arvand variety followed a significant decrement trend (11.9% and 36.5%, respectively) as the salinity level increased. However, SA application improved the grain number in Arvand variety (Table 2). Grain number per plantis a significant trait which determine the final grain yieldin wheat. The role of SA in provoking salinity tolerance mechanisms in plants has been well established for many crops (Stevens *et al*., 2006). Grain weight per plantwas significantly affected by salt stress and SA interaction in Tabasivariety (Table1). No beneficial effect of SA application was seen at control (no salinity stress) and 8 ds/m treatments. However, the maximum grain weight per plant (0.58 g) was achieved in Tabasi variety at 4 dS/m salinity concentration while sprayed by SA (Fig.1). By increasing the salinity levels in irrigation water, grain weight per plant in Arvand variety significantly decreased, however, SA application improved the grain weight by 12% (Table 2). The reduction in plant growth by salinity stress might be related to adverse effects of excess salt anion homeostasis, water balance, mineral nutrition, and photosynthetic carbon metabolism (Yusuf *et al*., 2007). Application of SA largely improved grain weight of wheat by enhancing plant height, spike length, and grain number per plant (Table 2). SA influences a wide variety of plant processes, including stomatal regulation, chlorophyll content, and photosynthesis (Yildirim *et al*., 2008). Our result supports the findings of El-Tayeb (2005) and Kadioglu *et al*., (2011) who documented that foliar application of SA enhanced biomass production in barley and wheat. They also reported that the increase in growth biomass in response to SA under salinity stress may be due to the protective role of SA on membranes that might be responsible for increasing plant salt tolerance. Ghassemi-Golezani and Lotfi (2015) found that exogenous application of SA enhances the maximum quantum efficiency of PSII (Fv/Fm) and performance index (PI) in mung bean plants. The exogenous application of SA prevented the lowering of IAA and Cytokine levels in salinity stressed wheat plants resulting in the betterment of cell division in root

apical meristem, thereby increasing growth and productivity of plants (Shakirova *et al*., 2003). Also, Nazar *et al*., (2015) reported that exogenous salicylic acid improves photosynthesis and growth through increase in ascorbate glutathione metabolism and S assimilation in mustard under salt stress. Interaction of salinity and SA showed a significant effect on chlorophyll a content in both studied variety (Table 1). The maximum chlorophyll a content in Tabasi and Arvand varieties (0.51 and 0.62 mg^{-1} FW, respectively) were observed in control (no salinity stress) and SAapplication in both wheat varieties (Fig. 2, 3).



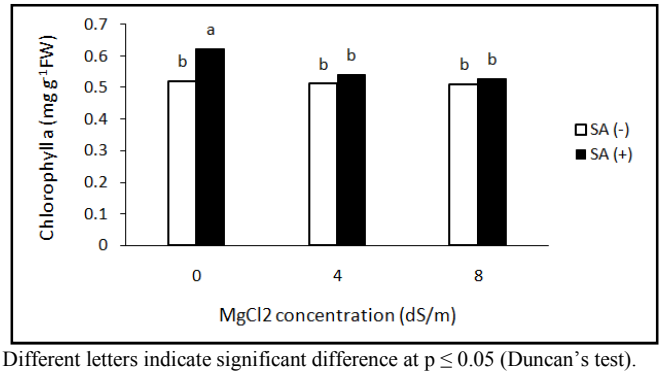
Different letters indicate significant difference at $p \leq 0.05$ (Duncan's test).

Fig. 1. Effect of salicylic acid application on grain weight per plant in Tabasi cultivar under salt stress



Different letters indicate significant difference at $p \leq 0.05$ (Duncan's test).

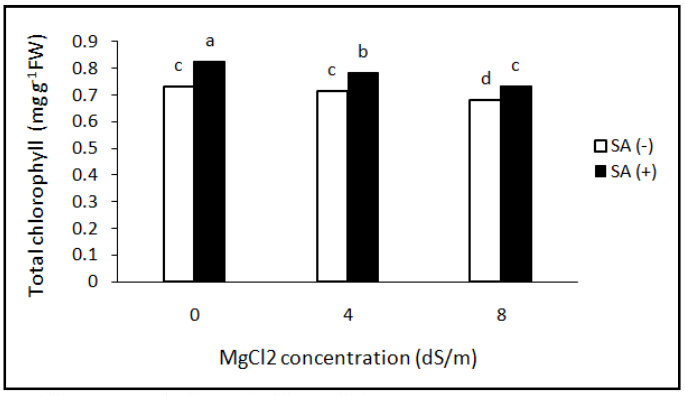
Fig. 2. Effect of salicylic acid application on chlorophyll a inTabasi cultivar under salt stress



Different letters indicate significant difference at $p \leq 0.05$ (Duncan's test).

Fig. 3. Effect of salicylic acid application on chlorophyll a inArvand cultivar under salt stress

SA Spraying improved chlorophyll a content across all the salinity levels in both varieties (especially in sensitive Tabasivariety).Chlorophyll b content was also adversely affected by salt stress in both varieties (Table 1). Chlorophyll b content decreased as salinity stress decreased (Table 2). Salt stress and SA application had a significant effect on total chlorophyll content in both varieties while the interaction of salt stress and the SA application was only significant on Arvand variety total chlorophyll. Salinity decreased total chlorophyll content in Tabasi variety while application of SAincreased it (Table 2). The maximum total chlorophyll content (0.82 mg g^{-1} FW) was observed in Arvand variety at control (no salinity stress)while sprayed by SA(Fig. 4). The total chlorophyll content decreased by increasing salinity levels, however, application of SA alleviated the adverse effects of salinity stress on total chlorophyll in Arvand variety (Fig. 4).



Different letters indicate significant difference at $p \leq 0.05$ (Duncan's test).

Fig. 4. Effect of salicylic acid application on total chlorophyll in Arvand cultivar under salt stress

Chlorophyll, as a natural pigment, plays a very important role in absorbing light energy for photosynthesis and promoting growth in plants. The decrease in chlorophyll content in saltaffected plants might be attributed to the possible oxidation of chlorophyll and other chloroplast pigments coupled with theinstability of the pigment protein complex under salt stress[47]. Reduction in chlorophyll contents was mitigated by the foliar application of SA. Results of the present study correspond with the findings of earlier researches where salt-induced reduction in the chlorophyll contents was alleviated by the foliar application of SA in crops such as wheat (Mohammadi *et al*., 2013), mung bean (Ghassemi Golezani and Lotfi 2015), alfalfa (Palma *et al*., 2013), and *Pisumsativum* ((Singh *et al*., 2016). Proline content was significantly affected by salt stress and SA application in both of varieties (Table 1). Both salinity levels of 4 and 8 ds/m significantly increased proline content in Tabasi (salt sensitive) variety compared to control.

However, inArvand (salinity tolerant) variety as the salinity increased from control to 8 ds/m, the proline content followed a significant increasing trend at each salinity level (15.9% and 28.7%, respectively) (Table 2).Increasing of salinity up to 8dS/m, increased the proline content in Tabasi and Arvand varieties (34.5% and 28.7%, respectively) compared to control. This is while application of SA decreased the proline content in both studied varieties. The response of Arvand (salinity tolerant) variety was more sever to SA application compared to Tabasi (salinity sensitive) in this regard (Table 2). Salt tolerance is believed to be affected by many different factors, such as transport selectivity and ion extrusion, ion compartmentation, synthesis of compatible solutes and activation of ROS scavenging mechanisms(Jiang *et al*., 2017; Munns and Tester 2008). Accumulation of large amounts of

osmolytes (proline) is an adaptive response in plants exposed to stressful environments(Yusuf *et al*., 2007).SA is a plant phenolic that is commonly present in plant tissues and plays an important role in regulating plant growth and development (Vicente and Plasencia 2011; Berkowitz *et al*., 2016).In addition to regulating numerous biological processes, SA has been recognized as an effective signal that mediates plantresponses to biotic and abiotic stresses (Vicente and Plasencia 2011). The RWC is a useful parameter when evaluating the physiological water status of plants. Relative water content decreased with increasing salinity levels in both of varieties. Also, application of SA improved the water content in both varieties (Table 2). SA application improved water content by 5.20% and 2.06% in Tabasi and Arvand variety, respectively. Tabasi variety showed the better response to application of SA in regard to RWC, Plant height, Chlorophyll content, grain weight in spike, and the number of grain per spike compared to Arvand variety (Table 2).Li *et al*.,(2014)reported positive effects of SA application on promoting relative water content in *Torreyagrandis* plants. Jiniand Joseph (2017) reported that the application of SA could alleviate the adverse effects of salt stress by the regulating the physiological mechanisms in rice plants.

Conclusion

The findings of thepresent experiment showed that saline water irrigation ($MgCl_2$) had aninhibitory effect on the growth and development of both sensitive and salt tolerant varieties of wheat (Tabasi and Arvand, respectively). Foliar application of SA mitigated the salinity induced adverse effects on the growth parameters, chlorophyll, proline and relative water content in both varieties. It was observed thatthe foliar application SA had stimulatory effects on wheat varieties under salt stress, especially in Tabasi (sensitive variety). Based on the results of this study spraying of SA on wheat plant is recommended under $MgCl_2$ salinity stress, especially for sensitive wheat varieties. The results of this research warrants an optimistic view of salicylic acid application in wheat production in saline condition.

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