



## Improving barley performance by proper foliar applied salicylic-acid under saline conditions

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### Abstract

Despite general effect of salicylic-acid (SA) in improving plant growth and productivity in saline conditions, there have not been unanimity about the best concentration. In this 2-yr field study the effect of different SA concentrations (0, 0.5, 1.0, 1.5 and 2.0 mM) was examined on growth, grain yield and yield components of barley under two non-saline (2 dS m<sup>-1</sup>) and saline (12 dS m<sup>-1</sup>) conditions. By using response curves and regression analyses the best concentration was also determined. The results showed that salt stress decreased barley plant height (22.7%), fertile tillers (19.0%), ear length (21.6%), grain number per ear (22.5%), thousand grain weight (19.9%), biological yield (29.6%) and grain yield (37.6%). Since salinity treatment when imposed the tillers were at their rapid growth phase; therefore, fertile tiller number per unit area was found to be the most sensitive trait to salt stress. Nonetheless, SA foliar application in different concentrations could ameliorate some of these negative impacts on growth, yield and yield components. Reduction percentage of grain yield due to salinity was the lowest at 1.5 mM in first and 1.0 mM SA concentration in second year corresponding to 27.3% and 33.8%, respectively; while those were highest at no-SA treatments (42.2% and 43.8% in first and second year, respectively). Modulating role of SA for adverse effect of salinity could be attributed to enhanced grain number. Based on the result of regression analysis, it can be concluded that SA foliar application at 2.0 mM under non-saline and at 1.41 mM under saline conditions could be considered as the best concentrations for improving barley performance.

**Keywords:** Grain yield; Plant growth regulators; Salinity; Yield components.

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## Introduction

Abiotic stresses are considered to be the main cause (71%) of yield reductions (Ashraf et al., 2008). The estimation of potential yield losses by individual abiotic stresses are 17% for drought, 20% for salinity, 40% for high temperature, 15% for low temperature and 8% for other factors (Ashraf and Harris, 2005). Nowadays, it has been well-known that salinity is a major environmental constraint to crop productivity worldwide. Under such conditions, the use of plant growth regulators (PGRs) to promote plant growth and production is becoming increasingly more common. Salt-stress generally causes reduced synthesis and in many cases also degradation of internal PGRs in plants (Kuiper et al., 1988). However, under saline conditions, it has been reported that exogenous application of PGRs may overcome much of the internal PGR deficiency and could lead to a reduction in inhibitory effects of salt stress (Ashraf and Foolad, 2007; Ashraf et al., 2008); as it has also been reported that SA levels in plant tissues can parallel the increase in SA concentration of tissues locally and systemically (Hayat et al., 2010). Therefore, application of PGRs such as SA offers a potential approach to mitigating the inhibitory effects of salt-stress on plant growth and crop productivity.

Salicylic acid, an ortho-hydroxybenzoic acid [ $C_6H_4(OH)COOH$ ], is a phenolic compound, first identified in the bark of willow tree (*Salix sp.*) in 1828 (Raskin, 1992). This PGR is part of a signaling pathway induced by several biotic and abiotic stresses (Pirasteh-Anosheh et al., 2012). Salicylic acid has been characterized in 36 diverse plant species. In plants such as rice, crabgrass, barley and soybean the level of salicylic acid is approximately  $1 \mu\text{g g}^{-1}$  FW (Hayat et al., 2010). There are numerous reports in the literature showing the beneficial effects of exogenous applications of SA in reducing the adverse effects of salt stress in different plant species. It has been reported that SA plays important roles in plant growth and development, photosynthesis-related processes, stomatal regulation and ion uptake and transport under saline conditions (Kaydan et al., 2007; Ashraf et al., 2010).

Enhanced tillers number, plant height, spikelet number per ear, grain number per ear, biological yield and grain yield have been reported as a result of SA application on two wheat genotypes under saline conditions (Jafar et al., 2012). In a field study, Pirasteh-Anosheh et al. (2012) also showed that although drought stress increased canopy temperature and decreased leaf area index and plant height in two wheat cultivars; exogenous applications of SA alleviated these harmful effects considerably.

Although large body of literature have documented positive role of SA in modulating adverse effect of salinity; it appears that the mode of action of SA may vary depending on its concentration. For example, Ashraf et al. (2010) found that SA had higher modulating effect at lower concentrations; contributing to enhanced antioxidant enzymes. They indicated that SA at higher concentrations may act as an antioxidant itself and could reduce antioxidant enzymatic activities. Also, Hayat et al. (2005) reported that higher leaf number as well as fresh and dry mass per plant were obtained in wheat plants when they were treated by lower concentration of SA. In the present 2-yr field study the effects of different concentrations of foliar applied SA on growth, grain yield and yield components of barley under salt stress conditions was examined. Determination of the optimal SA concentration to achieve the highest alleviation effect was also followed in this research.

## **Materials and Methods**

To evaluate the effect of SA foliar application on growth, yield and yield components of barley plants under salt stress conditions a 2-year study was conducted in Research Station of National Salinity Research Center, Yazd, Iran during 2012-2013 and 2013-2014 growing seasons. Cumulative amount of precipitation and irrigated water during the growing seasons as well as climatic conditions of the experimental site are shown in Figures 1 and 2, respectively. The treatments included irrigation with normal (tap water as control) and saline water (EC: 12 dS m<sup>-1</sup>) and SA application at five concentrations: 0 (without application), 0.5, 1.0 and 1.5 and 2.0 mM. This experiment was carried out in a split-plot based on randomized complete block design (RCBD) with three replicates. In each of the three replications, the main plots were randomly assigned to the two salinity regimes and each main plot consisted of five SA concentrations.

A commonly and new grown barley cultivars in Iran, Nosrat was used in this study. Tillage operation consisted of a moldboard plowing disturbing the soil to a 30-cm depth followed by two rounds of vertical tillage with harrow disking. Prior to sowing, the field was fertilized with ammonium phosphate and urea at the rate of 100 and 50 kg ha<sup>-1</sup>, respectively. During the growing season, additional urea fertilizer was applied at the stem elongation and flowering stages at the rate of 30 kg ha<sup>-1</sup> each time. Each experimental unit was a plot of 3×4 m. Row and plant spacing were 15 and 3 cm, respectively, resulting to 223 plants m<sup>-2</sup>. On November 20 in both years, barley seeds were

planted at a depth of 3-4 cm, using a hand dibber. Weeds were controlled manually. Chemical and physical properties of the soil at the experimental site in the depth of 0-30 cm are shown in Tables 1 and 2, respectively.

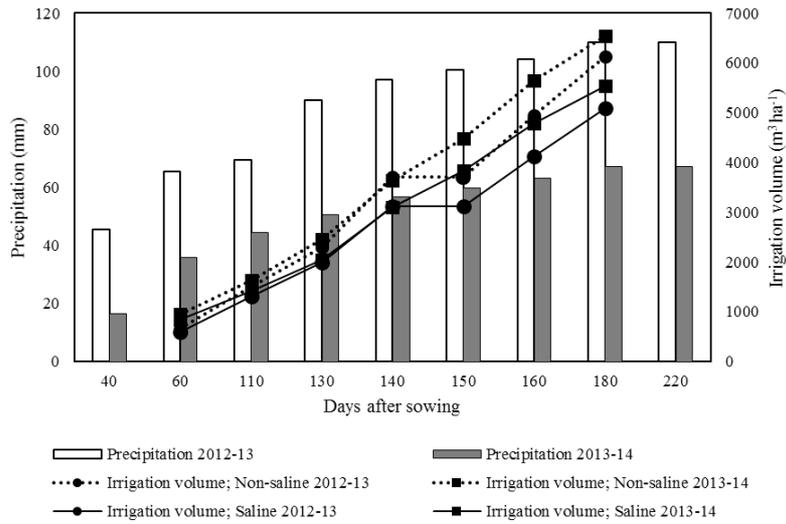


Figure 1. Cumulative quantity of precipitation and irrigation water volume during the growing seasons for two years.

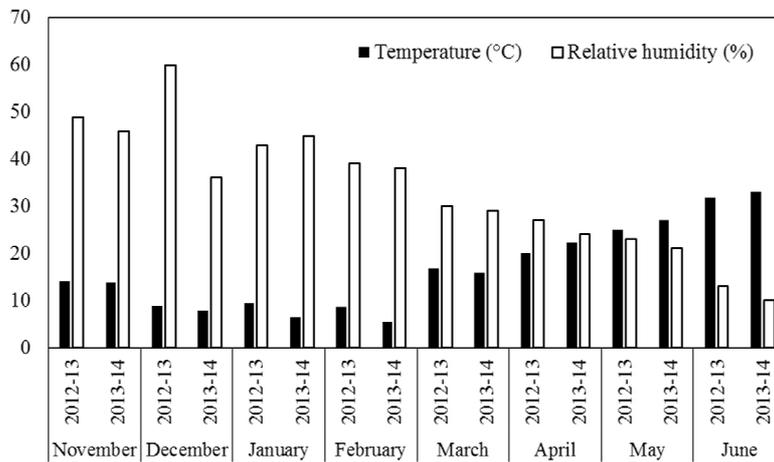


Figure 2. Temperature and relative humidity of experimental site during the growing seasons for two years.

Table 1. Chemical properties in 0-30 and 30-60 cm depths of soil before the experiment (Hosseinabad, Yazd, Iran).

Soil depth	pH	ECe (dS m <sup>-1</sup> )		(meq L <sup>-1</sup> )					(mg kg <sup>-1</sup> )			N (%)	O.C. (%)
		Cl <sup>-</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Na <sup>+</sup>	K <sup>+</sup>	CO <sub>3</sub> <sup>2-</sup>	HCO <sub>3</sub> <sup>2-</sup>	P	K	P		
0-30	7.37	101.2	23.0	27.4	71.30	0.76	2.91	2.76	16.2	142.0	0.032	0.46	
30-60	7.61	45.0	15.2	11.2	27.09	0.59	2.83	1.06	10.3	121.3	0.041	0.38	

Table 2. Physical properties of soil in the experimental site (Hosseinabad, Yazd, Iran).

Sand	Clay	Silt	Texture	Bulk density	Saturation	FC <sup>†</sup>	PWP
(%)				g cm <sup>-3</sup>	(%)		
55	18	27	Sandy loam	1.4	38.4	21.6	7.3

<sup>†</sup> FC: Field capacity, PWP: Permanent wilting point.

There was a saline water supply pipe system in the field delivering irrigation water with different levels of salinity, 2 to 14 dS m<sup>-1</sup>. Chemical properties of saline water used are shown in Table 3. To assure desired salinity level, EC of irrigation water was also monitored by a portable EC-meter in each plot entrance. The plots were irrigated by tap water (2.0 dS m<sup>-1</sup>) to raise the soil water to FC until the crop establishment; which included one irrigation in the first and two irrigations in the second year. Irrigation intervals were 15-16 days during autumn and winter and 10-12 days during spring in both years. The plants were subjected to saline irrigation water after complete establishment. The amount of water applied to each plot was measured using a water meter. Salicylic acid was applied in stem elongation (32 at Zadoks scale; Zadoks et al., 1974) as foliar application using a precision sprayer (AH-15N, Daegu, Korea; 0.3 MPa pressure) with the rate of 400 L ha<sup>-1</sup>. The plots not receiving SA treatment was similarly sprayed with equivalent volume of distilled water.

Table 3. Cations and anions of saline water (meq L<sup>-1</sup>) used in the field experiment (Hosseinabad, Yazd, Iran).

Salinity (dS m <sup>-1</sup> )	pH	HCO <sub>3</sub> <sup>-</sup>	Cl <sup>-</sup>	SO <sub>4</sub> <sup>2-</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Na <sup>+</sup>	K <sup>+</sup>
2	8.25	1.69	15.00	7.44	3.95	7.75	12.76	0.17
12	7.71	3.23	92.31	26.43	9.03	28.36	84.65	0.51

Plots were irrigated using surface poly ethylene tubes attached to a flow meter. For each plot, the timing and amount of irrigation water was determined by considering soil field capacity (F.C. %) at the depth of 0-90 cm according to the rooting depth. Before each irrigation event, soil samples were taken to determine their gravimetric water contents (Pw, %). Depth of net irrigation water (d<sub>n</sub>, cm) was calculated as follows:

$$\text{Equation 1: } d_n = \frac{[\theta_{FC} - (P_w \times A_s)] \times R_d}{100}$$

where  $\theta_{FC}$  is the volumetric soil water content (%) at field capacity,  $A_s$  is the apparent specific gravity in the soil profile in root depth defined as bulk density ( $\text{g m}^{-3}$ ) to water specific gravity ( $\text{g m}^{-3}$ ) ratio. Furthermore,  $R_d$  is the root depth (cm) varies during the growing season and was calculated as follows (Borg and Grimes, 1986):

$$\text{Equation 2: } R_d = P_d + R_{d_{\max}} [0.5 + 0.5 \sin(3.03 \frac{D_{ag}}{D_{tm}} - 1.47)]$$

where  $P_d$  is the planting depth (cm),  $R_{d_{\max}}$  is the maximum root depth (cm),  $D_{ag}$  is the days after germination,  $D_{tm}$  is the days from germination to maximum effective depth and the sine function is in radians. Based on FAO reports (Allen et al., 1998), maximum root depth for barley were assumed to be about 100 cm. The water application efficiency of all irrigation events was assumed as 70 percent (or 30% deep percolation). Therefore, the volume of water application for each main plot was calculated for the given plot area as follows:

$$\text{Equation 3: } V_g = \frac{d_n}{0.70} \times 10000 \times P_a$$

where  $V_g$  is the volume of water application ( $\text{m}^3$ ) for each plot and  $P_a$  is the given plot area ( $12 \text{ m}^2$ ). Irrigation volumes were 6128.2 and 5083.4  $\text{m}^3$  at first and 6546.4 and 5537.8  $\text{m}^3$  in second year for non-saline and saline conditions, respectively.

Sampling for growth and yield traits consisting of plant height (Ht), fertile tillers per unit area (FT), ear length (EL), grain number per ear (GN), thousand grain weight (TGW), grain yield (GY), biological yield (BY) were conducted at the end of growing season in both years. Plant height was measured from the soil surface, using ten randomly selected plants in each sub-plot. The average height from the ten plants was used for further analysis. All plants in each plot ( $20 \text{ m}^2$ ) were harvested on 13 and 15 June 2013 and 2014, respectively. After harvest, ten plants were randomly selected and ear length and grain number per ear were measured. Then thousand grain weight (TGW), grain yield (GY) and biological yield (BY) were determined. Water productivity was calculated as:

$$\text{Equation 4: } WP = \frac{GY}{WU}$$

where WP, GY and WU are the water productivity ( $\text{kg m}^{-3}$ ), the grain yield ( $\text{kg m}^{-2}$ ) and the water used ( $\text{m}^3 \text{m}^{-2}$ ), respectively.

Data were subjected to combined analysis of variance (ANOVA) using the computer software SAS v. 9.1 (SAS Institute, Cary, NC, USA). Since the interactions of year with the treatments were significant; so the result of each year were reported separately. Means were compared using standard error ( $\pm$ SE) values using software SAS v. 9.1. Stepwise regression and correlation coefficients were calculated using MINITAB 16. Response curve was also done using SAS v. 9.1.

## Results

Salt stress reduced plant height (Ht) significantly (Figures 3a and 3b). Salicylic acid foliar application increased Ht at all concentrations under saline and non-saline conditions in both years. Under non-saline conditions in both years the tallest plants (85.1 and 80.1 cm in first and second year, respectively) were observed at the highest SA concentration (2.0 mM); whereas, under saline conditions positive effect of SA on plant Ht was observed up to 1.0 and 1.5 mM in second (56.0 cm) and first (67.6 cm) year, respectively (Figures 3a and 3b). Saline irrigation water significantly reduced ear length (EL) of barley plants in both years (Figures 3c and 3d). Effect of foliar applied SA on EL in non-saline conditions was almost similar in two years; so that increasing in SA concentration up to 1.5 mM was associated with increasing positive effect on EL; while, in saline conditions increasing in SA concentration up to 1.5 and 1.0 mM enhanced EL of barley plants at first (6.0 cm) and second years (5.2 cm), respectively (Figures 3c and 3d). Increasing in SA concentration to 2.0 mM reduced EL under saline conditions only in second year. Salinity significantly decreased fertile tiller number per unit area (FTN) (Figures 3e and 3f). Increasing in EC of irrigation water from 2 to 12  $\text{dS m}^{-1}$  was associated with 15.9% to 16.4% and 21.1% to 22.4% reductions in FTN at first and second year, respectively.

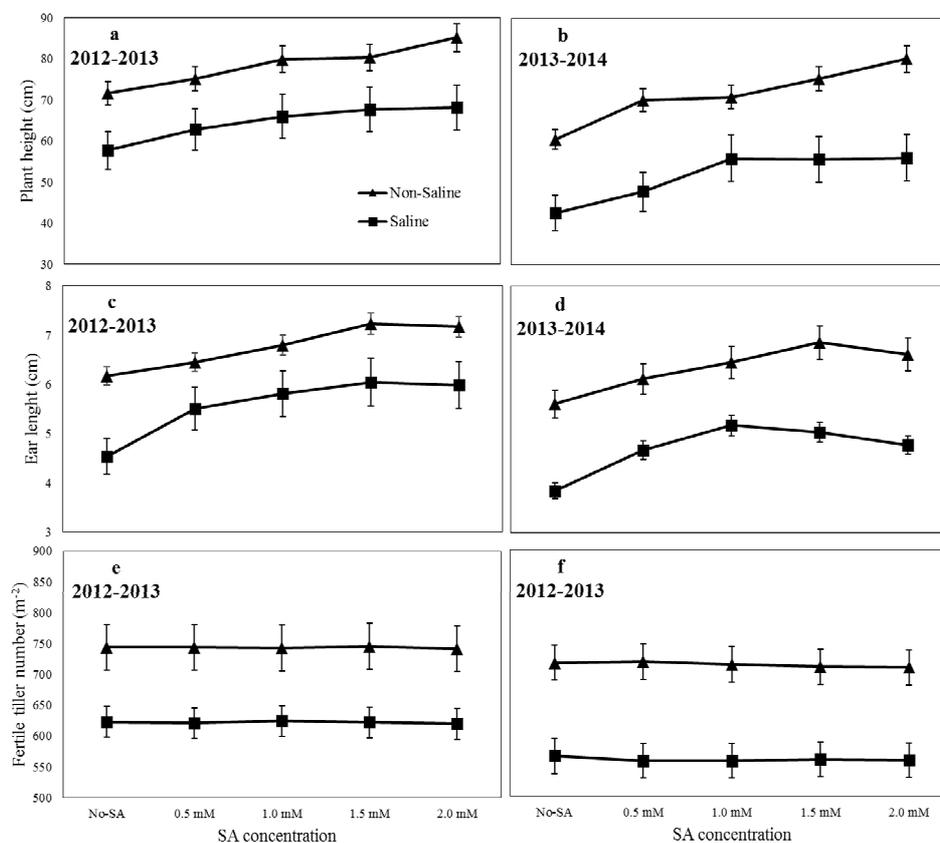


Figure 3. Effect of different concentrations of salicylic acid (SA) on barley growth parameters under saline and non-saline conditions. Vertical bars represent standard error ( $\pm$ SE).

Grain number per ear (GN) was significantly reduced under the influence of salt stress, that is to say stressed barley plants had lower GN in all SA treatments in both years (Figures 4a and 4b). Under non-saline conditions, increasing in SA concentration up to 2.0 mM was associated with enhanced GN in both years. While under saline conditions foliar applied SA increased GN up to 1.0 to 1.5 mM concentrations; however, greater concentration (i.e. 2.0 mM) reduced it (Figures 4a and 4b). Grains with lower mean weight were obtained in salt stressed barley plants at all SA levels; however SA foliar application altered this negative impact (Figures 4c and 4d). Salicylic acid improved thousand grain weight (TGW) with the highest observed

at 1.5 mM concentration under non-saline conditions; while at saline conditions it was achieved at 1.0 mM concentration. Greater SA concentration had no positively significant effect on TGW under non-saline conditions; whereas, stressed barley plants had lower TGW in the highest SA concentration (i.e. 2.0 mM) as shown in Figures 4c and 4d.

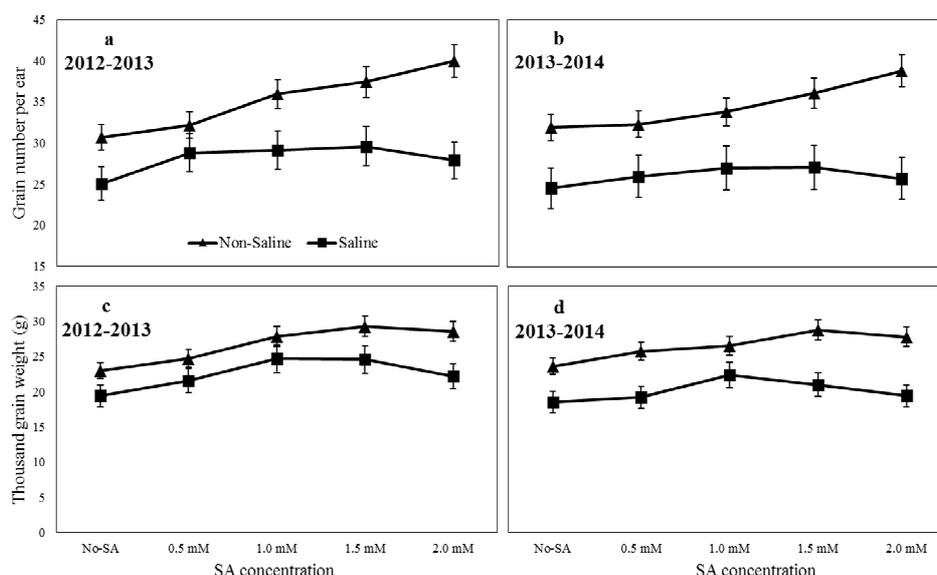


Figure 4. Effect of different concentrations of salicylic acid (SA) on yield components of barley under saline and non-saline conditions. Means with similar overlap had no significant difference ( $\pm$ SE).

Considerable reduction in grain yield (GY) was observed when EC of the irrigation water was increased from 2 to 12 dS m<sup>-1</sup> (Figures 5a and 5b), so that on average GY of stressed barley plants was lower by 34.3% and 40.8% compared to barley plants grown under non saline conditions at first and second year, respectively. Salicylic acid in all concentrations increased GY; however, there was a significant difference between the treatments. In both years, increasing SA concentration up to 2.0 mM was associated with enhanced GY under non-saline conditions; so that the highest GY was obtained from barley plants grown under non-saline conditions and treated with 2.0 mM SA as 7687.6 and 6581.0 kg ha<sup>-1</sup> in first and second year, respectively. Under saline conditions, the highest

GY was found in 1.5 mM SA in first year as 5155.0 kg ha<sup>-1</sup> (Figure 5a); while in the second year it was observed in 1.0 mM SA concentrations as 3904.7 kg ha<sup>-1</sup> (Figure 5b).

It was shown that the salt stressed barley plants had lower biological yield (BY) (Figures 5c and 5d); so that on average, salinity treatment was associated with 26.5% and 32.6% reduction in BY for the first and second year, respectively. Salicylic acid, even at the lowest concentration (i.e. 0.5 mM) increased BY; however, the best concentration was varied under different conditions. For example, under non-saline conditions increasing in SA concentration up to 2.0 mM enhanced BY in both years (on average 17348.4 kg ha<sup>-1</sup>); whereas at saline conditions, the highest BY was obtained in 1.5 and 1.0 mM in first (13580.2 kg ha<sup>-1</sup>) and second (10505.9 kg ha<sup>-1</sup>) year, respectively (Figures 5c and 5d).

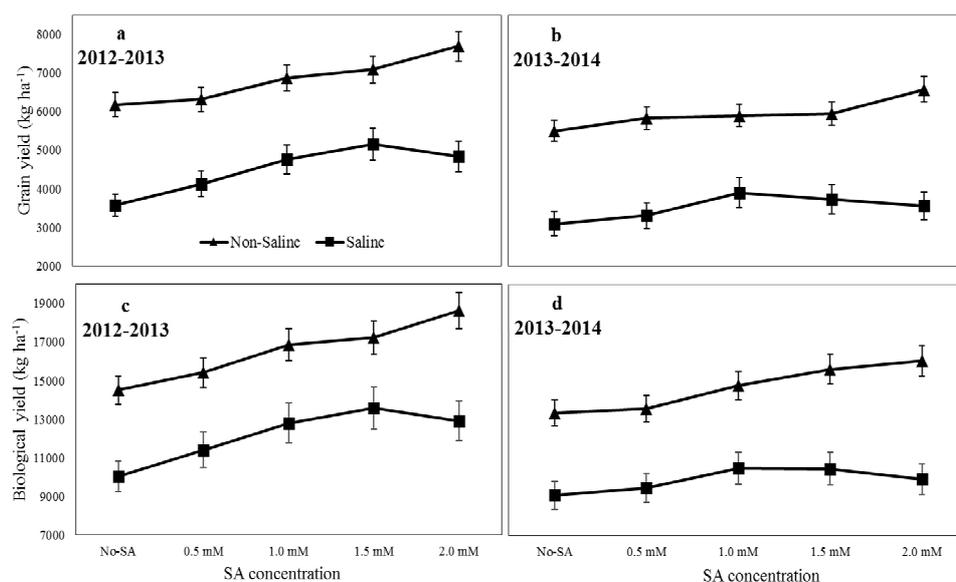


Figure 5. Effect of different concentrations of salicylic acid (SA) on biological and grain yield of barley under saline and non-saline conditions. Means with similar overlap had no significant difference ( $\pm$ SE).

The results of correlation analysis showed that under saline conditions the correlations between GY and measured agronomic traits were significantly higher than those under non-saline conditions (Table 4). In fact

Ht (P<0.01), EL (P<0.05), GN (P<0.01) and BY (P<0.01) were positively correlated with GY under non-saline conditions. On the other hand, under saline conditions, there were positive and significant correlations between EL (P<0.01), FTN (P<0.01), GN (P<0.01), TGW (P<0.01), BY (P<0.01) and HI (P<0.01) with GY. Stepwise regression also showed that BY and TGW in non-saline and BY, GN and FTN under saline conditions were the most effective traits on GY (Table 5). Under non-saline conditions, response curves revealed that liner equation was found to be the most fit relationship between GY and SA concentrations (SAC as mM) with F value=109.94\*\*, R<sup>2</sup>=0.87 and it is shown as follow:

$$GY = 606.53SAC + 5783.9$$

Table 4. Correlation of grain yield with other agronomic traits for both years.

	Ht <sup>†</sup>	EL	FTN	GN	TGW	BY	HI	GY <sub>p</sub>	GY <sub>s</sub>
Non-saline	0.809***	0.726*	0.564 <sup>ns</sup>	0.768*	0.561 <sup>ns</sup>	0.886**	0.264 <sup>ns</sup>	1.000	0.800*
Saline	0.529 <sup>ns</sup>	0.853**	0.817**	0.895**	0.822**	0.897**	0.902**	0.800*	1.000
Reduction (%)	-0.511 <sup>ns</sup>	-0.558 <sup>ns</sup>	-0.751*	-0.767*	-0.804**	-0.713*	-0.373 <sup>ns</sup>		-0.683*

<sup>†</sup> Ht: plant height, EL: ear length, FTN: fertile tiller number per area, GN: grain number per ear, TGW: thousand grain weight, BY: biological yield, HI: harvest index.

<sup>‡</sup> ns: no significant; \* and \*\* significant at 5% and 1% probability levels.

Table 5. Stepwise regression analysis between grain yield and other agronomic traits for both years.

	Variable entered	Partial R <sup>2</sup>	Model R <sup>2</sup>	F Value	Pr>F
Non-saline	BY <sup>†</sup>	0.8086	0.8086	721.88	<0.001
	TGW	0.1601	0.9687	285.10	0.012
Suggested model: GY=4.216BY+0.387TGW-2733					
Saline	BY	0.9036	0.9036	1048.91	<0.001
	GN	0.0241	0.9277	122.02	0.021
	FTN	0.0142	0.9419	65.93	0.043
Suggested model: GY=0.441BY+5.62GN+1.165FTN-853.3					

<sup>†</sup> FTN: fertile tiller number per unit area, GN: grain number per ear, TGW: thousand grain weight, BY: biological yield.

Furthermore, under saline condition, the most fit relationship between GY and SAC was quadratic regressions with F value=18.88\*\*,  $R^2=0.81$  and the equation is as follow:

$$GY = -499.31SAC^2 + 1489SAC + 2687.4$$

Therefore, it was concluded that the highest concentration used in this study (i.e. 2.0 mM) was the most appropriate concentration under non-saline conditions (Figure 6a). On the other hand, derivation of regression equation showed that 1.41 mM SA concentration was the best under saline conditions (Figure 6b).

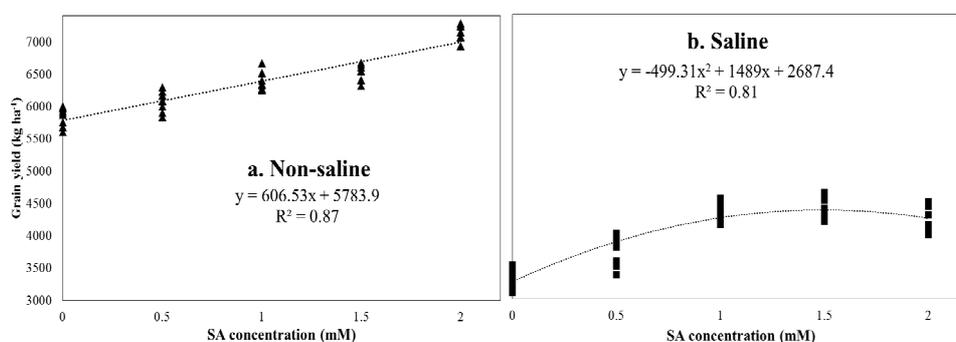


Figure 6. Relationship between SA concentration and grain yield under non-saline and saline conditions. Trending options for each condition were selected based on response curve results.

Salinity reduced water productivity (WP) in both years, so that WP was lower at saline conditions by 20.7% and 30.1% compared to non-saline conditions in first and second year, respectively (Figure 7). Salicylic acid foliar application increased WP in both years; however, under non-saline conditions increasing SA concentration up to 2.0 mM resulted to higher WP, whereas under saline conditions SA application enhanced WP only up to 1.5 and 1.0 mM in first (Figure 7a) and second year, respectively (Figure 7b). The above proper SA concentrations were associated with 24.9% and 31.2% (in first year) and 19.7% and 25.3% (in second year) increases in WP under non-saline and saline conditions, respectively. Overall, WP was higher in the first compared to the second year.

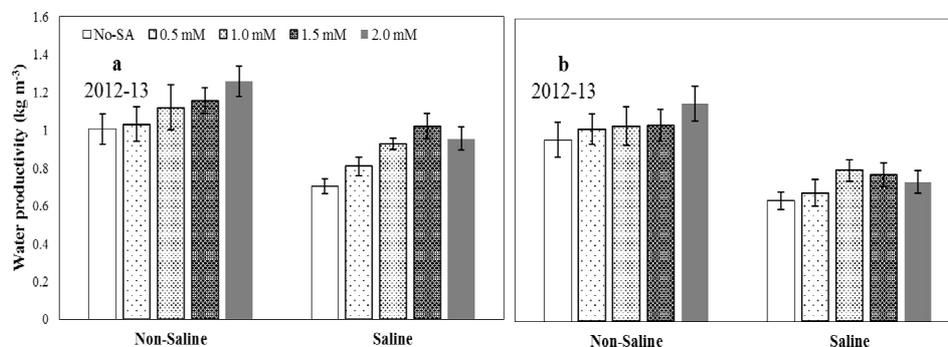


Figure 7. Water productivity of barley plants treated by different salicylic acid (SA) concentrations grown under non-saline and saline conditions. Means with similar overlap had no significant difference ( $\pm$ SE).

## Discussion

Salt stress led to significant reductions in growth and yield attributes, including plant height, fertile tiller per unit area, ear length, grain number per ear, thousand grain weight; which collectively lowered the grain and biological yields. The salinity-induced reductions in growth and yield parameters in barley plants are in agreement with the numerous previous reports on barley (Pancheva et al., 1996; Pakniyat et al., 2003; El Tayeb, 2005; Emam et al., 2013; Pakar et al., 2014) and other crops (Parida and Das, 2005; Sepaskhah and Beirouti, 2009; Sepaskhah and Yarami, 2010; Aftab et al., 2011). Salt stress has been reported to decrease the water imbibition by roots due to reduced osmotic potentials in root zone; resulting in alterations in metabolic activities leading to the reduction in crop growth and yield (Parida and Das, 2005; Aftab et al., 2011). Under salt stress conditions, water utilization ability is reduced and results in a reduction in growth (Munns, 2002; Emam et al., 2013). It has been well known that lowered GY was correlated with reduction in yield components; so that it was noted that reduced grain yield in bean plants under stressful conditions has been associated with reduced yield components in general and grain number in particular (Hay and Walker, 1989). Since, salt sensitivity of different organs in a plant is varied (Munns, 2002); therefore it could be argued that response of morpho-physiological traits may play a crucial role in reduction of resources efficiency use and hence lead to reduction in crop yield.

Although salinity influenced almost all measured traits in this study; reduction in grain yield upon salinity was significantly correlated to fertile tiller number per unit area, grain number per ear and grain weight (Table 4). Indeed, sensitivity of different plant organs to salinity differs; as also being noted by other researchers (Munns, 2002; Emam et al., 2013). In this study when salinity treatment was imposed the fertile tillers were at their rapid growth phase; therefore, fertile tiller number per unit area was considered to be the most sensitive trait to salt stress. This argument was also reflected in correlation (Table 4) and stepwise regression analyses (Table 5). Indeed, it was shown that in barley fertile tiller per unit area could be considered as a sensitive trait to salinity. However, other workers have reported different traits as the most sensitive in barley yield determination, for example emergence percentage and root dry weight have been noted by Emam et al. (2013). At the same time, the grain number per plant, known to be more effective in producing grain yield under normal conditions (Hay and Walker, 1989).

As have been reported previously, SA-application can improve Ht under both non-saline and saline conditions; foliar applied SA significantly modulated adverse effect of salt stress on plant height; which was varying among treatments. Pakar et al. (2014) also demonstrated that the most positive effect on Ht was related to foliar applied SA compared to control and even other PGRs (e.g. cycocel and jasmonic acid). It is reported that SA foliar application enhanced leaf area (Pancheva et al., 1996; Pirasteh-Anosheh et al., 2014), plant height (Pirasteh-Anosheh et al., 2012) and other growth traits (Ashraf et al., 2010). Altogether, the lowest negative effect of salt stress was observed in 1.5 and 1.0 mM SA concentrations (i.e. 15.8 and 21.1% reductions in Ht) in first and second year, respectively. Ashraf et al. (2010) indicated that SA foliar applications could alleviate adverse effects of salinity via promotion of seedling growth, restoring plant growth and promoting accumulation of proline, ABA, IAA and cytokinin.

In this research foliar applied SA considerably enhanced grain number via increasing both total floret number initiated as well as total fertile florets; so that the ear length and grain number per ear were increased upon SA-foliar application. Furthermore, under salinity foliar applied SA at various concentrations could alleviate reduction in grain number. The lowest reductions in EL due to salt stress were observed in 1.0 mM SA concentrations in both years (16.3%); while the highest positive effect of SA on GN was observed at 0.5 mM concentration for both years; so that reduction percentages due to salinity were 10.6% and 19.5% at first and

second year, respectively. Similar growth promoting responses have been reported in barley seedlings sprayed with SA (Pancheva et al., 1996; Shakirova, 2007; Pirasteh-Anosheh et al., 2014; Pakar et al., 2014). Since the GY is highly correlated with grain number in barley (Emam et al., 2013) and in this study it was shown that foliar applied SA improved grain number under salinity conditions. Therefore, the grain yield of barley could be significantly influenced by foliar applied SA. The beneficial effect of foliar applied SA on improvement in grain number might be achieved via improving such growth attributes as improved photosynthetic capacity and rubisco activity (Ashraf et al., 2010).

Improved grain number in both non- and saline conditions upon SA application was not associated with any reduction in grain weight. The lowest reduction percentages in TGW in salinity treatments in both years were found in 1.0 mM SA concentration (11.4% and 15.5% in first and second year, respectively). Ameliorative role of SA on adverse effects of salinity was clearly appeared in this study via less reduction in TGW. It might be due to the positive effect of SA foliar application on endogenous SA biosynthesis. Hence, exogenous application of PGRs overcomes much of the internal PGR deficiency that may be resulted by salt stress. This may lead to a reduction in the inhibitory effects on plant growth caused by salinity stress (Ashraf et al., 2008; Ashraf et al., 2010).

Although salt stress significantly decreased GY and BY, foliar applied SA compensated some of these losses. In first year, salt stress (12 dS m<sup>-1</sup>) was associated with 42.1%, 34.7%, 30.7%, 27.2% and 37.1% reduction in GY in SA- treated barley plants at 0.0 (no SA), 0.5, 1.0, 1.5 and 2.0 mM concentration, respectively. These values were 43.7%, 43.3%, 33.8%, 37.4% and 45.8%, respectively for second year. Thus, the lowest reduction in GY due to salinity was observed in 1.5 and 1.0 mM in first and second year, respectively. Reduction percentages in BY due to salt stress were 30.7% and 31.9% in 0.0 (no SA), 26.0% and 30.3% in 0.5 mM, 24.0% and 28.9% in 1.0 mM, 21.2% and 32.9% in 1.5 mM and 30.6% and 38.2% in 2.0 mM SA concentrations in first and second year, respectively. Since SA affected FTN, GN and TGW, as the main yield components, the increase in GY was not far from our expectation. This positive effect of foliar applied SA on GY and other traits might be due to stimulatory effect of SA on shoot growth resulting in greater photosynthesis rate, as being previously noted in the greenhouse experiment (Pirasteh-Anosheh et al., 2014) and allocation of more assimilates to grain (Noreen and Ashraf, 2008; Ashraf et al., 2010).

Modulating adverse effect of salinity on GY and BY by foliar applied SA was observed at 0.5-2.0 mM and 0.5-1.5 mM concentrations in first and second year, respectively. This suggests that SA, as an important PGR, has an effective role in protecting plants against salt stress. In earlier studies it has also been shown that SA application increased plant tolerance to salinity by offsetting GY and BY reduction in different crops, especially cereals such as barley and wheat (El-Tayeb, 2005; Noreen and Ashraf, 2008; Aftab et al., 2011; Pirasteh-Anosheh et al., 2012). Since salt stress restricts barley production by adverse effect on various physiological and biochemical processes including photosynthesis, antioxidant capacity and ion homeostasis, it is assumed that SA-induced enhancement in GY of salt stressed barley plants might have been due to SA-induced alteration in those biochemical or physiological processes (Noreen and Ashraf, 2008).

In current study, the most appropriate concentrations for achieving the highest grain yield were determined 2.0 and 1.41 under non-saline and saline conditions, respectively. In salt stressed barley plants, the highest concentration (i.e. 2.0 mM) of SA not only had no positive impact; but in some cases, for example BY in the first year as well as EL and TGW in the second year, had negative impact. Responses to different SA concentrations by cereals have been reported to be different by other workers (Fariduddin et al., 2003; Kaydan et al., 2007; Afshari et al., 2013). Similar to our finding, Fariduddin et al. (2003) also reported that dry matter accumulation in *Brassica juncea* was significantly increased when lower concentrations of SA were applied; however, at higher concentrations of SA an inhibitory effect was observed. Kaydan et al. (2007) and Afshari et al. (2013) noted that SA at lower concentrations had the best effect on growth of wheat and cowpea, respectively, compared to higher concentrations. It seems that inhibitory effect of SA at higher concentrations in previous works (e.g. Fariduddin et al., 2003; Kaydan et al., 2007; Hayat et al., 2010; Afshari et al., 2013) and especially under saline conditions in our research, might be attributed to reduced ribulose-1,5-biphosphate carboxylase/oxygenase as well as nitrate reductase activity (Hayat et al., 2010) and/or negative impact of SA as non-enzymatic antioxidant; which could lower the activity of naturally produced antioxidant enzymes (Hayat et al., 2005; Ashraf et al., 2010).

Despite lower volume of irrigation water used under saline conditions (Figure 1), WP was decreased as a result of salt stress. This was due to more reduction in GY under saline conditions. It was shown that evapotranspiration was reduced as salinity levels were increased from 0.5

to 15.5 dS m<sup>-1</sup> (Sepaskhah and Beirouti, 2009). Although there was no-significant difference between irrigation water used between No-SA and SA-treated treatments, SA enhanced WP via notable increase in GY. Fariduddin et al. (2003) reported that lower exogenous application of SA was found to enhance the net photosynthetic rate and internal CO<sub>2</sub> concentration resulting in an enhanced WP. In another study, foliar applied SA enhanced the WP in soybean (Kumar et al., 2000). Ashraf et al. (2010) speculated that foliar applied SA might result to stomatal closure and hence reduced transpiration which is beneficial in reducing water loss, especially under water deficit conditions. Noreen and Ashraf (2008) also reported that WP was increased due to SA applied as a foliar spray in both salt stressed and non-stressed sunflower plants.

## **Conclusion**

Increasing in EC of irrigation water from 2 to 12 dS m<sup>-1</sup> was associated with significant lowering in barley growth, yield and water productivity. Lower growth and yield of barley plants were found in second year which might have been due to lower temperature as well as precipitation. Herein, effect of salinity was intensified in second year as precipitation was decreased. Nonetheless, foliar applied SA in different concentrations could ameliorate some of these negative impacts on growth and yield. Overall, it can be concluded that: 1) fertile tiller number per unit area and grain weight were the most important yield components for salt stress tolerance in barley, 2) modulating role of SA for adverse effect of salinity could be attributed to enhanced grain number than other traits and finally 3) SA at 2.0 and 1.41 mM concentrations could be considered as the optimal used concentrations to improve barley performance under non-saline and saline conditions, respectively.

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