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The effect of salicylic acid on ion relations and some biochemical traits of two sugar beet cultivars under salinity stress

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Abstract

Salinity of soil and irrigation water reduces yield of the crops such as sugar beet. To study the effect of salicylic acid (SA) on salinity tolerance of sugar beet cultivars this study, an outdoor pot experiment, was conducted as split factorial based on randomized complete block design with three replications during growing seasons of 2012 and 2013. The studied factors were three levels of SA (0 mM, 0.5 mM and 1 mM), two levels of salinity (150 mm sodium chloride and control) and two sugar beet cultivars (Jolgeh and Shariff). SA was applied as foliar spray along with salinity in the 4-leaf stage. Results showed that salinity caused a significant increase in membrane permeability, proline, proline / potassium, sodium, potassium / sodium and total soluble sugars in the roots and leaves, while foliar application of SA was significantly decreasing these traits. High correlation between proline and root and shoot dry weight revealed foliar application and proline increasing enabled the plant to tolerate salinity and finally resulted in increase of the root and shoot dry weight. Regarding to the results of sensitivity index, Jolgeh and Shariff were determined as semi-tolerant and semi-sensitive, respectively.

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Introduction

The amount of agricultural production in arid and semi-arid climates is limited by saline soil and irrigation water which both are regarded as the most important barriers for plants growth (Siddiqui *et al.* 2006; Ashraf *et al.* 2008; Kausar *et al.* 2013). Salinity stress in each phase of plant growth causes a reduction of functional potential in related plants (Munns, 2002; Hameed *et al.* 2013). Also, some chemical, physiological and morphological changes in the plants can be resulted of salinity stress. This tension affects Growth, photosynthesis, protein synthesis, lipid metabolism, respiration and energy production (Parida and Das, 2005). Moreover, salinity makes disorder in absorption of minerals by interfering conveyers' activities and ion channels through root such as optional channels k^+ (competition between Na^+ and K^+), inhibition of root growth by osmotic effects of Na^+ or makes a disorder in water and minerals absorption by the effect of Na^+ on soil structure (Parida and Das, 2005; Tester and Venport, 2003). In addition, salinity affects Permeability of membrane by replacing Ca^{2+} by Na^+ (Sairam *et al.*, 2005). There are several ways in order to increase plants' resistance against salinity; one of these physiological methods in recent years having been used to reduce environmental stressses on different plants is external usage of stress-alleviating materials (Yuan and Leen, 2008). Among the materials, SA, which is one of the most important messenger molecules and causes the reaction of the plant against the tension and as a non-enzymatic antioxidant, plays remarkable role on regulation of plant physiological processes (Erfan *et al.*, 2007). SA or ortorhydroxy benzoic acid ($C_7H_6O_3$) belongs to a phenolic compounds group and is created by root cells (El-Tayeb, 2005; Popova *et al.*, 1997). SA absorption is affected by pH, as its inhibitory is increased with decreasing pH (El-Tayeb, 2005; Raskin, 1992). External use of SA can play a significant role on physiological processes of plants such as stomata closure, ion uptake and transportation (Gunes *et al.*, 2005), membrane integrity (Khan *et al.*, 2003), and photosynthesis (Nemeth *et al.*, 2002)

Some authors have shown that using SA can reduce deleterious effects of salinity stress in various plant species (Hussein *et al.*, 2007; Noreen and Ashraf, 2008; Ashraf *et al.*, 2010; Pirasteh-Anosheh and Emam, 2012; Pirasteh-Anosheh *et al.*, 2012). Samia *et al.* (2009) indicated that external usage of SA on corn had reduced the intensity of oxidative stress in the plant under salinity stress. Korkmaz *et al.* (2007) reported that using SA had alleviated drought stress of the plant by seed soaking and foliar spray.

Sugar beet, as an economic agricultural product, is widely planted in different areas of Iran. There has not been conducted a comprehensive study about the effect of salicylic acid on some major properties of various cultivars of sugar beet in Iran. Moreover, salinity stress is an important stress in sugar beet farms. Hence, the present study was aimed to evaluate the effect of salicylic acid on ion relations and some biochemical traits of two sugar beet cultivars under salinity stress in Iran.

Materials and methods

Study area

This study was conducted in the research station (48° 41′ E and 31° 20′ N; 20 m a.s.l.) of the Shahid Chamran University of Ahvaz during 2012-2013. In order to better control of soil salinity and correct application of treatments, the outdoor pot experiment (Benes *et al.*, 1996) was selected.

Methods

The present study was submitted in Factorial Split Plots based on Randomized Complete Block Design with three replications. Different salinity stresses including 25, 50, 75, 100, 125, 150 mm were applied. Salinity stresses were 150 mm sodium chloride solution and control. Foliar spray by SA contained 0 mM, 0.5 mM and 1 mM as the main plot and two selected cultivars of the sugar beet were Jolgeh and Shariff. In each pot, 10 seeds were cultivated in 2-3cm depth. During the growth period, irrigations were applied according to plant's need and soil's humidity. First thinning operation was performed in the stage four leaves, and the number of seedlings were thinned to five plants per pot and also the number of plants per pot was reduced to one plant in the stage 8 leaves. Applying salinity stress at stage 4 leaves began with 25 mm and gradually increased up to reaching the desired level of treatment (150 mm). Foliar spray with SA was launched simultaneously with salinity stress, in which each week the leaves were sprayed with a solution of SA, so that the inner and outer parts of leaves were quite sprayed. The surfactant was used in the amount of 0.01 percent for better absorption of foliar application of SA. Subsequently, to evaluate the effect of salinity on dry weight of shoots and roots, fresh samples of plants from each treatment were harvested and weighed. Moreover, to obtain the dry weight of the samples, they were placed in the oven at 70 ° C for 48 hours. Membrane permeability by Zhao *et al.* (1991) was calculated using the following formula:

$$\text{Membrane permeability} = \frac{EC_1\text{-}EC_0}{EC_2\text{-}EC_0} \times 100$$

In order to measure the amount of soluble sugars in leaves of Shlegil (1986) and proline content of Bates *et al.* (1973) was used. The amount of sodium and potassium in leaves were measured by Hamada and Elnay (1994). The methods used for soil properties are the texture of soil by Gee (2002), soil acidity by Thomas (1996) and soil electrical conductivity by Roades (1996). In addition, determination of organic matter and classification of plants based on the stress sensitivity index were measured by Walkey and Black (1934) and Fischer and Mourer (1987) (Table 1), respectively.

Results and discussion

Analysis of variance showed that the interaction of SA, cultivar, and salinity on root dry weight was significant at the 5% level (Table 3). As is shown by Fig. 1, the results were consistent with Table 1.

Table 1. The resistance of plants to drought stress susceptibility index.

tolerant	SSI= 0 - 0.5
semi-tolerant	SSI= 0.5 - 1
semi-sensitive	SSI= 1 – 1.5
sensitive	SSI= 1.5 – 2.5

Table 2. Descriptive statistics of the soil properties.

Texture	EC (ds/m)	PH	N (PPM)	P (PPM)	K (PPM)	OM (%)
Sandy loam	2	7.8	0.11	5	220	1.2

The interaction of SA, cultivar, and salinity on the leaf sodium, leaf potassium, potassium / sodium ratio, total soluble sugars, and leaf relative water was statistically significant (figs. 2 to 6). It is observed from the table 3 that salinity increases the amount of sodium and reduces potassium value. In sugar beet, sodium can replace potassium in metabolic and enzymatic processes, and even it is considered as a food source after establishing the plant. In this study the plant which was under salt stress, reduced the ratio of Na^+ / K^+. Similar findings by other researchers such as Das and Parida (2005), Azooz *et al.* (2011) have also been reported. Since the ratio of Na^+ / K^+ is an indicator for determining the degree of plant tolerance to salinity, generally in salt-sensitive plants, Na^+ was increased and K^+ decreased (Parida and Das, 2005). High concentration of salt, destroys homeostasis in water potential and makes a result in distribution of ions in leaves. These results are consistent with those obtained by Panda and Dash (2001). In this study, foliar application of SA in plants under salt stress reduced the sodium content of leaves, but the amount of potassium in leaves under salt stress increased. SA can increase photosynthetic pigments (as a factor which influences the production of biomass) and the amount of carotenoids (as a component of the antioxidant defense system), and improve photosynthetic parameters that its results can be observed in improvement of growth indexes. It is obvious that Proline and sodium have a significant role in osmoregulation. These results are consistent with those reported by Shakirova *et al.* (2003) and

Noreen *et al.* (2010). One of metabolic reactions to salinity stress is compatible osmolit synthesis. These organic compounds are involved in osmotic adjustment and protect plant cells from oxidative damage. There was found a direct relationship between the accumulation osmolit (proline and sugars) and increasing the stress tolerance in plants. Maintaining high levels of Absesic acid in plants treated with SA in terms of salinity, increases proline biosynthesis and immune proteins. In this study, while foliar application of SA decreased the accumulation of sodium, this reduction was not sufficient to eliminate the toxic effects of sodium. Hence, it seems that increasing the proline concentration under salinity stress reduces toxic effects of sodium through osmotic adjustment. Aftab *et al.* (2010) and Tari *et al.* (2002) showed that SA was able to alleviate sodium and chloride in plants, so their results support the findings of the present study. Increasing the concentration of soluble sugars under salinity stress may be involved in salt tolerance and adaptation, and also among various organic compounds, sugar creates the major part of the osmotic potential material. Soluble sugars increased salinity in the leaves of the semi-tolerant sugar beet (Jolgeh cultivar) than semi-sensitive one (Shariff cultivar). It means that the Jolgeh involved in osmoregulation and more water absorption by an increase in soluble sugars. Foliar application of SA leads to an increase in soluble sugars that this value was more significant in Shariff due to its semi-sensitive value. Foliar application of SA can take an active metabolic compound of soluble sugar in the formation of new cells, as a mechanism to increase growth under salinity stresse. Accordingly, similar results were reported by Ghader *et al.* (2011).

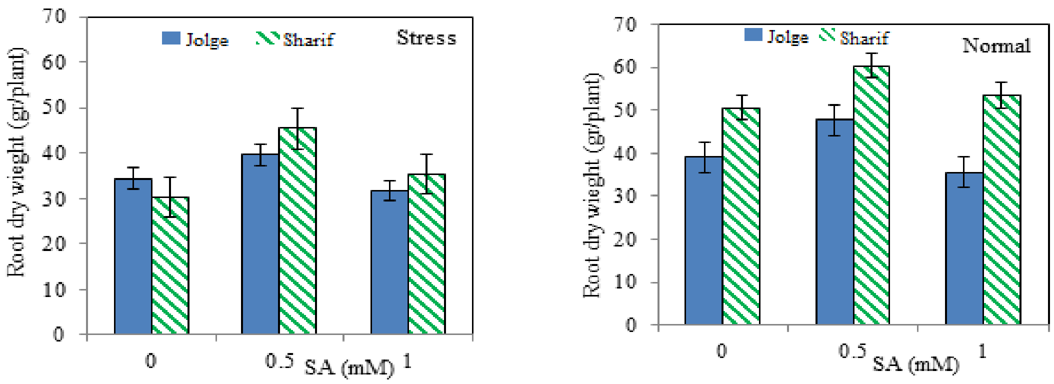

Fig. 1. Interaction between SA, Salinity and root dry weight.

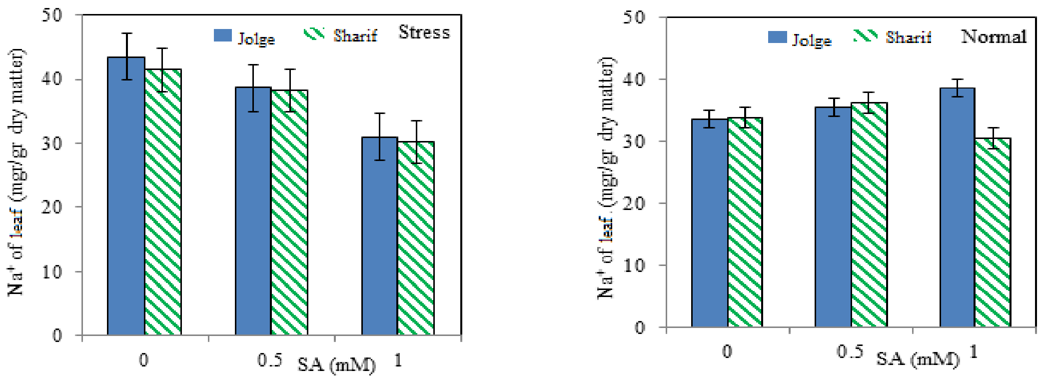

Fig. 2. Interaction between SA, Salinity and Na^+ of leaf.

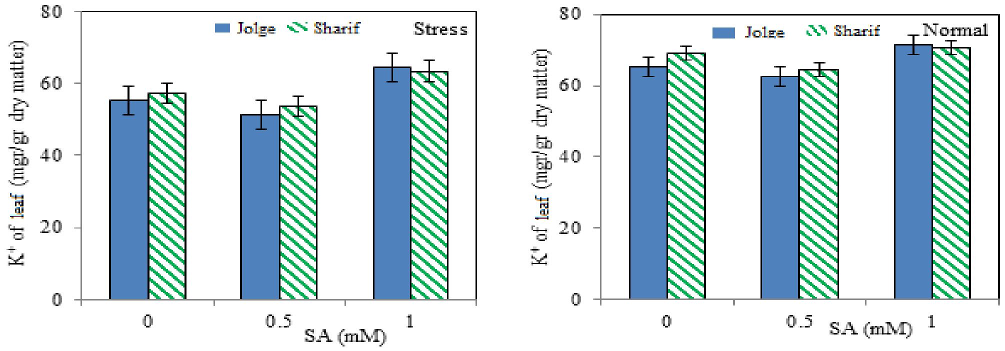

Fig. 3. Interaction between SA, Salinity and K^+ of leaf.

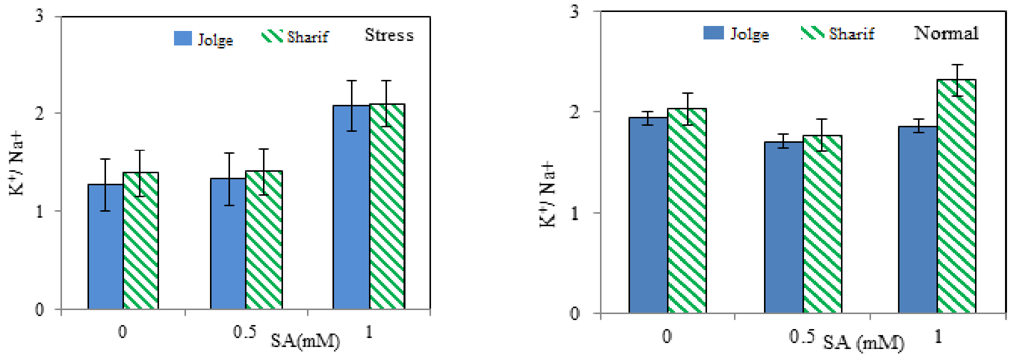

Fig. 4. Interaction between SA, Salinity and K^+ / Na^+.

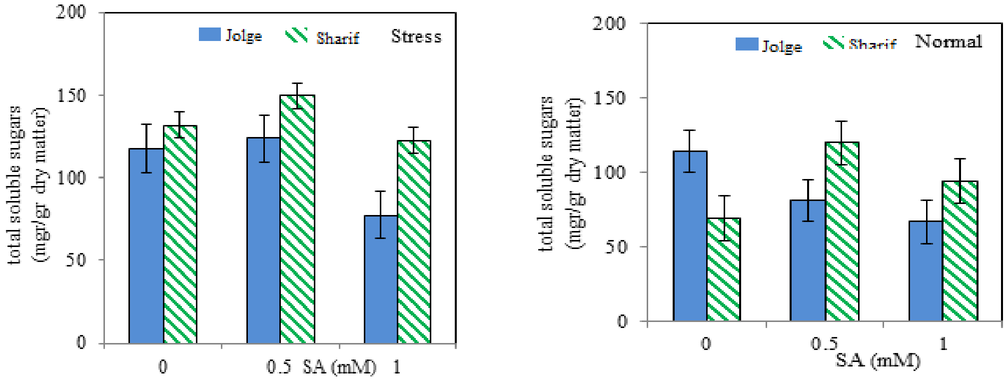

Fig. 5. Interaction between SA, Salinity and total soluble sugar.

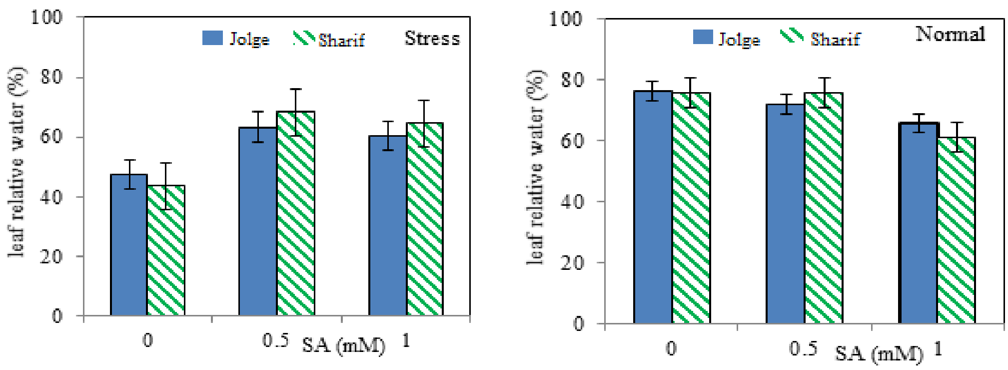

Fig. 6. Interaction between SA, Salinity and leaf relative water.

According to the results of the study using foliar application of SA, relative content of leaf water was increased through salinity condition. Reduction in leaf relative water content is the osmotic stress response in plants and is an appropriate indicators of water status in the plant. SA seems to affect plant water status and leads to increases leaf relative water content in the stress condition. Hayat *et al.* (2010) and Kadioglu *et al.* (2011) reported the similar results. The present study shows that salinity has led to increase membrane permeability. Percentage of electrolyte leakage shows a damage to the cell membrane. Loss of membrane damage caused by the use of SA known as salt tolerance in plants, may be associated with the production of the antioxidant response of the plant to produce antioxidant to reduce oxidation damage. The correlation results of the traits (Table 4) showed that the increase in root dry weight increased shoot dry weight, leaf proline, proline/potassium, and total soluble sugars ($r= 0.53^{**}$, $r=0.79^{**}$, $r=0.88^{**}$, and $r=0.61^{**}$, respectively). High correlation between proline and root dry weight and shoot dry weight showed that by foliar application of SA and increasing the proline, plants are more able to tolerate salinity and subsequently will obtain the higher root dry weight and shoot dry weight. There was found a significant correlation between shoot dry weight and leaf proline and proline/potassium ($r=0.72^{**}$ and $r= 0.74^{**}$, respectively). Potassium increase resulted in leaf sodium reduction and sugars of leaf soluble ($r=0.76^{**}$ and $r=0.64^{**}$, respectively). Potassium deficiency affects starch synthesis which leads to the accumulation of soluble sugars. Moreover, with the increase in leaf potassium potassium /sodium and root potassium were increased ($r=0.92^{**}$ and $r=0.73^{**}$, respectively). There was a significant negative correlation between leaf sodium and potassium /sodium and root potassium ($r= 0.95^{**}$ and $r= 0.62^{**}$, respectively). Also reduction of potassium / sodium has decreased the root sodium and whole soluble sugars ($r=0.50^{**}$ and $r=0.59^{**}$, respectively). The increase in leaf proline increased the potassium/proline ($r=0.96^{**}$). Proline / potassium made in augment in whole soluble sugars ($r=0.50^{**}$). The increase of whole soluble sugars made a significant growth of root sodium ($r= 0.70^{**}$) and reduction of root potassium ($r= 0.63^{**}$). However, rising up sodium accumulation in vacuoles of cells had no effects on the root and shoot dry weight. It seems that foliar application of SA to improve stress tolerance and neutralize the destructive effects of the sodium accumulation needs to uptake more potassium. Higher relative water content could be responsible for the maintenance of stomatal conductance and leads to high transpiration and photosynthesis, finally it results in higher dry weight of shoots and roots. The increase in dry matter of sugar beet in response to SA treatment in salinity conditions may be related to the protective role of membrane that increases plant tolerance to damage. The growth of root system and its healthy maintenance by SA caused to absorb more water and nutrients, which eventually led to the plant growth. In addition, relative permeability of the membrane decreased leaf relative water content ($r=0.94^{**}$).

Shoot weight has a substantial direct effect on the root performance that it can be considered as an effective factor in evaluating root efficiency. The results obtained by Younan *et al.* (1990) have supported this value. Generally in this study, SA application caused a reduction in the electrolyte leakage in high levels of salinity.

According to the results of sodium and potassium, it was found that foliar application of SA increased salinity tolerance of studied cultivars, so that the Jolgeh was changed from sensitive (Asadinasab *et al.*, 2012) to semi-tolerant. Regarding to sensitivity index, Jolgeh and Shariff were diagnosed as semi-tolerant and semi-sensitive cultivars. Foliar application of SA in the semi-sensitive cultivar, was more able to increase proline in salinity stress. These results were consistent with those obtained by Azooz *et al.* (2011).

Table 3. Analysis of variance (ANOVA) for studied parameters.

	df	root dry weight	shoot dry weight	K^+ of leaf	Na^+ of leaf	K^+ /Na+	Proline / K^+	K^+ of root	Na^+ of root	Proline	leaf relative water	Mem brane perme ability	Sensitivity index to stress
Block	2	4.98^{ns}	0.28^{ns}	0.10^{ns}	6.19^{ns}	0.02^{ns}	0.002^{ns}	0.08^{ns}	0.14^{ns}	8.77^{ns}	17^{ns}	1.53^{ns}	0.08^{ns}
SA	2	360^{**}	121^{**}	278^{*}	108^{*}	0.96^{**}	0.16^{**}	4.23^{**}	0.33^{*}	314^{**}	2742^{**}	502^{**}	264^{**}
Block*SA	4	4.03	1.37	0.03	1.10	0.003	0.002	0.02	0.002	7.42	22	0.72	0.15
Variety	1	563^{**}	21.68^{*}	19^{**}	30.8^{**}	0.17^{**}	0.000	0.11^{ns}	1.29^{**}	7.14^{ns}	3161^{**}	0.41^{ns}	4.35^{**}
Level salinity	1	1222^{**}	39.61^{**}	813^{**}	46^{**}	1^{**}	0.64^{**}	4.92^{**}	10^{**}	1366^{**}	8558^{**}	1208^{**}	1573^{**}
Variety *SA	2	42^{*}	4^{ns}	12.33^{**}	14.36^{**}	0.02^{*}	0.005^{ns}	0.48^{ns}	0.004^{ns}	19^{ns}	2682^{**}	7^{**}	33.6^{**}
salinity *SA	2	1.78^{ns}	0.40^{ns}	12.48^{**}	120*	0.33^{**}	0.04^{**}	0.13^{ns}	0.19^{*}	111^{**}	184^{**}	1200^{**}	734^{**}
salinity * Variety	1	342^{**}	0.08^{ns}	0.66^{*}	5.24^{ns}	0.04^{**}	0.01^{ns}	0.03^{ns}	4.01^{**}	59.4^{*}	831^{**}	86^{**}	10.2^{**}
salinity *SA* Variety	2	17^{*}	8.58^{ns}	1.03^{**}	38^{**}	0.05^{**}	0.008^{ns}	0.53^{ns}	0.005^{ns}	28.4^{ns}	1008.8^{**}	2.61^{ns}	2.61^{**}
Secondary error	18	6.42	4.76	0.53	1.86	0.005	0.005	0.18	0.07	16.42	13.44	1.04	0.09
Coefficient variation	-	6.04	14.16	1.03	3.78	3.88	15.92	7.23	4.87	15.32	3.49	2.12	0.48

ns, *, ** and ^{ns} mean significant at 5% ($P<0.05$), 1% ($P<0.01$) and not significant, respectively.

Table 4. The correlations among the studied parameters.

	root dry weight	Shoot dry weight	K^+ of leaf	Na^+ of leaf	K^+ /Na+	K^+ of root	Na^+ of root	Proline	Prol ine/ K^+	total soluble sugars	Mem brane perme ability	leaf relative water
root dry weight	1											
shoot dry weight	0.53^{*}	1										
K^+ of leaf	-0.58^{*}	-0.21^{ns}	1									
Na^+ of leaf	0.07^{ns}	-0.28^{ns}	-0.76^{**}	1								
K^+ /Na+	-0.31^{ns}	0.09^{ns}	0.92^{**}	-0.95^{**}	1							
K^+ of root	-0.45^{ns}	-0.26^{ns}	0.73^{**}	-0.62^{**}	0.72	1						
Na^+ of root	0.01^{ns}	0.03^{ns}	-0.35^{ns}	0.55^{*}	-0.50	-0.41^{ns}	1					
Proline	0.79^{**}	0.74^{**}	-0.24^{ns}	-0.23^{ns}	-0.52	-0.25^{ns}	-0.07^{ns}	1				
Proline/ K^+	0.88^{**}	0.72^{**}	-0.51^{*}	0.01^{ns}	0.02	-0.43^{ns}	0.03^{ns}	0.96^{**}	1			
total soluble sugars	0.61^{**}	0.42^{ns}	-0.64^{**}	0.48^{ns}	-0.25^{ns}	-0.63^{**}	0.70^{**}	0.37^{ns}	0.50^{*}	1		
Membrane permeability	-0.59^{**}	-0.71^{ns}	-0.04^{ns}	0.61^{**}	-0.59*	-0.03^{ns}	0.56^{*}	0.70^{**}	-0.63^{**}	0.13^{ns}	1	
leaf relative water	0.72^{**}	0.80^{**}	0.04^{ns}	-0.61^{**}	-0.39^{ns}	0.01^{ns}	-0.45^{ns}	0.78^{**}	0.69^{**}	0.08^{ns}	-0.94**	1
sensitivity index to stress	0.17ns	0.27^{ns}	0.12^{ns}	-0.11^{ns}	0.1^{ns}	-0.15^{ns}	0.57^{*}	0.22^{ns}	0.15^{ns}	0.64^{**}	0.12^{ns}	0.1^{ns}

**, *Correlation is significant at the 0.01 and level 0.05 level, respectively.

Conclusion

The use of SA in sugar beet plant under salinity stress can stimulate the growth and metabolism of carbohydrates and thus increase tolerance to salinity stress. In general, the results of the present study have showed that foliar application of SA as an economic and easy way can improve the growth characteristics under normal conditions (no stress) and reduce the destructive effects of salinity stress on plant growth. Based on the findings of this study, 0.5 mM concentration of SA is recommended. Moreover, the results have showed that there is a significant positive correlation between sensitivity to stress and root sodium and total soluble. Finally, after applying the SA, the Jolgeh moved from sensitive to semi-tolerant class and also the Sheriff was located in semi-sensitive class.

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