ALLEVIATION OF NaCl STRESS IN SUMMER SQUASH 'ESKANDRANI' BY FOLIAR APPLICATION OF SALICYLIC ACID

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ABSTRACT

The experiment was performed to assess the possibility of overcoming NaCl salinity stress by foliar sprays of summer squash 'Eskandrani' with salicylic acid (SA) at the concentration of 10^{-6} M. NaCl treatment caused reduction of shoot fresh weight, leaf number per plant, fruit yield, concentrations of potassium in aerial parts, and the concentration of chlorophyll in leaves. Plants grown under salt stress conditions had higher shoot sodium concentrations than plants untreated with NaCl. Foliar application of SA ameliorated partly the negative effect of NaCl treatment. The beneficial effect of SA was also observed in non-stressed plants, increasing the shoot potassium accumulation and leaf photosynthetic pigments status, and decreasing sodium accumulation in shoots.

Key words: salinity, salicylic acid, summer squash

INTRODUCTION

Soil fertility is decreasing globally due to enhanced degradation in the form of erosion, nutrient depletion, water scarcity, acidity, salinisation, organic matter depletion and poor drainage (Cakmak 2002). Salinity is one of the main environmental factors responsible for the decreased productivity of a wide variety of crops in arid and semi-arid regions. Each year about 40000 ha of land becomes unavailable for agricultural production worldwide due to salinisation, and approximately 50% of irrigated lands are currently affected by salinity (Hu & Schmidhalter 2005).

Salt stress affects plants metabolism, which results in decreased growth and yields. Excess of salts in the soil solution adversely affects plant growth either through inhibition of water uptake or specific ion effects. Specific ion effects may cause direct toxicity, while the insolubility and competitive absorption of ions may affect the nutritional imbalance of plants (Greenway & Munns 1980). In most cases, soil salinity has been associated with an increase in the uptake rate of Na^+ or reduction of the absorption

rates of K^+ and Ca^{2+} (Neel et al. 2002). Also, a high medium salinity induces serious metabolic perturbations in plants, as it generates reactive oxygen species (ROS), which disturb the cellular redox system in favour of oxidised forms thereby creating an oxidative stress that may damage DNA, inactivate enzymes and cause lipid peroxidation (Smirnoff 1993).

The strategies for alleviation of salt stress include breeding of salt-resistant cultivars, leaching the excess of soluble salts from upper to lower soil depths and flushing soils containing salt crusts at the surface (Qadir et al. 2000). Selection of salt tolerant plants is limited by high variability in soil salinity and environmental interactions. Leaching the excess of soluble salts from soil surface layer requires huge amounts of water and its impact on alleviation of salinity is not stable in growing season, at least in arid and semi-arid regions. Flushing method also does not have practical significance because efficiency of this treatment in alleviation of soil salinity is low. Therefore, the development of methods/strategies to ameliorate deleterious effects of salt tolerance on plants has received considerable

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attention. One of these strategies is the use of salicylic acid (SA) (Hayat et al. 2010).

SA naturally occurs in plants in very low concentration and participates in the regulation of stomatal closure, nutrient uptake, protein synthesis, inhibition of ethylene biosynthesis and transpiration (Khan et al. 2003; Shakirova et al. 2003). SA plays a protective role against plant stress (Güneş et al. 2007). Many studies have showed that SA induces increasing tolerance to salinity and osmotic stress in maize (Tuna et al. 2007; Agam 2013), wheat (Al-Hakimi & Hamada 2001; Arfan et al. 2007), barley (Fayez & Bazaid 2014), mung bean (Nazar et al. 2011; Khan et al. 2014), alfalfa (Palma et al. 2013), Indian mustard (Yusuf et al. 2008), *Arabidopsis* seedlings (Borsani et al. 2001), tomato (Tari et al. 2002, 2004; Szepesi et al. 2005, 2009) and cucumber (Dong et al. 2011; Hao et al. 2012). However, the effect of SA on plant growth and metabolism is still a matter of controversy in regards to different plant species, salt stress intensity and timing of application, as well as used rates (Horváth et al. 2007).

It has been reported that exogenous application of SA was able to enhance the photosynthetic rate and also maintain the stability of membranes, thereby improved the growth of salinity stressed barley (El Tayeb 2005), mung bean (Nazar et al. 2011) and alfalfa (Palma et al. 2013). Kaydan et al. (2007) observed that under both saline and non-saline conditions pre-sowing soaking treatment of seeds with SA positively affected the osmotic potential, shoot and root dry mass, K^+/Na^+ ratio and contents of photosynthetic pigments (chlorophyll a, b and carotenoids) in wheat seedlings. Foliar application of SA improved photosynthetic characteristics of leguminous plants and increased salt tolerance by increasing antioxidant metabolism (Nazar et al. 2011; Palma et al. 2013).

Summer squash is one of the most popular and widely used vegetable crops in the world. Approximately 1.8 million ha are planted yearly with squash, pumpkin and gourd all over the world and yielding 24.62 million tons with an average of $13.7 \text{ tons} \cdot \text{ha}^{-1}$. Egypt produced approximately 559600 tons of squash, pumpkin and gourd with an average of $18.2 \text{ tons} \cdot \text{ha}^{-1}$ (FAOSTAT 2012).

Large part of literature indicates that exogenous application of SA to the salinity-stressed plants can alleviate the toxic effects and improve vegetative and reproductive responses (Tari et al. 2004; Szepesi et al. 2005; El Tayeb 2005; Kaydan et al. 2007; Yusuf et al. 2008). However, there is little information concerning summer squash. Therefore, the aim of the study was to evaluate the effects of foliar application of SA on plants growth and fruits yield of 'Eskandrani' summer squash (*Cucurbita pepo* L.) grown under saline and non-saline conditions.

MATERIALS AND METHODS

The pot experiment was conducted under open-field conditions during the spring (mid of March to the end of May) of 2013 and repeated in 2014. Summer squash 'Eskandrani' seeds were sown in $30 \times 30 \text{ cm}$ (diameter $\times$ depth) plastic pots filled with sand with pH 8.2, electrical conductivity (EC) $0.46 \text{ dS} \cdot \text{m}^{-1}$, Ca^{2+} (0.4 mmol), Mg^{2+} (0.3 mmol), K^+ (0.3 mmol), Na^+ (3.0 mmol), HCO_3^- (1.6 mmol), Cl^- (3.0 mmol) and SO_4^{2-} (0.05 mmol). The environmental conditions during experiment were as follows: a 14 h photoperiod, temperature 35-40/23-26 °C in day/night, and a relative humidity 50-55%.

All the plants were fertigated four times a week, initially with 250 ml of the nutrient solution (20-20-20 NPK plus micronutrients) per pot and from the middle of April to the end of experiment with 500 ml. EC of nutrient solutions for combinations without use of NaCl was $2 \text{ dS} \cdot \text{m}^{-1}$, and $5 \text{ dS} \cdot \text{m}^{-1}$ for combinations with 50 mM NaCl. After 3 weeks of cultivation following treatments were initiated:

1. Plants fertigated with nutrient solution (20-20-20 NPK plus micronutrients) and sprayed twice a week with distilled water (control).
2. Plants fertigated with nutrient solution and sprayed with 10^{-6} M SA solution twice a week.
3. Plants fertigated with nutrient solution with addition of $5 \times 10^{-2} \text{ M}$ NaCl and sprayed twice a week with distilled water.
4. Plants fertigated with nutrient solution with addition of $5 \times 10^{-2} \text{ M}$ NaCl and sprayed twice a week with 10^{-6} M SA solution.

At the end of the experiment each salt-treated pot received 26 g NaCl.

SA concentration (10^{-6} M) was selected as earlier proved beneficial for growth of the same cultivar under field conditions (Elwan & El-Shatoury 2012). The volume of water or SA solution ranged from 20 to 50 ml per pot each time, depending on plant size. SA was initially dissolved in a few drops of dimethyl sulfoxide and the final volume was reached using distilled water. The pH was adjusted with KOH (1.0 N) to a value of 7.0. All sprays were applied in the morning (8:00-9:00 a.m.). Experiment was arranged in completely randomised design. Each treatment was replicated three times and each replicate included six pots.

Plant growth and yield

Fresh weights of fruits were recorded from the second week of April and continued for 4 weeks. Fruits were harvested when reached the size of over 10 cm. After the last harvest, plants were uprooted and the fresh weight of shoots and number of leaves per plant were determined.

Total chlorophyll concentration in leaves

Chlorophyll was determined according to Lichtenthaler and Wellburn (1983) in the third leaf of the top of the 6-week-old seedlings, in three samples per replicate. Results were expressed as $\text{mg} \cdot \text{g}^{-1}$ fresh weight.

Concentrations of K^+ and Na^+ in shoots

Three shoots per replicate were dried in an oven at 70°C for 3 days to determine K^+ and Na^+ concentration with flame photometer, according to Brown and Lilleland (1946). Powdered material was digested using a mixture of sulphuric acid and hydrogen peroxide.

Data analysis

The results were evaluated using two-ways ANOVA. The effects of salt and SA addition were evaluated by Fisher's *F*-test, followed by Duncan's multiple range test for comparing $\text{NaCl} \times \text{SA}$ combinations. All tests were performed at significance levels $p = 0.001, 0.01$ and 0.05 . Calculations were carried out using the software package Statistica TM for Windows version 6.1 (Statsoft 2001, Tulsa, OK, USA). Correlations were evaluated using Correlation Matrices (Statsoft 2001).

RESULTS

In both years of study NaCl-stressed plants had less numbers of leaves, lower fresh weight of shoots and lower fruit yield compared to control plants. Mature leaves of NaCl-treated plants turned from green to yellow and then brown colour, and died at the end of the experiment. Compared to control, plants sprayed with SA had 2.3 and 1.4 times more leaves and 3.2 and 2.5 times higher fresh weight in 2013 and 2014, respectively. NaCl-stressed plants did not yield fruits in 2013 and a few in 2014; thus, SA application enabled fruiting although at very low level (Table 1). Regarding the fruit yield, application of SA increased the yield from 59% to 65% (depending on year) under non-saline conditions. However, the efficiency of SA application on NaCl-stressed plants was more pronounced for increasing the yield in comparison to salt-stressed plants that were not treated with SA (Table 1).

In both seasons, salinity and SA affected leaf chlorophyll concentration, content of K^+ and Na^+ and K^+/Na^+ ratio in shoots (Table 2). Content of leaf chlorophyll and K^+ in shoots, and K^+/Na^+ ratio decreased as a result of NaCl application in both years of study (Table 2). Leaf chlorophyll and K^+ concentrations in shoots were about 30% lower in NaCl-stressed plants in comparison with control, whereas shoot concentration of Na^+ increased by about five times, therefore K^+/Na^+ ratio decreased by about three times. In both years of the study, SA application increased leaf chlorophyll and shoot K^+ concentrations in control plants but did not change shoot Na^+ level. Application of SA on NaCl-treated plants caused increase of leaf chlorophyll, shoot K^+ and K^+/Na^+ ratio, and decrease of Na^+ concentration in shoots but the alleviation did not reach contents present in control plants (Table 2).

Leaves number and fruits weight per plant were positively correlated with the concentration of the total chlorophyll in leaves, and with potassium and K^+/Na^+ ratio in aerial plant parts whereas negatively with Na^+ concentration in aerial plant parts (Table 3).

Table 1. Impact of NaCl and foliar application of salicylic acid (SA) on leaf number, fresh weight of shoots and fruit yield of summer squash 'Eskandrani'

NaCl	Salicylic acid	Leaf no. per plant		Fresh weight of shoots (g·plant ⁻¹)		Fresh weight of fruits (g·plant ⁻¹)	
		2013	2014	2013	2014	2013	2014
0	0	11.6 ab	12.6 ab	169 a	178 a	70 b	75 b
	10 ⁻⁶ M	13.2 a	14.8 a	179 a	184 a	116 a	119 a
5 × 10 ⁻² M	0	4.0 c	7.0 c	24.6 c	30.6 c	0.0 d	4.0 d
	10 ⁻⁶ M	9.2 b	10.0 b	80.5 b	88.8 b	20.0 c	23.3 c
Significance of tested effects							
NaCl		***	***	***	***	***	***
Salicylic acid (SA)		*	*	**	**	***	***
NaCl*SA		NS	NS	*	**	*	*

Note: weight of died plants and no fruits was counted as zero

Values are the means of 6 samples per replicate (three replicates). Values followed by the same letter within a column are not significantly different at the 0.05% level of probability according to Duncan's multiple range test

***, ** and * significant at 0.1%, 1% and 5%; NS: not significant

Table 2. Impact of NaCl and foliar application of salicylic acid (SA) on total chlorophyll in leaves and potassium and sodium concentration in shoots of summer squash 'Eskandrani'

NaCl	Salicylic acid	Total chlorophyll (mg·g ⁻¹ FW)		K ⁺ (mg·g ⁻¹ DW)		Na ⁺ (mg·g ⁻¹ DW)		K ⁺ /Na ⁺ ratio	
		2013	2014	2013	2014	2013	2014	2013	2014
0.0	0.0	0.85 b	0.91 b	32.0 b	33.5 b	0.95 c	1.05 c	33.8 b	32.1 b
	10 ⁻⁶ M	1.04 a	1.09 a	36.5 a	37.5 a	0.85 c	0.95 c	43.1 a	39.6 a
5 × 10 ⁻² M	0.0	0.61 c	0.64 d	21.5 d	22.5 d	5.75 a	6.25 a	3.7 d	3.6 d
	10 ⁻⁶ M	0.76 b	0.79 c	28.0 c	28.5 c	4.25 b	4.75 b	6.6 c	6.0 c
Significance of tested effects									
NaCl		**	***	***	***	***	***	***	***
Salicylic acid (SA)		*	**	**	**	**	**	***	**
NaCl*SA		NS	*	*	*	*	*	**	*

Values are the means of three samples per replicate (three replicates). Values followed by the same letter within a column are not significantly different at the 0.05% level of probability according to Duncan's multiple range test

***, ** and * significant at 0.1%, 1% and 5%; NS: not significant

Table 3. Correlation coefficients between the morphological traits and chlorophyll content in leaves, and K⁺ and Na⁺ contents in the shoots

	Chlorophyll	K ⁺	Na ⁺	K ⁺ /Na ⁺
Leaf No.	0.8***	0.8***	-0.7**	0.7**
Shoot fresh weight	0.8***	0.9***	-0.9***	0.9***
Fruit fresh weight	0.9***	0.9***	-0.9***	0.9***

*** and ** significant at 0.1% and 1%

DISCUSSION

Morphological traits – leaves number and fruits yield in addition to plant fresh weight and chemical parameters, such as chlorophyll and K⁺ contents as well as K⁺/Na⁺ ratio decreased as a result of soil application of 50 mM NaCl, whereas Na⁺ content increased. This is in accordance with findings in experiments on strawberry (Kaya et al. 2001), cucumber (Kaya et al. 2003; Dong et al. 2011), melon (Kaya et al. 2007), eggplant (Elwan

2010), maize (Agami 2013), mung bean (Khan et al. 2014) and barley (Fayez & Bazaid 2014). It is well known that salt stress suppresses plant growth due to reduction in water availability or to excessive Na^+ ion accumulation in plant tissues (Güneş et al. 1995) and reduce the availability of nutrients, especially K^+ (Liebersbach et al. 2004; Cakmak 2005). The reduction in photosynthesis has been associated with the disturbance in homeostasis of Na^+ ions and essential mineral nutrients (Güneş et al. 2007; Keutgen & Pawelzik 2009), stomatal closure (Steduto et al. 2000) and the increased production of ROS in chloroplasts (Meneguzzo et al. 1999), which causes membrane damage.

Amelioration of the adverse effects of salinity stress on plants growth and yield by application of SA on summer squash was not reported. In our experiment, the foliar spraying with SA was beneficial for plants growth, increased fresh weight and chlorophyll content that might be due to the selective uptake and accumulation of more potassium and less sodium. Therefore, the adaptive mechanism of plants treated with 10^{-6} M SA for salinity may be explained in part by more selective accumulation of nutrients, especially K^+ . These results are in agreement with the findings of Binzel and Reuveni (1994), Güneş et al. (2005) and Al-Hakimi and Hamada (2001) who reported that salt-tolerant plants exhibit greater K^+/Na^+ ratio than susceptible plants. In our study selectivity in ions accumulation was also connected with higher tolerance to salt stress caused by SA application. The results of Azooz (2009) and Kha et al. (2014) showed that SA application modifies Na^+ and K^+ selectivity uptake and decreases Na^+/K^+ ratio, which helps in lowering membrane damage. The protective role of SA in membrane integrity and regulation of ion uptake has also been reported by El-Tayeb (2005), Güneş et al. (2007), Nazar et al. (2011). SA might be involved in mobilisation of internal tissue nitrate and chlorophyll biosynthesis that strengthens the functional state of the photosynthetic machinery in plants (Shi et al. 2006; Elwan & El-Shatoury 2012; Nazar et al. 2011; Khan et al. 2014; Fayez & Bazaid 2014). The above statements were confirmed in our study by increasing the leaf number, plants fresh weight and chlorophyll content under saline and non-saline conditions.

In conclusion, salinity stress caused by NaCl inhibited plants growth and fruits yield of summer squash 'Escandrani'. Foliar application of SA at the concentration of 10^{-6} M alleviated the salt stress. The foliar application of SA was also beneficial for non-stressed plants. It caused the K^+ accumulation in shoots, increased chlorophyll concentration and decreased Na^+ accumulation in shoots.

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