



Influence of salicylic acid pre-treatment on emergence and early seedling growth of cucumber (*Cucumis sativus*) under salt stress

B. Baninasab^{*}, M.R. Baghbanha

Department of Horticulture, College of Agriculture, Isfahan University of Technology, Isfahan 84156-83111, Iran.

^{*}Corresponding author. E-mail: bbanin@cc.iut.ac.ir

Received 13 March 2012; Accepted after revision 28 September 2012; Published online 1 February 2013

Abstract

Salicylic acid (SA) is a common, plant-produced signal molecule that is responsible for inducing tolerance to a number of biotic and abiotic stresses. An experiment was therefore conducted to test whether the application of SA at various concentrations (0, 0.25, 0.50 or 1.0 mM) through a seed-soaking method would protect cucumber (*Cucumis sativus* L.) seedlings subjected to salt stress. After soaking, seeds were sown in plastic trays filled with washed fine sand. The trays were then placed in the greenhouse and watered with different NaCl solutions (0, 50, 100 and 150 mM), for a period of 4 weeks. SA improved the majority of physiological (relative leaf chlorophyll content, leaf relative water content and chlorophyll fluorescence ratio) and morphological parameters (total emergence, mean emergence time, shoot and root dry weights) of cucumber seedlings subjected to salt stress. SA improved the injuries caused by salt stress by promoting K⁺ accumulation and preventing increases in leaf electrolyte leakage and Na⁺ contents of leaves and roots. The best protection appeared to be obtained from SA applied by seed-soaking method at 1.00 mM.

Keywords: Cucumber; Salt stress; Salicylic acid; 2-hydroxybenzenecarboxylic acid.

Introduction

Salt stress is an important growth-limiting factor for most non-halophytic plants. High level of salts cannot be tolerated by most crops, a fact that

severely limits the use of salt-affected soils for crop production (Tiwari et al., 2010). The United Nations Food and Agriculture Organization and the United Nations Environment Programme estimate that there are currently 4 million square kilometers of salinized land whereas approximately 20% of agricultural land and 50% of cropland in the world is salt-stressed threatening agricultural productivity (Ravindran, 2007; Rozena and Flowers, 2008). Natural boundaries imposed by soil salinity also limit the caloric and the nutritional potential of agricultural production (Yokoi et al., 2002). Estimates suggest that about 34 million ha, including 4.1 million ha of irrigated land, are salt-affected in Iran as the consequence of naturally occurring phenomena (causing primary or fossil salinity and/or sodicity) and anthropogenic activities (Qadir et al., 2008).

High external NaCl concentrations in the soil limit plant growth by exerting both osmotic and ionic stresses, which results in nutritional deficiencies and metabolic imbalance (Zhu, 2002). Osmotic stress is caused primarily by water deficit in plant tissues. Ionic stress is caused by the accumulation of Na⁺ and Cl⁻ ions and by the disturbance of the K⁺/Na⁺ ratio in plant cells (Blumwald et al., 2000). Na⁺ ions not only diminish potassium uptake by roots (Sairam and Tyagi, 2004) but are also toxic to enzymes when accumulated at high levels in the cytoplasm (Hasegawa et al., 2000). Salt stress leads to inhibition of growth and development, reduction in photosynthesis, respiration and protein synthesis and disturbs nucleic acid metabolism (Bray et al., 2000). In many crop plants, seed germination and early seedling growth are the most sensitive stages to abiotic stresses such as salinity (Sivritepe et al., 2003).

The development of methods to induce stress tolerance in plants is vital and should receive considerable attention. Approaches to develop stress-tolerant plants include genetic engineering (McKerise et al., 1988), breeding (Vettakkorumakankav et al., 1999), *in vitro* selection (Shankhdhar et al., 2000) and the use of growth regulators (Baninasab, 2009).

Salicylic acid (SA), a plant hormone-like substance, has been shown to be an important signal molecule for modulating plant responses to environmental stress (Breusegem et al., 2001). SA may play an important role in the seed germination (Korkmaz, 2005), stomatal closure, ion uptake and transport (Gunes et al., 2005), membrane permeability (Hayat and Ahmad, 2007) and photosynthetic, transpiration and growth rates (Khan et al., 2003). The role of SA in defense mechanisms under biotic and/or abiotic stress suggests that it

could also alleviate salt stress in plants (Stevens et al., 2006). Exogenous application of SA was reported to have effects on a wide range of physiological processes including increased cold germination tolerance in *Capsicum annuum* (Korkmaz, 2005), salinity tolerance in *Hordeum vulgare* (El-Tayeb, 2005), improved heat shock tolerance in *Sinapis alba* (Dat et al., 1998) and decreased inhibitory effects of drought stress on *Cucumis sativus* (Baninasab, 2010). SA is known to play an important role in modulating the redox balance across membranes, thereby counteracting the negative effects of reactive oxygen species (ROS) generated by oxidative stress (Yang et al., 2004) by increasing the activity of anti-oxidant enzymes such as superoxide dismutase (Singh and Usha, 2003). SA is also involved in the functioning of different anti-stress programmes in plants under osmotic stress, for example by increasing the accumulation of lectins in *Triticum aestivum* (Shakirova and Bezrukova, 1997) and the rapid activation of a 48-kDa protein kinase in suspension cell cultures of *Nicotiana tabacum* (Mikolajczyk et al., 2000). However, these results were contradictory and depended on the developmental phase of the plants (Borsani et al., 2001) or on the experimental conditions used (Nemeth et al., 2002).

Cucumber (*Cucumis sativus*) is an important vegetable crop for human nutrition in the world and has been classified as salt sensitive (Stepien and Klobus, 2006). Salinity is common in many soils of Iran where cucumbers are grown extensively and affect the emergence and growth of plants and reduces the yield of marketable fruit. Therefore, the objectives of this work were to: (1) determine the effect of salt stress on emergence and early seedling growth of cucumber plants and (2) test whether seed soaking of cucumber seeds in varying concentrations of SA could mitigate the adverse affect of salt stress.

Materials and Methods

Plant material and cultural practice

Cucumber seeds (*Cucumis sativus* L. cv Super Aston; As grow Vegetable Seeds, Saticoy, CA, USA) were surface-sterilized in a 1% (v/v, active ingredient) sodium hypochlorite solution for 10 min to eliminate possible seed-borne micro-organisms, then rinsed for 1 min under running water prior to drying for 30 min at room temperature.

Seeds were soaked in an aerated solution of SA at 0 (control), 0.25, 0.50 or 1.0 mM for 24 h at 23 ± 2 °C under dark conditions. After soaking, seeds were put in a wire mesh strainer and washed with tap water for 3 min and then rinsed with distilled water. Following this, seeds were dried between two filter papers and sown in plastic trays filled with washed fine sand. The trays were then transferred to the greenhouse with average temperature of 27/19 °C (day/night) and natural light (December and January) for a period of 4 weeks.

The layout was a 4×4 factorial experiment in a complete randomized design with four replications and one hundred seeds per replication (plastic tray).

Imposition of salt stress

Salinity treatments were established by adding 0, 50, 100 and 150 mM of NaCl to a half-strength Hoagland's solution (Hoagland and Arnon, 1950). Electrical conductivities of these solutions after adding 0, 50, 100 and 150 mM NaCl were 1.32, 6.41, 11.18 and 15.53 ds m⁻¹, respectively. After the trays were placed in the greenhouse, the plastic trays were irrigated daily with different saline solutions up to field capacity. To prevent of salt accumulation, trays were leached with distilled water (after every three irrigations) (Hajihashemi et al., 2007). At the end of 4 weeks plants were harvested and evaluated for their response to salinity.

Emergence parameters

The plastic trays were inspected daily and seedling emergence recorded as the appearance of the cotyledons. Total number of emerged seedlings in each replicate was determined and evaluated as percentage, in calculation of total emergence (TE). Mean emergence time (MET) was calculated according to the equation of Ellis and Robert (1981).

Shoot and root dry weights

At the end of the experiment, the seedlings were harvested from trays and the media carefully washed from the root systems. Shoot and root dry weights (SDW and RDW, respectively) were determined after 48 h of drying plant material at 80 °C.

Relative leaf chlorophyll content (RLCC)

Immediately before seedlings were harvested, RLCC of the youngest fully-expanded leaf was determined using a chlorophyll content meter (Hansatech Instrument Ltd., King's Lynn, UK). Chlorophyll meter readings were used as relative values for chlorophyll content.

Electrolyte leakage

Electrolyte leakage was used to assess membrane permeability. This procedure was based on Lutts et al. (1996). Electrolyte leakage was measured using an electrical conductivity meter (CC-501, Elmetron, Zabrze, Poland). Ten leaf discs of five randomly chosen plants per replicate sample were taken from the youngest fully-expanded leaf before harvesting and drying. After three washes with distilled water to remove surface contamination, the leaf discs were then placed in a test tube containing 10 ml distilled water. These samples were incubated at room temperature, on a shaker, for 24 h. Electrical conductivity (EC) of the solution (EC_1) was then read after incubation. The same samples were then placed in a boiling water bath for 20 min and a second EC reading (EC_2) was taken after cooling the solution to room temperature. Electrolyte leakage was then calculated as: EC_1/EC_2 and expressed as a percentage.

Leaf relative water content (LRWC)

Two leaves were collected from the young fully expanded leaves of three plants per replicate. Individual leaves were first detached from the stem and then weighed to determine fresh weight (FW). In order to determine turgid weight (TW), leaves were floated in distilled water inside a closed petri dish. Leaf samples were weighed periodically, after gently wiping the water from the leaf surface with the tissue paper until a steady state achieved. At the end of the imbibition period, leaf samples were placed in a pre-heated oven at 80 °C for 48 h, in order to determine dry weight (DW). Values of FW, TW and DW were used to calculate LRWC using the equation below (Kaya et al., 2003):

$$LRWC (\%) = [(FW - DW) / (TW - DW)] \times 100$$

Chlorophyll fluorescence

Measurements of the maximum efficiency of photosystem II photochemistry (F_v/F_m) were performed by using a Plant Efficiency Analyzer (Hansatech Instrument Ltd, King's Lynn, Norfolk, UK) after 30 min of dark adaptation. The F_v/F_m ratio was calculated as:

$$F_v/F_m = (F_m - F_o) / F_m$$

Where F_m and F_o are the maximum and basal fluorescence yields, respectively, of dark-adapted leaves.

Analysis of ion concentration

To determine ion concentration, leaf and root samples were oven-dried at 80 °C for 48 h, then ground to pass through a 30-mesh screen. The ground material (0.5 mg) were ashed in a muffle furnace at 550 °C for 5 h and the ash was then dissolved in 10 ml 2 M HCl and made up to 100 ml with distilled water. Na^+ and K^+ were determined using a flame photometer (PEP7, Jenway, Dunmow, UK).

Statistical analysis

Data were analysed for significant differences using a factorial analysis of variance, with NaCl level and SA concentration as the main factors. Statistical analysis was performed using the MSTATC statistical version 1.4 software programme (Michigan State University, East Lansing, MI, USA) and means were compared using the least significant differences (LSD) test at $P=0.05$.

Results and Discussion

Our results showed that TE was significantly affected by salt stress and SA pre-treatment (Table 1). Salinity significantly decreased TE, with the largest decrease (49.4%) in TE with 150 mM of NaCl (Table 2). Application of SA significantly increased TE. The most TE was obtained from seeds soaked with 0.50 mM SA (78.9%), which was 17.24% more than the control

(Table 2). However, a significant interaction occurred between salinity and SA concentration. The maximum TE values observed with the 0.50 mM SA in 50 mM of NaCl (90.0%) and with the 1.00 mM SA in 100 and 150 mM of NaCl (76.2 and 56.9%, respectively) (Table 2). Increased salinity also significantly increased MET and delayed the emergence of cucumber seedlings (Table 2). SA had a positive effect on MET of seedlings. Seedlings from soaked seeds with SA emerged earlier than control seeds. However the earliest seedling emergence was obtained from seeds soaked with 0.50 and 1.00 mM SA (5.9 days) which was 24.36% less than control seeds (Table 2). There was a significant interaction between salinity and SA concentration (Table 1). The least MET value observed with the 0.50 mM SA in 50 mM of NaCl (5.2 days), while the emergence observed with the 1.00 mM SA in 100 and 150 mM of NaCl (6.3 and 6.4 days, respectively) (Table 2). A negative effect of salinity on germination and emergence has been reported for several vegetables species (Sivritepe et al., 2003; Jamil et al., 2006). Such a response might be related to the inhibitory effect of the solution low osmotic potential and/or to ionic toxicity (Zhu, 2002). Earlier studies have shown that salinity reduces protein hydration (Kramer, 1983) and induces changes in the activities of many enzymes (Dubey and Rani, 1990) in germinating seeds. However, according to the above results, pre-treatment of cucumber seeds with SA improved seedlings emergence and decreased the emergence time under salinity conditions. This result is in agreement with these reported by Shakirova et al. (2003) and El-Tayeb (2005), who found that SA treatment increased seedling emergence percentage and decreased the emergence time under salinity conditions. This might be explained by the fact that SA enhances the activity of hydrolases, which increased the reserve breakdown and earlier start of germination (Shakirova et al., 2003). Karthiresan et al. (1984) and Zhang et al. (1999) also reported that increase in emergence percentage in seeds primed with SA under saline conditions may be due to enhanced oxygen uptake, increased α -amylase activity and the efficiency of mobilizing nutrients from the cotyledons to the embryonic axis and increased the contents of soluble sugar, protein and free amino acids.

Table 2. Effect of SA pre-treatment on total emergence (TE) and mean emergence time (MET) of cucumber seedlings under salt stress.

NaCl salinity (mM)	SA (mM)	TE (%)	MET (days)
0	0	96.6 ^{a*}	5.2 ^{hi}
	0.25	96.0 ^a	5.1 ⁱ
	0.50	97.7 ^a	5.3 ^{hi}
	1.00	94.9 ^a	5.2 ^{hi}
50	0	82.7 ^d	7.0 ^c
	0.25	88.6 ^{bc}	5.7 ^{gh}
	0.50	90.0 ^b	5.2 ^{hi}
	1.00	85.1 ^{cd}	5.6 ^{g-i}
100	0	54.0 ^{gh}	8.6 ^b
	0.25	70.0 ^f	6.8 ^{c-e}
	0.50	73.8 ^e	6.10 ^{fg}
	1.00	76.2 ^e	6.3 ^{ef}
150	0	36.0 ⁱ	10.3 ^a
	0.25	50.7 ^h	7.10 ^c
	0.50	54.0 ^{gh}	6.9 ^{cd}
	1.00	56.9 ^g	6.4 ^{d-f}
Means for NaCl salinity			
0		96.3 ^a	5.2 ^d
50		86.6 ^b	5.9 ^c
100		68.5 ^c	6.9 ^b
150		49.4 ^d	7.7 ^a
Means for SA concentration			
	0	67.3 ^c	7.8 ^a
	0.25	76.3 ^b	6.2 ^b
	0.50	78.9 ^a	5.9 ^c
	1.00	78.3 ^a	5.9 ^c

* Values followed by the same lower-case letters within a column are not significantly different at $P \leq 0.05$.

RLCC was significantly affected by both salinity and SA pre-treatment (Table 1). RLCC values were significantly decreased with the increasing salinity stress (Table 3). These observations are consistent with those of Stepien and Klobus (2006) and Yildirim et al. (2008) who indicated that chlorophyll content considerably decreased in the leaves of cucumber plants with increasing NaCl concentration. The decrease may be due to the formation of proteolytic enzymes such as chlorophyllase, which is responsible for the chlorophyll degradation (Sabater and Rodriguez, 1978). All concentrations of SA increased RLCC values significantly compared with the control. However, 1.00 mM SA was most effective (12.48) (Table 3).

There was a significant interaction between salinity and SA concentrations (Table 1). The highest RLCC value was obtained from seedlings pre-treated with 0.50 mM SA in 50 mM NaCl (14.53), while the maximum RLCC values observed with the 1.00 mM SA in 100 and 150 mM of NaCl (12.83 and 8.45, respectively) (Table 3). This result is in agreement with those reported by El-Tayeb (2005) in barley, Gunes et al. (2007) in maize and Yildirim et al. (2008) in cucumber, who found that SA-treated plants had less decrease in chlorophyll concentration than the non-SA-treated plants under salt stress. The higher RLCC of SA-treated cucumber leaves may be related to its influence on the endogenous cytokinin content. The SA-treated plants synthesized more cytokinin (Sakhabutdinova et al., 2003) that in turn enhances chloroplast differentiation and chlorophyll biosynthesis and prevents chlorophyll degradation Fletcher et al. (1982).

An increase in percent conductivity indicates leakiness of ions due to a loss of membrane integrity. This is an inherent feature of plants which are exposed to stresses. Salinity significantly increased leaf electrolyte leakage, with the largest increase (41.49%) in leaf electrolyte leakage with 150 mM of NaCl (Table 3). Similar reports were presented by Parida and Das (2005) and Yildirim et al. (2008) for several crops. High concentrations of NaCl caused membrane disorganization (Hasegawa et al., 2000). Mansour et al. (2002) pointed out that molar percentages of sterols and phospholipids decreased with increasing salinity. Electrolyte leakage enables cell membrane injury to be assessed when plants are subjected to salinity stress. Maintaining integrity of the cellular membranes under salt stress is considered an integral part of the salinity tolerance mechanism (Stevens et al., 2006). Application of SA significantly decreased leaf electrolyte leakage. However the lowest leaf electrolyte leakage was obtained from seedlings pre-treated with 0.50 mM SA (18.39%) which was 38.31% less than control (Table 3). There was a significant interaction between salinity and SA concentrations (Table 1). The lowest leaf electrolyte leakage value was obtained from seedlings pre-treated with 0.50 mM SA in 50 and 100 mM NaCl (10.06 and 18.39%, respectively), while the minimum leaf electrolyte leakage values observed with the 1.00 mM SA in 150 mM of NaCl (33.14%) (Table 3). This result is consistent with that reported by Stevens et al. (2006) for tomato and Yildirim et al. (2008) for cucumber, who determined that SA facilitated the maintenance membrane functions. Data also exist showing that SA causes increases in the activities of anti-oxidant enzymes which, in turn protect plants against the generation of ROS and membrane injury or may result in the synthesis of other substances which have a protective effect on plants growing under stress (Xu et al., 2008).

Table 3. Effect of SA pre-treatment on relative leaf chlorophyll content (RLCC), electrolyte leakage (EC_1/EC_2), leaf relative water content (LRWC), chlorophyll florescence ratio (F_v/F_m), shoots dry weight (SDW) and root dry weight (RDW) of cucumber seedlings under salt stress.

NaCl salinity (mM)	SA (mM)	RLCC	EC_1/EC_2 (%)	LRWC (%)	F_v/F_m	SDW (mg)	RDW (mg)
0	0	13.53 ^{c*}	8.64 ^{ij}	89.21 ^a	0.805 ^c	1532 ^{bc}	162 ^{b-f}
	0.25	13.47 ^c	8.17 ⁱ	89.55 ^a	0.817 ^a	1566 ^{bc}	168 ^{b-e}
	0.50	14.80 ^{ab}	8.47 ^{ij}	88.61 ^{ab}	0.820 ^a	1822 ^{ab}	180 ^{a-c}
	1.00	15.16 ^a	8.80 ^{ij}	89.36 ^a	0.810 ^b	1933 ^a	208 ^a
50	0	11.47 ^c	19.11 ^{fg}	81.51 ^c	0.735 ^f	1138 ^{d-f}	145 ^{d-f}
	0.25	12.73 ^d	12.13 ^h	86.45 ^{bc}	0.760 ^e	1379 ^{cd}	158 ^{c-f}
	0.50	14.53 ^b	10.06 ^{h-j}	87.49 ^{ab}	0.775 ^d	1562 ^{bc}	174 ^{b-d}
	1.00	13.47 ^c	10.85 ^{hi}	86.52 ^{bc}	0.772 ^d	1550 ^{bc}	190 ^{ab}
100	0	8.47 ^f	34.17 ^{cd}	74.31 ^{fg}	0.615 ^j	870 ^{ef}	112 ^{gh}
	0.25	10.94 ^e	23.18 ^e	81.45 ^e	0.662 ⁱ	1158 ^{de}	131 ^{fg}
	0.50	11.39 ^e	18.39 ^g	82.61 ^{de}	0.670 ^h	1369 ^{cd}	150 ^{c-f}
	1.00	12.83 ^d	21.58 ^{ef}	84.60 ^{cd}	0.712 ^g	1362 ^{cd}	175 ^{b-d}
150	0	4.14 ⁱ	57.31 ^a	65.47 ^h	0.497 ^l	511 ^g	67.00 ⁱ
	0.25	6.49 ^h	38.90 ^b	72.45 ^g	0.565 ^k	823 ^{fg}	86 ^{hi}
	0.50	7.20 ^g	36.62 ^{bc}	73.45 ^g	0.615 ^j	962 ^{ef}	109 ^{gh}
	1.00	8.45 ^f	33.14 ^d	76.43 ^f	0.662 ⁱ	1009 ^{ef}	137 ^{e-g}
Means for NaCl salinity							
0		14.24 ^a	8.52 ^d	89.18 ^a	0.813 ^a	1713 ^a	180 ^a
50		13.05 ^b	13.04 ^c	85.50 ^b	0.761 ^b	1407 ^b	167 ^a
100		10.91 ^c	24.33 ^b	80.74 ^c	0.665 ^c	1190 ^c	142 ^b
150		6.57 ^d	41.49 ^a	71.95 ^d	0.585 ^d	826 ^d	100 ^c
Means for SA concentration							
	0	9.40 ^d	29.81 ^a	77.63 ^c	0.663 ^d	1013 ^c	122 ^c
	0.25	10.91 ^c	20.59 ^b	82.48 ^b	0.701 ^c	1231 ^b	136 ^c
	0.50	11.98 ^b	18.39 ^c	83.04 ^b	0.720 ^b	1429 ^a	154 ^b
	1.00	12.48 ^a	18.59 ^c	84.23 ^a	0.739 ^a	1463 ^a	178 ^a

* Values followed by the same lower-case letters within a column are not significantly different at $P \leq 0.05$.

LRWC was significantly affected by both salinity and SA pre-treatment (Table 1). Increasing the concentrations of NaCl from 0 to 150 mM lowered LRWC in cucumber plants (Table 3). LRWC in leaves is known as an alternative measure of plant water status, reflecting the metabolic activity in tissues (Gonzalez and Gonzalez-Vilar, 2001). Decrease in LRWC indicated a loss of turgor that resulted in limited water availability for the cell extension process in sugar beet (Katerji et al., 1997). Similar reports have been made for many plant species under salinity stress conditions (Srivastava et al., 1998; Stepien and Klobus, 2006). This decrease in LRWC

could be because of lower water availability under stress conditions (Shalhevet, 1993), or root systems which are not able to compensate for water lost by transpiration through a reduction of the absorbing surface (Gadallah, 2000). All concentrations of SA increased LRWC significantly compared with the control. However, 1.00 mM SA was most effective (84.23%) (Table 3). There was a significant interaction between salinity and SA concentrations (Table 1). The highest LRWC was obtained from seedlings pre-treated with 0.50 mM SA in 50 mM NaCl (87.49%), while the highest LRWC values observed with the 1.00 mM SA in 100 and 150 mM of NaCl (84.60 and 76.43%, respectively) (Table 3). In agreement with the present results, barley seedlings treated with SA showed higher LRWC than the control seedlings following salt stress (El-Tayeb, 2005). Szepesi et al. (2005) also found that exogenous SA increased water potential and LRWC of salt stressed tomato plants. This phenomenon may be attributed that SA can increase leaf diffusive resistance and lower transpiration in plants (Yildirim et al., 2008).

The F_v/F_m was significantly affected by salinity and SA pre-treatment (Table 1). The F_v/F_m significantly decreased with the increasing salinity stress (Table 3). SA treatment significantly increased F_v/F_m in the cucumber leaves. The most F_v/F_m was obtained from leaves of the seedlings pre-treated with 1.00 mM SA (0.739), which was 11.52% more than the control (Table 3). There was a significant interaction between salinity and SA concentrations (Table 1). The highest F_v/F_m was obtained from seedlings pre-treated with 0.50 mM SA in 50 mM NaCl (0.775), while the highest F_v/F_m values observed with the 1.00 mM SA in 100 and 150 mM of NaCl (0.712 and 0.662, respectively) (Table 3). The F_v/F_m ratio in SA-treated plants was higher than in control plants during salt stress, indicating that SA reduced salt-induced photoinhibition by protecting photosystem II. The chlorophyll fluorescence ratio (F_v/F_m) is correlated with the efficiency of leaf photosynthesis. A decline in this ratio provides an indicator of photo-inhibitory damage caused by the incident photon flux density when plants are subjected to a wide range of environmental stresses (Bjorkman and Demming, 1987). Maintenance of the F_v/F_m ratio in SA-treated plants under stress has been observed in previous studies (Szepesi et al., 2005; Baninasab, 2010).

The SDW and RDW were significantly affected by salinity and SA pre-treatment, but not their interaction (Table 1). SDW and RDW were significantly decreased with the increasing salinity stress (Table 3). Earlier studies have shown that NaCl treatment decreased the some growth parameters such as fresh weight of shoot and root of plants (Zhu et al., 2004;

Yildirim et al., 2008; Mori et al., 2011). Application of SA significantly increased SDW and RDW. The most SDW and RDW were obtained from seedlings pre-treated with 1.00 mM SA (1463 and 178 mg, respectively), which was 44.42 and 45.90% more than the controls, respectively (Table 3). From the RLCC results, it can be inferred that the higher chlorophyll contents in leaves, leading to higher rates of photosynthesis, might have increased SFW and RFW in the SA pre-treated seedlings (Table 2). Khan et al. (2003) also reported that SA increase carbon dioxide assimilation and photosynthetic rate. Our results in agreement with those of Stevens et al. (2006) in tomato and Gunes et al. (2005) in maize who showed that SA treatments ameliorated the negative effect of salt stress on fresh and dry weight of plants. Shakirova et al. (2003) indicated that SA treatments reduced the damaging action of salinity on wheat seedlings growth, rising indoleacetic acid content and enhancing of cell division and extension of root cell. Khan et al. (2003) reported that SA stimulated the root formation of some crops. Gunes et al. (2005) recorded that increases in fresh and dry matter of salt stressed plants in response to SA might be related to the induction of antioxidant response and protective role of membranes that increase the tolerance of plant to damage.

The salinity and SA pre-treatment significantly affected Na^+ and K^+ contents in leaves and roots (Table 1). The applied NaCl induced Na^+ accumulation in leaves and roots of the cucumber seedlings, the highest Na^+ accumulation was consistently displayed in plants subjected to the highest salinity (17.51 and 20.37 mg g^{-1} , respectively) (Table 4). All concentrations of SA decreased Na^+ accumulation in leaves and roots significantly compared with the controls. However, 1.00 mM SA was most effective (9.03 and 10.73 mg g^{-1} , respectively) (Table 4). Simultaneously, the accumulation of K^+ in leaves and roots decreased gradually with the rise of salinity (Table 4). SA pre-treatment increased K^+ accumulation in leaves and roots significantly compared with the controls. However, the most K^+ accumulation in leaves and roots were observed in seedlings pre-treated with 1.00 mM SA (34.85 and 9.29 mg g^{-1} , respectively), which was 21.94 and 6.05% more than the controls, respectively (Table 4). Many studies have demonstrated that NaCl salinity increased Na^+ and decreased K^+ in plant tissue of crops (Sivritepe et al., 2003; Parida and Das, 2005; Asgari et al., 2012). Na^+ is highly water soluble and is readily taken up by plants and transported into the shoots, most likely Na^+ is acting as osmotica, but only moderate concentrations can be tolerated before growth and photosynthesis are reduced (McCree, 1986). A secondary effect of high concentrations of Na^+ and Cl^- in the root media is the suppression of uptake of essential nutrients such as K^+ (Perviaz et al., 2002).

Na⁺ Competes with K⁺ for uptake through common transport systems and this happened effectively since the Na⁺ in saline environments is usually considerably greater than K⁺ (Maathuis et al., 1996). In agreement with the present results, seedlings of barley treated with SA had lower Na⁺ and higher K⁺ in the shoots and roots under salt stress (El-Tayeb, 2005). Similarly, Gunes et al. (2007) determined that SA supply inhibited Na⁺ accumulation, but stimulated K⁺ uptake by salt stressed maize plants compared to non-treated ones. The antagonistic relation between Na⁺ and K⁺ as a result of SA pre-treatment indicates that, SA could play a role in modifying K⁺/Na⁺ selectivity under salt stress, which is reflected in lowering membrane damage and higher water content.

Table 4. Effect of SA pre-treatment on Na and K contents of leaves and roots of cucumber seedlings under salt stress.

NaCl salinity (mM)	SA (mM)	Leaf		Root	
		Na (mg g ⁻¹)	K (mg g ⁻¹)	Na (mg g ⁻¹)	K (mg g ⁻¹)
0	0	3.27 ^h	42.45 ^b	3.42 ⁱ	12.17 ^b
	0.25	3.12 ^h	42.88 ^b	3.56 ^f	12.33 ^b
	0.50	2.94 ^h	44.42 ^{ab}	3.20 ^f	12.38 ^{ab}
	1.00	3.45 ^h	46.75 ^a	3.36 ^f	12.77 ^a
50	0	12.23 ^{d-i}	30.06 ^{ef}	13.27 ^{de}	9.62 ^d
	0.25	8.29 ^{fg}	33.48 ^{cd}	10.72 ^e	9.97 ^{cd}
	0.50	8.32 ^{fg}	35.69 ^c	9.48 ^e	10.15 ^c
	1.00	8.12 ^g	36.41 ^c	9.14 ^e	10.18 ^c
100	0	17.60 ^b	24.14 ^h	19.79 ^{bc}	7.93 ^f
	0.25	12.88 ^{c-e}	27.43 ^{fg}	15.64 ^{cd}	8.26 ^{ef}
	0.50	10.13 ^{e-g}	29.86 ^{ef}	13.38 ^{de}	8.30 ^{ef}
	1.00	10.48 ^{e-g}	31.40 ^{de}	13.16 ^{de}	8.40 ^e
150	0	24.00 ^a	17.67 ^j	26.82 ^a	5.30 ^h
	0.25	16.77 ^{bc}	20.11 ^{hi}	20.36 ^b	5.67 ^{gh}
	0.50	15.20 ^{b-d}	22.05 ^{hi}	18.47 ^{bc}	5.63 ^{gh}
	1.00	14.07 ^{b-e}	24.83 ^{gh}	17.26 ^{b-d}	5.82 ^g
Means for NaCl salinity					
0		3.19 ^d	44.12 ^a	3.38 ^d	12.41 ^a
50		9.24 ^c	33.91 ^b	10.65 ^c	9.98 ^b
100		12.77 ^b	28.21 ^c	15.49 ^b	8.22 ^c
150		17.51 ^a	21.17 ^d	20.73 ^a	5.61 ^d
Means for SA concentration					
	0	14.27 ^a	28.58 ^d	15.83 ^a	8.76 ^c
	0.25	10.26 ^b	30.97 ^c	12.57 ^b	9.06 ^b
	0.50	9.15 ^b	33.00 ^b	11.13 ^b	9.11 ^{ab}
	1.00	9.03 ^b	34.85 ^a	10.73 ^b	9.29 ^a

* Values followed by the same lower-case letters within a column are not significantly different at P≤0.05.

In this study, the correlations between and among various physiological indices (e.g., RLCC, leaf electrolyte leakage, LRWC, F_v/F_m and Na^+ and K^+ contents of leaves and roots) and various morphological parameters (e.g., TE, MET, SDW and RDW) in cucumber seedlings subjected to salt stress was analysed (Table 5). Our results showed that significant correlations existed between and among these physiological indices and morphological parameters. These correlations suggested that emergence and early seedling growth were positively correlated with RLCC, F_v/F_m ratio, LRWC and K^+ contents of leaves and roots, but negatively correlated with leaf electrolyte leakage and Na^+ contents of leaves and roots. Therefore, we conclude that SA ameliorated the negative effects injury caused by salt stress by preventing decreases in RLCC and F_v/F_m ratio, by increases in K^+ contents of leaves and roots and by inhibiting increases in leaf electrolyte leakage and Na^+ contents of leaves and roots. Higher RLCC may have caused higher SDW and RDW. Since all these variables are closely related to photosynthetic capacity.

Conclusion

The response of cucumber seedlings to SA pre-treatment outlined in this study suggests that the application of SA would protect cucumber seedlings partially against salt stress. SA applied by seed soaking at 1.00 mM was most effective in providing salt tolerance especially at higher concentrations of NaCl. The fact that SA, readily available, could be used to prevent crop losses due to salt stress may have a significant practical application.

Acknowledgements

We thank Mr. M. Hakimi Fard and Mr. R. Mohammadi for their valuable help with this experiment. This research was supported by Isfahan University of Technology.

Table 5. Correlations between total emergence (TE), Mean emergence time (MET), relative leaf chlorophyll content (RLCC), leaf electrolyte leakage (EC₁/EC₂), leaf relative water content (LRWC), chlorophyll fluorescence ratio (F_v/F_m), shoot dry weight (SDW), root dry weight (RDW), Na content of leaves (NaL), K content of leaves (KL), Na content of roots (NaR) and K content of roots (KR) of cucumber seedlings under salt stress^a.

	TE	MET	RLCC	EC ₁ /EC ₂	LRWC	F _v /F _m	SDW	RDW	NaL	KL	NaR	KR
TE	1	-0.84**	0.96**	-0.96**	0.93**	0.97**	0.81**	0.80**	-0.93**	0.93**	-0.94**	0.94**
MET	-	1	-0.84**	0.87**	-0.84**	-0.85**	-0.74*	-0.73*	0.88**	-0.77**	0.86**	-0.72*
RLCC	-	-	1	-0.96**	0.94**	0.95**	0.83**	0.85**	-0.90**	0.90**	-0.91**	0.90**
EC ₁ /EC ₂	-	-	-	1	-0.92**	-0.95**	-0.83**	-0.81**	0.94**	-0.88**	0.93**	-0.89**
LRWC	-	-	-	-	1	0.71*	0.79**	0.82**	-0.87**	0.87**	-0.89**	0.87**
F _v /F _m	-	-	-	-	-	1	0.82**	0.82**	-0.94**	0.93**	-0.96**	0.92**
SDW	-	-	-	-	-	-	1	0.82**	-0.85**	0.84**	-0.83**	0.77**
RDW	-	-	-	-	-	-	-	1	-0.79**	0.81**	-0.77**	0.73*
NaL	-	-	-	-	-	-	-	-	1	-0.91**	0.97**	-0.89**
KL	-	-	-	-	-	-	-	-	-	1	-0.93**	0.94**
NaR	-	-	-	-	-	-	-	-	-	-	1	-0.93**
KR	-	-	-	-	-	-	-	-	-	-	-	1

^a Pearson correlation coefficient, * Significant at P≤0.05, ** Significant at P≤0.01.

References

- Asgari, H.R., Cornelis, W., Damme, Van P., 2012. Salt stress effect on wheat (*Triticum aestivum* L.) growth and leaf ion concentrations. *Int. J. Plant Production*, 6, 195-208.
- Baninasab, B., 2009. Amelioration of chilling stress by paclobutrazol in watermelon seedlings. *Sci. Hort.* 121, 144-148.
- Baninasab, B., 2010. Induction of drought tolerance by salicylic acid in seedlings of cucumber (*Cucumis sativus* L.). *J. Hort. Sci. Biotechnol.* 85, 191-196.
- Bjorkman, O., Demming, B., 1987. Photon yield of oxygen evolution and chlorophyll fluorescence characteristics at 77 °K among vascular plants of diverse origin. *Planta*. 170, 489-504.
- Blumwald, E., Aharon, G.S., Apse, M.P., 2000. Sodium transport in plant cells. *Biochim. Biophys. Acta.* 1465, 140-151.
- Borsani, O., Valpuesta, V., Botella, M.A., 2001. Evidence for a role of salicylic acid in the oxidative damage generated by NaCl and osmotic stress in *Arabidopsis* seedlings. *Plant Physiol.* 126, 1024-1030.
- Bray, E.A., Bailey-Serres, J., Weretilnyk, E., 2000. Responses to abiotic stresses. In: Buchanan, B.B., Gruissem, W., Jones, R.L. (Eds.), *Biochemistry and Molecular Biology of Plants*. ASPP, Rockville, pp. 1158-1203.
- Breusegem, F.V., Vranova, E., Dat, J.F., Inze, D., 2001. The role of active oxygen species in plant signal transduction. *Plant Sci.* 161, 405-414.
- Dat, J.F., Lopez-Delgado, H., Foyer, C.H., Scott, I.M., 1998. Parallel changes in H₂O₂ and catalase during thermotolerance induced by salicylic acid or heat acclimation in mustard seedlings. *Plant Physiol.* 116, 1351-1357.
- Dubey, R.S., Rani, M., 1990. Influence of NaCl salinity on the behavior of protease, aminopeptidase and carboxyl-peptidase in rice seedlings in relation to salt tolerance. *Aust. J. Plant Physiol.* 17, 215-224.
- Ellis, R.H., Roberts, E.H., 1981. The quantification of ageing and survival in orthodox seeds. *Seed Sci. Technol.* 9, 373-409.
- El-Tayeb, M.A., 2005. Response of barley grains to the interactive effect of salinity and salicylic acid. *Plant Growth Regul.* 45, 215-224.
- Fletcher, R.A., Kallidumbil, V., Steele, P., 1982. An improved bioassay for cytokinins using cucumber cotyledons. *Plant Physiol.* 69, 675-677.
- Gadallah, M.A.A., 2000. Effects of indole-3-acetic acid and zinc on the growth, osmotic potential and soluble carbon and nitrogen components of soybean plants growing under water deficit. *J. Arid Environ.* 44, 451-467.
- Gonzalez, L., Gonzalez-Vilar, M., 2001. Determination of relative water content. In: Reigosa, M.J. (Ed.), *Handbook of Plant Ecophysiology Techniques*. Kluwer Academic, Dordrecht, pp. 207-212.
- Gunes, A., Inal, A., Alpaslan, M., Cicek, N., Guneri, E., Eraslan, F., Guzelordu, T., 2005. Effects of exogenously applied salicylic acid on the induction of multiple stress tolerance and mineral nutrition in maize (*Zea mays* L.). *Arch. Agron. Soil Sci.* 51, 687-695.
- Gunes, A., Inal, A., Alpaslan, M., Eraslan, F., Guneri Bagci, E., Cicek, N., 2007. Salicylic acid induced changes on some physiological parameters symptomatic for oxidative stress and mineral nutrition in maize (*Zea mays* L.) grown under salinity. *J. Plant Physiol.* 164, 728-736.

- Hajjhashemi, S., Kiarostami, K., Saboora, A., Enteshari, S., 2007. Exogenously applied paclobutrazol modulates growth in salt-stressed wheat plants. *Plant growth Regul.* 53, 117-128.
- Hasegawa, P.M., Bressan, R.A., Zhu, J.K., Bohnert, H.J., 2000. Plant cellular and molecular responses to high salinity. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 51, 463-499.
- Hayat, S., Ahmad, H., 2007. *Salicylic acid: A Plant Hormone*. Springer, Dordrecht, The Netherlands.
- Hoagland, D.R., Arnon, D.I., 1950. *The Water-Culture Method for Growing Plants Without Soil*. Circular 347. Agricultural Experiment Station, University of California, Berkeley.
- Jamil, M., Lee, D.B., Jung, K.Y., Ashraf, M., Lee, S.C., Rha, E.S., 2006. Effect of salt (NaCl) stress on germination and early seedling growth of four vegetables species. *J. Cent. Eur. Agric.* 7, 273-282.
- Katerji, N., Van Hoorn, J.W., Hamdy, A., Mastrorilli, M., Mou Karzel, E., 1997. Osmotic adjustment of sugarbeets in response to soil salinity and its influence on stomatal conductance, growth and yield. *Agric. Water Manage.* 34, 57-69.
- Kathiresan, K., Kalyani, V., Gnanarethium, J.L., 1984. Effect of seed treatments on field emergence, early growth and some physiological processes of sunflower (*Helianthus annuus* L.). *Field Crops Res.* 9, 255-259.
- Kaya, C., Higgs, D., Ince, F., Amador, B.M., Cakir, A., Sakar, E., 2003. Ameliorative effects of potassium phosphate on salt-stressed pepper and cucumber. *J. Plant Nutr.* 26, 807-820.
- Khan, W., Prithiviraj, B., Smith, D.L., 2003. Photosynthetic responses of corn and soybean to foliar application of salicylates. *J. Plant Physiol.* 160, 485-492.
- Korkmaz, A., 2005. Inclusion of acetyl salicylic acid and methyl jasmonate into the priming solution improves low temperature germination and emergence of sweet pepper seeds. *HortScience*, 40, 197-200.
- Kramer, P.J., 1983. *Water Relations of Plants*. Academic Press, New York, USA.
- Lutts, S., Kinet, J.M., Bouharmont, J., 1996. NaCl-induced senescence in leaves of rice (*Oryza sativa* L.) cultivars differing in salinity resistance. *Ann. Bot.* 78, 389-398.
- Maathuis, F.J.M., Verlin, D., Smith, F.A., Sandres, D., Fernea Bandez, J.A., Walker, N.A., 1996. The physiological relevance of Na⁺-coupled K⁺ transport. *Plant Physiol.* 12, 1609-1616.
- Mansour, M.M.F., Salama, K.H.A., Al-Mutawa, M.M., Abou Hadid, A.F., 2002. Effect of NaCl and polyamines on plasma membrane lipids of wheat roots. *Biol. Plant.* 45, 235-239.
- McCree, K.J., 1986. Whole-plant carbon balance during osmotic adjustment to drought and salinity stress. *Aust. J. Plant Physiol.* 13, 33-43.
- McKersie, B.D., Senaratna, T., Walker, M.A., Kendall, E.J., Hetherington, P.R., 1988. Deterioration of membranes during aging in plants: Evidence for free radical mediation. In: Nooden, L.D., Leopold, A.C. (Eds.), *Senescence and Aging in Plants*, Academic Press, London, pp. 442-464.
- Mikolajczyk, M., Awotunde, O.S., Muszynska, G., Klessig, D.F., Dobrowolska, G., 2000. Osmotic stress induces rapid activation of a salicylic acid-induced protein kinase and a homolog of protein kinase ASK1 in tobacco cells. *Plant Cell*, 12, 165-178.
- Mori, M., Di Mola, I., Quaglietta Chiaranda, F., 2011. Salt stress and transplant time in snap bean: growth and productive behavior. *Int. J. Plant Production*, 5, 49-63.

- Nemeth, M., Janda, T., Hovarth, E., Paldi, E., Szali, G., 2002. Exogenous salicylic acid increases polyamine content but may decrease drought tolerance in maize. *Plant Sci.* 162, 569-574.
- Parida, A.K., Das, A.B., 2005. Salt tolerance and salinity effects on plants: a review. *Ecotoxicol. Environ. Safety*, 60, 324-349.
- Perviaz, Z., Afzal, M., Xiaoe, Y., Ancheng, L., 2002. Selection criteria for salt-tolerant in wheat cultivars at seedling stage. *Asian J. Plant Sci.* 1, 85-87.
- Qadir, M., Qureshi, A.S., Cheraghi, S.A.M., 2008. Extent and charateristion of salt-affected soils in Iran and strategies for their amelioration and management. *Land Degrad. Dev.* 19, 214-227.
- Ravindran, K.C., Venkatesan, K., Balakrishnan, V., Chellappan, K.P., Balasubramanian, T., 2007. Restoration of saline land by halophytes for Indian soils. *Soil Biol. Biochem.* 39, 2661-2664.
- Rozena, J., Flowers, T., 2008. Crops for a salinized world. *Science*, 322, 1478.
- Sabater, B., Rodriguez, M.I., 1978. Control of chlorophyll degradation in detached leaves of barley and oat through effect of kinetin on chlorophyllase levels. *Physiol. Plant.* 43, 274-276.
- Sairam, R.K., Tyagi, A., 2004. Physiology and molecular biology of salinity stress tolerance in plants. *Curr. Sci.* 86, 407-421.
- Sakhabutdinova, A.R., Fatkhutdinova, D.R., Bezrukova, M.V., Shakirova, F.M., 2003. Salicylic acid prevents the damaging action of stress factors on wheat plants. *Bulg. J. Plant Physiol.* 29, 314-319.
- Senaratna, T., Touchell, D., Bunn, E., Dixon, K., 2000. Acetyl salicylic acid (Aspirin) and salicylic acid induce multiple stress tolerance in bean and tomato plants. *Plant Growth Regul.* 30, 157-161.
- Shakirova, F.M., Bezrukova, M., 1997. Induction of wheat resistance against environmental salinization by salicylic acid. *Biol. Bull.* 24, 109-112.
- Shakirova, F.M., Sakhabutdinova, A.R., Bezrukova, M.V., Fatkhutdinova, R.A., Fatkhutdinova, D.R., 2003. Changes in the hormonal status of wheat seedlings induced by salicylic acid and salinity. *Plant Sci.* 164, 317-322.
- Shalhevet, J., 1993. Plants under salt and water stress. In: Fowden, L., Mansfield, T., Stoddart, J. (Eds.), *Plant Adaptation to Environmental Stress*. Chapman and Hall, London/Glasgow/New York/Tokyo/Melbourne/Madras, pp. 133-154.
- Shankhdhar, D., Shankhdhar, S.C., Mani, S.C., Pant, R.C., 2000. In vitro selection for salt tolerance in rice, 43, 477-480.
- Singh, B., Usha, K., 2003. Salicylic acid induced physiological and biochemical changes in wheat seedlings under water stress. *Plant Growth Regul.* 39, 137-141.
- Sivritepe, N., Sivritepe, H.O., Eris, A., 2003. The effects of NaCl priming on salt tolerance in melon seedlings grown under saline conditions. *Sci. Hortic.* 97, 229-237.
- Srivastava, T.P., Gupta, S.C., Lal, P., Muralia, P.N., Kumar, A., 1998. Effect of salt stress on physiological and biochemical parameters of wheat. *Ann. Arid Zone*, 27, 197-204.
- Stepien, P., Klobus, G., 2006. Water relations and photosynthesis in *Cucumis sativus* L. leaves under salt stress. *Biol. Plant.* 50, 610-616.
- Stevens, J., Senaratna, T., Sivasithamparam, K., 2006. Salicylic acid induces salinity tolerance in tomato (*Lycopersicon esculentum* cv. Roma): associated changes in gas exchange, water relations and membrane stabilization. *Plant Growth Regul.* 49, 77-83.

- Szepesi, A., Csiszar, J., Bajkan, S., Gemes, K., Horvath, F., Erdei, L., Deer, A.K., Simon, M.L., Tari, I., 2005. Role of salicylic acid pre-treatment on the acclimation of tomato plants to salt-and osmotic stress. *Acta Biol. Szegediensis*, 49, 123-125.
- Tiwari, J.K., Munshi, A.D., Kumar, R., Pandey, R.N., Arora, A., Bhat, J.S., Sureja, A.K., 2010. Effect of salt stress on cucumber: Na⁺-K⁺ ratio, osmolyte concentration, phenols and chlorophyll content. *Acta Physiol. Plant.* 32, 103-114.
- Vettakkorumakankav, N.N., Falk, D., Saxena, P., Fletcher, R.A., 1999. A crucial role for gibberellins in stress protection of plants. *Plant and Cell Physiol.* 40, 542-548.
- Xu, Q., Xu, X., Zhao, Y., Jiao, K., Herbert, S.J., Hao, L., 2008. Salicylic acid, hydrogen peroxide and calcium-induced saline tolerance associated with endogenous hydrogen peroxide homeostasis in naked oat seedlings. *Plant Growth Regul.* 54, 249-259.
- Yang, Y.N., Qi, M., Mei, C.S., 2004. Endogenous salicylic acid protects rice plants from oxidative damage caused by aging as well as biotic and abiotic stress. *Plant J.* 40, 909-919.
- Yildirim, E., Turan, M., Guvenc, I., 2008. Effect of foliar salicylic acid applications on growth, chlorophyll and mineral content of cucumber grown under salt stress. *J. Plant Nutr.* 31, 593-612.
- Yokoi, S., Bressan, R.A., Hasegawa, P.M., 2002. Salt stress tolerance of plants . Japan International Research Center for Agricultural Sciences Working Report No. 23. In: Iwanaga, M. (Ed.), *Genetic Engineering of Crop Plants for Abiotic Stress*. Japan International Research Center for Agricultural Sciences Publishing, Tsukuba, pp. 25-33.
- Zhang, S., Gao, J., Song, J., Zhang, S.G., Gao, J.Y., Song, J.Z., 1999. Effects of salicylic acid and aspirin on wheat seed germination under salt stress. *Plant Physiol. Commun.* 35, 29-32.
- Zhu, Z., Wei, G., Li, J., Quin, Q., Yu, J., 2004. Silicon alleviates salt stress and increases antioxidant enzymes activity in leaves of salt-stressed cucumber (*Cucumis sativus* L.). *Plant Sci.* 167, 527-533.
- Zhu, J.K., 2002. Salt and drought signal transduction in plants. *Annu. Rev. Plant Biol.* 53, 247-273.