

COMPARATIVE EFFECTS OF VARIOUS SALICYLIC ACID DERIVATIVES ON KEY GROWTH PARAMETERS AND SOME ENZYME ACTIVITIES IN SALINITY STRESSED MAIZE (*ZEA MAYS* L.) PLANTS

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Abstract

Salicylic acid, 5-Sulfo Salicylic acid and Acetylsalicylic acid are Salicylic acid derivatives. They differ in their substitution on the benzene ring and may have different effects on plant membranes. The effects of the derivatives of various Salicylic Acid [Salicylic acid (SA), 5-Sulfo Salicylic Acid (SSA) and Acetylsalicylic Acid (ASA)] on antioxidant enzyme activities, mineral element uptake, growth and some stress related parameters of maize (*Zea mays* L. cv. DK 684) plant grown in containers under salinity stress were investigated. Salicylic acid were applied by foliar treatments at five days interval. Treatments were: 1-) control, 2-) salt treatment 125 mM NaCl, 3-)125 mM NaCl + 1 mM Salicylic Acid, 4-)125 mM NaCl + 1 mM Sulfo Salicylic Acid, 5-)125 mM NaCl + 1 mM Acetylsalicylic Acid, 6-) 125 mM NaCl + 2 mM Salicylic Acid, 7-)125 mM NaCl + 2 mM Sulfo Salicylic Acid and 8-) 125 mM NaCl + 2 mM Acetylsalicylic Acid. Salt treatment reduced the plant growth, chlorophyll content, relative water content and ear of corn weight, but increased antioxidative enzymes and membrane permeability. Besides compared with the control group, nutrient uptakes of leaves and roots were inhibited by salt treatment. Tested parameters were generally positively affected by the applications of the salicylic acid derivatives compared to the salt treatment. For example, total chlorophyll, shoot dry matter, relative water content and ear of corn weight were ameliorated by 1 and 2 mM SSA, 1 mM ASA and 1 mM SSA treatments. The macro and micro element content of leaves and roots were generally increased by salicylic acid treatments compared to the salt treatment. Salicylic acid application seems to be more effective in the element uptakes than other ones. Salicylic acid treatments decreased antioxidant enzyme activities compared to the salt treatment. The data clearly shows that, the various derivatives of salicylic acid could protect maize plant from the detrimental effects of salt stress by improving physiological parameters tested such as relative water content, membrane permeability and nutrient status of plant.

Introduction

Salinity is one of the limiting environmental factors for crop production, and is becoming more serious issue as the intensity of agriculture increases. All around the world, about 100 million ha, or 5% of arable land has already been adversely affected by high salt concentrations which reduce crop growth and yield (Ghassemi *et al.*, 1995; Gunes *et al.*, 2007).

Salicylic acid (SA) (*o*-hydroxybenzoic acid), which belongs to a group of plant phenolics, is widely distributed in plants and is now considered as a hormone-like substance, which plays an important role in the regulation of plant growth and development (Raskin, 1992; Klessig & Malamy, 1994). During the last 20 years this

substance has drawn the attention of researchers because of its ability to induce systemic acquired resistance (SAR) in plants. At the present, considerable interest has been aroused by the ability of SA to produce a protective effect on plants under the action of stress factors of different abiotic nature. Thus considerable data have been obtained concerning the SA induced increase in the resistance of wheat seedlings to salinity (Shakirova & Bezrukova, 1997), and water deficit (Bezrukova *et al.*, 2001), of tomato and bean plants to low and high temperature (Senaratna *et al.*, 2000), as well as the injurious action of heavy metals on rice plants (Mishra & Choudhuri, 1999). Gomez *et al.*, (1993) and Rajasekaran & Blake (1999) reported a positive effect of SA on photosynthesis and plant growth under stress. Gomez *et al.*, (1993) observed greater economic yield of wheat cultivars grown under water stress when treated with SA. In maize plants, pretreatment with SA caused a decrease in net photosynthesis under normal growth conditions, but it activated some antioxidant enzymes (POX and GR), which in turn increased chilling tolerance in subsequent 2°C stress (Janda *et al.*, 2000). However, its exogenous application to the plants generates diverse physiological effects, such as inhibition of dry mass accumulation (Schettel & Balke, 1983), and control of ion uptake and their transport (Harper & Balke, 1981).

Salicylic acid (SA) has been reported to cause a multitude of effects on the morphology and physiology of plants (Pierpoint, 1994; Pancheva *et al.*, 1996) and to induce a protective mechanism enhancing resistance to biotic and abiotic stresses (Lopez-Delgado *et al.*, 1998). There is also evidence that SA can alter the antioxidant capacity in plants (Chen *et al.*, 1997; Rao *et al.*, 1997). Many studies support the SA-induced increases in the resistance of wheat and maize to salinity (Gunes *et al.*, 2007; Sakhabutdinova *et al.*, 2003; Shakirova & Bezrukova, 1997; Shakirova *et al.*, 2003) and osmotic stress (Bhupinder & Usha, 2003) and of rice on heavy metal stress (Mishra & Choudhuri, 1999).

In the present study, the effects of salicylic acid and its derivatives as a typical plant hormone, on the growth of the maize plant, nutrient uptake, antioxidant enzyme activities and some other stress related parameters were investigated.

Materials and Methods

Zea mays L. cv. DK 647, was used and the study was designed as three replicates according to trial plot in line with random plots design. Treatments were designed as follows: 1-) Control (only nutrient solution), 2-) NaCl 125 mM, 3-) NaCl 125 mM plus 1 mM SA, 4-) NaCl 125 mM plus 1 mM SSA, 5-) NaCl 125 mM plus 1 mM ASA, 6-) NaCl 125 mM plus 2 mM SA, 7-) NaCl 125 mM plus 2 mM SSA, 8-) NaCl 125 mM plus 2 mM ASA

Three seeds of maize were sown directly in plastic pots containing 8.0 kg of peat, perlite and sand mixture in equal ratios; following germination, plants were thinned to one plant per pot. The plants were fed with modified Hoagland-Arnon solution. The ratios of the minerals in this solution are as follows mg/l): 270 N, 30 P, 240 K, 200 Ca, 60 S, 50 Mg, 3 Fe, 0.5 Mn, 0.5 B, 0.02 Cu, 0.05 Zn. Until their height became 40cm, they had only been treated with water and nutritional substance and the nutritional solution was given to the control plants. When they became longer than 40 cm, Salicylic acid derivatives were sprayed to the leaves with five day-intervals. Each treatment was replicated three times and each replicate included 5 pots (i.e. 15 pots per treatment). The pH of the nutrient solution was adjusted to 6.5 with 0.1 mM KOH during the entire growing period. The volume of the nutrient solution applied to the root zone of the plants

ranged from 200 to 500 ml from the end of March to the middle of June each day depending on plant age. Plants were harvested 90 days after seedling emergence.

Chlorophyll determination: Prior to extraction, fresh leaf samples were cleaned with deionized water to remove any surface contamination. Chlorophyll extraction was carried out on fresh fully expanded leaf material; one g leaf sample was ground in 90% acetone using a pestle and mortar. The absorbance was measured with a UV/Visible spectrophotometer (Pye Unicam SP6-550, UK) and chlorophyll concentrations were calculated using the equation proposed by Strain & Svec (1966).

Chl. a (mg ml^{-1}) = $11.64 \times (A_{663}) - 2.16 \times (A_{645})$

Chl. b (mg ml^{-1}) = $20.97 \times (A_{645}) - 3.94 \times (A_{663})$

where (A_{663}) and (A_{645}) represent absorbance values read at 663 and 645 nm wavelengths, respectively.

Electrolyte leakage: This parameter was included in order to have more information on membrane stability and thereby on the relative ion content in the apoplastic space. Electrolyte leakage was assessed as described by Lutts *et al.*, (1996) using 9 young leaf discs for each treatment. Samples were washed three times with deionized water to remove surface-adhered electrolytes. Leaf discs were placed in closed vials containing 10 ml of deionized water and incubated at 25°C on a rotary shaker for 24 h; subsequently electrical conductivity of the solution (L_t) was determined. Samples were then autoclaved at 120°C for 20 min., and the last electrical conductivity (L_0) was obtained after equilibration at 25°C. The electrolyte leakage was defined as follows:

$$\text{Electrolyte leakage (\%)} = (L_t/L_0) \times 100$$

Proline determination: Proline was determined according to the method described by Bates *et al.*, (1973). Approximately 0.5 g of fresh leaf material was homogenized in 10 ml of 3% aqueous sulfosalicylic acid and filtered through Whatman's No. 2 filter paper. Two ml of the filtrate was mixed with 2 ml acid-ninhydrin and 2 ml of glacial acetic acid in a test tube. The mixture was placed in a water bath for 1 h at 100°C. The reaction mixture was extracted with 4 ml toluene and the chromophore containing toluene was aspirated, cooled to room temperature, and the absorbance was measured at 520 nm with a Shimadzu UV 1601 Spectrophotometer. Appropriate proline standards were included for the calculation of proline in the samples.

Leaf relative water content: Leaf relative water content (LRWC) was calculated based on the methods from Yamasaki & Dillenburg (1999). Two leaves of two randomly chosen plants per replicate were collected from the mid-sections of the plants in order to minimize age effects. Individual leaves were first removed from the stem and then weighed to obtain fresh mass (FM). In order to determine the turgid mass (TM), leaves were floated on distilled water inside a closed Petri dish. Maximum turgidity was determined by weighing leaves (after gently wiping the water from the leaf surface with tissue paper) until no further weight increase occurred. 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 obtain dry mass (DM). All mass measurements were made using an analytical scale, with a

precision of 0.0001 g. Values of FM, TM and DM were used to calculate LRWC using the equation below:

$$\text{LRWC (\%)} = [(FM-DM)/(TM-DM)] \times 100$$

Protein content: Protein content in the enzyme extracts was determined according to Bradford (1976) using Bovine Serum Albumin V as a standard.

Enzyme Determination: Leaves (0.5 g) were homogenized in 50 mM sodium phosphate buffer (pH 7.0) containing 1% soluble polyvinyl pyrrolidone (PVP). The homogenate was centrifuged at 20,000 g for 15 min at 4° C and the supernatant used for assays of the activities of POX and SOD.

The activity of SOD was assayed by monitoring its ability to inhibit the photochemical reduction of NBT (Beauchamp and Fridovich, 1971). One unit of SOD was defined as the amount of enzyme necessary to inhibit the reduction of cytochrome c by 50%.

The activity of POD was assayed by adding aliquot of the tissue extract (100 µl) to 3 ml of assay solution, consisting of 3 ml of reaction mixture containing 13 mM guaiacol, 5 mM H₂O₂ and 50 mM Na-phosphate (pH 6.5) (Chance and Maehly, 1955). An increase of the optical density at 470 nm for 1 min at 25°C was recorded using a spectrophotometer. POD activity was expressed as change in absorbance min⁻¹ mg⁻¹ protein. The increase in A₄₇₀ was measured for 3 min and activity expressed as ΔA₄₇₀/mg protein/min.

Polyphenol oxidase (PPO) activity was assayed with 4-methylcatechol as a substrate according to the method of Zaubermann et al. (1991). Half gram of fresh leaf was ground with 10 ml of 0.1 mol/l sodium phosphate buffer (pH 6.8) and 0.2 g of polyvinylpyrrolidone (PVP, insoluble). After centrifugation at 19,000 g for 20 min, the supernatant was collected as the crude enzyme extract. The assay of the enzyme activity was performed using 1 ml of 0.1 mol/l sodium phosphate buffer (pH 6.8), 0.5 ml of 100 mmol/l 4-methylcatechol, and 0.5 ml enzyme solution. The increase in absorbance at 410 nm at 25 °C was recorded automatically for 5 min. One unit of enzyme activity was defined as an increase of 0.01 in absorbance per min per mg protein.

Statistics: Each pot was considered to be an experimental unit. Treatments were replicated three times and each replicate included 5 pots (i.e. 15 pots per treatment). The data for all parameters were statistically analyzed using the Statview-ANOVA test on computer. Statistically different groups were compared using an LSD test ($P < 0.05$).

Results and Discussion

Key growth parameters: In the plants treated with salt, the amount of proline increased by %150 when compared to the control plants (Table 1). In NaCl treatment with the application of 1mM dose of SA, the increase in the proline continued; however, it decreased with 2mM SA dose. It is believed that 2mM dose of SA and its derivatives cause decrease in proline content by supporting the defense system of the plant. Proline indirectly causes increase in metabolic activation by providing osmoregulation for the plants under stress (Pesserakli & Huber, 1987). Proline amino acid has gained an

important position as a research subject in plant stress physiology. Although it is still under discussion, this compound has a defense mechanism supporting role for the plants under stress (Hare & Cress, 1997).

Relative water content (RWC) decreases with the application of salt compared to control treatment, but SA application increased it either moderately or remained stable (Table 1). This could be explained by transpiration decreasing effects of SA and its derivatives on leaves and epidermis (Aktaş, 2001). Agarwal *et al.*, (2005) reported that RWC capacity increased in wheat with SA treatment. It is known that salinity conditions decreases water uptake. For instance, in a study investigating plant-water relations in tomato plant and the ways of water uptake, it was found that when the tomato plant is watered with saltwater, a decrease both in its growth and its water uptake is observed (Romero-Aranda *et al.*, 2001).

Table 1. The effects of salicylic acid derivates on dry matter, proline and relative water content of maize plants grown at high NaCl.

| Treatments | Shoot dry matter (%) | Root dry matter (%) | Proline μ moles/g FW | RWC % |
|---------------|----------------------|---------------------|--------------------------|---------|
| Control | 17.40a* | 12.90a | 7.44e | 79.90a |
| NaCl | 14.76b | 11.76abc | 18.35bc | 67.46b |
| NaCl+ 1 mM SA | 14.83b | 11.33abc | 20.25b | 72.73ab |
| NaCl+1 mM SSA | 14.03b | 11.03c | 22.85a | 71.70ab |
| NaCl+1 mM ASA | 15.60b | 12.66ab | 18.00c | 68.36b |
| NaCl+2 mM SA | 15.33b | 11.23bc | 11.77d | 69.53b |
| NaCl+2 mM SSA | 15.36b | 11.96abc | 11.07d | 73.40ab |
| NaCl+2 mM ASA | 15.30b | 11.16bc | 16.60c | 72.10ab |

SA: Salicylic Acid, SSA: 5-sulfo Salicylic Acid, ASA: Acetylsalicylic Acid, EC: Electrical conductivity
*Within each column, same letter indicates no significant difference between treatments ($P<0.05$)

Table 2. The effects of salicylic acid derivates on stem height, stem diameter and ear of corn weight-number of maize grown at high NaCl.

| Treatments | Stem height (cm) | Stem diameter (mm) | Ear of corn number (unit/plant) | Ear of corn weight (g/plant) |
|---------------|------------------|--------------------|---------------------------------|------------------------------|
| Control | 230.8a* | 18.433 | 1.88b | 274.35a |
| NaCl | 162.9c | 16.313 | 2.44ab | 158.13bc |
| NaCl+ 1 mM SA | 164.5bc | 17.443 | 2.77a | 164.15bc |
| NaCl+1 mM SSA | 159.8c | 14.457 | 2.55a | 184.51b |
| NaCl+1 mM ASA | 165.8bc | 16.923 | 2.55a | 148.53c |
| NaCl+2 mM SA | 168.8bc | 18.467 | 2.66a | 160.25cb |
| NaCl+2 mM SSA | 179.3b | 17.630 | 2.77a | 169.61cb |
| NaCl+2 mM ASA | 175.1bc | 16.937 | 2.55a | 160.02cb |

SA: Salicylic Acid, SSA: 5-sulfo Salicylic Acid, ASA: Acetylsalicylic Acid, EC: Electrical conductivity
*Within each column, same letter indicates no significant difference between treatments ($P<0.05$)

NaCl treatment reduced root and shoot dry matter. ASA treatment at 1 mM resulted in a moderate increase in root and shoot dry matter, but SA treatments did not result in significant change in this parameter (Table 1). The positive impact of SA on dry and fresh weight of the plant has been cited. For instance, 0.75 mM SA applied to wheat plant under stress increased both root and shoot length and dry and fresh weight. Moreover, SA given in the same dose, increased the yield in unsalted environment and had positive impacts on the physiological properties of the spike of the wheat plant. The chlorophyll a content of leaves under salinity conditions increased with 0.25 and 1 mM SA treatment (Arfan *et al.*, 2007).

In parallel with the vegetative growth deteriorations under stress, some physical parameters are also adversely affected (Blumwold, 2000). At the foremost of these come the stem height, stem diameter and productivity-related characteristics of the plant. Stem height and diameter were reduced by high salinity, but both stem height and stem diameter of the plant were positively affected by 2 mM dose of SA and its derivatives (Table 2). Another parameters adversely affected by salinity were ear of corn number and ear of corn weight. Salinity increased ear of corn number, but reduced ear of corn weight. Application of different most of chemical compounds used did not change those parameters except for 2mM SSA where slight increases were obtained. Supporting of rooting and speeding of vegetative growth by Salicylic acid and some compounds which are close analogues of it can explain the reason behind the positive impact seen on plant height and yield-related parameters as can be seen from Table 3 (Aktas, 2001; Agarwal *et al.*, 2005).

Salinity impaired membrane permeability increasing electrolyte leakage. However, application of salicylic acid (SA) partly maintained membrane permeability (Table 3). It has been noted that maintaining integrity of cellular membranes under stress conditions is considered an integral part of salinity tolerance mechanisms. Present study showed that SA reduced the amount of ion leakage in salt stressed maize plants indicating that SA treatment has facilitated the maintenance of membrane functions under stress conditions. Supporting evidence was shown when SA reduced electrolyte leakage salt stressed tomato leaves and in corn leaf, rice leaf, cucumber hypocotyl under chilling stress (Sztveit & Kang, 2001; Stevens *et al.*, 2006). Salinity reduced chlorophyll and carotenoid contents in maize plant compared to the control plants, but application of 1mM doses of SSA and ASA increased total chlorophyll when compared to salt group. The highest carotenoid content was reached in 1mM SSA dose (Table 3). There is a relation among chlorophyll content, photosynthesis activity and CO₂ fixation. The decreases seen in chlorophyll and carotenoid contents are the result of the indirect effect of NaCl. The decrease in net CO₂ fixation is the result of water shortage, closure of stomatas, accumulation of apoplast salt and mesophyll cells losing turgor and direct toxicity of salt ions. All these negative situations are the indicators of the fact that general metabolic activity has been deteriorated (Pessarakli & Huber, 1987).

Enzyme activities: Salinity increased the concentrations of all antioxidant enzymes tested (SOD, PPO and POX), but in most cases, application of mentioned chemicals reduced those enzyme activities.

Among the investigated antioxidant enzymes, SOD increased with salt treatment but decreased with SA treatments (Table 4). Similar case is also true for PPO and POX. However, application of SA in 2 mM dose considerably increased POX activity different

effects of SA on anti-oxidant activity of plants have been reported. For example, in a study where the effects of SA on apoplastic enzyme activity in wheat plant leaves, it was found that with SA treatment, while apoplastic catalase enzyme activity decreased, apoplastic peroxidase and polyphenol oxidase activities increased (Tasgin *et al.*, 2006). Plants have antioxidants and antioxidative enzyme in varying amounts which protect (Asada & Takahashi, 1987; Ye *et al.*, 2000). Chloroplasts have antioxidative defense mechanism against toxic oxygen derivatives, and the foremost of these antioxidants are: vitamin E, vitamin C, glutathione, beta carotene and zeaxanthin. Enzymes such as superoxide dismutase (SOD), ascorbat peroxidase (APx), glutathione reductase (GR) and catalase (CAT) are known to be the most effective enzymes in the destruction of these radicals (Cakmak & Marschner, 1992; Cakmak, 1994; Gosset *et al.*, 1994; Dionisio-Sese & Tobita, 1998; Sreenivasulu *et al.*, 1999; Sreenivasulu *et al.*, 2000).

In another study, the effects of SA on oxidative stress and antioxidant enzyme activity in different wheat genotypes were investigated. SA, when compared to control plants, increased catalase, superoxide dismutase and ascorbat peroxidase enzyme activities. According to the authors; the reason behind the increase seen in antioxidant enzyme activities and decrease in stress is the increase in contents of chlorophyll, carotenoid and relative water, and this increased the total biomass of the plant (Agarwal *et al.*, 2005).

Nutrient content in leaf and root: It is known that SA and its derivatives may cause ion uptake thwarting effect in some plants. But the truth of this has not been tested in uncontrolled conditions. As complex bio-chemical events take place in the plant metabolism under stress, and as every plant has different degree of tolerance for stress, the mechanism of ion uptake may be under the impact of other factors, too. As can be seen in Table 5, 6, 7 and 8, separate macro and micro element levels were detected in the leaf and the root. According to the data provided in the literature, while usually in plants under NaCl stress macro element uptake and transportation are reduced, there is not sufficient information on micro element uptake and transportation. (Pancheva *et al.*, 1996; Pierpoint, 1994; Raskin, 1992). Lopez & Satti (1996) reported that the NaCl added to nutrition solution considerably decreased Ca and K contents in the leaves of tomato plants.

Table 3. The effects of Salicylic acid derivatives on membrane permeability (MP), chlorophyll and carotenoid contents in maize plants grown at high NaCl.

| Treatments | MP % | Carotenoid (mg/g FW) | Chlorophyll a (mg/g FW) | Chlorophyll b (mg/g FW) | Total Chlorophyll (mg/g FW) |
|----------------|---------|----------------------|-------------------------|-------------------------|-----------------------------|
| Control | 29.80d* | 2.54ab | 1.64ab | 0.580a | 2.22ab |
| NaCl | 50.23a | 2.37ab | 1.48ab | 0.496ab | 1.97b |
| NaCl+ 1 mM SA | 39.40c | 2.17b | 1.56ab | 0.510ab | 2.07ab |
| NaCl +1 mM SSA | 38.80c | 2.78a | 1.76a | 0.583a | 2.34a |
| NaCl +1 mM ASA | 42.07bc | 2.50ab | 1.79a | 0.590a | 2.38a |
| NaCl +2 mM SA | 44.97b | 2.43ab | 1.54ab | 0.500ab | 2.04b |
| NaCl +2 mM SSA | 45.00b | 2.34ab | 1.54ab | 0.486ab | 2.02b |
| NaCl +2 mM ASA | 38.70c | 2.29b | 1.36b | 0.446b | 1.81b |

SA: Salicylic Acid, SSA: 5-sulfo Salicylic Acid, ASA: Acetylsalicylic Acid, EC: Electrical conductivity

*Within each column, same letter indicates no significant difference between treatments ($P < 0.05$)

Table 4. The effects of salicylic acid derivates on some antioxidant enzymes activities of maize grown at high NaCl.

| Treatments | SOD enzyme activity (Unit/ mg Prot.) | PPO enzyme activity (Unit x100/mg prot.) | POX enzyme activity (ΔA_{470} /min/mg prot.) |
|----------------|-----------------------------------------|---------------------------------------------|----------------------------------------------------------|
| Control | 30.32b | 4.79b | 47.84c |
| NaCl | 38.10a | 6.34a | 61.15b |
| NaCl+ 1 mM SA | 35.71a | 5.87b | 52.38c |
| NaCl +1 mM SSA | 28.02b | 5.26b | 43.57d |
| NaCl +1 mM ASA | 27.05b | 5.55b | 60.68b |
| NaCl +2 mM SA | 15.36c | 3.30c | 115.59a |
| NaCl +2 mM SSA | 29.11b | 5.60b | 60.34b |
| NaCl +2 mM ASA | 26.30b | 5.65b | 41.21d |

SOD: Superoxide dismutase, PPO: Polyphenol oxidase, POX: Peroxidase

SA: Salicylic Acid, SSA: 5-sulfo Salicylic Acid, ASA: Acetylsalicylic Acid, EC: Electrical conductivity

*Within each column, same letter indicates no significant difference between treatments ($P < 0.05$)

Table 5. The effects of salicylic acid derivates on macro elements (%) in the leaves of maize plants grown at high NaCl.

| Treatments | N | P | K | Ca | Mg | Na |
|----------------|--------|------|--------|-------|---------|--------|
| Control | 3.08a* | 0.49 | 2.02b | 1.14a | 0.64a | 0.018c |
| NaCl | 2.47e | 0.42 | 1.17f | 0.54b | 0.45c | 0.27ab |
| NaCl + 1 mM SA | 2.97b | 0.49 | 1.92cd | 0.65b | 0.54abc | 0.25b |
| NaCl +1 mM SSA | 2.82c | 0.44 | 1.79e | 0.60b | 0.58ab | 0.36a |
| NaCl +1 mM ASA | 2.97b | 0.43 | 1.87d | 0.55b | 0.49bc | 0.3ab |
| NaCl +2 mM SA | 2.97b | 0.46 | 2.11a | 0.62b | 0.50bc | 0.24b |
| NaCl +2 mM SSA | 2.63d | 0.41 | 1.75e | 0.55b | 0.52abc | 0.30ab |
| NaCl +2 mM ASA | 2.53de | 0.44 | 1.97bc | 0.51b | 0.45c | 0.32ab |

SA: Salicylic Acid, SSA: 5-sulfo Salicylic Acid, ASA: Acetylsalicylic Acid, EC: Electrical conductivity

*Within each column, same letter indicates no significant difference between treatments ($P < 0.05$)

Table 6. The effects of salicylic acid derivates on micro elements (mg kg⁻¹) in the leaves of maize plants grown at high NaCl.

| Treatments | Cu | Fe | Mn | Zn |
|-----------------|--------|------|-------|-------|
| Control | 16.5a | 308a | 162cd | 170ab |
| NaCl | 15.1b | 221d | 179b | 154c |
| NaCl + 1 mM SA | 13.6cd | 229c | 189a | 162bc |
| NaCl +1 mM SSA | 14.1c | 233b | 195a | 172ab |
| NaCl +1 mM ASA | 14.2bc | 229c | 144e | 173ab |
| NaCl + 2 mM SA | 13.9cd | 228c | 158d | 173ab |
| NaCl + 2 mM SSA | 13.4cd | 236b | 189a | 177a |
| NaCl + 2 mM ASA | 13.1d | 229c | 165c | 175ab |

SA: Salicylic Acid, SSA: 5-sulfo Salicylic Acid, ASA: Acetylsalicylic Acid, EC: Electrical conductivity

*Within each column, same letter indicates no significant difference between treatments ($P < 0.05$)

Table 7. The effects of salicylic acid derivates on macro elements in the roots of maize plants grown at high NaCl.

| Treatments | N | P | K | Ca | Mg | Na |
|----------------|--------|-------|---------|--------|-------|-------|
| Control | 1.35a* | 0.55a | 3.24a | 1.73a | 2.11a | 2.17g |
| NaCl | 1.17b | 0.17b | 0.32bc | 0.35e | 0.42d | 4.76a |
| NaCl + 1 mM SA | 1.19ab | 0.22b | 0.37b | 0.69c | 0.59c | 3.15d |
| NaCl +1 mM SSA | 1.24ab | 0.20b | 0.35b | 0.57cd | 0.43d | 4.14b |
| NaCl +1 mM ASA | 1.19ab | 0.16b | 0.21de | 0.49d | 0.43d | 3.52c |
| NaCl +2 mM SA | 1.20ab | 0.22b | 0.28bcd | 0.92b | 0.88b | 2.54f |
| NaCl +2 mM SSA | 1.21ab | 0.16b | 0.18e | 0.58cd | 0.82b | 3.21d |
| NaCl +2 mM ASA | 1.11b | 0.19b | 0.23cde | 0.58cd | 0.48d | 2.84e |

SA: Salicylic Acid, SSA: 5-sulfo Salicylic Acid, ASA: Acetylsalicylic Acid, EC: Electrical conductivity

*Within each column, same letter indicates no significant difference between treatments ($P < 0.05$)**Table 8. The effects of salicylic acid derivates on the micro elements in the roots of maize plants grown at high NaCl.**

| Treatments | Cu | Fe | Mn | Zn |
|-----------------|-------|------|--------|------|
| Control | 28.2a | 505c | 45abc | 160a |
| NaCl | 15.6b | 263e | 33bcd | 28b |
| NaCl + 1 mM SA | 10.4b | 191g | 50a | 31b |
| NaCl +1 mM SSA | 12.5b | 550b | 44abcd | 19b |
| NaCl +1 mM ASA | 10.5b | 249f | 39abcd | 19b |
| NaCl + 2 mM SA | 15.9b | 474d | 48ab | 29b |
| NaCl + 2 mM SSA | 27.0a | 640a | 30cd | 23b |
| NaCl + 2 mM ASA | 33.0a | 190g | 28d | 28b |

SA: Salicylic Acid, SSA: 5-sulfo Salicylic Acid, ASA: Acetylsalicylic Acid, EC: Electrical conductivity

*Within each column, same letter indicates no significant difference between treatments ($P < 0.05$)

As can be understood from the investigation of the tables, while SA and its derivatives given in addition to NaCl remain below the control, they are generally influential on micro element contents of the leaves and roots. This situation is more evident in the leaf in terms of N and K and in the root in terms of Ca and Mg. Among the SA derivatives, the one best competing with Na is Salicylic acid. When micro elements are looked into, while the best reaction in the leaf is given by Zn, the other micro elements reveals a fluctuating pattern in both the leaf and root.

There are studies showing that Salicylic acid improves the quality in plants. There is an indirect relation between the quality and nourishment. In a study conducted on *Prunus persica* (L.) it was found that with 1 mM SA treatment, the destruction caused by cold decreases and rotting index and fruit hardness are improved. As known, especially in fruits, Ca element is responsible for cellular stabilization and quality. In this study SA served a function similar to that of Ca (Wang *et al.*, 2006).

In another study, the effects of SA treatments ranging from 0.1 to 1 mM on the plant's element uptake, its growth and membrane permeability in maize plant under salinity stress were investigated. With 1mM SA treatment, the membrane permeability considerably decreased when compared to NaCl group. While with SA treatment, Na and Cl uptake of the plant was strongly inhibited, N, Mg, Fe, Mn and Cu uptake was considerably stimulated. According to the researchers, SA took the role of an important hormone fostering the resistance against stress in the plant (Gunes *et al.*, in press).

In few studies, it has been reported that SA exhibits a synergisms with Ca in the plant cell. In a study conducted on grape plant, under normal circumstances, while the electrolyte leakage ratio decreased, cytosolic contents were found to be higher compared to the control. According to the researchers' statements, inner-cell Ca balance (homeostasis) and antioxidant system improves with the exogenous application of SA in plants under normal circumstances or under stress (Wang & Li, 2006).

Various physiological and biochemical effects of SA on plant systems have been well documented (Raskin, 1992; Cameron, 2000). However, studies related to ion concentration and uptake are relatively lacking. Aly & Soliman (1998) studied the effect of SA on iron uptake in soybean genotypes. They found that SA was effective in correcting iron chlorosis in soybean genotypes grown in calcareous soils. Al-Hakimi & Hamada (2001) also observed similar effects of SA in the Na, K, Ca and Mg content of wheat plants grown under salinity. Positive effects of SA on the ion uptake and inhibitory effects on Na and Cl uptake should also be responsible for managing salinity of maize plants (Gunes *et al.*, in press).

Conclusions

In the maize plant under the salinity stress, growth-related properties were adversely affected and anti-oxidant enzyme activities increased. SA derivatives given through the leaf, especially in lower doses, positively affected the growth parameters when compared to the control plants. Macro and micro element uptake of the maize plant generally increased by application of SA derivatives compared to salinity-stressed plants. Furthermore, SA treatments generally reduced antioxidant enzyme activities compared to plants at high NaCl. There seems to be no significant difference with regards to their effects on the plant among salicylic acid derivatives.

The obtained data reveal that salicylic acid derivatives which are found in the plants very commonly, and having hormone-like effect, can decrease the adverse effects of salinity stress, especially in low dose applications.

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