Indian Journal of Plant Sciences ISSN: 2319–3824(Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/jps.htm
2015 Vol. 4 (1) January-March, pp.69-77/Singh et al.
Research Article

EFFECTS OF SALT STRESS ON PHYSICO-CHEMICAL CHANGES IN MAIZE (*ZEA MAYS* L.) PLANTS IN RESPONSE TO SALICYLIC ACID

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ABSTRACT
Biosynthesis and accumulation of secondary metabolites in plants under abiotic stresses are the key point in view of sustainable plant productivity. The aim of the present work was to investigate the accumulation of phenolic acids in 2 weeks old maize (Zea *mays* L.) plants grown under salt stress (0, 50, 100, 150 and 200 mM NaCl) in presence and absence of 0.5 mM SA. The results showed sever reduction in plant dry weight, leaf relative water content and photosynthetic pigments. Content of total phenolics was increased by 64%, in the plants after salt treatment. The exogenous application of SA significantly alleviated the growth inhibition of plants caused by NaCl, and was accompanied by higher leaf relative water contents, photosynthetic pigments, and lower total phenolics. Further, ferulic acid dominated among all phenolic acids under normal conditions but salicylic acid content increased significantly and was the dominant phenolic acid under salinity stress. Taken together, these results suggested that the presoaking application of SA was an effective way to improve the salt tolerance of maize plants.

Keywords: *Salinity Stress; Phenolic Profiles; Relative Water Content; Salicylic Acid; Salinity Acclimation; Zea Mays L*

INTRODUCTION
Plants regularly face abiotic stress conditions during growth and development, such as drought, chilling, freezing, high temperatures and salinity. Stress condition can delay growth and development, reduce productivity, and, in extreme cases cause plant death (Krasensky and Jonak, 2012). Salinity imposes detrimental effects on plant growth through low osmotic potential of soil solution and nutritional imbalance (Munns and Tester, 2008). As a consequence of these primary effects of salt stress, caused by its hyperosmotic effect, secondary stresses, such as oxidative damage, often occur (Zhu, 2001). In such conditions reactive oxygen species (ROS) are excessively produced in plants, these ROS if not sufficiently reduced, through antioxidant systems cause irreversible intracellular damage through oxidation (Apel and Hirt, 2004). In general, plants possess an anti-oxidant system including anti-oxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) etc. to protect their cells against the damages caused by ROS (Mittler *et al.*, 2004).
Plant cells contain a range of protective and repair systems, which control the metabolism under adverse environmental conditions. Induction of secondary metabolism is one of the regulatory systems of the plants involved in defense system (Dixon and Pavia, 1995). Accumulation of phenolic acids is a well-known symptom of adverse environmental conditions and the production of different classes of phenolic acids produced via the phenylpropanoid system is dependent on the nature of stress exposed to plants (Weisskopf *et al.*, 2006).
SA is one of the naturally occurring phenolic acids known as signaling molecule that regulates plant responses to a variety of abiotic stresses such as low and high temperature, salts and oxidative conditions (Gunes *et al.*, 2007). The application of exogenous SA has been shown to protect against several types of stresses such as salinity, temperature, radiation etc. The exogenous application of salicylic acid has been suggested to be an effective approach in improving crop salt tolerance in wheat (Singh and Usha, 2003), barley (Tayeb, 2005), maize (Gautam and Singh, 2009) and tomato (Szepsi *et al.*, 2009). However, the mechanism of SA action in plants is not fully understood.

The present study was conducted to determine the role of salicylic acid in alleviating salt stress in maize by measuring the plant dry weight, leaf relative water content (RWC), synthesis of photosynthetic pigments in the plants supplied with and without SA. We tried to test, whether the supply of SA could improve the plant water status by maintaining higher leaf relative water content, and photosynthetic pigments. Present aim was also designed to know the accumulation total phenolics and changes in the profile of phenolic acids in maize plants under salinity stress.

MATERIALS AND METHODS

2.1 Plant Growth Conditions

Maize (*Zea mays* L.) var. Jaunpuri seeds were procured from Plant Breeding Department, Institute of Agricultural Sciences, B.H.U., and Varanasi, India. Seeds were surface sterilized with 0.01% $HgCl_2$ and 0.1% of cetramide solution followed by distilled water washing. Homogenous seeds were presoaked in all treatments for 6 h as follows: control in distilled water; 0.5 mM SA in distilled water; 50, 100, 150 and 200 mM of NaCl and 0.5 mM SA with each level of salinity. Treated seeds were placed on moistened Whatman no. 1 filter paper in acid-washed Petri dishes in dark at 27°C for germination and thereafter transferred to pots with perforated plastic tops. Plants were grown in a glasshouse under natural light conditions (in the range of 27-35°C air temperature, 450–500 µmol/m^2s light intensity and 75% relative humidity). Each pot [10×3→ total 30 pots] contains six plants and supplied with 20 ml of 50% of Hoagland's nutrient solution at alternate days (Hogland and Arnon, 1950).

Two week-old plants were harvested after the germination and dried in a thermo-ventilated oven at (70-75) °C until reaching a constant mass for dry weight determination. Growth parameters, such as root length, shoot length, leaf area; fresh weight and dry weight were calculated according to the standard methods. Various biochemical analyses were performed in the leaf samples of 2 week-old maize plants.

2.1 Plant Growth Analyses

Plants were uprooted carefully and washed in distilled water. Shoot and root length was measured with the help of scale. Plant fresh weight was noted by electric balance. Plant samples were placed in oven at 75°C. After 4-days plant dry weight was calculated with the help of electric balance. Both fresh and dry weights were expressed as g $plant^{-1}$ basis.

2.2 Determination of Photosynthetic Pigments and Relative Water Content (%)

Photosynthetic pigments (chlorophyll a, b and carotenoids) were measured in fresh leaf samples before harvesting according to the method of Lichtenthaler (1987). The leaf relative water contents (RWC) were calculated according to Beadle *et al.*, (1993).

2.3 Determination of Total Phenolics

Total Phenolics were determined spectro-photometrically following the method described by Swain and Hills, (1959) using gallic acid as a standard.

2.4 Measurement of Phenolic Acids by HPLC

2.4.1 General

Phenolic acids content were determined according to the HPLC method of Zhou *et al*., (2004).

2.4.2 Extraction of Phenolic Acids

10 g of maize leaves were macerated in pestle – mortar with 80 % aqueous ethanol (80:20, v/v, 10 ml), filtered with Whatman No.1 filter paper, repeated above step and extracted materials were kept over night. Filtrates were centrifuged at 1500 rpm for 15 minute. Clear supernatants were evaporated under vacuum at room temperature. The residue was dissolved in 1.0 ml HPLC grade methanol, filtered through membrane-filter (Millipore, 0.45 µ) and stored at 40C for HPLC analysis. Internal Standards (Sigma chemicals) was used for comparisons. The samples were analyzed under the same conditions. Retention time and spectrum was compared with extracts was measured from the peak height obtained at 280 nm and 290 nm and was expressed in terms of milligrams per gram fresh weight.

2.4.3 HPLC Analysis

High performance liquid chromatography of the samples were performed by HPLC system (Shimadzu Corporation, Kyoto, Japan) comprising LC-20 ATVP reciprocating pumps, a variable SPD-20A VP UV-

VIS detector, C-18 reverse phase HPLC column, 250 x 4.6 mm I'd. Particle size 5µ C-18, (Phenomenex USA) at 360C under isocratic condition.
Operating conditions were: injected volume - 10 µl; mobile phase - MeOH: 0.4% acetic acid in water (66:34, v/v); flow rate - 1ml/min and UV detector was set at 280 and 290 nm. Gallic, Caffeic, Ferulic, Trans cinnamic and Salicylic acid were identified by comparing the retention time (Rt in min.) of standards. Quantification of each phenolic acid was calculated by comparing peak areas of reference compounds with those in the samples run under the same eluting conditions.

$$\text{Amount of phenolic} = \frac{\text{Peak area of sample x amount of standard X 20}}{\text{Peak area of standard (1000 µg of sample)}}$$

2.5 Statistical Analysis
The experiments were repeated twice with three replicates (n = 5) and the data presented are mean ± standard errors (SE). The results were subjected to analysis of variance and means were compared by the least significant difference test at the 0.05% level of significance.

RESULTS AND DISCUSSION

Result

3.1 Growth Measurements
Influence of SA on growth measurements of maize plants under salinity stress were analyzed by accumulation of root shoot weight. Dry matter of salt stressed plants gradually decreased with increasing concentrations of NaCl and the reduction was severe at 150 mM NaCl without exogenous supply of SA. Exogenously applied SA (0.05mM) increased dry yield in both saline and non saline conditions. However, this effect of SA was more pronounced and significant in saline conditions (*Table 1*). Initial concentration of NaCl (50 mM) did not appear damaging while at 150 mM of NaCl dry weight of maize plants reduced by 24 % of aqueous control. Interestingly, this reduction in dry weight is completely encountered by the treatment of exogenous SA (*Table 1*). Root length of two-week old maize plants decreased upto 28 % at 200 mM of NaCl in comparison to aqueous control whereas exogenous SA application alleviated the damaging effects of deleterious salt concentration by 16 % of aqueous control (*Table 1*). Similar trend was observed in the case of shoot length; however reduction in shoot length was high in comparison to that of root. Shoot length decreased up to 60.4 % at 200 mM of NaCl in comparison to aqueous control plants. SA control plants showed 9 % increase in shoot length as compared to SA deficient control. At higher doses of salinity exogenous SA application alleviated the effect of salt and the decrease was 47 % at 200 mM of NaCl.
Leaf area decreased gradually with increasing salt concentrations and the reduction was alarming (53 %) at 200 mM of NaCl treatment as compared to aqueous control plants. SA application to salt stressed plants alleviated the decrease in leaf area and it was upto 43% at 200 mM of NaCl. Whereas, supply of exogenous SA alone showed 14 % increase in leaf area as compared to aqueous control. Present study stated that SA affects as growth hormone and its impact was more significant under saline conditions (*Table 1*).

3.2 Relative Water Content
The relative water content in the leaves decreased gradually with the increasing concentration of NaCl and the decrease was 17 % and 25 % at 150 mM and 200 mM of NaCl respectively, whereas the subsequent treatment of SA significantly increased its level over the control (an increase of 4 %) and also overcome the toxic effects generated by NaCl (7 % and 15 % at 150 mM and 200 mM of NaCl respectively) (*Table 2*).

3.3 Photosynthetic Pigments
The content of photosynthetic pigments (chlorophyll a, b and carotenoids), especially chlorophyll a decreased sharply with increasing stress levels. High doses of NaCl appeared with deleterious effects (81.20 % at 150 mM and 92.86 % at 200 mM) on the content of chlorophyll a in the absence of exogenous SA as compared to the aqueous control. Although in presence of 0.5 mM SA, effects of NaCl

stress were counteracted and pigments level were increased significantly 72 % (*Table 2*). This restoratory effect of SA pretreatment on the reduction in the content of chlorophyll a in maize seedlings under saline condition was 9 % at 150 mM and 14 % at 200 mM of NaCl (*Table 2*).

Chlorophyll b also decreased sharply with increasing stress levels. High doses of NaCl appeared with deleterious effects (79 % at 150 mM and 94 % at 200 mM) on the content of chlorophyll b in the absence of exogenous SA as compared to the aqueous control. Although in presence of 0.5 mM SA, effects of NaCl stress were counteracted and pigments level were increased significantly 76 % (*Table 2*). This restoratory effect of SA pretreatment on the reduction in the content of chlorophyll b in maize seedlings under saline condition was 32 % at 150 mM and 4 % at 200 mM of NaCl (*Table 2*). Similar trend was observed with carotenoids content (*Table 2*).

3.4 Total Phenolics Content

NaCl-stressed accumulation of total phenolics was 2 fold high (96 %) at 200 mM concentration of NaCl than that of control in absence of salicylic acid. At 100 mM concentration of NaCl there was only slight increase of 25 % in total phenolics content, which then increased to 64 % at 150 mM of NaCl. Whereas, SA pretreated salt stressed plants showed significant reduction in accumulation of total phenolics as compared to salt stressed plants without SA (*Table 2*). The reduction in total phenolics content was 11 %, 40 % and 48 % at 100, 150 and 200 mM concentrations of NaCl respectively (*Table 2*).

3.5 Phenolic Acids Content

Contents of phenolic acids in leaves of two weeks old maize plants were determined by HPLC (Shimadzu LC-20 ATVP, Japan). HPLC analysis was conducted to ascertain the metabolism of phenolic acids in salt stressed maize leaves supplied with exogenous salicylic acid (0.5mM). Results of HPLC analyses referring to isolated phenolic acids fraction present in two weeks old *Zea mays* L. seedlings are analyzed through Chromatograms. Different phenolic acids quantitized from leaves of *Zea mays* L. were Gallic, Caffeic, Ferulic, Cinnamic and Salicylic acid (*Table 3*). Total content of these phenolic acids fractions (determined using HPLC technique) in maize leaves amounted to 20.01 µg g-1 FW [including caffeic (0.40 µg g-1), ferulic (2.27 µg g-1), gallic (13.99 µg g-1), trans- cinnamic (1.65 µg g-1), and salicylic acids (1.70 µg g-1)] under non-saline condition, while under saline conditions accumulation of phenolic acids was increased significantly (52.78 µg g-1 FW). It is worth mentioning that gallic acid dominated among all phenolic acids under normal conditions but under stress conditions salicylic acid content increased significantly and was the dominant phenolic acid under salinity stress (18.14 µg g-1). Unexpectedly, level of gallic acid was much higher in the present experiment under both saline and non-saline conditions. However, there is no such report of its presence (as the dominant phenolic acid) in cereal crops. To know the effect of NaCl-stress on phenolic acids biosynthesis in maize plants reverse-phase HPLC chromatogram was developed using Shimadzu LC-20. The level of endogenous salicylic acid (SA) was sharply increased (10 fold) under NaCl-stress in comparison to aqueous control. This induction in SA biosynthesis was increased upto 150 mM of NaCl treatment but then decreased at 200 mM NaCl treatment (Table 3). Exogenous SA (0.5 mM) application decreased SA level with increasing NaCl concentrations in the present study.

Endogenous trans- cinnamic acid level increased slightly (2 fold) in 100 and 150 mM concentrations of NaCl as compared to aqueous control but decreased at 200 mM of NaCl treatment in absence of exogenous SA. However, in the presence of 0.5 mM SA, no significant changes in the level of trans-cinnamic acid were observed under NaCl treatments (Table 3). Similar trend was observed in the case of ferulic and caffeic acids. Concentration of ferulic acid increased significantly at 50 mM of NaCl treatment in maize plants but then decreased gradually with further increasing NaCl concentrations. Exogenous SA (0.5 mM) treatment led to slight increase in endogenous ferulic acid content with 50 and 150 mM concentrations of NaCl, whereas it increased significantly with 200 mM NaCl treatment. Endogenous caffeic acid level increased significantly at 50 mM of NaCl treatment, then decreased at higher concentrations of NaCl treatment in absence of exogenous SA. However, under the supply of 0.5 mM salicylic acid content of caffeic acid was initially increased at higher doses of NaCl treatment (50 and 100 mM) and then decreased with 150 and 200 mM of NaCl treatment. Endogenous gallic acid content

increased significantly in both saline and non-saline conditions in two weeks old maize leaves; however accumulation of gallate was higher in saline condition in comparison to non-saline condition. Accumulation of gallic acid was also reduced upto 16.6 % under the application of 0.5 mM exogenous salicylic acid (Table 3).

Table 1: Effects of salicylic acid on growth characteristics of 2-week-old maize plants under salt stress

NaCl (mM)	Dry weight (g)		Root length (cm)		Shoot length (cm)		Leaf area (cm^2)	
	SA-	SA+	SA-	SA+	SA-	SA+	SA-	SA+
0	0.720[a] (±0.16)	0.740[b] (±0.16)	6.5[a] (±1.45)	7.1[a] (±1.59)	9.1[a] (±2.03)	10[a] (±2.24)	25.13[a] (±5.62)	29.24[a] (±6.54)
50	0.710[b] (±0.22)	0.720[c] (±0.16)	6.2[b] (±1.39)	6.6[b] (±1.48)	8.2[b] (±1.83)	9.2[b] (±2.06)	23.71[b] (±5.30)	24.56[b] (±5.49)
100	0.700[c] (±0.16)	0.690[d] (±0.15)	6.1[c] (±1.36)	6.4[c] (±1.43)	7.4[c] (±1.65)	7.9[c] (±1.77)	20.32[c] (±4.54)	22.69[c] (±5.07)
150	0.550[e] (±0.12)	0.770[a] (±0.17)	5.8[d] (±1.30)	6.1[d] (±1.36)	5.1[d] (±1.14)	6.2[d] (±1.39)	16.16[d] (±3.61)	19.27[d] (±4.31)
200	0.650[d] (±0.15)	0.720[c] (±0.16)	4.7[e] (±1.05)	5.5[e] (±1.23)	3.6[e] (±0.81)	4.8[e] (±1.07)	11.71[e] (±2.62)	16.81[e] (±3.76)
LSD	(0.012)	(0.001)	(0.022)	(0.153)	(0.022)	(0.024)	(0.024)	(0.020)

Data presented are mean ± SE (n = 5). Values a, b, c, d, e represent significant differences compared to controls at $P < 0.05$ according to Duncan's multiple range test. LSD values were determined at $P < 0.05$.

Table 2: Effects of salicylic acid on biochemical changes in 2week-old maize plants under salt stress.

(mM)	(mg g^{-1} FW)		(mg g^{-1} FW)		(mg g^{-1} FW)		Content (%)		(mg g^{-1} FW)	
	SA-	SA+	SA-	SA+	SA-	SA+	SA-	SA+	SA-	SA+
0	2.56[a] (±0.57)	4.43[a] (±0.99)	1.38[a] (±0.31)	2.51[a] (±0.56)	1.75[a] (±0.39)	2.87[a] (±0.64)	80.1[a] (±17.91)	83.2[a] (±18.60)	0.121[e] (±0.03)	0.127[c] (±0.03)
50	2.14[b] (±0.48)	4.10[b] (±0.92)	1.06[b] (±0.12)	2.42[b] (±0.54)	1.27[b] (±0.28)	2.48[b] (±0.55)	79.6[b] (±17.79)	81.1[b] (±18.14)	0.128[d] (±0.03)	0.132[c] (±0.03)
100	1.16[c] (±0.26)	3.36[c] (±0.75)	0.51[c] (±0.11)	2.21[c] (±0.49)	0.86[c] (±0.19)	2.14[c] (±0.48)	76.1[c] (±17.02)	80.3[c] (±17.96)	0.151[c] (±0.03)	0.134[c] (±0.03)
150	0.50[d] (±0.11)	2.84[d] (±0.64)	0.31[d] (±0.07)	1.97[d] (±0.44)	0.70[d] (±0.15)	2.00[d] (±0.45)	66.5[d] (±14.87)	73.9[d] (±16.53)	0.199[b] (±0.16)	0.169[b] (±0.24)
200	0.21[e] (±0.05)	2.28[e] (±0.51)	0.10[e] (±0.02)	1.39[e] (±0.31)	0.51[e] (±0.11)	1.74[e] (±0.39)	60.3[e] (±13.48)	67.7[e] (±15.14)	0.237[a] (±0.05)	0.179[a] (±0.04)
LSD	(0.021)	(0.023)	(0.020)	(0.022)	(0.022)	(0.022)	(0.024)	(0.020)	(0.007)	(0.001)

NaCl Chlorophyll a Chlorophyll b Carotenoids Relative water Total Phenolics

Data presented are mean ± SE (n = 5). Values a, b, c, d, e represent significant differences compared to controls at P < 0.05 according to Duncan's multiple range tests. LSD values were determined at P < 0.05

Table 3: HPLC quantification of different phenolic acids (µg g^{-1} FW) in leaves of two weeks old maize (*Zea mays* L.) plants grown under salt stress

NaCl	Gallic acid		Caffeic acid		Ferulic acid		Cinnamic acid		Salicylic acid		Sum	
(mM)	-SA	+SA	-SA	+SA	-SA	+SA	-SA	+SA	-SA	+SA	-SA	+SA
0	13.99^{e} (±3.13)	13.08^{e} (±2.92)	0.40^{c} (±0.09)	0.56^{c} (±0.13)	2.27^{c} (±0.51)	0.36^{c} (±0.08)	1.65^{d} (±0.37)	0.88^{b} (±0.20)	1.70^{c} (±0.38)	8.45^{a} (±1.88)	20.01^{e} (±4.47)	23.33^{e} (±5.21)
50	26.49^{d} (±5.92)	24.43^{c} (±5.46)	1.29^{a} (±0.29)	0.86^{b} (±0.19)	3.27^{a} (±0.73)	0.51^{b} (±0.11)	1.59^{e} (±0.36)	0.95^{a} (±0.21)	nd	2.39^{c} (±0.53)	32.64^{c} (±7.30)	29.14^{c} (±6.52)
100	26.87^{c} (±6.01)	24.26^{d} (±5.42)	0.08^{e} (±0.02)	1.09^{a} (±0.24)	2.60^{b} (±0.58)	nd	2.24^{b} (±0.50)	0.73^{c} (±0.16)	nd	2.18^{d} (±0.49)	31.79^{d} (±7.11)	28.26^{d} (±6.32)
150	30.69^{a} (±6.86)	25.90^{b} (±5.79)	0.49^{b} (±0.11)	0.33^{d} (±0.07)	0.94^{d} (±0.21)	0.50^{b} (±0.11)	2.52^{a} (±0.56)	0.55^{d} (±0.12)	18.14^{a} (±4.06)	2.04^{e} (±0.45)	52.78^{a} (±11.8)	29.32^{b} (±6.56)
200	30.04^{b} (±6.72)	26.02^{a} (±5.82)	0.19^{d} (±0.04)	0.32^{d} (±0.07)	0.91^{e} (±0.20)	1.21^{a} (±0.27)	1.96^{c} (±0.44)	0.75^{c} (±0.17)	9.89^{b} (±2.21)	2.49^{b} (±0.56)	42.66^{b} (±9.54)	30.79^{a} (±6.89)
LSD	(0.023)	(0.023)	(0.017)	(0.020)	(0.028)	(0.022)	(0.021)	(0.022)	(0.018)	(0.023)	(0 .020)	(0.022)

Data presented are mean ± SE (n = 5). Values a, b, c, d, e represent significant differences compared to controls at P < 0.05 according to Duncan's multiple range tests. LSD values were determined at P < 0.05

Discussion

The growth parameters (dry mass of roots and shoots, their lengths and the leaf area) of maize plants decreased significantly with the rise of stress level, compared with that of aqueous control (Table 1). These results are in agreement with those of Ghoulam *et al.,* (2002). They showed that salinity caused a marked reduction in growth parameters of sugar beet plants. The plants subjected to NaCl and subsequently treated with SA, possessed higher dry mass compared to those grown without SA treatment (Table 1). The results of the present study clearly indicated that the dry matter of maize seedlings decreased significantly with exposure to NaCl and was severe at concentration of 200 mM. Exogenously applied 0.5 mM of SA shows growth restoratory action and increased dry matter both in saline and non-saline conditions. However, this effect of SA was more pronounced and significant in saline conditions. Gutierrez-Coronado *et al.,* (1998) have also reported a similar increase in the growth of shoots and roots of soybean plants under normal conditions in response to SA treatment. The damage caused by salt stress can also be attributed to the water stress or a kind of physiological drought generated by NaCl (Das *et al.,* 1990), as evident from the decrease in water use efficiency and relative water content in the present study (*Table 2)*. These growth inhibitory effects of NaCl were antagonized significantly with supply of 0.5mM SA in maize plants. The earlier studies strongly favour these observations, such as, SA and acetyl SA proved effective in protecting tomato (Stevens *et al.,* 2006) and wheat (Singh and Usha, 2003) plants against salinity and drought stress. Anyhow, most of the workers have attributed the involvement level of phytohormones and osmolytes to the development of anti-stress reactions, induced by SA. Content of photosynthetic pigments, chlorophyll a, b and carotenoids decreased significantly under salinity stress; whereas, in the presence of 0.5 mM SA, effects of NaCl stress on the content of photosynthetic pigments was reduced up to 50%. SA is supposed to increase the functional state of the photosynthetic machinery in plants either by the mobilization of internal tissue nitrate or chlorophyll biosynthesis (Khodary, 2004). SA has also been reported to have stimulatory effects on photosynthetic capacity in maize plants through the induction of Rubisco activity under NaCl stress conditions (Gunes *et al.,* 2007).

NaCl-stressed accumulation of total phenolics was very high than that of aqueous control (2 fold) in absence of salicylic acid. Phenolics constitute a part of cellular solutes and provide a reducing

environment to the system (Dixon and Pavia, 1950). Salt stress exerts its effect through membrane peroxidation, which indicates that oxygen free radicals are formed during stress. Thus, more phenol accumulation could be a cellular adaptive mechanism for scavenging the free radicals of oxygen and preventing sub-cellular damages during stress. Whereas, in the presence of exogenous salicylic acid, phenolics content was reduced significantly (*Table 2*). The ameliorative effect of SA might be due to its involvement in ROS scavenging mechanisms or by the protection of membrane deterioration through the reduction in the hydrolysis of membrane lipids. These results are in agreement with the findings of Gunes *et al.,* (2007) who have reported the salicylic acid induced acclimation in maize plants under salinity stress.

Induction of the phenolic acids biosynthesis potentiates the survival strategies of plants under various stress conditions. It might be due to enlargement of cellular pool of SA, which induces the scavenging mechanisms of oxidative injury in plants under adverse environmental conditions (Sawada *et al.,* 2006). The results of present study also revealed the above hypothesis for the role of SA in adaptive mechanism of maize plants under salinity stress. Content of phenolic acids increased with increasing salinity stress and maximum accumulation was at 150 mM NaCl treatment. It is evident from the data that biosynthesis of salicylic acid is induced in maize plants exposed to NaCl-stress and the induction was concentration based. It could be stated that fraction of phenolic acids increased with increasing salinity stress and maximum accumulation was at 150 mM NaCl treatment (52.78 µg g^{-1} FW) whereas at 200 mM of salinity it decreased to 42.66 µg g^{-1} FW. The presence of highest amount of ferulic acid among all phenolic acids, under normal conditions may determine biological activity of extracts achieved from *Zea mays* L. leaves, whereas the accumulation of salicylic acid as the dominant phenolic acid under salinity stress pointed out towards its role in salinity stress tolerance. Several studies have reported the protective effect of exogenous SA during salt stress through increased survival rate, shoot growth and photosynthesis in tomato and barley plants (Stevens *et al.,* 2006; Tayeb *et al.,* 2005). However, *in vivo* level of SA during stress and its biosynthesis pathway under stress conditions need to be elucidated. Borsani *et al.,* (2001) have observed that endogenous SA amplified the effect of initial ROS levels and involved in the plant responses to salt and osmotic stress in *Arabidopsis.*

Molina *et al.,* (2002) have reported in their study that tomato cells adapted to 100 mM NaCl contained lower amounts of free and conjugated SA than non-adapted ones. Above reports favour our findings that accumulation rate of SA is dependent on the salinity level and adaptability capacity of the plant species. According to Sawada *et al.,* (2006) salicylic acid (SA) is an important signal molecule of the plant defense responses and is involved in regulation of the anti-oxidative system.

They stated that endogenous SA content is increased by various environmental stresses, including salinity due to the higher activity of benzoic acid 2-hydroxylase (BA2H) a rate limiting enzyme of SA biosynthesis in rice seedlings. Therefore, induction and control of BA2H under stress conditions are important for the anti-oxidative system. When rice seedlings were exposed to salt stress and treated with uniconazole *in vivo*, accumulation of SA was suppressed. These results suggest that inhibition of BA2H can control the endogenous response to salt stress and prevent SA accumulation in rice seedlings under oxidative stress.

Phenolic acids are considered to be powerful antioxidants, widely present in plants. In particular, hydroxy-cinnamic acids, a group of phenolics highly abundant in cereals, exhibit good antioxidant properties. As the antioxidant ability of hydroxyl-cinnamic acids was demonstrated by their ability to quench singlet molecular oxygen, Janda *et al.,* (2007) have also been reported that an increase in the oHCA content in winter wheat plants could be induced independently of SA biosynthesis, and might play a role in the antioxidative response to plants under adverse environmental conditions. In the present study, an increase in the level of phenolic acids was found with increasing concentrations of salt treatment, and so the increased phenolic acids level during salt stress could also be the result of adaptation. SA level may have elevated the ROS level, because increased level of phenolic acids were observed, which could have had an antioxidant role. It can be concluded from the data that phenolic acids may have an antioxidant role during salt stress and could play a role in adaptation processes.

Conclusion

Induction of secondary metabolism under abiotic stress conditions in plants is a vital point to develop a stress tolerance crop plants. The results of this study signify the role of SA in regulating the salt stress response of maize, and suggest that SA could be used as a potential growth regulator to improve plant growth and development under salt stress. These effects of SA may depend on the plant species, and further research is needed to confirm our results.

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