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EFFECTS OF SALICYLATE ON GROWTH AND BIOCHEMICAL CHANGES IN MAIZE SEEDLINGS UNDER SALT STRESS

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ABSTRACT

Protective effects of exogenous salicylic acid (SA) on maize (Zea mays L.) seedlings under salinity stress were studied. Pre-soaking treatments of NaCl (0, 50, 100, 150 and 200mM) were given to maize seeds in presence as well as absence of 0.5 mM salicylic acid. The injurious effects of salinity on growth and development were manifested by decreased dry weight, leaf area, number of roots, and percentage water content along with reduction in biochemical components. Activity of both superoxide dismutase (SOD) (EC 1.15.1.1) and catalase (CAT) (EC 1.11.1.6) increased during saline conditions, however SA pretreatment increased SOD activity further and the activity of catalase was decreased in antagonistic manner. Degree of lipid peroxidation declined through the significant reduction in MDA content in maize seedlings. Results suggests that exogenous Salicylic acid reduced the detrimental effects of salinity and controlled growth, development and stress responses in maize plants.

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KEY WORDS

Antioxidant enzymes; Lipid peroxidation; Salicylic acid; Salinity; Zea mays

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[I] INTRODUCTION

Salt stress is one of the major problems for agriculture as it prevents plants from realizing their yield potential and has become more prevalent as the intensity of agriculture increases [1]. Salinity stress-induced metabolic changes are enhanced due to accumulation of toxic compounds in cells that include excess ions and reactive oxygen species (ROS). ROS are cleared from the cell by the action of superoxide dismutase (SOD), catalase, and peroxidases [2] and /or involved in oxidative signal transduction which in turn trigger the antioxidant defense system associated with the mechanisms by which plant cells sense the environment and make appropriate adjustments to gene expression [3], metabolism [4] and physiology [5]. Development of methods to induce salt stress tolerance in plants is vital and receives considerable attention to increase plant productivity.

Salicylic acid (SA) has appeared as a new phytohormone biosynthesized from the phenylalanine in plant metabolism and considered as an important signaling molecule modulating plant responses to abiotic stresses [6]. Studies have shown that plant dry mass under abiotic stresses was significantly higher with the application of SA than without SA [7, 8]. However, effects of exogenous SA on physiology and biochemistry of plant species under saline condition are still not understood.

In the present work it is demonstrated that SA pretreatment caused protection against salinity in 2-week-old maize seedlings under saline and non-saline conditions.

[II] MATERIALS AND METHODS

Seeds of maize (Zea mays L.) var. Jaunpuri were procured from Plant Breeding Department, Institute of Agricultural Sciences, B.H.U., India. Seeds were surface sterilized with 0.01% $HgCl_2$ followed by thorough washing with glass-distilled water. Homogenous lots of surface sterilized seeds were presoaked in different treatments for 6 h as follows: Distilled water (Control); 0.5 mM SA; 50, 100, 150 and 200 mM of NaCl and 0.5mM 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 acid washed sand in polybags. Plants were grown in a glasshouse under natural light conditions (in range of 27-35º C air temperature, 450-500 µmol/m2 s light intensity and 75% relative humidity). Each polybag contains 6 plants and supplied with 20 ml of 50% of Hoagland's nutrient solution at alternate days [9].

Plants were harvested two weeks after the germination and dried in a thermoventilated oven at 70ºC until constant weight. Growth parameters like number of roots, % water content, leaf area and dry weight were calculated according to the standard methods. Various biochemical analyses were performed in the leaf samples of two weeks old maize plants. Pigment contents,soluble protein and total phenolic content were estimated as per Lichtenthaler [10], Lowry *et al*. [11] and Farkas and

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Kiraly [12] respectively. MDA content, Catalase activity and Superoxide dismutase activity were determined as per Heath and Packer [13], Kar and Mishra [14] and Beauchamp and Fridovich [15] respectively. The experiments were repeated twice with three replicates (n=5) and the data presented are mean ± standard errors (SE). The results were subjected to one-way ANOVA and means were compared by the least significant difference (LSD) test and Tukey's multiple range test at the 0.05 and 0.01 percent level of significance.

[III] RESULTS AND DISCUSSION

Plant growth: Growth pattern of maize plants was adversely affected with exposure to NaCl salinity. 0.5 mM SA application resulted in significant increase in dry matter yield both in saline and non-saline conditions **[SupplementaryTable–1]**, however, effect was more pronounced under saline condition as compared to non-saline conditions. Shakirova *et al.* [16] also reported similar results. Increase in dry matter of salt stressed plants in response to SA may be attributed to antioxidants and protective role of membranes that alleviate the plants tolerance to damage.

Pigments, soluble protein and phenolics: Data presented in **Supplementary Table–2**. show that pigments content of NaCl-treated maize plants was significantly lower compared to controls. Whereas, in presence of 0.5 mM salicylic acid, effects of NaCl-stress on the pigment content was reduced upto 50%. Soluble protein content decreased sharply with increasing concentrations of NaCl-stress **[Supplementary Table–2]**. Retardation in soluble protein content was significantly observed at 150 mM, reaching maximum at 200 mM of salt stress. The reduction in the level of soluble protein content under salinity may be due to breakdown/ degradation of chlorophylls or due to inhibition of foliar proteins required for the genesis of photosynthetic pigments. While, in presence of 0.5mM salicylic acid effects of NaCl stress were counteracted and soluble protein levels were increased significantly. Salicylic acid is supposed to increase the functional state of photosynthetic machinery in plants either by the mobilization of internal tissue nitrate or chlorophyll biosynthesis [17]. This may lead to increased soluble protein. NaCl-stressed accumulation of total phenolics was very high than that of control (2 fold) in absence of salicylic acid. Phenolics constitute a part of cellular solutes and provide a reducing environment to the system [18]. Whereas, in the presence of exogenous salicylic acid, phenolics content was reduced significantly **[Supplementary Table–2]**. Salicylic acid modulates plant responses to a wide range of oxidative stresses [19].

Lipid peroxidation: Degree of lipid peroxidation was measured as accumulation of MDA content in leaf tissues of 2 weeks old maize seedlings. Accumulation of MDA in NaCl stressed (200mM) maize seedlings was very high (2 fold) than that of control in absence of SA, whereas in the presence of 0.5mM exogenous SA content of MDA was reduced significantly **[Figure–1]**. MDA content could reflect the degree of membrane lipid peroxidation with increasing concentrations of NaCl-stress because lipid peroxidation is caused by the reaction between •OH and the methylene groups of polyunsaturated fatty acids, which are the main components of membrane lipids [20]. Present findings are in agreement with those of Gunes *et al.* [21].

Antioxidant enzymes: ROS scavenging enzymes (SOD, catalase), activity increased with exposure to NaCl salinity (0, 50, 100,150, 200mM) in maize seedlings in the presence of 0.5 mM SA. In absence of SA, activity of catalase increased significantly while activity of superoxide dismutase (SOD) was found as good as control **[Figure– 2 A and B]**. After 0.5 mM of SA pretreatment a decrease in catalase activity was observed which exhibit the inhibitory properties of SA to catalase activity in several plant species [22]. In the present case a pronounced decrease was found in the catalase activity after 0.5 mM SA pretreatment to maize seeds. This decrease could be due to a secondary effect of SA rather than to a direct catalase-binding affinity resulting in enhancement of H_2O_2 production and subcellular localization in the cells [23]. Increasing NaCl (50-200 mM) treatments (14 days) increased SOD activity with respect to the control values. Moreover, application of 0.5 mM SA induced higher SOD activity than those of salt stressed maize plants. This implies that SA might be involved in the positive amplification loops in ROS signaling pathways, which results in enhanced production and amplification of the ROS signals [24]. Accumulation of ROS in cells might activate the ROS-scavenging pathways (leading to increasement in SOD and other antioxidant enzymes) and result in suppression of ROS [25]

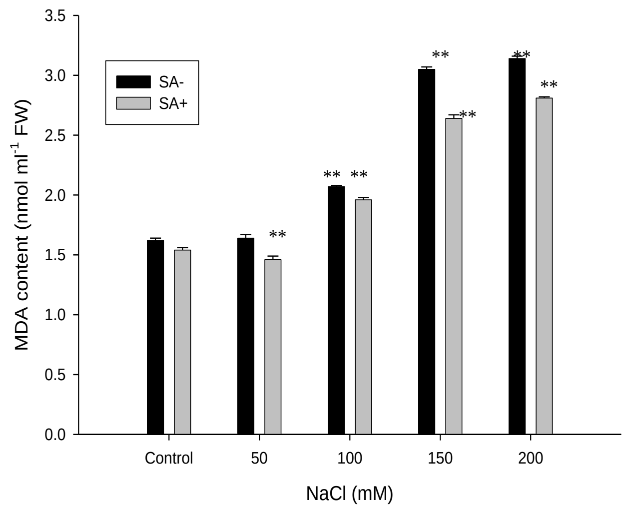

Fig: 1. Effects of salicylic acid on MDA content in 2-week-old maize seedlings under increasing concentration of NaCl. 0.5 mM SA was given as presoaking seed treatment. Data presented are mean ± S.E. (n=5). ** represent significant differences compared to controls at P < 0.01 according to Tukey's multiple range test. LSD values were determined at *P* < 0.05.

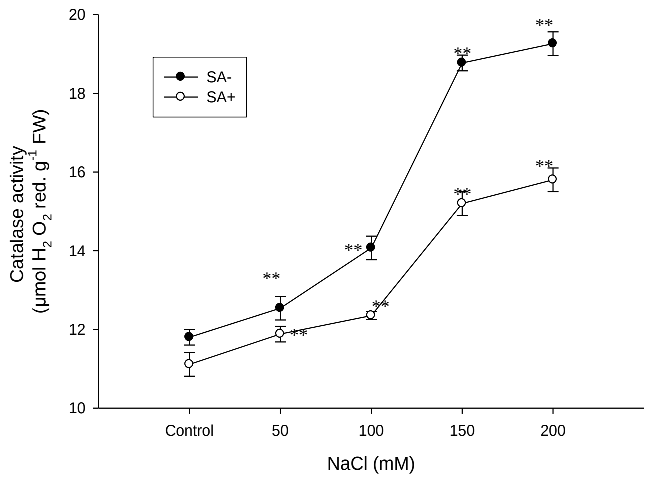

Fig: 2(A). Effects of salicylic acid on changes in (A) CAT and (B) SOD activity in 2- week-old maize seedlings under increasing concentration of NaCl. 0.5 mM SA was given as presoaking seed treatment. Data presented are mean ± S.E. (n=5). ** represent significant differences compared to controls at P < 0.01 according to Tukey's multiple range test. LSD values were determined at *P* < 0.05

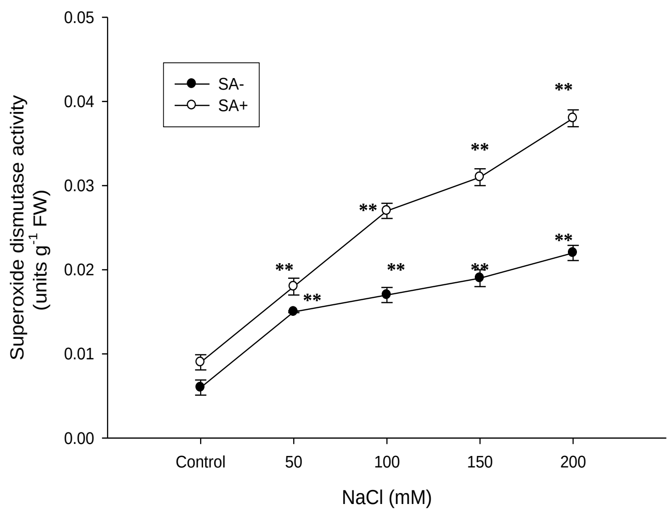

Fig: 2(B). Effects of salicylic acid on changes in (A) CAT and (B) SOD activity in 2- week-old maize seedlings under increasing concentration of NaCl. 0.5 mM SA was given as presoaking seed treatment. Data presented are mean ± S.E. (n=5).** represent significant differences compared to controls at P<0.01 according to Tukey's multiple range test. LSD values were determined at *P* < 0.05

[IV] CONCLUSION

In conclusion salinity induced oxidative stress in maize plants, as evidenced by the decline in growth, increase in lipid peroxidation and changes in antioxidant defence mechanism. The potentiating effect of SA was observed in present study after pretreating maize seeds with 0.5 mM SA, where the deleterious effects of salinity was restored as growth, lipid peroxidation and pattern of antioxidant enzymes in maize plants.

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Supplementary Tables (As supplied by author)

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

NaCl (mM)	Dry weight (g)		Leaf area (cm2)		No. of roots		Percentage water content	
	SA-	SA+	SA-	SA+	SA-	SA+	SA-	SA+
0	2.97 (±0.66)	3.56 (±0.80)	28.1 (±7.43)	42.2 (±8.26)	14 (±3.57)	19 (±4.03)	75.58 (±16.90)	76.28 (±17.05)
50	2.56** (±0.57)	3.09** (±0.69)	23.2** (±5.80)	40.5** (±7.63)	11** (±2.91)	17 (±3.57)	78.68** (±17.60)	78.77* (±17.42)
100	2.18** (±0.49)	2.69** (±0.60)	18.7** (±5.24)	34.4** (±6.37)	8** (±2.24)	14** (±2.91)	78.56** (±17.50)	78.96** (±17.96)
150	1.90** (±0.42)	2.09** (±0.47)	12.2** (±4.50)	30.1** (±5.73)	6 (±1.79)	12 (±2.46)	76.66** (±17.10)	82.54** (±18.46)
200	1.54** (±0.34)	2.00** (±0.45)	6.1** (±2.41)	24.2** (±4.09)	3** (±1.12)	10** (±2.01)	75.77** (±16.90)	78.74** (±17.61)
LSD	(0.019)	(0.021)	(0.022)	(0.023)	(1.072)	(1.433)	(0.028)	(0.023)

SupplementaryTable: 2. Effects of salicylic acid on changes in biochemical components of 2-week-old maize plants under NaCl stress

NaCl (mM)	Total chlorophyll (mg g^{-1} FW)		Carotenoids (mg g^{-1} FW)		Soluble protein (mg g^{-1} DW)		Total phenolics (mg g^{-1} FW)	
	SA-	SA+	SA-	SA+	SA-	SA+	SA-	SA+
0	4.15 (±0.92)	7.19 (±1.61)	1.84 (±0.41)	2.98 (±0.44)	1.44 (±0.32)	1.91 (±0.43)	0.121 (±0.03)	0.127 (±0.03)
50	3.33** (±0.74)	6.56** (±1.47)	1.36** (±0.30)	2.57** (±0.34)	1.07** (±0.24)	1.82** (±0.41)	0.128** (±0.03)	0.132 (±0.03)
100	1.82** (±0.41)	5.71** (±1.27)	0.90** (±0.23)	2.23** (±0.28)	1.00** (±0.22)	1.34** (±0.29)	0.151** (±0.03)	0.134 (±0.03)
150	0.81** (±0.18)	4.88** (±1.09)	0.70** (±0.18)	2.01** (±0.23)	0.73** (±0.16)	1.09** (±0.24)	0.199** (±0.04)	0.169** (±0.04)
200	0.28** (±0.06)	3.73** (±0.83)	0.50** (±0.17)	1.81** (±0.21)	0.54** (±0.12)	0.98** (±0.22)	0.237** (±0.05)	0.179** (±0.04)
LSD	(0.020)	(0.021)	(0.020)	(0.015)	(0.017)	(0.014)	(0.0001)	(0.020)

Data presented are mean ± SE (n=5). * and ** represent significant differences compared to controls at $P < 0.05$ and $P < 0.01$ respectively according to Tukey's multiple range test LSD values were determined at $P < 0.05$