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Effect of Salicylic Acid on the Growth, Photosynthesis and Carbohydrate Metabolism in Salt Stressed Maize Plants

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ABSTRACT

The effect of 10^{-2} M salicylic acid (SA) on the counteracting of the NaCl (50, 100 and 150 mM)-induced deleterious effects on maize (*Zea mays* L.) cultivar was studied. Effects of SA on salt tolerance of maize were determined by measuring the growth parameters: shoot and root lengths, shoot and root fresh and dry weights and leaf area. The activity of ribulose 1,5-bisphosphate carboxylase (Rubisco), photosynthetic pigments (chlorophylls *a*, *b* and carotenoids) content, the rate of $^{14}CO_2$-fixation and sugars level were investigated in response to the interactive effects of SA and NaCl treatment. NaCl significantly reduced all growth parameters measured, Rubisco activity, photosynthetic efficiency and pigments, as well as sugar contents. The effects of NaCl on the previous parameters were increased with NaCl concentrations. Exogenous application (foliar spray) of SA counteracted the NaCl deleterious effects on maize cultivar. SA enhanced the maize salt tolerance in terms of improving the measured plant growth criteria. SA appears to stimulate maize salt tolerance by activating the photosynthetic process.

Key Words: Salicylic acid; Photosynthesis; *Zea mays* L.; Salinity; $^{14}CO_2$-Fixation

INTRODUCTION

Environmental stresses as salinity and drought reduce growth and agricultural productivity more than other factors (Karakas *et al.*, 1997). Higher salinity levels caused significant reduction in growth parameters like leaf area, leaf length and root and shoot dry weight (Ashrafuzzaman *et al.*, 2002). Waterlogging and seawater treatments decrease carotenoids in *Zea mays* seedlings and induce reduction in chlorophyll and photosynthetic activity (Hill reaction and $^{14}CO_2$-light fixation) (El-Shihaby *et al.*, 2002).

El-Shihaby *et al.* (2002) reported that the activity of ribulose 1,5-bisphosphate carboxylase (Rubisco) decreased in maize plants in saline conditions. The authors also stated that the reduction in Rubisco activity resulted in a drop of net formation of carbohydrates.

Salicylic acid (SA) and related compounds have been reported to induce significant effects on various biological aspects in plants. These compounds influence in a variable manner; inhibiting certain processes and enhancing others (Raskin, 1992). Different levels of acetylsalicylic acid appeared to function as antitranspirant in leaves of *Phaseolus vulgaris*, and inhibiting the opening of stomata in epidermal strips of *Commelina communis* (Larqué-Saavedra, 1978, 1979). Salicylic acid has also been recorded to reverse the closure of stomata caused by abscisic acid (ABA) (Rai *et al.*, 1986). Obvious effects on yield of various crop species have been achieved following exogenous application of salicylic acid: an increase in yield and number of pods has been observed in mung bean (Singh & Kaur, 1980) and *Phaseolus vulgaris* (Rendon, 1983; Lang, 1986). Salicylic acid treatment resulted in retarding ethylene synthesis, interfering with membrane depolarization, stimulating photosynthetic machinery, increasing the content of chlorophyll as well as blocking wound response in soybeans (Leslie & Romani, 1988; Zhao *et al.*, 1995). More recently, it has been recognized that salicylic acid is required in the signal transduction for inducing systemic acquired resistance against some pathogenic infections (Gaffney *et al.*, 1993; Métraux *et al.*, 1990; Vernooij *et al.*, 1994).

The objective of this work was to investigate whether salicylic acid could be a protectant to ameliorate the influence of salt stress on maize and thereby increasing its salt tolerance.

MATERIALS AND METHODS

A homogenous lot of seeds of *Zea mays* L. (S.C. 10) were obtained from the Crop Institute, Agricultural Research Center, Giza, Egypt. Seeds were sterilized with sodium hypochlorite solution (5%) for five minutes, washed thoroughly with distilled water before use. The seeds were transferred into polyethylene containers (20 cm internal diameter and 50 cm long), each filled with 5 kg sandy soil. Six seeds were sown in each container and after emergence; three homogenous seedlings were left in each container. Irrigation was carried out using 50% strength Hoagland nutrient solution (Hoagland & Arnon, 1950) with or without the desired concentrations of NaCl (0.0, 50, 100 and 150 mM) for 7 d. One container represented one replicate. Salicylic acid was initially dissolved in a few drops of dimethylsulfoxide and the final volume was reached, using distilled water. The pH was adjusted at 5.5 with KOH (1.0

N), Tween40 was added to evoke spreading of the applied solutions on the plant-leaf surface. The solutions were sprayed once on the leaves in the early morning, when the plants had their fourth leaf completely expanded. A constant volume was sprayed in all cases with a manual pump. All determinations were carried out seven days after salicylic acid treatment and 15 d after salt exposure. The plants were divided into four groups: (1) control (50% strength Hoagland nutrient solution), (2) plants treated with salicylic acid (10^{-2} M), (3) plants received NaCl solutions at 50, 100 and 150 mM, (4) plants treated with SA (10^{-2} M) plus each of the salinity levels.

All treatments were replicated three times. Half of the samples were rapidly dried in an oven at 80°C to constant weight and then ground to fine powder, which was used for determination of dry weight and sugar fractions. The other half was used for growth data, extraction of pigments, Rubisco and photosynthetic activity. Determination of photosynthetic efficiency ($^{14}CO_2$-fixation) using ^{14}C-technique was carried out in the Atomic Energy Authority, Radioisotopes Department, Cairo, Egypt. According to the procedure of Moussa (2001), 80% acetone extract of $^{14}CO_2$ was assayed using liquid scintillation counter. The activity of Rubisco was assayed as described by (Vu *et al.*, 1997).

Estimation of pigment contents was achieved by application of the method of Metzner *et al.* (1965): 80% acetone extract was colorimetrically assayed at 452, 644 and 655 nm.

The procedure of Naguib (1964) was used to determine the sugar fractions: plant extract plus arsenomolybdate solution develops the color, which was colorimetrically measured at 700 nm.

Statistical analysis. The results presented in the tables are the mean of three replications. The data were statistically analyzed using the least significant difference (LSD) test as described by (Snedecor & Cochran, 1981).

RESULTS AND DISCUSSION

Application of the test levels of NaCl to maize plants adversely influenced their growth pattern (shoot and root length, fresh and dry weights of shoots and roots and leaf area), as compared with control plants (Table I). These results are in agreement with those of Ghoulam *et al.* (2001), who showed that NaCl salinity caused a marked reduction in growth parameters (leaf area, fresh and dry weight of shoots and roots of sugar beet plants. Salicylic acid-treated maize plants exhibited an increase in tolerance to salt treatment. This increase in salt tolerance was reflected in the measured growth criteria: fresh, dry and length of shoots and roots as well as leaf area were increased comparing with plants received NaCl only (Table I). Gutierrez *et al.* (1998) also reported a similar increase in the growth of shoots and roots of soybean plants in response to salicylic acid treatment. Dhaliwal *et al.* (1997) and Zhou *et al.* (1999) also indicate that SA increases the leaf area in sugarcane plants, which is consistent with our results in maize plants.

Data presented in Table II show that Rubisco and photosynthetic activities significantly reduced in salinized maize plants under NaCl levels compared with controls. Water stress decreased Rubisco in sugarcane leaves (Du *et al.*, 1998). Wattana and Monica (1999) showed that salinity-stressed plants had a consistently lower photosynthetic rate than those of the control. The decrease in CO_2-fixation induced by NaCl treatments was as follows: 12% at 50 mM, 33% at 100 mM and 62% at 150 mM NaCl. SA solely or in combination with NaCl levels significantly stimulated Rubisco and photosynthetic activities in all tested plants (Table II). Liu *et al.* (1999) also demonstrated that salicylic acid enhanced the photosynthetic ability of apple leaves. The ameliorative effects of SA on NaCl-induced reduction in CO_2-fixation were 34% at 50 mM, 48% at 100 mM and 19% at 150 mM NaCl.

Table III shows that the pigments (chlorophyll *a*, *b* and carotenoids) content of NaCl-treated maize plants was significantly decreased below that of the controls. Similarly, Dela-Rosa and Maiti (1995) found an inhibition in chlorophyll biosynthesis in sorghum plants because of salt stress. Enhancing effects of SA on photosynthetic capacity could be attributed to its stimulatory effects on Rubisco activity and pigment contents reported in this study. Salicylic acid-treated plants exhibited higher values of pigment concentration than those of control or salinity-

Table I. Growth characteristics of *Zea mays* seedlings in response to treatment with NaCl in presence or absence of salicylic acid. Each value is the mean of three replicates. All treatments are significant at 1% level of their controls

Salicylic Acid (M)	NaCl (mM)	Lengths (cm)		Fresh weight (g)		Dry weight (g)		Leaves area/plant (cm^2)
		Shoot	Root	Shoot	Root	Shoot	Root	
	0.0	43.0	13.1	16.28	3.03	2.63	0.40	193
	50	42.5	14.0	15.07	2.80	2.45	0.36	182
0.0	100	37.0	10.1	13.17	2.64	2.09	0.33	168
	150	30.3	6.7	10.09	1.89	1.52	0.25	160
	0.0	47.3	15.8	20.07	6.18	3.35	0.81	215
	50	45.5	16.0	18.74	7.01	3.00	0.90	210
10^{-2}	100	44.7	13.8	17.40	4.75	2.74	0.53	200
	150	38.9	9.2	14.82	3.82	2.28	0.47	194
L.S.D. 1%		1.4	1.3	1.2	0.9	1.3	0.05	2.6

Table II. Changes in rubisco and photosynthetic activity of *Zea mays* seedlings in response to treatment with NaCl in presence or absence of salicylic acid. Each value is the mean of three replicates. All treatments are significant at 1% level of their controls

Salicylic Acid Concentration (M)	NaCl Concentrations (mM)	Rubisco Activity (**nKat/g fresh weight)	Photosynthetic Activity (*dpm/mg fresh weight)
	0.0	13	7975
	50	12	7037
0.0	100	10	5352
	150	7	3078
	0.0	19	11381
	50	20	9417
10^{-2}	100	15	7935
	150	13	5882
L.S.D 1%		1.42	

* Disintegration per minute; ** Mol/sec × 10^{-9}

Table III. Changes in pigments and carbohydrate contents of *Zea mays* seedlings in Response to treatments with NaCl in presence or absence of salicylic acid. Each value is the mean of three replicates. All treatments are significant at 1%level of their controls

Salicylic Acid (M)	NaCl (mM)	Pigments(mg/g fresh weight) Chlorophyll *a*	Chlorophyll *b*	Carotenoids	Carbohydrates (mg/g dry weight) Soluble Sugars	Polysaccharides	Total
0.0	0.0	4.13	1.92	0.97	77.9	178.6	256.5
	50	3.71	1.64	0.71	83.4	170.3	253.7
	100	3.02	1.13	0.48	90.1	158.9	249.0
	150	1.91	0.98	0.30	98.7	144.7	243.4
	0.0	6.04	3.12	2.15	72.3	195.0	267.3
10^{-2}	50	5.12	2.71	1.73	75.7	188.4	264.1
	100	4.51	2.02	1.42	81.5	179.6	261.1
	150	3.90	1.71	1.01	87.9	158.9	246.8
LSD 1%		0.28	0.18	0.12	2.1	2.5	2.0

treated samples (Table III). In soybean plants, treatment with salicylic acid, increased pigments content as well as the rate of photosynthesis (Zhao *et al*., 1995). Sinha *et al.* (1993) pointed out that chlorophyll and carotenoid contents of maize leaves were increased upon treatment with SA. Taking together, the results of the previous authors support our findings.

Maize plants submitted to NaCl salinity treatment, showed a progressive increase in their soluble sugar content with increasing the salinity level, while an opposite trend was obtained with respect to polysaccharide concentration (Table III). Moreover, SA treatment caused a significant decrease in the content of soluble sugars below that of untreated samples (Table III). SA increased, however, the polysaccharide contents as compared with salinized or the control plants (Table III). In this regard, soluble sugar content was also increased in tomato plants in relation to salt stress (Maria *et al*., 2000). It is suggested that SA application might activate the metabolic consumption of soluble sugars to form new cell constituents as a mechanism to stimulate the growth of maize plants reported in this study. SA treatment might also be assumed to inhibit polysaccharide-hydrolyzing enzyme system on one hand and/or accelerate the incorporation of soluble sugars into polysaccharides. Our assumption could be supported by the result that SA increased polysaccharide level on the sake of soluble sugars. In this connection, Sharma and Lakhvir (1988) postulated that foliar spray of SA to ray plants, resulted in decreasing their soluble sugar level.

In summary, it might be concluded that SA treatment of salt stressed maize could stimulate their salt tolerance via accelerating their photosynthesis performance and carbohydrate metabolism.

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