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Salicylic Acid and Salinity Effects on Growth of Maize Plants

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Abstract: A Pot experiment was conducted at the greenhouse of the National Research Centre at Dokki, Cairo, Egypt. During the winter season of 2004/2005 to evaluate the effect of salinity and salicylic acid on growth of maize plants c.v. Single Hybrid 10. The treatments of salinity by irrigation with diluted Mediterranean sea water: tap water (250 ppm), 2000 and 4000 ppm and spraying salicylic acid in 200 ppm twice after 3 weeks from sowing and two weeks later. A negative relationship was detected between vegetative growth parameters and the increase in salt concentration in irrigation water. The area of green leaves were mostly decreased from 5102 cm2 in the plants irrigated by water contains 250 ppm salts (control) to be 2389 cm^2 in those irrigated by water contained 4000 ppm (53.18 %). Moreover, dry weight of stem, leaves and whole plant showed approximately similar response. The depression on stem, leaves and whole plants dry weight when irrigated by saline water of 4000 ppm amounted by 57.29, 47.43 and 51.43 % compare to the control. Spraying plants with salicylic acid in the rate of 200 ppm improved all growth characters i.e. plant height, number and area of green leaves, stem diameter and dry weight of stem, leaves and whole plant. The highest increment was shown in stem dry weight and the lowest in stem diameter. All amino acid concentrations were lowered by salinity except for proline and glycine. All determinate amino acid concentrations (except methionine) were increased with the application of salicylic acid (200ppm). On the other hand, methionine was negatively responded which slightly lowered. For plants irrigated with fresh water, SA gave its higher effect on cystine followed by that in arginine and tyrosine while the other amino acids were slightly affected. Serine phenyalanine and tyrosine showed approximately similar response. However, at 4000 ppm treatment, salicylic acid improved the concentration of arginine, lycine, serine and glutamic acid. Moreover, proline concentration increased when using salicylic acid foliar application and under salt stress.

Key words: Maize- Salinity-Diluted seawater-Salicylic acid-Growth-Dry matter-Amino acids.

INTRODUCTION

Consequent to population growth and high limiting standards in several areas in arid and semi arid regions competition for fresh water among different water sectors is expected to increase vis -a-vis its decreased allocation to irrigation. Non conventional water resources such as saline or sodic drainage and under ground water represent complimentary supply to narrow the gab between fresh water availability and demand[35].

Negative effect was observed in growth and yield of maize plants irrigated with salt water or increased the salt concentration in the soil solution: Li, *et al.*[30]; Wichern, *et al.*[51] and Rodríguez, *et al.*[40].

Salicylic acid (SA) is an endogenous growth regulators of phenolic nature, which participates in the regulation of physiological processes in plant[44], postulate to play a role as a natural indicator of the thermogenesis to indicate flowering in a range of plants, to control ion uptake by roots and stomatal conductivity[39]. Also participation in signal regulation of genes expression in the course of leaf senescence[34]. Moreover, it might serve as a regulator of gravitropism[32], inhibition of fruit ripening[48] and many other processes.

Several studies have shown that salicylic acid is an essential component of the plant resistance to pathogens and participates in the plant response to adverse environmental conditions[9]. Salicylic acid used to increase plants tolerance against the adverse effects of biotic and abiotic stresses: Abduo, *et al.*[1], Shakirova, *et al.*[44], Sawada, *et al.*[42], Shalaby[45], Bosch, *et al.*[9], Kim, *et al.*[28] and Chen, *et al.*[12].

Therefore, this study was designed to evaluate the influences of salicylic acid in growth and amino acid profile in the leaves of maize plants and the possibility to ameliorate salt stress.

MATERIALS AND METHODS

A Pot experiment was conduced in the greenhouse of the National Research Centre at Dokki, Cairo, Egypt. During the winter season of 2004/2005 to evaluate the effect of salinity and/or salicylic acid on growth of

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maize plants c.v. Single Hybrid 10. The treatments were saline irrigation water (diluted Mediterranean sea water) tap water (250 ppm), 2000 and 4000 ppm and spraying with salicylic acid in 200 ppm concentration twice, after 3 weeks from sowing and two weeks later.The experiment included 4 levels of salinity in combination with 2 levels salicylic acid i.e. 6 treatments in 6 replicates arranged in completely randomized design. Metallic tin pots 35 cm in diameter and 50 cm in depth were used. Every pot contained 30 kg of air dried clay loam soil. The inner surface of the pots was coated with three layers of bitumen to prevent direct contact between the soil and metal. In this system, 2 kg of gravel, (particles about 2-3 cm. in diameter) was used to cover the bottom of the pot. Irrigation water was poured through a vertical tube (2.5 cm. diameter), so the movement of water was from the base upward.

Seeds of maize (*Zea mays*, *L.*) c.v. Single Hybrid 10 were sown in 15th July, plants were thinned twice one twenty days after sowing and another two weeks later leaving three plants/pot. Calcium super phosphate (15.5 % P_20_5) and potassium sulfate (48.5 % K_2O) in the rate of 2.29 and 1.14 g/pot were added before sowing. Ammonium sulfate (20.5 % N) in the rate of 6.86 g/pot were added in two equal portions the 1st after two weeks from sowing and the second two weeks latter. Salicylic acid was sprayed twice at 21 and 35 days in the rate of 200 ppm. Samples were gathered to estimate different vegetative characters and detection of amino acids analysis.

Amino acids composition of samples was determined according to the methods of Moore, *et al.* (1958). Dry and defatted samples containing 50 mg protein were weighed in the ampoules and 5 ml of 5.7 N HCL was added.Ampoules was sealed under vacuum and the contents were digested at 110° c for 24 hr. The sealed tubes were then opened and samples were filtered and the residue was washed with distilled water and the filtered volume was completed to 50 ml with double distilled water. Five ml of the filtrate were evaporated under vacuum at room temperature. The residue was dissolved in 5 ml sodium citrate buffer (PH 2.2) and filtered trough 0.22um membrane. Twenty ul` of the filtered hydrolyzate was used for the amino acids fractions.

Operating Parameters:

Instrument: EPPDROF-Geramany LC 3000 amino acid analyzer.

Condition:

Flow rate: 0.2 ml/min

Pressure of buffer: 0 to 50 bar

Pressure of reagent: 0 to 150 bar

Reaction temperature: 123°c

The data collected were statistically analyzed as described by Snedecor and Cochran[46].

RESULTS AND DISCUSSIONS

Irrigation by Diluted Sea Water:

a)-Growth: A negative relationship was detected between vegetative growth parameters and the increasing salt concentration in water of irrigation. The highest effect was shown in the area of green leaves which decreased from 5102 cm2 in the plants irrigated by tap water to be 2389 cm2 in those irrigated by water contained 4000 ppm (53.18 %). Moreover, dry weight of stem, leaves and whole plant showed approximately similar response. The depression on stem, leaves and whole plants dry weight when irrigated with high salt concentration amounted by 57.29, 47.43 and 51.43% compared to the control treatment (Table 1). Salicylic Acid is thought by some investigators to be a new plant growth hormone. Also, some researchers use Aspirin in cut flowers to make the flowers last longer. Murtaza, *et al.*[35] noticed that irrigation by saline and/or sodic water lowered growth and yield of wheat. Mittova, *et al.*[33] found that salinity stress marginally decreased the rate of photosynthesis, ribulose-bisphosphate carboxilase activity (RUBPC) and chlorophyll content in the two salt tolerant varieties CSR-13 and Pokali, however, salt sensitive variety MI-48 showed greater reduction. Yamane, *et al.*[53] reported the reduction in chlorophyll by salt stress and concluded that these results induced injury in chloroplasts is dependant on light and that H_20_2 and 0H are responsible for the deleterious effects of salt stress on chlorophyll content and chloroplast ultra structure.

b)-Amino Acids: The amino acid concentrations)g/100 g protein (as affected by salinity were illustrated min Table (4). All amino acid concentrations were lowered by salinity except proline and glycine. Salinity level at 4000 ppm caused a depression which exceeded that obtained at 2000 ppm salt concentration treatment except for tyrosine. The highest depression was found in the leaves of plants irrigated with moderate saline water were shown in cystine (53.57%) and methaionine (39.71 %) while the lowest was shown in lucine (1.97%) and alanine (2.14 %). Furthermore, on raising the concentration of salts in diluted seawater to be 4000 ppm the highest depression was obtained in methionine (89.71%) and cystine (75.00%) followed by that in lycine (44.17%), arginine (40.48%) valine (35.47%). Meanwhile, the lowest depression was shown in the concentration of lucine (6.43 %) and tyrosine (8.45%) compare to that in maize plants irrigated regularly by fresh water. On the contorary, proline increased by 2.17 and 6.86 % glycine by 10.70 and

Table 1: Effect of different salinity levels on growth of maize.

Salinity levels ppm	Plant height cm	Stem diameter cm	No. of leaves	Area of leaves	Dry weight Stem	Leaves	Whole plant
Tap water	70.0	2.13	11.0	5102	25.94	26.07	52.01
2000	57.3	1.70	9.0	2595	14.59	18.06	32.65
4000	47.8	1.60	8.0	2389	11.08	14.18	25.26
L.S.D. at 5 % level	N.S.	N.S.	0.23	1453	N.S.	N.S.	9.65

Table 2: Effect of salicylic acid on growth of maize.

Salysalic acid ppm	Plant height cm	Stem diameter cm	No. of leaves	Area of leaves	Dry weight Stem	Leaves	Whole plant
0	54.80	1.72	8.7	2857	13.72	16.05	29.77
200	61.80	1.90	10.0	2867	20.68	22.82	43.44
L.S.D. at 5 % level	2.90	N.S.	0.11	714	N.S.	5.75	3.40

Table 3: Effect of Salicylic acid on growth of maize under different salinity levels.

Salinity levels ppm	Salicylic acid ppm	Plant heightcm	Stem diameter cm	No. of leaves	Area of leaves	Dry weight Stem	Leaves	Whole plant
Tap water	0	65.6	1.85	10	4058	19.19	20.14	39.33
	200	74.5	2.40	12	6146	32.69	32.00	64.69
2000	0	55.0	1.75	9	2676	12.38	16.02	28.40
	200	59.5	1.65	9	2514	16.80	20.09	36.89
4000	0	44.0	1.59	7	1837	9.59	11.99	21.58
	200	51.5	1.65	9	2941	12.56	16.37	28.93
L.S.D. at 5 % level		N.S.	N.S.	0.19	1236	N.S.	N.S.	5.89

16.61% in leaves of plants irrigated with diluted seawater of 2000 and 4000 ppm, compared to that of the control. Deficiency of K+ induced by salinity increased the level of free amino acids especially of proline, aspartic and glutamic acids[11]. They also added that proline contribute up to 27 % to total osmolality. It is hypothesized that strong proline accumulation indicates the exceeding of a critical salinity level. Nadjafa and Mirmasum[36] revealed that salinity) 2-15 dS/m(increased alanine and proline and decreased glycine and glutamuic acids in soybean plants.

The effect of salt stress on the nitrogenous compounds are fairly well known (Mansour,2000). Prakash *et al.*[37]. Na_2SO_4 treatment brought about considerable decline in labeling of sugars and an increase in labeling of amino acids and sugar phosphates in peanuts plants. Salt stressed resulted in an elevation of amino acid levels in plants[19,22,15,24]. Amino acids accumulation in salt stressed plant species has been studied by several authors: Causido *et al.*[11]; Wiladino *et al.*[52]; Hartzendorf and Rolletschek[22]; Radi, *et al.*[38] and Hussein *et al.*[24]. Proline accumulation was proposed to be associated with tolerance to osmotic and saline stress[31,38,36,50]. El-Samad and Shaddad[16] concluded that that the tolerance to salinity of soybean varieties was related to the accumulation of soluble protein, amino acids, proline, K and Ca and the sensitivity was associated with a decrease in water, sugar, protein, K and Ca. The amino acids level under salt stress conditions are directly related to the alterations of the enzymatic activities caused by feed back and / or depressive mechanisms[47,15]. Also, the increase in amino acid concentration in stressed tissues of different plants may be related to the breakdown of protein (Kramer, 1998).

Data in Table (4) cleared that salt stress depressed the concentration of essential and non essential amino acids. The depression induced by the highest level of salt used reached two fold of that caused by the moderate level of salt compared to un-stressed plants. Concerning the total amino acid decreased by the both salt solutes but decrement more with 4000 ppm and reached to be two folds of that in 2000 ppm treatment. The total essential and nonessential amino acid concentrations showed the same response. El-Tayeb,[17] found that soluble sugars, soluble proteins, free amino acids including proline content and lipid peroxidation level and peroxidase activity were increased in the two barley plant organs root and shoots with increasing of NaCl.

El-Basseouny and Bakheta[15] reported that salinity increased the total amino acid content in Gemmaza 9 while it decreased this content in Giza 168 wheat variety. This finding confirmed results obtained by: Aziz *et al.*[5] and Solcum and Wienstein[47].

Salicylic Acid:

a) - Growth: Data recorded in Table (2) showed the effect of salicylic acid on growth of maize plant. Presented data indicated that spraying salicylic acid in the rate of 200 ppm improved all growth characters i. e. plant height, number and area of green leaves, stem diameter and dry weight of stem, leaves and whole plant. The highest increment was shown in stem dry weight and the lowest in stem diameter. Gunes, *et al.*[25] reported that it has been proposed that salicylic acid acts as endogenous signal molecule responsible for inducing abiotic stress tolerance in plants. They emphasized that exogenous application of SA increased plant growth significantly both in saline and non saline conditions. H202 concentrations were increased by SA treatment (0 - 0.10 mM). Arfan, *et al.*[4] stated that changes in photosynthetic rate due to stomatal inhibition but was associated with metabolic factors other than photosynthetic pigments or leaf carotenoids. Plants produce proteins in response to abiotic and biotic stress and many of these proteins are induced by phytohormones such as ABA[27] and salicylic acid[23]. Salicylic acid is an endogenous growth regulator of phenolic nature, which influence a range of diverse processes in plants, including seed germination[13], stomatal closure[29], ion uptake and transport, membrane permeability[7], photosynthetic and growth rate[26]. SA is also important signal molecule for modulating plant responses to environmental stress. SA is known to provide protection against a number of abiotic stresses[43]. Khan, *et al.*[26] reported that applied salicylic acid, acetyl salicylic acid and genicic acid enhanced photosynthetic rate. Stomatal conductance and transpiration also increased however, chlorophyll did not altered in both soybean (a C3 plant) and corn (a C4 plants). They added that, in most cases, treatment with these compounds increased leaf areas and plant dry mass, while, plant height and root length were not affected. Moreover, Gunes, *et al.*[20] demonstrated that exogenously applied SA increased plant growth significantly both in saline and non-saline conditions and this may be related to the strongly to its inhibiting effect on Cl- and Na+ and improving the uptake of N, Mg, Fe, Mn and Cu and / or due to its effect on lipid peroxidation, measured in terms of malondialdehyde (MDA) content and membrane permeability.

b)- Amino Acids: All determinate amino acid concentrations were increased with the application of salicylic acid (200 ppm). On the other hand, methionine was reversely responded which slightly lowered (5.00 %). Tyrosine lysine, arginine concentration were markedly increased (31.16, 22.30 and 20.68 %), however, the concentration of alinine and lucine increased by 2.65 and 2.78% (compared to unstressed plant (Table 4). The effect of salicylic acid on amino acid status in plants were studied by: Burkhanova, *et al.*[10]; Fidena, *et al.*[18] and Sakhabutdinova, *et al.*[41].

Diluted Seawater X Salicylic Acid:

a) - Growth: The interaction of salinity and salicylic acid on growth of maize plants were illustrated in Table (3). Number and area of leaves and whole plant dry weight were significantly affected by the interaction,while, differences in plant height, stem diameter and dry weight of leaves and stem were not significant. Similar responses were detected in number and area of leaves and dry weight of whole plant in which salicylic acid treatment increased both characters under saline irrigation water of 4000 ppm salinity level as well as the unstressed plants but the percentage of increment under the high salt stress used slightly more than that caused under non stress condition. However, this organic acid did not exert any significant effect on number or area of green leaves of maize plants subjected to 2000 ppm salt stress. Furthermore, salicylic acid foliar application increased number as well as area of leaves and dry weight of whole plant by: 64.48, 29.89 and 34.06 % compared to control plants. Presented date proved that salicylic acid improved dry matte under saline and non saline conditions but act more in plants grown under normal condition.

From the above mentioned data, it could be concluded the possibility of salicylic acid using in ameliorate the adverse effect of salinity on maize plant. Concerning this phenomenon, Sakhabutdinova, *et al.*[41] concluded that the SA treatment reduced the damaging action of salinity and water deficit on wheat seedling growth and accelerated a restoration of growth processes. Afzal, *et al.*[3] measured growth of wheat seedlings grown under normal (4 dS/ cm) and saline (15 dS/cm) conditions, reported that root and shoot length, fresh and dry weight of seedlings were significantly increased by 50 ppm ascorbic acid and 50 ppm salicylic acid treatments under both normal and saline conditions. It was concluded that hormonal priming has reduced the severity of the effect of salinity but the amelioration was better due to 50 ppm SA and 50 ppm ascorbic acid treatments as these showed best

Table 4: Effect of salicylic acid and salinity on amino acid profile induces of maize plants (g/100 g protein).

	Tap water (250ppm)		2000 (ppm)		4000 (ppm)	
	Saliyclic acid ppm					
	0	200	0	200	0	200
Aspartic	8.84	9.14	7.54	8.32	5.99	6.60
Thrionine	4.36	5.02	3.59	4.15	3.05	3.43
Serine	5.02	6.42	4.68	5.84	3.97	4.69
Glutamic	13.52	14.69	12.88	13.32	11.61	12.92
Proline	8.36	9.04	8.59	9.29	8.77	9.90
Glycine	2.64	2.77	2.94	3.05	3.07	3.24
Alanine	2.74	9.89	9.55	9.66	7.09	8.35
Cystine	0.09	0.18	0.06	0.07	0.03	0.04
Valine	7.13	7.24	6.32	6.50	4.39	4.88
Muthionine	0.75	0.61	0.38	0.44	0.06	0.08
Isolycine	5.06	5.38	4.23	4.57	3.70	3.84
Lucine	13.78	14.02	13.03	13.29	11.98	12.55
Tyrosine	3.17	4.29	2.78	3.92	2.33	2.64
Phynilalanine	6.02	6.38	4.64	5.46	4.38	4.79
Histidine	3.02	3.27	2.57	3.01	2.08	2.29
Lycine	3.94	4.12	3.08	3.52	1.58	2.91
Arginine	4.26	4.98	3.12	3.58	2.33	3.17
TEAA	37.17	40.00	31.69	35.42	27.09	30.33
TNEAA	62.61	67.44	50.29	62.48	50.11	56.01
TAA	99.78	107.44	89.98	97.90	77.20	86.34

TAA= Total amino acids
TEAA = Total essential amino acids
TNEAA = Total nonessential amino acids

results on seedling growth, fresh and dry weights under non-saline and saline conditions whereas hormonal priming with ABA as not effective in inducing salt tolerance under present experimental material and conditions. Hamada[21] revealed that salicylic acid increased with decreasing soil moisture. The soluble proteins and proline in roots and proline and other free amino acids in shoots increasing with water stress but the presence of vitamins or aspirin during water stress lowered this effect. Bezrukova, *et al*.[8] pointed out that treatment of wheat by SA (0.05mM) either by soaking seeds or placing 3-days-old sprouts for 24 h in a solution of SA reduced the changes caused by the osmosis in the hormonal balance of roots and the associated inhibition in growth of wheat. Sakhabutdinova, *et al*.[41] concluded that the SA treatment reduced the damaging action of salinity and water deficit on wheat seedling growth and accelerated a restoration of growth processes. Gunes, *et al*.[20] demonstrated that salicylic acid inhibited strongly Na+ and CL+ accumulation but stimulate N, Mg, Fe, Mn and Cu concentration of stressed maize plants. These results suggest that SA could be used as a potential growth regulator to improve plant salinity stress tolerance. Arfan, *et al*.[4] indicated that exogenous application of SA (Salicylic acid) promoted growth and yield and counteracted the salt stress-induced inhibitory of salt tolerant for C.V. S-24, whereas, for MH-97 durum wheat varity there was no improvement in growth or

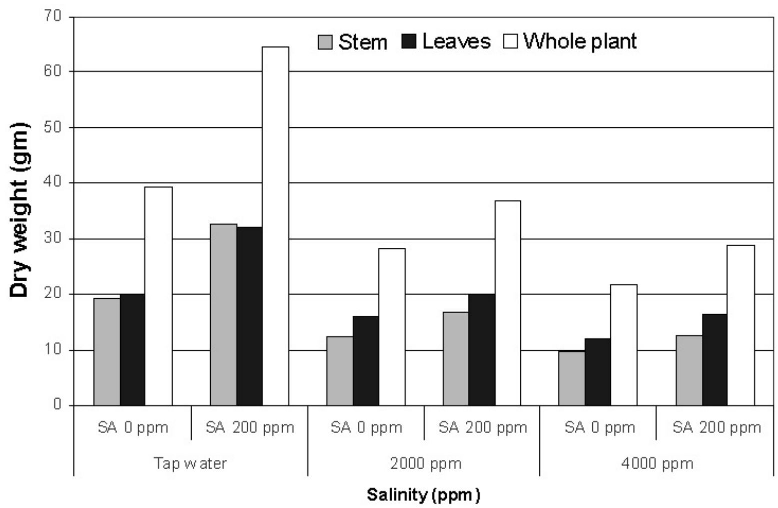

Fig. 1: Effect of salicylic acid (SA) and salinity on dry matter of maize plants.

grain yield with SA application. The most effective level of SA for promoting growth and grain yield were 0.75 or 0.25 mM under normal and saline conditions, respectively. Moreover, Gunes *et al*.[20] proposed that salicylic acid (SA) acts as an endogenous signal molecule responsible for inducing abiotic stress tolerance in plants.

b) - Amino Acids: The interactive effect of spraying Salicylic acid and irrigation by diluted seawater on the amino acid profile in leaves of maize plants was given in Table (4). Regardless the salicylic acid treatment, in plants grown in soil irrigated by fresh water, SA showed its best effect on cystine followed by that in arginine and tyrosine were the concentration of other amino acids were slightly affected. Serine phenyalanine and tyrosine showed approximately similar response. However, in the case of highest saline water used, salicylic acid improved the concentration of arginine, lycine, serine and glutamic acid. Moreover, proline concentration increased by either salicylic acid foliar spraying or salt stress.

The interaction effect of salinity and water stress and phytohormones on amino acids in plants were reported by many authors: Stewart and Voetberg[49]; Fidena, *et al*.[18].

Concerning this Hamada *et al*. [21] revealed that since proline is one of the important components of defense reactions of plants to salinity it might be expected that pretreatment with SA contributes to accumulation of this amino acid under stress through maintaining an enhanced level of ABA in seedlings. Salinity and water deficit induces accumulation of proline in seedlings. Sakhabutdinova, *et al*.[41] on their study for the effect of salicylic acid on decreasing the damages of salt stress on wheat seedlings data suggests that proline is an important component in the spectra of SA-induced ABA-mediated protective reactions of wheat plants in response to salinity and water deficit, which contribute to a reduction of injurious effects of stress factors and acceleration of restoration processes during the period after action of stress, which might be a manifestation of the protective action of SA on wheat plants.

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