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Physiological Response of *Zea mays* to NaCl Stress with Respect to *Azotobacter chroococcum* and *Streptomyces niveus*

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Abstract: A pot experiment was conducted to evaluate the impact of NaCl salinity (20, 40 and 60 mM) and salt-tolerant *Azotobacter chroococcum* and *Streptomyces niveus* on some physiological traits of the salt sensitive cultivar Giza 122 of *Zea mays* plants grown for 3 months in the greenhouse. Irrigating plants with saline water decreased N, P, K and Mg concentrations, but increased Na concentration in both shoots and roots. Raising salinity decreased chlorophyll (Chl) concentrations of leaves, increased Chl a to b ratio, but did not affect the carotenoid concentration. Salinity induced a marked increase in total-soluble sugars, total free amino acids and proline concentrations of both shoots and roots, whereas the total-soluble proteins, DNA and RNA concentrations were reduced. Shoot growth (length, fresh and dry masses) and indole-3-acetic acid (IAA) biosynthesis were inhibited by increasing salinity, while both root growth, IAA concentration and root to shoot ratio were increased. Applying *A. chroococcum* and/or *S. niveus* to the experimental soil, influenced most test characters by increasing the salt-tolerance of the plant. This response was more evident in case of applying both microorganisms than *A. chroococcum* or *S. niveus*. However, the number of these microorganisms was reduced under saline conditions. In most cases, the interactive effects of salinity and inoculum seemed to be insignificant. It was concluded that applying *A. chroococcum* and *S. niveus* slightly improved the salt-tolerance of the test cultivar. Therefore, the test cultivar of maize is not promising to be cultivated in salinized soils even in the presence of both *A. chroococcum* and *S. niveus*.

Key words: Chlorophyll, growth, maize, microorganism inoculation, minerals, nucleic acids, proline, proteins, salt-tolerance, sugars

INTRODUCTION

Excessive soil salinity occurs in many semi-arid regions of the world inhibiting plant growth by water deficiency and salinity effects (Neumann, 1997). In drying saline soils, plants are exposed to elevated levels of both water and osmotic stresses because of a simultaneous decrease in matrix and osmotic potentials with decreasing soil moisture (Levitt, 1980; Lovato *et al*., 1999).

Water stress induced by salinity could be regarded as a major factor exerting considerable alterations in plant growth and metabolism (Khan *et al*., 1994). The degree of these alterations, which can be regarded as salinity resistance, mainly depends on the plant species and the growth stage as well as the salt level (Turner and Kramer, 1980). According to the electrical conductivity (EC in mmhos cm^{-1}) of the irrigation water, Ebrahim and Abu-Grab (1997) classified the soil salinity into 3 orders: (1) none "EC < 0.75", (2) moderate "EC from 0.75 to 3.0" and (3) severe "EC > 3.0". The severe salinity induces detrimental effects on plant growth and yield (Abdel Razek *et al*., 1991). The salt tolerance is linked through a common mechanism of salt uptake for osmotic adjustment (Lovato *et al.,* 1999). Salinity affects growth of plants by: (1) decreasing the availability of water to roots due to the osmotic effect of external salt and (2) exerting toxic effects of excessive salt accumulation within the plant (Munns, 1993). The above mentioned effects may directly or indirectly influence other physiological processes such as germination, photosynthesis, respiration and metabolite accumulation (Turner and Kramer, 1980; Almansouri *et al.,* 2001).

Rhizosphere bacteria, such as *Azotobacter, Arthrobacter* and *Streptomyces*, in turn exert strong beneficial effects on plant growth and health by nutrient solubilisation, nitrogen–fixation, or by the production of plant hormones (El-Shanshoury, 1991; Gomes *et al.,*2001). The beneficial responses of crops to inoculation with *Azotobacter* and *Streptomyces* have been reported by El-Shanshoury (1991, 1995). Production of plant growth substances by both bacteria (El-Shanshoury, 1995), nitrogen fixing–capacity of *Azotobacter* and antibiotic

production by *Streptomyces* (El-Shanshoury, 1991, 1995), all are considered of great importance to both bacteria and associated plants.

Maize is one of the most important economic crops in many countries, particularly, in those characterized by a tropical semi-arid climate. The plant response to salinity was evaluated during germination (Farah *et al*., 1981) only a few genotypes however have been evaluated during growth (Abdel–Razek *et al*., 1991). The above authors recorded significant effects of salinity on dry matter yield of roots and shoots, but no effects on the percentage and rate of germination.

Despite several studies on plant responses to salinity and to inoculation with *Azotobacter* (Brown *et al*., 1964; Dewan and Rao, 1979; El-Shanshoury, 1995) and *Streptomyces* (El-Shanshoury, 1989; El-Abyad *et al*., 1993; El- Shanshoury *et al*., 1996), there is no information about the interactive effects of both factors on the vegetative growth and productivity of *Zea mays* plants. In this work, we hypothesized that soil inoculation with *Azotobacter chroococcum* and *Streptomyces niveus* might eliminate or decrease the detrimental effects of salinity and consequently increase the salt tolerance of the test plant. Therefore, this study aimed at: (1) studying the role of *Azotobacter chroococcum* and *Streptomyces niveus* (added singly or in combination) to reduce or eliminate the harmful effects of salinity on maize plants, (2) finding an explanation for the above role based on the test characters, (3) evaluating the enhancement of salt–tolerance of maize plant and (4) finding a recommendation for the possibility of maize cultivation under saline conditions.

MATERIAL AND METHODS

A pot experiment was conducted, under greenhouse conditions [Kafr El-Sheikh (geographical coordinates 31° 07′ N, 30° 56′ E), North Delta, Egypt], to evaluate the salt–tolerance of maize plant grown for 3 months under the impact of saline irrigation water and biofertilization, using *Azotobacter chroococcum* (Beijerinck, 1901) and *Streptomyces niveus* (Smith *et al*., 1956).

Bacteria: *Azotobacter chroococcum (A.)* was isolated from saline Egyptian soils, collected from Borg El-Arab and identified according to Krieg and Holt (1984).

Streptomyces niveus (S.) was isolated from marine water of the Mediterranean sea in Egypt (Balteem city, Kafr El-Sheikh). It was cultured on starch–nitrate agar (Shirling and Gottlieb, 1966) containing 25 μg cm^{-3} nystatin and 25 μg cm^{-3} cycloheximide, as described by Agwa *et al*. (2000). The plates were incubated at 28°C for 7-10 d, where different streakings were made on the starch– nitrate agar containing different concentrations of NaCl (5-14%).

Plant material and growth conditions: Maize grains (*Zea mays* L. cv. Giza 122) were obtained from the Agricultural Research Center, Sakha, Egypt. The grains were disinfected in 2% (v/v) Na-hypochlorite solution for 10 min followed by washing with sterile water.

Clay–loam soil (collected from fields, water content = 26.4%, field capacity = 41.57%, EC of 1:5 soil extract at 25°C = 2.05 mmohs cm^{-1}, pH of 1 : 2.5 soil suspension = 7.8 and available NPK = 33, 12 and 435 mg kg^{-1}, respectively) was used and dispensed in plastic pots (28 cm diameter, 20 cm depth, 4 kg soil pot^{-1}). Pots were divided into four groups and each group was further subdivided into four subgroups. The four groups represented the used concentrations of NaCl (control, 20, 40 and 60 mM), while the four subgroups indicated the inoculum treatment (-, *A*., *S*. and *A*.+ *S*.). Ten seeds were sown per pot, then thinning was carried out to three at 15 d post sowing. The sowing date was May 22, 2000 and the experiment was conducted for about 3 months. Pots were irrigated with tap water (EC = 0.39 mmhos cm^{-1}, pH = 6.7 and ion concentration of Na^+, K^+, Ca^{2+}, Mg^{2+} and Cl^- = 1.78, 0.22, 2.34, 2.74 and 2.48 mmol l^{-1}, respectively) whenever they needed, for 15 d post sowing. NPK fertilizers were applied at rates of 0.6 g urea pot^{-1}, 0.75 g Ca-superphosphate pot^{-1} and 0.25 g K-sulphate pot^{-1}. Phosphorous was added during soil preparation and before sowing. Nitrogen and K were applied, in two equal doses, at thinning and two weeks post thinning. Test bacteria were inoculated after the thinning process, then pots were irrigated with the saline irrigation water (200 cm^3 pot^{-1}, twice weekly) according to the treatments described above.

Soil inoculation: *A. chroococcum* and *S. niveus* were cultured on nitrogen free and starch nitrate media, respectively, for 7 d at 30°C and 220 rpm. Thereafter, the cells were collected and washed several times with distilled water, then 20 ml of the bacterial suspensions ($4x10^6$ cell cm^{-3}) were used to inoculate the root region of each pot. In case of inoculation with *A. chroococcum* + *S. niveus*, 20 cm^{-3} of each bacterial suspension were used.

Physiological measurements: At 3 months post inoculation, plants were harvested and oven–dried at 60°C until constant masses (4 d). The dry matter (d.m.) of both shoots and roots was used for determination of mineral composition as well as most of the chemical constituents. Relatively few attributes were determined in

the fresh matter (f.m.) and expressed on dry matter (d.m.) basis.

Total–nitrogen concentration was estimated using the micro-Kjeldahl method (Jacobs, 1958). Phosphorus concentration was determined by molybdenum–blue method (Page, 1982). Potassium, Na and Mg were determined according to Allen *et al.* (1974). Flamephotometer (Corning-Scientific Instruments, Model 400) was used for determination of K and Na, while Atomic–Absorption Spectrophotometer (Perkin–Elmer, 2380) was used for Mg determination. Photosynthetic pigments of green plant leaves were extracted in 85% cold acetone and quantified according to Metzner *et al.* (1965). Total soluble sugars (TSS) were estimated, using the phenol–sulphuric acid method as described by DuBois *et al.* (1956). Total–soluble proteins (TSP) were extracted by borate buffer (pH 8) and determined according to the method of Lowry *et al.* (1951). Total free amino acids (TAA) and proline, in ethanol extracts, were quantified according to the methods described by Rosen (1957) and Bates *et al.* (1973), respectively. Nucleic acids (DNA and RNA) were extracted and determined following the method described by Bassett *et al.* (1988). Indole-3-acetic acid (IAA) was extracted as described by Stoessl and Venis (1970), and estimated according to the method of Kengt and Bruinsma (1973). Root depth and shoot length as well as fresh and dry weights (FW and DW) of shoots and roots were also recorded.

Total counts of soil microflora in addition to *Azotobacter* and *Streptomyces* were also determined in the rhizosphere and non-rhizosphere soils. This was performed, using agar plate techniques containing nutrient agar for bacteria, starch nitrate agar for actinomycetes and *Streptomyces*, nitrogen free medium for *Azotobacter* and Czapeck's Dox medium for fungi.

Statistical analysis: Data were averaged and statistically analyzed, whenever possible, using two-way analysis of variance. The least significant difference (LSD) at 5% level was used to compare the mean values (Steel and Torrie, 1980).

RESULTS

Bacterial examination: Examination of *Azotobacter (A.),* isolated on nitrogen-free medium, revealed that they were aerobic, Gram-negative, coccoid and produce brown pigments. This confirmed that it is *A. chroococcum* (Krieg and Holt, 1984). The microscopic examination of *Streptomyces* (*S.*) indicated that it is *S. niveus* as reported by Williams *et al.* (1989).

Mineral concentration: Increasing the level of NaCl in the irrigation water induced a progressive absorption of Na^+. In contrast, N,P,K and Mg concentrations decreased within shoots and roots by rising the level of NaCl (Table 1). Applying the bacterial inocula had significantly increased the concentration of magnesium in shoots. The trend for nitrogen was more pronounced in case of inoculation with *A.*+*S.* than *A.* than *S.*.

Photosynthetic pigments: Although non-significant differences were detected in the carotenoid concentration, the Chl concentration of leaves was significantly reduced by the high salinity (40 and 60 mM NaCl). Furthermore, Chl a to b ratio was increased by salinity indicating that Chl a is more stable to salinity than Chl b (Table 2). On the contrary to salinity, the inoculation of both *A. chroococcum* and *S. niveus* mostly improved the Chl concentration, but the carotenoid concentration was not affected (Table 2).

Chemical constituents: Both NaCl level and the bacterial inocula caused significant effects on all metabolites investigated, whereas the interactive effects were insignificant, except in case of total free amino acids and proline concentrations (Table 3). Irrigating maize plants with saline water for 3 months induced a marked increase in total soluble sugars (TSS), total free amino acids (TAA) and proline in both shoots and roots. In contrast, total soluble proteins (TSP), DNA and RNA concentrations declined under the same conditions. Irrespective of salinity, the inoculation with *A. chroococcum* and/or *S. niveus* decreased TSS, TAA and proline concentrations, whereas the concentrations of TSP, DNA and RNA were increased (Table 3).

Endogenous IAA and plant growth: NaCl treatments significantly reduced the shoot growth (length, FW and DW) and its content of the endogenous IAA, while the reverse was true in case of the root. Such response was more pronounced at the elevated levels of NaCl. However, the inoculum alleviated the adverse effect of salinity in the shoot but not in the root, particularly, when plants were inoculated with both bacteria (*A.*+*S.*) (Table 4).

Counts of microorganisms: The counts of rhizosphere and non-rhizosphere microorganisms were evidently affected by salinity as well as the nature of the inoculum (Table 5). The rhizosphere counts were relatively higher than those of non-rhizosphere. Also, the count of bacteria appeared to be more than that of fungi followed by actinomycetes. Increasing NaCl level, generally, decreased the number of all micro-organisms either in the

Table 1: Mineral concentration [mg g^{-1} (d.m.)] of shoots and roots of *Zea mays* plant grown for 3 months under different concentrations of NaCl with respect to *Azotobacter chroococcum* (*A.*) and *Streptomyces niveus* (*S.*)

Treatment		Shoot					Root				
NaCl conc. (mM)	Inoculum	N	P	K	Na	Mg	N	P	K	Na	Mg
Control	-	20.3	12.5	13.6	3.57	5.7	18.1	10.0	11.0	5.21	4.21
	A.	21.8	12.8	14.0	3.79	6.3	19.9	10.4	11.9	5.04	4.33
	S.	20.9	12.7	13.8	3.88	5.9	18.7	10.4	11.0	5.19	4.11
	A. + *S.*	22.1	12.9	14.3	3.74	6.4	20.3	10.6	11.9	4.91	4.42
20	-	18.7	11.4	12.5	5.00	4.6	16.1	9.42	11.5	7.02	4.00
	A.	19.6	11.7	12.7	4.82	5.2	16.9	9.61	11.7	6.92	3.80
	S.	19.2	11.5	12.6	4.69	4.8	16.7	9.60	11.6	6.89	3.79
	A. + *S.*	20.2	11.9	13.0	4.91	5.4	17.5	9.73	11.6	6.78	3.91
40	-	16.6	10.2	11.7	6.10	3.5	14.8	8.01	9.88	7.99	3.39
	A.	17.9	10.5	11.9	5.89	4.1	15.4	8.20	10.1	7.82	3.42
	S.	17.1	10.4	11.8	5.76	3.6	15.0	8.22	9.67	7.61	3.33
	A. + *S.*	18.5	10.7	12.2	5.98	4.4	15.9	8.37	10.3	7.78	3.51
60	-	15.0	9.49	10.5	7.21	2.2	13.0	7.97	9.12	9.00	3.22
	A.	15.9	9.71	11.1	6.94	2.8	13.1	8.08	10.0	8.89	3.16
	S.	15.3	9.69	10.9	6.84	2.5	13.1	7.99	9.82	8.99	3.14
	A. + *S.*	16.4	9.77	11.5	6.89	3.0	13.5	8.19	10.1	8.92	3.01
Factor		LSD at 5% level									
NaCl conc.		0.81	0.58	0.92	0.35	0.19	0.74	0.34	0.43	0.29	0.17
Inoculum		0.66	0.44	0.75	0.27	0.15	0.62	0.26	0.35	0.22	0.13
Interaction		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS: non significant

Table 2: Chlorophyll (Chl) and carotenoids concentrations [mg g^{-1} (d.m.)] of leaves of *Zea mays* plant grown for 3 months under different concentrations of NaCl with respect to *Azotobacter chroococcum* (*A.*) and *Streptomyces niveus* (*S.*)

Treatment						
NaCl conc. (mM)	Inoculum	Chl a	Chl b	Chl a+b	Chl a/b ratio	Carotenoids
Control	-	8.1	2.5	10.6	3.24	0.81
	A.	9.2	2.7	11.9	3.41	0.76
	S.	8.3	2.5	10.8	3.32	0.76
	A. + *S.*	9.5	2.8	12.3	3.39	0.74
20	-	7.9	2.4	10.3	3.29	0.79
	A.	9.1	2.6	11.7	3.50	0.75
	S.	8.5	2.5	11.0	3.40	0.76
	A. + *S.*	9.3	2.6	11.9	3.58	0.74
40	-	7.6	2.1	9.7	3.62	0.75
	A.	8.4	2.2	10.6	3.82	0.70
	S.	7.8	2.0	9.8	3.90	0.71
	A. + *S.*	8.5	2.3	10.8	3.70	0.72
60	-	6.9	1.7	8.6	4.06	0.71
	A.	7.2	1.8	9.0	4.00	0.68
	S.	6.9	1.7	8.6	4.06	0.66
	A. + *S.*	7.4	1.8	9.2	4.11	0.71
Factor		LSD at 5% level				
NaCl conc.		0.24	0.11	0.58		NS
Inoculum		0.21	0.09	0.49		NS
Interaction		NS	NS	NS		NS

NS: non significant

Table 3: Total-soluble sugars (TSS) and proteins (TSP), total-free amino acids (TAA), proline, and nucleic acids (DNA) and (RNA) contents [mg g^{-1} (d.m.)] in shoots and roots of *Zea mays* plant grown for 3 months under different concentrations of NaCl with respect to *Azotobacter chroococcum* (*A.*) and *Streptomyces niveus* (*S.*)

Treatment		Shoot						Root					
NaCl conc (mM)	Inoculum	TSS	TSP	TAA	Proline	DNA	RNA	TSS	TSP	TAA	Proline	DNA	RNA
Control	-	161	131	8.07	0.49	4.47	3.12	113	117	11.4	0.54	2.53	2.40
	A.	141	138	7.12	0.38	4.63	3.27	109	123	10.1	0.54	2.74	2.47
	S.	149	134	7.99	0.33	4.41	3.24	113	118	10.7	0.52	2.63	2.44
	A. + *S.*	138	139	6.97	0.31	4.65	3.39	112	124	8.49	0.51	2.64	2.49
20	-	190	116	19.0	2.04	3.91	3.01	124	100	31.4	3.06	2.30	2.04
	A.	172	122	16.3	1.91	4.24	3.14	117	105	25.8	2.92	2.41	2.10
	S.	181	120	16.0	1.82	4.12	3.02	117	102	28.2	2.84	2.32	2.18
	A. + *S.*	164	124	15.0	1.42	4.27	3.28	121	107	21.9	2.94	2.42	2.28
40	-	209	101	26.1	3.86	3.41	2.91	141	92.4	41.6	6.01	2.22	1.91
	A.	196	107	22.6	2.99	3.52	2.93	137	96.2	38.0	5.48	2.30	2.03
	S.	198	104	24.1	3.11	3.51	2.92	139	96.0	38.4	5.09	2.30	2.00
	A. + *S.*	191	110	21.7	2.74	3.63	3.07	136	97.8	33.9	4.98	2.34	2.14

Table 3: Continued

Treatment		Shoot						Root					
NaCl conc (mM)	Inoculum	TSS	TSP	TAA	Proline	DNA	RNA	TSS	TSP	TAA	Proline	DNA	RNA
60	-	241	95.0	35.7	6.04	2.90	2.70	169	81.4	52.2	10.1	2.01	1.64
	A.	220	95.2	31.6	5.11	3.12	2.82	159	82.0	51.0	8.67	2.20	1.83
	S.	227	94.7	33.2	5.88	2.92	2.71	164	79.2	50.3	9.72	2.02	1.73
	A. + *S.*	219	97.1	30.3	4.99	3.24	2.93	160	83.4	47.9	8.24	2.36	1.84
Factor		LSD at 5% level											
NaCl conc.		6.64	3.86	0.81	0.12	0.16	0.14	4.64	3.39	1.48	0.16	0.11	0.11
Inoculum		5.05	3.13	0.64	0.09	0.14	0.11	3.52	2.64	1.25	0.13	0.09	0.07
Interaction		NS	NS	1.62	0.24	NS	NS	NS	NS	NS	0.32	NS	NS

NS: non significant

Table 4: Endogenous indole-3-acetic acid (IAA) concentration and growth criteria of shoots and roots of *Zea mays* plant grown for 3 months under different concentrations of NaCl with respect to *Azotobacter chroococcum* (*A.*) and *Streptomyces niveus* (*S.*)

Treatment		Shoot				Root				Root/
NaCl conc. (mM)	Inoculum	IAA [μg g^{-1} (f.m.)]	Length (cm $plant^{-1}$)	FW (g $plant^{-1}$)	DW (g $plant^{-1}$)	IAA [μg g^{-1} (f.m.)]	Depth (cm $plant^{-1}$)	FW (g $plant^{-1}$)	DW (g $plant^{-1}$)	Shoot (DW basis)
Control	-	6.4	62.1	21.9	3.08	3.2	6.74	2.31	0.94	0.31
	A.	10.0	71.7	23.0	3.21	6.2	7.05	2.45	0.96	0.30
	S.	7.6	66.9	22.0	3.08	4.0	6.86	2.36	0.95	0.31
	A. + *S.*	14.0	77.1	23.4	3.27	5.5	7.22	2.63	1.11	0.34
20	-	5.9	59.0	19.8	2.57	3.9	8.04	3.12	1.42	0.55
	A.	9.6	65.2	21.7	2.83	6.7	7.93	2.94	1.34	0.47
	S.	6.9	60.1	20.2	2.63	6.4	7.62	2.82	1.29	0.49
	A. + *S.*	11.0	73.0	22.0	2.86	6.2	7.81	2.95	1.31	0.46
40	-	4.6	45.2	17.0	2.35	4.5	9.53	4.21	1.70	0.72
	A.	9.1	54.9	19.2	2.51	7.9	8.75	3.63	1.41	0.56
	S.	5.0	51.4	17.4	2.40	6.7	8.51	3.42	1.34	0.56
	A. + *S.*	8.0	68.2	19.6	2.55	7.9	8.67	3.71	1.52	0.60
60	-	4.4	32.9	13.8	1.96	4.9	11.29	4.90	1.89	0.96
	A.	5.0	48.4	15.1	1.97	8.2	10.72	4.52	1.84	0.93
	S.	4.5	42.1	14.4	1.98	8.0	11.01	4.71	1.85	0.93
	A. + *S.*	7.3	57.8	16.9	2.36	9.2	10.77	4.43	1.88	0.80
Factor		LSD at 5% level								
NaCl conc.		0.36	2.0	0.57	0.09	0.25	0.3	0.18	0.09	
Inoculum		0.29	1.66	0.46	0.06	0.22	0.25	0.14	0.08	
Interaction		0.71	4.0	NS	NS	0.5	NS	0.36	NS	

NS: non-significant

Table 5: Number of microorganisms (cells g^{-1} dry soil) associating roots of *Zea mays* plant grown for 3 months under different concentrations of NaCl with respect to *Azotobacter chroococcum* (*A.*) and *Streptomyces niveus* (*S.*)

Treatment		Bacteria x 10^4		Actinomycetes x 10^3		Fungi x 10^4		*A.* x 10^3		*S.* x 10^3	
NaCl conc. (mM)	Inoculum	Rhiz.	Non-rhiz.	Rhiz.	Non-rhiz.	Rhiz.	Non-rhiz.	Rhiz.	Non-rhiz.	Rhiz.	Non-rhiz.
Control	-	19.0	8.2	9.4	4.0	6.2	3.4	None	None	None	None
	A.	19.9	9.9	4.9	4.0	7.0	6.0	3.2	2.0	None	None
	S.	20.2	9.6	9.8	6.0	6.6	4.2	None	None	3.0	1.7
	A. + *S.*	21.1	12.4	10.9	4.3	7.1	7.2	4.9	2.2	3.4	2.4
20	-	7.3	4.6	7.8	2.4	4.4	3.2	None	None	None	None
	A.	12.4	4.4	4.0	3.6	4.4	2.9	1.7	2.0	None	None
	S.	9.9	4.0	6.3	4.7	4.0	2.3	None	None	2.2	2.0
	A. + *S.*	10.2	8.9	8.2	3.0	5.2	2.6	3.0	1.6	2.0	1.0
40	-	3.9	2.2	4.3	2.0	2.1	2.2	None	None	None	None
	A.	6.9	3.3	3.2	2.8	3.2	1.4	1.0	1.1	None	None
	S.	4.8	2.9	5.4	4.9	3.2	2.0	None	None	1.4	1.4
	A. + *S.*	8.1	6.5	4.9	2.2	4.3	1.5	2.2	1.0	1.0	0.6
60	-	2.2	1.9	2.9	1.6	1.3	1.7	None	None	None	None
	A.	4.2	2.5	2.1	2.0	3.0	1.0	0.9	0.6	None	None
	S.	2.4	2.0	3.2	2.0	2.2	1.0	None	None	1.2	1.0
	A. + *S.*	4.4	4.0	3.4	2.1	2.7	1.0	1.0	0.8	0.9	0.2

Rhiz.: rhizospher. Non-rhiz.: non-rhizosphere

rhizosphere or the non-rhizosphere soil. In all treatments, inoculation with *A.* plus *S.* increased both counts of *A. chroococcum* and *S. niveus*. These observations certify the beneficial role of both *A.* and *S.* for alleviation of the harmful effects of salinity. This finding might be taken into consideration to interpret the effects of bacterial inocula on all test characters (Tables 1, 2, 3 and 4).

DISCUSSION

A. chroococcum and *S. niveus* were identified according to Krieg and Holt (1984) and Williams *et al.* (1989), respectively. *Stroptomyces niveus* was salt-tolerant, growing in the presence of NaCl (15% v/v) at 28°C. Similar results were recorded for *S. sulphureus* which could grow up to 13% (w/v) of NaCl (Williams *et al*., 1989).

In the present investigation, maize plant might be suffering from problems originated from high levels of NaCl, Na^+ and Cl^- in the soil solution. NaCl exclusion minimizes ion toxicity, but accelerates water deficit in plants (Gunes *et al*., 1996). Salt absorption facilitates osmotic adjustment, but can lead to ion toxicity and nutritional imbalance (Marschner, 1995).

NaCl salinity increased the absorption of Na^+, whereas N, P, K and Mg uptake was decreased. These results are in general or partial agreement with several authors (Gunes *et al.,* 1996; Abdel–Ghaffar *et al.,* 1998; Aldesuquy *et al.,* 1998; Morant-Avice *et al.,* 1998; Del Zoppo *et al.,* 1999) and could be due to the salinity problems described above. Increasing Na^+ concentration disturbs the nutrient balance, osmotic regulation and causes specific ion toxicity (Munns, 1993). The decrease in K and N concentrations, in plant, by salinity was ascribed to the antagonism between Na^+ and K^+ and between Cl^- and NO_3^-, respectively (Gunes *et al.,* 1996). Although the fertilizers increased the accumulation of N, P, K and Mg; Na^+ concentration was reduced. This finding is in line with results of Krieg and Holt (1984) and El-Shanshoury (1995) and could be attributed to a decrease in Na^+ concentration by bacterial inoculation (Table 1). The role of *A. chroococcum* and *S. niveus* in producing growth promoting substances and nitrogen fixation by *A. chroococcum* might be also taken into consideration (El-Shanshoury, 1995). Moreover, Webster (1959) stated that both protein and N concentrations in plants are dependent on N fertilizer and the activity of roots for N absorption. Increasing salinity may also decreases the P absorption (Ebrahim and Abu-Grab, 1997).

The decrease in Chl concentration by salinity has been reported (Gunes *et al.,* 1996; Abdel-Ghaffar *et al.,* 1998; Del-Zoppo *et al.,* 1999) and it was attributed to a salt induced weakening of protein–pigment–lipid complexes (Gunes *et al.,* 1996), increased chlorophyllase activity (Sivtsev *et al.,* 1973) and/or decreased concentrations of N and Mg (Table 1). However, enhancing Chl concentration by *A. chroococcum* might be due to its role in: (1) decreasing the absorption of sodium and (2) increasing both N and Mg concentrations of maize shoots (Table 1).

Salinity induced a marked increase in TSS, TAA and proline concentrations in both shoots and roots, while a contrary trend was achieved in case of TSP, DNA and RNA. Similar results were reported by several workers (e.g., Dubey and Pessarakli, 1995; Gunes *et al.,* 1996; Aldesuquy *et al.,*1998; Morant–Avice *et al.,* 1998; Rajesh *et al.,* 1999). In our work, the correlation between proline and Na ion concentrations confirms the reported role of proline in osmoregulation which enables plants to tolerate or adapt to saline conditions (Levitt, 1980; Chowdhury *et al*., 1993). Also, the reductions in DNA and RNA concentrations could be attributed to the inhibition of their biosynthesis and/or to the stimulation of their breakdown through enhancing the activities of DNAase and RNAase (Dubey, 1983). The influence of both bacteria on these characters is still relatively obscure.

The inhibition of plant growth and IAA biosynthesis, by salinity treatment, was reported by several authors (Neals and Sharky, 1981; Bejaoui, 1985; Abdel-Ghaffar *et al*., 1998). Suppression of plant growth by salinity could be attributed to an inhibitory effect on both meristematic activity and biosynthesis of IAA (Table 4), whereas the alleviation of this effect by inoculation with bacteria was ascribed to the role of bacterial inocula in nitrogen fixation and production of growth promoting substances and antibiotics (El-Shanshoury, 1989, 1995; Gomes *et al*., 2001). In the present work, the positive correlation between the plant growth and Chl concentration in leaves, metabolite accumulation and enhanced levels of IAA in both shoots and roots (Tables 2, 3 and 4) could be taken into consideration for explaining the variation of maize growth and productivity with salinity and the bacterial inoculation. Also, increasing the root to shoot ratio with salinity (Table 4) could be ascribed to an attempt of maize plants to: 1) decrease the transpired water and 2) increase the absorbed water. Consequently, the plant can resist water stress resulting from growth under saline conditions.

The number of both rhizosphere and non-rhizosphere micro-organisms showed a significant decrease with salinity, but a drastic depletion was shown at 60 mM NaCl. This depletion might be attributed to the salinity problems, which could induce a spontaneous efflux of cell water and the accumulation of osmo-regulators in the cell.

Irrespective of salinity, the bacterial inoculum increased the counts of all soil microflora, but the response was more pronounced in case of *A.* plus *S.* than *A.* than *S.* Similar observations were recorded by Krieg and Holt (1984). This increment might be ascribed to the secondary metabolites secreted by the inoculated bacteria, but such interpretation requires further investigations.

In summary, salinity retarded maize growth and caused a drastic increase in proline concentration, which has been correlated with the level of Na^+ in both shoots and roots. Inoculating control plants with *A. chroococcum* and/or *S. niveus* improved the plant growth. However, inoculation of the NaCl–stressed plants alleviated the adverse effect (s) of salinity, particularly, when plants were inoculated with both bacteria. This alleviation was not enough for the plant to be able to efficiently resist the harmful effects of salinity. Therefore, we recommend that the test cultivar of maize is not promising to be cultivated in salinized soils even in the presence of both *A. chroococcum* and *S. niveus* (i.e., the present data did not satisfactorily support our hypothesis described in the introduction section.

REFERENCES

Abdel-Ghaffar, B.A., M.N. El-Shourbagy and E.M. Basha, 1998. Responses of NaCl stressed wheat to IAA. Proceedings, Sixth Egypt. Bot. Conf., Cairo Univ., Giza, Egypt, 6: 79-88.

Abdel-Razek, A.A., S.E. Sadek and I.M. El-Naggar, 1991. Evaluation of some maize genotypes (*Zea mays* L.) with regard to their salt tolerance. Minufiya J. Agric. Res., 16: 919-933.

Agwa, H., A.R. El-Shanshoury, M. Aly and R. Bonaly, 2000. Isolation and characterization of two *Streptomyces species* produce antifungal agents. J. Union Arab Biol., Cairo, Egypt, 9: 283-303.

Aldesuquy, H.S., A.M. Badawy and S.M. El-Dohlob, 1998. Physiological studies on adaptation of wheat plants to irrigation with sea water. III. Regulation of mineral, carbohydrate and proline content by the combined effects of phytohormones and sea water. J. Union Arab Biol., Cairo, Egypt, 6: 267-297.

Allen, S.E., H.M. Grimshaz, J.A. Parkinson and C. Quarmby, 1974. Chemical Analysis of Ecological Materials. Blackwell Scientific Publishing, Oxford.

Almansouri, M., J.M. Kinet and S. Lutts, 2001. Effect of salt and osmotic stresses on germination in durum wheat (*Triticum durum* Desf.). Plant Soil, 231: 243-254.

Bassett, C.L., J.R.Y. Pawson and J.A.N. Stedt, 1988. DNA and RNA levels in bundle sheath and mesophyll cells of pearl millet (*Pennisetum americanum*). Plant Physiol., 87: 307-310.

Bates, L.S., R.P. Waldren and I.D. Teare, 1973. Rapid determination of free proline under water stress studies. Plant Soil, 39: 205-207.

Beijerinck, N.W., 1901. Ueber oligonitrophile Mikroben. Zentralbl. Bakteriol Parasitenk. Infektionsk. Hyg. Abt. II., 7: 561-582.

Bejaoui, M., 1985. Interaction between NaCl and some phytohormones on soybean growth. J. Plant Physiol., 120: 95-110.

Brown, M.G., S.K. Birlingham and R.M. Jackson, 1964. Studies on *Zotobacter* species in soil. III. Effects of artificial inoculation on crop yields. Plant Soil, 20: 194-214.

Chowdhury, J.B., S. Jain and R.K. Jain, 1993. Biotechnological approaches for developing salt tolerant crops. J. Plant Biochem. Biotech., 2: 1-7.

Del Zoppo, M., L. Galleschi, A. Onnis, A. Pardossi and F. Saviozzi, 1999. Effect of salinity on water relations, sodium accumulation, chlorophyll content and proteolytic enzymes in a wild wheat. Biol. Plant., 42: 97-104.

Dewan, G.I. and N.S. Rao, 1979. Seed inoculation with *Azospirillum brasilense* and *Azotobacter chroococcum* and the root biomass of rice (*Oryza sativa* L.). Plant Soil, 53: 295-302.

Dubey, R.S., 1983. Biochemical changes in germinating rice (*Oryza sativa*) seeds under saline stress. Biochem. Physiol. Pflanz., 177: 523-527.

Dubey, R.S. and M. Pessarakli, 1995. Physiological mechanisms of nitrogen absorption and assimilation in plants under stressful conditions. In: M. Pessarakli (Ed.), Handbook of Plant and Crop Physiol., Marcel Dekker, New York, pp: 605-625.

DuBois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith, 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem., 28: 350-356.

Ebrahim, M.K.H. and O.S. Abu-Grab, 1997. Effect of water quality and alar or cycocel on yield, chemical constituents and water-use efficiency of onion bulb. J. Union Arab. Biol., Cairo, Egypt, 4: 179-196.

El-Abyad, M.S., M.A. El-Sayed, A.R. El-Shanshoury and N.H. El-Batanony, 1993. Inhibitory effects of UV mutants of *Streptomyces corchorusii* on bean and banana wilt pathogen. Can. J. Bot., 71: 1080-1086.

El-Shanshoury, A.R., 1989. Growth promotion of wheat seedlings by *Streptomyces atroolivaceus*. J. Agron. Crop Sci., 163: 109-114.

El-Shanshoury, A.R., 1991. Biosynthesis of indole-3-acetic acid in *Streptomyces atroolivaceus* and its changes during spore germination and mycelial growth. Microbios, 67: 159-164.

El-Shanshoury, A.R., 1995. Interactions of *Azotobacter chroococcum*, *Azospirillum brasilense* and *Streptomyces mutabilis* in relation to their effect on wheat development. J. Agron. Crop Sci., 175: 119-127.

El-Shanshoury, A.R., S.M. El-Sououd, O.A. Awadalla and N.B. El-Bandy, 1996. Effect of *Streptomyces corchorusii*, *Streptomyces mutabilis*, pendimethalin and metribuzin on the control of bacterial and *Fusarium* wilt of tomato. Can. J. Bot., 74: 1016-1022.

Farah, M.A., M.F. Soliman and L.M. Anter, 1981. Seed germination and root growth of corn and cotton seedlings as affected by soil texture and salinity of irrigation water. Agric. Res. Rev., 59: 157-163.

Gomes, N.C.M., H. Heuer, J. Schonfeld, R. Costa, L. Mendonca-Hagler and K. Smalla, 2001. Bacterial diversity of the rhizosphere of maize (*Zea mays*) grown in tropical soil studied by temperature gradient gel electrophoresis. Plant Soil, 232: 167-180.

Gunes, A., A. Inal and M. Alpaslan, 1996. Effect of salinity on stomatal resistance, proline and mineral composition of pepper (*Capsicum annuum* L.). J. Plant Nutr., 19: 389- 390.

Jacobs, M.B., 1958. The Chemical Analysis of Food and Food Products. D. Van Nostrand Co. Inc., New York.

Kengt, E. and J. Bruinsma, 1973. A rapid, sensitive accurate determination of indole-3-acetic acid. Phytochem., 22: 91-97.

Khan, M.G., M. Silberbush and S.H. Lips, 1994. Physiological studies on salinity and nitrogen interaction in alfalfa. II. Photosynthesis and transpiration. J. Plant Nutr., 17: 669-682.

Krieg, N.R. and J.G. Holt, 1984. Bergy's Manual of Systematic Bacteriology. Vol. 1, Williams and Wilkins Co., Baltimore.

Levitt, J., 1980. Responses of Plants to Environmental Stresses. Academic Press, New York, London, 2: 365-454.

Lovato, M.B., J.P. De-Lemos Filho and P.S. Martins, 1999. Growth responses of *Stylosanthes humilis* (Fabaceae) populations to saline stress. Environ. Exp. Bot., 41: 145-153.

Lowry, O.H., N.J. Rosebrough, L.A. Farr and C. Randall, 1951. Protein measurements with the Folin-phenol reagent. J. Biol. Chem., 193: 265-275.

Marschner, H., 1995. Mineral Nutrition of Higher Plants. 2nd Edn., Academic Press, London.

Metzner, H., H. Rau and H. Senger, 1965. Ultersuchungen zur Synchronisierbarkeit einzelner Pigmentmangel-Mutanten von *Chlorella*. Planta, 65: 186-194.

Morant-Avice, A., E. Pradier and R. Houchi, 1998. Osmotic adjustment in triticales grown in presence of NaCl. Biol. Plant., 41: 227-234.

Munns, R., 1993. Physiological processes limiting plant growth in saline soils: some dogmas and hypotheses. Plant Cell Environ., 16: 15-24.

Neales, T.F. and T.P. Sharkey, 1981. Effect of salinity on growth, mineral and organic constituents of the halophyte *Disphyma australe*. Aust. J. Plant Physiol., 8: 165-169.

Neumann, P., 1997. Salinity resistance and plant growth revisited. Plant Cell Environ., 20: 1193-1198.

Page, A.L., 1982. Chemical and microbiological properties (Part 2). In: A.L. Page, D.E. Baker, J. Roscoe-Ellis, D.R. Keeney, R.H. Miller and J.D. Rhoades (Eds.), Methods of Soil Analysis. 2nd Edn., Madison, Wisco, USA.

Rajesh, A., R. Arumugam and V. Venkatesalu, 1999. Responses of *Ceriops roxburghiana* to NaCl stress. Biol. Plant., 42: 143-148.

Rosen, H., 1957. A modefied ninhydrin colourimetric analysis for acid nitrogen. Arch. Biochem. Biophys. 67: 10-15.

Shirling, E.B. and D. Gottlieb, 1966. Methods for characterization of *Streptomyces* species. Int. J. Syst. Bact., 16: 313-340.

Smith, C.G., A. Dietz, W.T. Sokolski and G.M. Savage, 1956. Streptonivicin, a new antibiotic. I. Discovery and biological studies. Antibiot. Chemother, 6: 135- 142.

Steel, R.G. and J.H. Torrie, 1980. Principals and Procedures of Statistics. 2nd Edn., McGraw-Hill Book Company Inc., New York, London.

Sivtsev, M.V., S.A. Ponomareva and E.A. Kuznetsova, 1973. Effect of salinization and a herbicide on chlorophyllase activity in tomato leaves. Fiziol. Rast., 20: 62-65.

Stoessl, A. and M.A. Venis, 1970. Determination of submicrogram levels of indole-3-acetic acid. A new highly specific method. Anal. Biochem., 34: 344-351.

Turner, N.C. and P.J. Kramer, 1980. Adaptation of Plants to Water and High Temperature Stress. John Wiley and Sons, New York.

Webster, G.C., 1959. Nitrogen Metabolism in Plants. Biological Monographs. Row Peterson and Co., New York.

Williams, S.T., M.E. Sharpe and J.G. Holt, 1989. *Streptomyces* and Related Genera. In: Bergey`s Manual of Systematic Bacteriology, Krieg, N.R. and J.G. Holt (Eds.). Vol. 4, Williams and Wilkins, Baltimore, pp: 2451-2508.