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INTERACTION BETWEEN SODIUM CHLORIDE AND STREPTOMYCES ATROOLIVACEUS AND THEIR EFFECTS ON MAIZE (ZEA MAYS L. DIHYBRID 204)

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ABSTRACT

Inoculated and non inoculated seedlings of maize (Zea mays L. dihybrid 204) were grown in sterile or non-sterile, artificial salinized or non salinized sand soils. Plants inoculated with a salt-tolerant locally isolated Streptomyces atroolivaceus grown in non-sterile either salinized or non salinized soil grew better than control plants. The growth was significantly higher in non salinized soil when compared with the salinized inoculated soil. Monosaccharides of both root and shoot systems and disaccharides of the root were significantly enhanced by inoculation under all the experimental conditions. Endogenous indole-3-acetic acid (IAA) of the experimental plant was increased by inoculation of sterile or non sterile soils, and increasing salinization compared with the control. Under the experimental conditions potassium, phosphorus and magnesium of the shoot were higher than of the root system. A marked increase of sodium/calcium and monovalent/divalent of both shoot and root systems was recorded under salinization. Inoculation resulted in an increase of the number of S. atroolivaceus cells in soil and rhizosphere which were significantly higher in non salinized (sterile or non-sterile) cultures than in the salinized.

Delta J. Sci. 12 (3) 1988

The increase of bacterial number was correlated to growth response and IAA of maize.

INTRODUCTION

Much attention has been paid recently to salinity, the excess of soluble salts (Jensen 1981; Kawasaki et al., 1983 ; Jeschke et al, 1986; Kingsbu and Epstein 1986). Salt stress disturbs endogenous hormones and the exogenous application of phytohormones implicates growth promotion (Rao et al.,1984 ; Abdel- Ghaffar et al.,1984).

It has been stated that epiphytic bacteria may increase the IAA content in plants (Libbert et al.,1966, 1969) . The direct uptake by plants of IAA produced by bacteria has been also observed (Libbert and Silhengst, 1970) The bacteria are also known to affect the morphology of plant roots and plant development in the same way as gibberellic acid and IAA (Brown et al.,1968; Brown, 1972, 1974; El-Shourbagy et al, 1979).

It has been suggested that microorganisms might enhance salt-tolerance of selected varieties (Menge et al.,1978) . The objective of the present study was to determine the effects and interactions between sodium chloride and Streptomyces atroolivaceus ; the salt tolerance and producer-organism of natural plant growth regulators on Zea mays L. dihybrid 204

Delta J. Sci. 12 (3) 1988
El-Shanshoury and Hamada

MATERIALS AND METHODS

Corn grains (Zea mays L. dihybrid 204) were soaked for 15-20 minutes in 1 percent sodium hypochlorite . Sterile grains were germinated in wooden bow , 4-days-old seedlings were transplanted to 10 cm pots containing HCL-prewashed sand, either steam-sterilized or non-sterilized with one of the following treatments:

1- Soil without any inoculation and irrigated with basic nutrient solution (20 mosmol $kg^{-1}H_2O$).
2- Soil inoculated with Streptomyces atroolivaceus and irrigated with 20 mosmol kg^{-1} H_2O.
3- Soil inoculated with S. atroolivaceus and irrigated with 98 mosmol $kg^{-1}H_2O$.
4- Soil inoculated with S. atroolivaceus and irrigated with 150 mosmol $kg^{-1}H_2O$.

To inoculate maize seedlings with S. atroolivaceus the following procedure was employed: six-day-old cultures of Streptomyces in a medium contained: glucose, 30g; peptone, 10g ; $CaCO_3$, 3g ; tryptamine, 1g ; water make 1 litre, pH 6 (El-Shanshoury 1985) . The culture contained $2x10^3$ cells/ml. Three ml of this suspension were added to the root region at the time of transplanting. Control seedlings were treated with dilute sterile culture medium. The pots were kept at room temperature (20-30°C) and received 150 ml of Hoagland

nutrient solution twice per week. Each pot was watered to field capacity every second day.

When the plants were 75 days old (from transplanting), they were harvested and the following parameters were measured: root depth, shoot height, fresh and dry weights of roots and shoots, mineral elements, mono- and disaccharides, IAA and the population of S. atroolivaceus in soil and rhizosphere.

Mono- and disaccharides were extracted with hot water (80°C) and estimated using a modified Nelson solution as described by Naguib(1964) as mg g^{-1} fresh weight.

IAA was determined by slightly modifying the method of Kengt and Bruinsma (1973) as ng g^{-1} fresh weight.

Mineral elements were extracted with hot water and measured according to Allen et al. (1974). The hot water extract was also used for measuring electrical conductivity and osmolality using an electrical conductivity meter (WTW LF 56, Germany) and an Advanced Wide-Range Osmometer (3 W II) respectively.

Microbial count was estimated from three replicate samples of rhizosphere and soil apart from the root. roots of the control treatment, Streptomyces - inoculated plants and soil were shaken in starch nitrate liquid medium

and suitable dilutions were plated on starch nitrate soild medium to give a count of Streptomyces. Washed roots were dried and weighed and the soil suspension was evaporated to dryness. The number of Streptomyces were related to dry weight of roots or soil.

Data were computed statistically to find out any significant differences in each estimate among concentrations, between sterilization and non sterilization and the interaction between sterilization and concentration. Statistical treatments of results (the two-way analysis of variance-Anova-2) followed procedures outlined in Steel and Torrie (1980).

RESULTS

Under salinization both sterilized and non sterilized, inoculated sand cultures showed that the increase in osmolality of external solution from 20 to 98 mosmol Kg^{-1} H_2O decreased root depth, shoot height and fresh weight of both parts (Table 1). The root depth and root fresh weight increased with increasing osmolality (from 98 to 150 mosmol $Kg^{-1}H_2O$) of the external solution in sterile soil while a reverse effect was observed in plants of non sterile soils. The shoot height decreased in sterile soil and increased in the non sterile, compared to the non inoculated control while the shoot fresh weight decreased in the former and

increased in the latter.

Statistical analysis indicated that variations in root depth were not significant due to treatments, sterilization or the treatment by sterilization. The variations of shoot height were not significant due to sterilization, but significant due to treatment or treatment by sterilization.

Monosaccharides and disaccharides of Zea (Table 2) decreased by increasing osmolality of the external solution till 150 mosmol $kg^{-1}H_2O$. Higher sugar contents were found in shoots of both sterile and non sterile soils than in roots.

The results of IAA contents (Table 2) in sterile soil revealed an enhancement of the growth regulator of both shoot and root systems of Zea by inoculation of the soil and raising the osmolality of the external solution. In non sterile soil, inoculation of the experimental organism to the soil and increasing salinity of the applied solution increased IAA. However IAA content decreased by applying solution of 150 mosmol $kg^{-1}H_2O$ but the values attained still higher than the non inoculated plants.

Statistical analysis revealed that variations in monosaccharides were not significant due to sterilization

but significant due to treatments and interactions between the treatments and sterilization. Disaccharides varied significantly due to treatments, sterilization and the interaction of treatments and sterilization. Analysis of variance (2-way) indicated that variations in IAA were not significant due to treatments or sterilization but significant due to the interactions between them.

EC, osm, Na, K, Ca and P (Table 3) increased by increasing osmolality of the external solution from 20-98 mosmol kg^{-1} H_2O for both plant parts, and this was true for inoculated sterile and non sterile cultures. Na, K and Ca of root recorded lower values in non sterile salinized soil. The trend of element content was in the order: Na> K> Ca> P in shoot and root systems. Na, K and Ca of root and K contents of shoot decreased with increasing osmolality from 98-150 mosmol $kg^{-1}H_2O$ in both sterile and non sterile soils.

Statistical analysis indicated that the differences in EC, osm, Mg and P of root; and EC and K of shoot were not significant due to changes of treatments, sterilization and treatment by sterilization.

Table 4 showed that Na/K ratio of root ranged from 2.6-4.6 in sterile and from 2.1-4.3 in non sterile soil. Lower Na/K ratios were recorded in shoots relative to roots.

Salinization increased Na/Ca ratios of shoots in comparison to roots. The Na+K/Ca+Mg ranged from 3.0-5.5 and from 2.1-6.2 for roots and shoots respectively. The shoot/root K approached 2.0 in sterile and non sterile conditions. The shoot/root Na exceeded 1.0 under saline conditions and was lower than 1.0 in non salinized soils.

Examination of non inoculated pots revealed that Streptomyces atroolivaceus commonly occurred under maize cultivation. They were encountered in appreciable densities in soil and rhizosphere. The bacterial count was fluctuating throughout the experimental treatments (Table 5). The lowest counts of S. atroolivaceus were found in non inoculated sterile and non sterile soils and rhizosphere. The highest counts were recorded in the inoculated sterile and non sterile soils and rhizosphere. Considering the treatment 20 mosmol $kg^{-1}H_2O$, it was found that the population of Streptomyces increased 31.3 and 44.6 folds for sterile and non sterile soil. The increase was 40.5 and 21.3 folds for rhizosphere of sterile and non sterile soil compared to the non inoculated pots. The population of S. atroolivaceus in the pots treated with 98 and 150 mosmol $kg^{-1}H_2O$ had increased 3.9 and 3.2 ; 25.1 and 27.7 times respectively in sterile conditions in the soil and rhizosphere over those the non inoculated pots. The Strepto

myces number of soil and rhizosphere was enhanced in pots treated with 98 and 150 mosmol $kg^{-1}H_2O$ and inoculated under non sterile conditions; the increases were respectively 139 and 30.7 ; 30.4, 25.2 and 13.7 times than those in the non inoculated control.

Statistical analysis indicated that variations in the population of S. atroolivaceus in the soil were not significant due to soil sterility buth highly significant due to treatment and sterility by treatment. The variations in bacterial number in the rhizosphere were significant due to sterility and highly significant due to both treatment and sterility by treatment.

DISCUSSION

Various procedures for improving the salt tolerance of plants have been developed on the basis of hormonal applications (Awad and Kamel, 1983; Balki and Padol ,1982; Bastianpillia et al, 1982; Khan and Unger ,1988; Roth, 1981, 1985) or using microorganisms (Menge et al.,1978; Hirrel and Gerdeman ,1980; Pond and Menge ,1984). A locally isolated organism (Streptomyces atroolivaceus) was reported to produce plant growth regulators; IAA, GA_3 and kinetin (El-Shanshoury, 1985) and other different indole compounds in the synthetic medium (El- Sayed et al., 1987). This organism was used instead of the synthetic growth regulators.

Delta J. Sci. 12 (3) 1988

Inoculation of plants fed by Hoagland only enhanced the root depth shoot height and fresh weight of both root and shoot systems in accordance with Brown (1974) and El-Shourbagy et al. (1979). On the other hand inoculating the experimental organism to plants fed by Hoagland and saline solution increased the root depth of plants grown under sterile or non sterile conditions but increased the shoot height and fresh weights of plants grown under non sterile conditions only. This may be due to the increase of bacterial population and also their metabolites which affect plant growth. In this connection, Libbert et al. (1966 , 1969) stated that epiphytic bacteria may increase IAA contents. Libbert and Silhengst (1970) observed a direct uptake by plants of IAA produced by bacteria. The decrease of fresh weight of both root and shoot systems in the sodium chloride treated plants compared to the control, in spite of the increase of IAA contents may be ascribed to the increase of growth inhibitors which completely neutralized the presence of growth promoting substances. However Khan et al (1976) found that sodium chloride salinity as compared to non saline controls increased the level of growth inhibitor.

The accumulation of monosaccharides in both root and shoot systems of Zea, inoculated with Streptomyces under sterile, non sterile and non saline conditions may be due to the effect of the population of the experimental

organism and the endogenous IAA which were in parallel with these sugars (Tables 2 and 5). The decrease of soluble sugars in Zea grown in the presence of sodium chloride may be due to the increase of salinity (Bernstein, 1962 ; Kasim 1986) or to the stimulation of the high osmolality of the external solution to the experimental organism to exhaust the sugars. However the decrease of these sugars is clear in non sterile soils and parallel with the increase of the number of bacteria and IAA. These results agree with those of Barber and Martin (1976).

Mineral ion contents of Zea can be arranged in the order; Na > K > Ca > P, this means that the experimental species prefer monovalent cations than divalents.

In the inoculated non salinized sterile or non sterile soil P,Ca,Na,EC and osmolality of the root system were decreased. By increasing salinization Na,K and Ca decreased. This can be explained by the increase in the population of the experimental organism, exhaution and competition between these bacteria and the plant (Barber et al, 1976) or by increasing of salinity levels (Ahmad, 1982 ; Kawasaki et al, 1983). Sodium contents were enhanced by salinization in parallel to the results obtained by other investigators (Salama et al., 1981 ;

Kawasaki et al., 1983 ; Abd El-Rahman and Abdel-Hadi, 1984). Magnesium levels of Zea plant grown in inoculated or non inoculated soils showed no definite trend in accordance with Salama et al (1981). The Phosphorus contents were reduced in the roots of the inoculated plants whether under sterile or non sterile soil conditions in agreement with Barber et al. (1976). They stated that in old barley plants the microorganisms compete with the roots for phosphate and thus reduce its uptake into plants.

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Delta J. Sci. 12 (3) 1988

Table 1 : Root depth, shoot height (cm), root fresh weight and shoot fresh weight (g^{-1} plant) of 75-day-old *Zea mays* L. (dihybrid 204) plants as affected by inoculation with *Streptomyces atroolivaceus*, under saline condition.

Treatments	Root		Shoot	
	Depth	Fresh weight	Height	Fresh weight
Sterile soil				
Non inoculated				
+ 20 mosmol $kg^{-1}H_2O$	28.0	3.78	30.0	2.23
Inoculated				
+20 mosmol $kg^{-1}H_2O$	36.0	3.59	26.0	1.14
+98 mosmol $kg^{-1}H_2O$	21.0	1.85	25.0	1.10
+150mosmol $kg^{-1}H_2O$	31.3	2.81	24.0	1.21
Non sterile soil				
Non inoculated				
+20 mosmol $kg^{-1}H_2O$	20.0	2.38	21.5	0.94
Inoculated				
+20 mosmol $kg^{-1}H_2O$	33.0	3.78	26.7	1.63
+98 mosmol $kg^{-1}H_2O$	28.7	2.47	23.5	1.38
+150 mosmol $kg^{-1}H_2O$	20.0	1.18	22.0	1.56
Statistical analysis				
sterility (st)	ns		ns	
Treatment (T)	ns		*	
st x T	ns		*	

ns = not significant
* = significant at 5 %
** = significant at 1 %

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Table 2 : Monosaccharides, disaccharides (mg g^{-1} fresh weight), IAA (ng g^{-1} fresh weight) of 75-day-old Zea mays (dihybrid 204) plants as affected by inoculation with Streptomyces atroolivaceus, under saline conditions.

Treatments	Root			Shoot		
	Monosacch-arides	Disacch-arides	IAA	Monosacch-arides	Disacch-arides	IAA
Sterile soil						
Non inoculated						
+ 20 mosmol $kg^{-1}H_2O$	2.2	0.6	23.0	8.1	25.1	15.0
Inoculated						
+ 20 mosmol $kg^{-1}H_2O$	9.7	1.7	23.7	79.6	94.6	15.8
+ 98 mosmol $kg^{-1}H_2O$	3.0	0.8	41.7	28.3	16.1	20.0
+150 mosmol $kg^{-1}H_2O$	3.0	0.6	149.0	20.0	9.4	28.5
Non sterile soil						
Non inoculated						
+ 20 mosmol $kg^{-1}H_2O$	1.2	0.2	55.8	28.7	32.6	30.0
Inoculated						
+ 20 mosmol $kg^{-1}H_2O$	18.1	30.4	104.2	65.8	23.7	31.0
+-98 mosmol $kg^{-1}H_2O$	17.0	10.1	160.0	44.8	23.5	86.7
+150 mosmol $kg^{-1}H_2O$	1.9	3.1	118.2	9.6	7.0	42.5
Statistical analysis						
sterility (st)	ns	*	ns	ns	*	ns
Treatment (T)	**	**	ns	*	**	ns
st x T	**	**	*	**	**	*

ns = not significant , * = significant at 5 % , ** = significant at 1 %

Table 3 : Electrical conductivity (EC mhos^{-1} cm), osmolality (Osm mosmol kg^{-1}H$_2$0), Na, K, Ca, Mg, total cation content (TCC) and P of 75-day-old Zea mays L. (diybrid 204) plants as affected by inoculation with S. atroolivaceus, under saline conditions.

Treatments	Root								Shoot							
	EC	Osm	Na	K	Ca	Mg	TCC	Px10^{-3} ppm	EC	Osm	Na	K	Ca	Mg	TCC	Px10^{-3} ppm
	mg g^{-1} fresh weight								mg g^{-1} fresh weight							
Sterile soil																
Non inoculated																
+ 20 mosmol kg^{-1}H$_2$0	9.3	260	3.90	1.31	1.31	0.39	6.91	29	19.4	360	1.58	3.12	1.02	0.90	6.62	76
Inoculated																
+ 20 mosmol kg^{-1}H$_2$0	7.6	165	3.35	1.31	0.86	0.16	5.68	14	7.6	340	1.59	3.38	1.07	1.06	7.10	87
+ 98 mosmol kg^{-1}H$_2$0	9.4	420	7.41	2.49	1.47	0.33	11.70	50	28.0	420	8.48	5.44	1.36	0.89	16.17	123
+150 mosmol kg^{-1}H$_2$0	8.6	350	5.15	1.10	1.11	0.23	7.59	14	10.6	735	6.96	2.34	1.40	0.85	11.55	77
Non sterile soil																
Non inoculated																
+ 20 mosmol kg^{-1}H$_2$0	11.4	325	4.68	1.16	1.43	0.07	7.34	33	10.2	380	4.37	2.37	1.27	1.27	9.28	49
Inoculated																
+ 20 mosmol kg^{-1}H$_2$0	8.1	315	4.05	1.94	1.20	0.45	7.64	28	8.7	377	2.98	3.47	1.23	1.89	9.57	90
+ 98 mosmol kg^{-1}H$_2$0	12.9	465	5.55	2.22	1.38	0.54	9.69	42	19.4	610	5.49	4.98	1.31	1.33	13.11	93
+150 mosmol kg^{-1}H$_2$0	16.5	600	4.65	1.08	1.02	0.88	7.63	123	20.2	810	15.00	4.37	2.35	1.43	23.15	146
Statistical analysis																
	*	*	ns	ns	ns	*		*	*	ns	ns	*	ns	ns		ns
	**	**	ns	ns	**	**		**	**	ns	ns	**	**	**		**
	**	**	ns	*	*	**		**	*	ns	ns	**	*	*		**

ns = non significant , * = significant at 5 %. ,** = significant at 1 %.

Table 4 : Sodium/potassium (Na/K), sodium/calcium (Na/Ca), $\frac{\text{sodium + potassium}}{\text{calcium+magnesium}}$ (MDR) ratios for root and shoot, and $\frac{\text{shoot Na}}{\text{root Na}}$ (S/R Na) and $\frac{\text{shoot K}}{\text{root K}}$ (S/R K) ratios of 75-day-old *Zea mays* L. (dihybrid 204) plants as affected by inoculation with *S. atroolivaceus*, under saline conditions

Treatments	Root			Shoot			$\frac{S}{R}$ Na	$\frac{S}{R}$ K
	Na/K	Na/Ca	MDR	Na/K	Na/Ca	MDR		
Sterile soil								
Non inoculated								
+ 20 mosmol $kg^{-1}H_2O$	3.0	3.0	3.1	0.51	1.6	2.4	0.4	2.38
Inoculated								
+ 20 mosmol $kg^{-1}H_2O$	2.6	3.9	4.6	0.47	1.5	2.3	0.47	2.56
+ 98 mosmol $kg^{-1}H_2O$	3.0	5.0	5.5	1.56	6.3	6.2	1.15	2.17
+150 mosmol $kg^{-1}H_2O$	4.6	4.7	4.7	3.00	4.9	4.1	1.35	2.12
Non sterile soil								
Non inoculated								
+ 20 mosmol $kg^{-1}H_2O$	4.0	3.3	3.9	1.8	3.4	2.7	0.93	2.04
Inoculated								
+ 20 mosmol $kg^{-1}H_2O$	2.1	3.4	3.6	0.9	2.4	2.1	0.71	1.66
+98 mosmol $kg^{-1}H_2O$	2.5	4.0	4.1	1.1	4.2	4.0	1.0	2.22
+150 mosmol $kg^{-1}H_2O$	4.3	4.6	3.0	3.4	6.4	5.1	3.23	4.0

Delta J. Sci. 12 (3) 1988

Table 5 : Colony counts of Streptomyces atroolivaceus after 75 days of inoculating Zea mays L. (dihybrid 204) plants under saline conditions.

Treatments	Number of S. atroolivaceus ($\times 10^3$)g of dry matter	
	Soil	Rhizosphere
Sterile soil		
Non inoculated		
+ 20 mosmol $kg^{-1}H_2O$	1.30	0.40
Inoculated		
+ 20 mosmol $kg^{-1}H_2O$	40.65	16.20
+ 98 mosmol $kg^{-1}H_2O$	5.13	10.03
+150 mosmol $kg^{-1}H_2O$	4.10	11.06
Non sterile soil		
Non inoculated		
+ 20 mosmol $kg^{-1}H_2O$	0.79	2.85
Inoculated		
+ 20 mosmol $kg^{-1}H_2O$	35.25	60.65
+ 98 mosmol $kg^{-1}H_2O$	10.95	71.90
+150 mosmol $kg^{-1}H_2O$	24.25	39.17
	Statistical analysis	
sterility (st)	ns	*
Treatment (T)	**	**
st x T	**	**

ns = not significant
* = significant at 5 %
** = significant at 1 %

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التفاعل بين كلوريد الصوديوم واستربتوميسيس أنترو أوليفاسيس وتأثيرهما على نبات الذرة (هجين ٢٠٤)

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يهدف هذا البحث الى تبيان التأثير المشترك لكلوريد الصوديوم وبكتيريا استربتوميسيس أترو أوليفاسيس المعزولة محليا ، المقاومة للملوحة والمنتجة لمنظمات النمو النباتية على بادرات نبات الذرة (هجين ٢٠٤) ومحتواها من السكريات أندول ـ٣ـ حمض الخليك والعناصر المعدنية ، كما يهدف البحث الى دراسة التغير فى تعداد الكائن المختبر وعلاقة ذلك بنمو النبات والتغير فى محتواة من المواد سابقة الذكر ، وقد دلت نتائج الدراسة على أن بادرات الذرة أظهرت نموا واضحا فى التربة الملقحة ـ الغير معقمة سواء مملحة أو غير مملحة وكانت الزيادة معنوية فى التربة الغير ملقحة مقارنة بالتربة المملحة ، كما دلت النتائج على زيادة معنوية فى محتوى المجموع الجذرى والخضرى من السكريات الاحادية ومحتوى الجذر من السكريات الثنائية فى النباتات الملقحة ، وازداد المحتوى الداخلى للنبات من أندول ـ٣ـ حمض الخليك بتلقيح النبات سواء فى التربة المعقمة أو غير المعقمة وذلك بزيادة الملوحة بالمقارنة بالتربة الغير مملحة. وجدت زيادة فى محتوى المجموع الخضرى من البوتاسيوم ، الفسفور والماغنسيوم عما هو فى المجموع الجذرى تحت ظروف التمليح لوحظت زيادة واضحة فى نسبة الصوديوم/الكالسيوم والعناصر أحادية التكافؤ/ثنائية التكافؤ فى كل من المجموع الخضرى والجذرى ، كما دلت النتائج على أن التلقيح أدى الى زيادة معنوية فى تعداد خلايا استربتوميسيس اتروأوليفاسيس فى كل من التربة والتربة الجذر المحيطية وكان ذلك أكثر وضوحا فى التربة الغير مملحة (سواء معقمة أو غير معقمة) كما أوضحت النتائج وجود ارتباط بين تعداد الكائن المختبر ونمو نبات الذرة وكذلك محتواه الداخلى من اندول ـ٣ـ حامض الخليك.