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MAXIMIZING THE YIELD OF MAIZE GROWN ON A SALINE-SODIC SOIL THROUGH MINERAL AND BIOFERTILIZATION AND SPRAYING WITH PROLINE

Mohaseb, M. I[1].; M. F. Abd El-Aziz[1] and Kh. A.Shaban[1] .
1 Soils, Water and Environment Research Institute, ARC, Giza.

ABSTRACT

This research was conducted to study the effect of different nitrogenous fertilizers (urea, urea formaldehyde and sulfur coated urea) whether applied solely or combined with the nitrogen fixing bacteria Azospirillum sp.in presence or absence of the growth osmo-regulator proline on maize plants grown on a saline-sodic soil located at Sahl El-Tina,NorthSaini.Calcium superphosphate was added during the soil preparation at a rate of 309.4 kg P/ha^{-1} whereas potassium was added in the form of K_2 SO_4 ata rate of 166.6 kg K ha^{-1}intwo equal doses after 21 and 45 days of planting. The experimental design was a randomized complete block factorial in three factors i.e. the nitrogenous fertilization, biofertilization and spraying with proline.The results showed that maize grain yield increased significantly due to application of N and sulfer coated urea whereas the control treatment was of the least effect on maize grain yield. Biofertilization and spraying plants with proline maximized effect of the nitrogenous fertilizers on grain yield. The applied nitrogen fertilizers especially the sulfur coated urea significantly increased weight of 100 grains. Spraying with proline and biofertilization were of significant effects on weight of 100 grains. All the applied nitrogenous fertilizers with special concern of the sulfur coated urea increased plant uptake of NPK and the effect became more obvious with the biofertilization and proline application.
Keywords : Saline-sodic soil, maize plant, N fertilizers, biofertilization, proline.

INTRODUCTION

High levels of salts in soil can often cause serious limitations to agricultural production and land development (Aroiee*et al*.,2009).These effects could be due to high osmotic potential of soil solution, specific ion effects, nutritional imbalance or a combined effect of all these factors (Balba,1995).For overcoming salt stress, plants have evolved complex mechanisms that contribute to the adaptation to osmotic and ionic stress caused by high salinity.Proline accumulation is one of the most frequently reported mechanisms. Its possible roles have been attributed to stabilizing the structure of macromolecules through stabilizing proteins and membranes againstdenaturating effect of high concenterations of salts and other harmful solutes (Yurekli et al 1996 andAshraf and Fooad 2007). Exogenous addition of proline was very effective in counteracting the effect of salts (Torello and Rief.,1986and Troeh and Thompson, 1993)Yurekli et al.(1996)showed that bio-fertilizers alleviated adverse effects of high levels of salinity through accumulation of more polyamins.

Rhizosphere bacteria such as Azotobacterexerts strongbeneficial effects on plant growth (Ali,2011)however, the significance of proline accumulation in osmotic adjustment is still debated and varies according tothe plant species (Lutts et al., 1996 and Rodriguez et al., 1997).

The role of proline in cell osmotic adjustment, membrane stabilization and detoxification of injurious ions in plants exposed to salt stress is widely reported(Ashraf and Fooad 2007). Colmer*et al*.(1995) found that proline content was higher in sensitive wheat plants than in tolerant wheat plants .Reducing the hazardous effects of soil salinity on maize plants grown on saline –sodic soil will be tried in this investigation through some mineral andbio- fertilization treatments andproline foliar application.

MATERIALS AND METHODS

The current study aims at investigation the effect of some fertilization treatments i.e. single application of different nitrogenous fertilizers, combined with bio-fertilization in presence or absence of spraying proline on maize plants grown on a saline-sodic soil located at Sahl-El-Tina Plain, North Sinai. A representative surface soil sample (0-30 cm) was collected from the studied area, dried, crushed, sieved through a 2mm sieve and analyzed physically and chemically according to the standard methods outlined by Page *et al*.(1982) and Klute(1986). Table 1 shows some physical and chemical properties of the investigated soil.

Table1.Somephysical and chemical properties of soil the used in the study.

Soil property	Value	Soluble ions (m molc L^{-1})	
C.sand (%)	14.17	Ca^{2+}	10.2
F. sand (%)	55.83	Mg^{2+}	20.4
Silt (%)	7.36	Na^{+}	53.5
Clay (%)	22.64	K^{+}	0.9
Texture	Sandy clay	HCO^{-}	7.5
O M (g kg^{-1})	6.1	Cl^{-}	60.0
$CaCO_3$ (g kg^{-1})	103	SO^{2-}_4	17.5
pH (1:2.5 wv^{-1})	8.10	CO^{2-}_3	0.0
EC (dSm^{-1})	7.2	ESP	15.89
Available nutrients (mg kg^{-1})			
N		38.0	
P		6. 9	
K		181	
Fe		3.1	
Mn		1.7	
Zn		1.1	
Cu		0.01	

EC was determined in soil paste extract

This soil is irrigated from El-Salam canal water (Nile water mixed with drainage water at a ratio of 1:1). The chemical characteristics of the irrigation water are shown in Table 2.

Table 2. Chemical characteristics of El-Salam canal irrigation water.

Property	Value
pH	8.21
EC (dS m^{-1})	1.30
SAR	4.46
NO_3-N(mgL^{-1})	8.75
NH_4-N(mgL^{-1})	13.93
P (mgL^{-1})	5.10
K (mgL^{-1})	6.79
Fe (mgL^{-1})	2.75
Mn(mgL^{-1})	1.56
Zn (mgL^{-1})	1.10

Materials of study

1-An inoculum of the salt tolerant "*Azospirillumbraisilense* No.40" bacteria in a water suspension supplied by the Microbiology Department, Institute, of the Agriculture Reseach Center " ARC ", Giza, Egypt .

2- Proline as a growth osmo-regulator .

3- Sources of nitrogen fertilizer: urea (460 g N kg^{-1}), urea formaldehyde (400 g N kg^{-1}) and sulfur coated urea (400 g N and 170 g S kg^{-1}).

4- Seeds of maize(Zea mays) supplied by the Field Crops Research Institute, ARC.

The experimental work.

The experimental design was a "Randomized Complete Block , factorial"; in three factors.The factors and the treatments are as follows:

A: N-fertilization (N):

Four treatments; N_0, N_1, N_2, N_3 represented by control, urea (460 g N kg^1) formaldehyde (400 g N kg^1) and sulfur coated urea (400 g N and 170 g S kg^1) which were applied at a rate of 285.6 kg N/ha (120 kg N/fed) in 3 equal doses applied after 21, 45 and 60 days from seeding.

B: Biofertilization (B):

Two treatments were used namely B_0 and B_1 i.e. no biofertilization and N-fixing salt-tolerant *Azospirillumbrasilense* No .40 inocula at a rate of 2.4 kg ha^{-1}(1 kg fed^{-1}) and then sprayed on the soil beside the plant roots at 30, 55 and 65 days after seeding at a rate of 12 L ha^{-1}(5 L fed^{-1})(1 mL contains 3 x 10^9 bacterial cell)

C: Proline (P) :

Two treatments of proline namely, P_0 (no addition of proline) and P_1(30 mg proline L^{-1}) with a total volume of 953 L ha^{-1} (400 L fed^{-1}). Spraying was done at20, 45 and 60 days after sowing.

Therefore, the total number of treatments covering the different combinations of the abovementioned factors is 16 (4 N fertilizationtreatments X 2 biofertilization treatmentsX2 prolinetreatmeants).

Calcium super-phosphate (67.7 g P kg^{1}) was added at a rate of 309.4 kg P ha^{-1} (130 kg P fed^{-1}) during soil preparation,while potassium sulphate (398.4 g K kg^{1}) at a rate of 166.6 kg ha^{-1} (70 kgfed^{-1}) was applied in two doses after 21 and 45 days of planting.

At maturity maize plants were harvested and grain yield, weight of 100 grains, total carbohydrate content and NPK uptake values were estimated

Methods ofplant analysis:

Representative plants were sampled from the plots area under investigation . Grains of maize in plant samples were oven dried at 70 ^{0}c for 48h and the corresponding dry weights were recorded.

Total carbohydrates were determined according to Yemmand Willis(1954).A portion of 0.2 g of each dried plant sample was wet digested using a mixture of concentrated H_2SO_4/$HClO_4$ acids (1:1) Nitrogen, phosphorus and potassium were determined in the digested solution as follows.

1-Nitrogen: was determined by the microkjeldahl method according to A.O.A.C. (2000).

2-Phosphorus: was determined colormeterically according to the method described by Frei*et al.* (1964).

3-Potassium: was determined as described by Brown and Lilliand (1964) using a flame photometer.

RESULTS AND DISCUSSION

Grain yield of maize (Mg ha^{-1}) as affected by N-fertilizer sources, bio-fertilizer and proline:

Data presented in Table 3 show that significant increases in grain yield were occurred with N- application; however, the responses of maize were significantly different under the different sources of applied N-fertilizers.This finding stands in well agreement with those of Siam *et al.*(2008).The highest increases in grain yields were recorded with application of sulfur coated urea followed by urea formaldehyde whereas, the lowest increases were recorded with no fertilization (control) treatment.

The rapid hydrolysis of urea in soil might led to ammonia volatilization(Troeh and Thompson 1993). Both sulfurcoated urea and urea formaldehyde are the common forms of N used to eliminate Ntransformations in soilby coating urea granules with sulfurfor the first form andformaldehyde in the second form and thusincrease N-use efficiency. Both forms are used in this study as slow release fertilizers to supply plants with their N requirements. Such anapproachseemed acceptable as the recorded grain yield wasrelativelyhigh; however, itshigh cost stands against recommending this fertilizerin the areaof studybutstill consideredasone of the best choices to attain high grain yield under the saline conditions found therein.

The increases in grain yield become more obviousespecially with spraying plants with proline and/ or inoculating seeds with *Azospirillum sp*. Such results verify the importance ofproline as a plant anti-drought (*Yurekli, et al., 1996*). Proline accumulates in roots at high concentrations and thus, decreases the water potential of roots to increase their ability to absorb water from soil (*Torello, and Ricf1986*) and also reduces transpiration through affecting stomata. *Azospirillumsp* is considered a plant growth promoting bacteria (*Stamford et al., 2002*) even under saline condition (*Lutts et al., 1996)*. Altering the sensitivity of plants to Na^+, Ca^{2+} and Mg^{2+} is one of the suggested mechanisms *(Sayd et al., 2004*).

Table 3. Grain yield of maize (Mg ha^{-1}) as affected by N-fertilizer sources, bio- fertilizer and proline.

Proline	Bio- fertilizer	N – Source				Mean
		N_0	N_1	N_2	N_3	
P_0	B_0	2.13	3.46	3.72	4.03	3.34
	B_1	3.11	3.70	3.87	4.26	3.73
	Mean	2.62	3.58	3.80	4.14	3.53
P_1	B_0	3.09	3.62	3.82	4.15	3.67
	B_1	3.13	3.77	3.95	4.42	3.82
	Mean	3.11	3.69	3.89	4.28	3.74
Grand mean		2.86	3.64	3.84	4.21	

	Means of Bio fertilizer (B)				Mean
B_0	2.61	3.54	3.77	4.09	3.50
B_1	3.12	3.73	3.91	4.34	3.77

N sources :N_0 : no N, N_1 :urea, N_2: urea formaldehyde, N_3: sulfur coated urea & B_0 no-biofertilization& B_1biofertilization with inoculation of seeds with *Azosirillumbraiseleuse*& P_0 : no proline addition & P_1 : proline addition at a rate of 953 L ha^{-1}i.e 400 L fed^{-1} (each L contain 30 mg Proline).

LSD:0.05:-N=0.011, B=0.0066, P=0.0066, NB=0.0149, NP=0.0149, BP=0.0094, NBP=0.0212.

100 maize grain weight (g) as affected by N source, biofertilizer and proline.

Table 4reveals that all sources of N-fertilizers significantly increased the 100-grain weight especially the sulfur coated urea.Significant increases in 100-grain weight also occurred with proline treatment. Likewise, inoculating plants with *Azospirillumbrasilense* significantly increased 100- grain weight.Similar results were obtained by El-Douby*et al*.,(2001) and Siam *et al*(2008).

Total carbohydrate content in maize grain (g kg^{-1}) as affected by N-source,biofertilizer and proline.

Concerning, the effect of N source on carbohydrate accumulation in maize grains, data presented in Table 5indicats that sulfur coated urea recorded the highest increases in total carbohydrate accumulation in maize grains. This effect could be ascribed to the acidity effect of sulfur coated urea which consequently increased availability of nutrients and their uptake by plants.Theincreases in total carbohydrates in maize grain were significant and more obvious when plants were treated with bio-fertilizer and/or proline.Thebiofertilizer might contributed to improvement of soil physical and chemical properties beside of its role in fixation of N and providing the plant with it in available from . Proline might enabled the plant to grow well under salinity condition since it contributed to cell osmotic adjustment (Ashraf and fooad, 2007 and Chookgampaeng,2011)

Table 4. 100- maize grain weight (g) as affected by N-source, bio-fertilizers and proline.

Proline	**Bio-fertilizer**	**N – Source**				**Mean**
		N_0	**N_1**	**N_2**	**N_3**	
P_0	B_o	31.90	31.99	34.16	36.14	33.55
	B_1	32.36	33.12	36.33	38.25	35.01
	Mean	32.13	32.55	35.24	37.20	34.28
P_1	B_o	32.14	33.03	35.06	37.81	34.51
	B_1	32.59	38.45	36.79	40.13	36.99
	Mean	32.36	35.74	35.93	38.97	35.75
Grand mean		32.25	34.15	35.58	38.08	
		Means of Bio fertilizer (B)				**Mean**
B_o		32.02	32.51	34.61	36.97	34.02
B_1		32.47	35.78	36.56	39.19	36.00

See footnotes of Table 3
LSD :0.05:- N=0.018, B= 0.011 , P=0.011, NB=0.025 , NP=0.025, BP= 0.016 , NBP= 0.035

N uptake (kg ha^{-1}) by maize as affected by N-source , bio-fertilizer and proline.

Data in Table 6 illustrate that N-fertilization increased significantly N uptake by maize; however, the amount of N uptake differed significantly with the source of N-fertilizer. Similar results were reported by Siam *et al*.(2008) who found that the addition of N significantly increased N uptake by maize plants. El-Rys (2012) went almost to a similar finding.The highest increases in N were recorded for sulfur coated urea, while the lowest ones were recorded when urea was applied as a source for N .

The acidifying effect of sulfur coated urea may account for increasing availability of N in soil and hense its uptake by plants(ScottPerin etal.,1998).Inoculation of maize grains by*Azospirillumbrasilense*improved N uptake by maize plants. Similar results were reported by Dalla Santa*et al*.(2004) who found significant increases in N uptake and N-use efficiencies owing to inoculating maize with *Azospirillumbrasilense*. *Azospirillumbrasilense*probably stimulated N uptake by roots which resulted in higher N uptake and grain yield,proline treatment caused further significant increases in values of N-uptake. Proline is considered a nitrogen containing compound applied to increase plant adaptation to salinity stress (Mansour, 2000).

Table 5. Total carbohydrates in maize grains (g kg^{-1})as affected by N-source, bio- fertilizer and proline.

Proline	Bio-fertilizer	N – Source				Mean
		N_0	N_1	N_2	N_3	
P_0	B_o	124.13	145.17	166.83	171.27	151.85
	B_1	136.97	165.00	181.93	188.10	168.00
	Mean	130.55	155.08	174.38	179.68	159.92
P_1	B_o	133.20	154.57	178.07	179.57	161.35
	B_1	144.57	177.03	186.30	197.07	176.24
	Mean	138.88	165.80	182.18	188.32	168.79
Grand mean		134.72	160.44	178.28	184.00	

	Means of Bio fertilizer (B)				Mean
B_o	128.67	149.87	172.45	175.42	156.60
B_1	140.77	171.02	184.12	192.58	172.12

See footnotes of Table 3.
LSD :0.05:- N=1.17 , B=0.74 , P=0.74 , NB= 1.65 , NP= 1.65 , BP=n.s , NBP= 2.33

Table 6. N uptake (kg N ha^{-1}) by maize as affected by N-source, bio-fertilizer and proline.

Proline	Bio-fertilizer	N – Source				Mean
		N_0	N_1	N_2	N_3	
P_0	B_o	27.18	44.68	50.65	58.86	45.34
	B_1	41.83	53.27	57.59	69.35	55.51
	Mean	33.63	46.43	52.72	61.09	48.47
P_1	B_o	40.08	48.18	54.80	63.32	51.59
	B_1	42.96	55.86	62.91	77.91	59.91
	Mean	42.39	54.57	60.25	73.63	57.71
Grand mean		38.01	50.50	56.49	67.36	

	Means of Bio fertilizer (B)				Mean
B_o	34.50	48.98	54.12	64.11	50.43
B_1	41.52	52.02	58.86	70.62	55.75

See footnotes of Table 3.
LSD :0.05:- N= 0.707 , B=0.447, P=0.447, NB=0.999 , NP= 0.999, BP=n.s , NBP=1.413

P uptake (kg P ha^{-1}) by maize as affected by N- source, bio-fertilizer and proline.

Table 7 reveals that N-fertilizers caused significant increases in P-uptake; however, the amount of absorbed P differed significantly according to the source of the applied N-fertilizer. The highest increases in P-uptake were found in treatments which received sulfur coated urea, while the lowest ones were achieved with urea application. These results agree with those of Siam et al .(2008) who reported that N-fertilizer up to 285kg/ha increased P uptake.

*Azospirillumbrasilense*and/ or proline significantly increased P uptake by plants. *Azospirillumbrasilense* bacteria acidify the rhizosphere and thus increase P availability in soil .

Table 7. P uptake (kg P ha^{-1}) by maize as affected by N-source , bio-fertilizer andproline.

Proline	Bio-fertilizer	N – Source				Mean
		N_0	N_1	N_2	N_3	
theseP_0	*B_o*	9.08	16.16	18.62	19.49	15.84
	B_1	16.26	20.60	21.64	23.54	20.51
	Mean	12.67	18.38	20.13	21.51	18.17
P_1	*B_o*	13.81	18.84	21.67	20.60	18.73
	B_1	17.21	20.96	23.72	27.40	22.32
	Mean	15.51	19.90	22.69	24.00	20.52
Grand mean		14.09	19.14	21.41	22.46	
		Means of Bio fertilizer (B)				**Mean**
B_o		11.44	17.50	20.14	20.04	17.28
B_1		16.73	20.78	22.68	25.47	21.42

See footnotes of Table 3.

LSD :0.05:- N=0.577, B=0.365 , P=0.365 , NB= 0.817 , NP= n.s , BP=0.516 , NBP= 1.155

K uptake (kg K ha^{-1}) by maize as affected by N- source, bio-fertilizer and proline.

Data presented in Table 8 indicate that application of fertilizer N increased K-uptake by plants; however, such increases differed significantly with the source of applied N-fertilizer. The increases in K-uptake were as follows: sulfur coated urea >urea formaldehyde >urea.

Table 8. K uptake (kg K ha^{-1}) by maize as affected by N-source, bio - fertilizer andproline.

Proline	Bio-fertilizer	N – Source				Mean
		N_0	*N_1*	*N_2*	*N_3*	
P_0	B_o	45.91	76.55	84.91	95.69	75.76
	B_1	69.78	88.05	91.35	106.10	88.81
	Mean	57.84	82.30	88.13	100.90	82.29
P_1	B_o	68.82	83.19	89.59	101.90	85.88
	B_1	70.80	91.89	95.81	112.52	92.75
	Mean	69.81	87.54	92.70	107.20	89.31
Grand mean		63.82	84.91	90.41	104.04	
		Means of Bio fertilizer (B)				Mean
B_o		57.37	79.57	87.25	98.79	80.74
B_1		70.29	90.00	93.58	109.30	83.26

See footnotes of Table 3.

LSD :0.05:- N=0.521, B=0.330, P= 0.330, NB= 0.737, NP=0.737, BP=0.466 , NBP=1.0

The relative low uptake of K upon application of urea might ascribed as mentioned by Irshad*et al.(2002)*to competition among cationson the transporters and canals of K under saline conditions.

CONCLUSION

This study recommend, therefore,fertilization of saline-sodic soil with the sulfur coated urea together with biofertilization and spraying with proline to achieve the highest crop yield of maize and, at the same time, to avoid the harmful effect of salts on plant growth.

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تعظيم محصول الذرة النامية على أرض ملحية- صودية من خلال التسميد المعدنى والحيوى والرش بالبرولين

محمد ابراهيم محسب - محمد فؤاد عبدالعزيز و خالد عبده شعبان
معهد بحوث الأراضى والمياه والبيئة – مركز البحوث الزراعية –الجيزه

اجرى هذا البحث لدراسةأثر الأسمدة النيتروجينية المختلفة (يوريا & يوريا فورمالدهيد &يوريا مغطاة بالكبريت) سواء كانت منفردةأو متحدة مع البكترياالمثبتة للنيتروجين Azospirillum sp.فى غياب أو وجود البرولين على نباتات الذرة تحت ظروف ارض صودية ملحية بمنطقه سهل الطينة بشمال سيناء.

حيث تم اضافه سماد سوبر فوسفات الكالسيومبمعدل 4 .309 كجم P/ هكتار

بينما اضيف سماد كبريتات البوتاسيوم (k_2SO_4) بمعدل 166.6 كجم K/ هكتارعلى دفعتين متساويتين الدفعه الاولى بعد 21 يوم والدفعه الثانيه بعد 45 يوم من الزراعه.

وصممت التجربه فى تصميم قطاعات كاملة العشوائيه ذات 3 عوامل وهى التسميد النيتروجينى والتسميد الحيوى والرش بالبرولين.

وقد اوضحت النتائج زيادة محصول حبوب الذرة زياده معنوية باضافة النيتروجين وكانت اليوريا المغلفة بالكبريت الاكثر تأثيرا بينما كانت المعاملة الكونترول هى الاقل أثرا على محصول حبوب الذرة.

أدى رش النباتات بالبرولين والتسميد الحيوى الى تعظيم أثر المعاملات النيتروجينية على محصول الحبوب.

أدت الاسمده النيتروجينية المضافة خاصه اليوريا المغلفة بالكبريت الى زياده معنوية فى وزن ال 100 حبة.

وكان للرش بالبرولين مع التسميد الحيوى أثرا معنويا فى زياده وزن المائه حبه.

أدت جميع الاسمده النيتروجينيه المضافه وخاصه اليوريا المغلفة بالكبريت الى زياده امتصاص النبات من عناصر ال NPK وكان التأثير أكثر وضوحا مع التسميد الحيوى والرش بالبرولين .

وتوصى الدراسه باستخدام اليوريا المغلفة بالكبريت مع التسميد الحيوى والرش بالبرولين فى حالة الأرض الملحية الصودية للحصول على أكبر محصول ممكن من الذرة وتجنب التأثير الضار للاملاح على النباتات.