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RESPONSE OF SOYBEAN PLANTS TO ROCK PHOSPHATE APPLICATION COMBINED WITH PHOSPHATE DISSOLVING BACTERIA UNDER SALINITY STRESS CONDITION

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ABSTRACT: Greenhouse pot experiments were carried out on soybean (Glycin max) to study the effect of fertilization with rock phosphate (RP) at 14.5mg P kg^{-1} soil and phosphate dissolving bacteria (PDB) on a clay loam soil under irrigation with different grades of saline water (1000, 2000, 4000,and 8000mgL^{-1}) as compared with tap water (400 mgL^{-1}) on nodulation, nitrogenase activity (N_2-ase), available P, total counts of bacteria and phosphate dissolving bacteria, CO_2 evolved as well as some macronutrients content of soybean plants.

Dry matter yield of soybean plants decreased with increasing salinity but this was less marked in presence of RP+PDB. N, P, K and Ca concentration in plants decreased with increasing salinity. The soybean plants fertilized with RP and /or inoculated with PDB were enhanced. Inoculation of PDB with application of RP gave high nodulation, nitrogenase activity, available-P and CO_2 evolved from the rhizosphere up to the irrigation water was 4000 mgL^{-1}. Na concentration in plants increased with increasing salinity. Total counts of bacterial and phosphate dissolving bacteria decreased by increasing salinity; the highest number of total count of bacteria and PDB were obtained with the RP+PDB.

Key words: Rock phosphate, phosphate dissolving bacteria, soybean, salinity.

INTRODUCTION

The importance of soybean as source of plant protein has become vital. To ensure high yields, the plant requirements should be furnished. Inoculation of legumes with rhizobium and phosphate dissolving bacteria in the presence of phosphate fertilization increases plant growth and yield (Mehasen and El-Ghozoli, 2003).

Natural materials such as rock phosphate may be used along with bio fertilizers, which are forms of materials supplying crops with their nutrients via their biological activities. Seeds or soil inoculation with phosphate dissolving bacteria and simultaneous application of rock phosphate to soil may be a substitute for super-phosphate application (Gaur *et al.*, 1980 and Mehasen and El-Ghozoli, 2003).

The phosphate dissolving bacteria utilize organic compounds as energy source and produce organic acids which would solubilize insoluble inorganic phosphates (Pareek and Gaur, 1973; Gaur *et al.*, 1979; Gained and Gaur, 1991). The possibilities for saving half dose of N and replacing super phosphate with rock phosphate in the presence of phosphate dissolving bacteria were discussed by El- Sayed (1998). Abd El-Salam (2007) found that inoculation with phosphate dissolving bacteria (*B. megatherium*) in presence of rock phosphate increased plant growth, P uptake by plant and available P in soil.

As increasing the demand for irrigation water in Egypt, utilization of ground, sea and well water has become a logic and important component of total water of these resources are generally saline and can be used to supplement agricultural water supplies under very special conditions. El-Haddad (1989) and Amer *et al.* (1993) found that the plant growth parameters and uptake of N, P and K were adversely affected by increasing salinity stress.

The availability of nutrients under salinity stress is directly or indirectly dependent on salt concentration, activity of ions in the solution and exchangeable phase (Amer *et al.*, 1993).

The present investigation was undertaken to evaluate the response of soybean plants to inoculation with phosphate dissolving bacteria separately and/or with rock phosphate under irrigation with different levels of saline water.

MATERIALS AND METHODS

Two pot experiments were carried out in the Training Center for Recycling of Agricultural Residues at Moshtohor during 2006 and 2007 seasons to study the effect of fertilization with rock phosphate separately with or without inoculation with phosphate dissolving bacteria (PDB) under irrigation with saline water. The experiment was factorial, involving two factors. Factors and their treatments were as follows: 1- factor "A" salinity: it includes 5 treatments as followed water salinity: 400 (tap water), 1000, 2000, 4000 and 8000 mgL^{-1}, 2- factor "B" fertilization treatment: it includes 4 as follows: no fertilization, fertilization with RP; biofertilization with PDB and fertilization with RP in combination with biofertilization with PDB. The plant was soybean (Glycin max) cv. Giza 22. All treatments were inoculated with N fixing bacteria.

The physical and chemical properties of the soil used in the study were determined according to Piper (1950) and Jackson (1967) and are shown in Table 1.

Each experiment included 20 treatments which were the combination of the 5 salinity grades of irrigation water.

Bradyrhizobium japonicum strain USDA 110 and *Bacillus megatherium* var. phosphaticum (local strain) used during the current study were obtained from Agric. Microbiology. Dept., Soils, Water and Environment Res. Inst., Agric. Res. Center, Giza, Egypt.

Chemical characterization of sea water was determined according to Richard's (1954) and reported in Table 2.

Preparation of Bacterial Inocula

For preparation of bacterial inocula, yeast manitol broth medium (Vincent, 1970) and medium described by Bunt and Rovira (1955) and modified by Abdel-Hafez (1966) were inoculated by *Bradyrhizobium japonicum* and *Bacillus megatherium*, respectively, then incubated at 30°C for 7-days.

Rock phosphate (total P=7.4g/kg, total N= 0.027% and available-P =30.1mg kg^{-1}) was added

Table 1. Physical and chemical properties of the experimental soil

Particle size distribution (%):		CO_3^{2-}	0.0
Sand	46.1	HCO_3^-	5.5
Silt	25.0	Cl^-	51.6
Clay	28.9	SO_4^{2-}	4.2
Textural class	Clay loam	Soil moisture constants (%):	
Organic matter %	1.37	Field capacity	14.0
PH (1:2.5 suspension)	7.92	Wilting point	7.4
EC (dSm^{-1})	5.53	Available water	6.7
Soluble ions ($mmol_c L^{-1}$):			
Ca^{2+}	21.0		
Mg^{2+}	13.5		
Na^+	24.6		
K^+	2.2		

Table 2. Sea water characterization

EC(dSm^{-1})	**pH**	**Soluble ions ($mmol_c L^{-1}$)**							
		Ca^{2+}	**Mg^{2+}**	**Na^+**	**K^+**	**CO_3^{-2}**	**HCO_3^-**	**CL^-**	**SO_4^{2-}**
45.8	7.05	27.1	148.0	552	12.1	0.0	4.20	607.0	128.0

at a rate of 14.5 mg P kg. Inoculation of seeds was done (to pre-washed seeds with water, then air dried) by socking the seeds in a suspension of *Bacillus megatherium* ($1.2X10^8$ viable cell/ ml) for 30min. Then, seeds were inoculated socking in a suspension of *Bradyrhizobium Japonicum* ($8X\ 10^7$ viable cell /ml) for 30 min. Gum Arabic was used in the socking operations.

The pots were arranged in a randomized complete block desing with three replicates for each treatment. After two weeks of germination, seedlings were thinned to three plants per pot and the moisture content was kept at field capacity. Nitrogen fertilizer was applied as ammonium sulphate (20.5%) at a rate of 30kg N/fed. at two equal doses.

At flowering stage, soil samples were taken from the rhizosphere area to determine CO_2 evolved according to Page *et al.* (1982). The available –P was extracted by 0.5 $MNaHCO_3$ solution of pH 8.5 (Olsen *et al.*, 1954) and total counts of bacteria and phosphate dissolving bacteria were estimated by using plates count technique. The dry weight and the number of nodules / plant were recorded. The N_2 – ase was estimated according to Hardy *et al.* (1973).

After 90 days from germination, the plants were harvested, dried at 70°C and the dry weight was recorded. Samples were digested by a mixture of conc. H_2SO_4 and $HCLO_4$ to determine total N by the micro-Kjeldahl method, as well as P according to Murphy and Riley (1962) as modified by John (1970) and K by using a flamephotometer according to Jackson (1967).

Data were subjected to statistical analysis according to Ryan and Joiner (1994).

RESULTS AND DISCUSSION

Dry Matter Yield

Data presented in Table 3 show that the dry matter yield (DM) of soybean plants was significantly decreased by applying saline water and the decrease was progressive with increasing salinity of irrigation water. The percentage of reduction in dry weight of soybean plants from salinity of irrigation water of 1000, 2000, 4000 and 8000 mgL^{-1} were 5.2%, 6.9%, 12.6% and 22.9%, respectively. This reflects the negative effect of salinity on photosynthesis and transpiration (Tornabene *et al.*, 1979 and Amer *et al.*, 1993) and absorption of nutrients (Shukla and Mukhi, 1985).

On the other hand, soybean receiving either RP or the biofertilizer of PDB or both gave yields higher than the non fertilized treatment record. The highest values were obtained with the treatment of RP+PDB. This illustrates the combined stimulating effect of phosphate dissolving bacteria and rock phosphate. These results are in agreement with those obtained by El-Sayed (1998) and Mehasen and El-Ghozoli (2003).

The regression equation and correlation coefficient DM yield (as Y) and salinity (as X) is as follows:

D.M. yield (g plant^{-1}) = 2.29-0.000066 X (mgL^{-1}) (r = - 0.207*)

N, P and K Concentrations of Soybean Plants

It is clear from data presented in Table 3 that the N,P and K concentrations of soybean plants were negatively affected with increasing salinity of irrigation water. The percentage decrease in the concentration of N caused by increased salinity to 1000, 2000, 4000 and 8000 mgL^{-1} were 10.7, 21.3, 36.0 and 37.8%, respectively.

The regression equation and correlation coefficient relating N concentration with salinity is as follows:

N- concentration%=3.05-0.000152 X (mgL^{-1}) (r= - 0.661***)

The crosspounding figures in P-concentration were 9.52, 16.7, 26.2 and 31.0%, respectively. The regression equation and correlation coefficient are as follows :

P-concentration (%) =0.394-0.000014 X (mgL^{-1}) (r= - 0.426**)

Regarding K-concentration of soybean plants was reduced by 10.7, 21.3, 36.0 and 37.8% by applying irrigation water of the aforementioned salinity levels respectively. The regression equation and correlation coefficient of the relationships between K-concentration and water salinity (X) are as follows:

K-concentration % = 2.68-0.000130 X (r= -0.750***)

Response to Fertilization

N, P and K concentrations in shoots of soybean plants were positively affected by application of RP and/or PDB. The treatment of RP+PDB was superior as compared to any other treatment. The increase in N content is a direct effect of the high N fixation and phosphate dissolving bacteria.

Table 3. Dry matter yield, N, P, K, Na and Ca concentrations of soybean plants as affected by rock phosphate and phosphate dissolving bacteria under salinity of irrigation water conditions

Salinity mgL^{-1} (S)	Fertilization (F)														
	Dry weight (g/plant)					N%					P %				
	None	RP	PDB	RP+ PDB	mean	None	RP	PDB	RP+ PDB	mean	None	RP	PDB	RP+ PDB	mean
400	1.54	2.60	2.13	2.98	2.31	2.85	3.12	3.15	4.01	3.28	0.337	0.450	0.359	0.520	0.420
1000	1.26	2.27	2.29	2.94	2.19	2.80	2.89	2.98	3.05	2.93	0.324	0.347	0.341	0.438	0.380
2000	1.26	1.90	2.55	2.89	2.15	1.82	2.79	2.82	2.89	2.58	0.303	0.363	0.338	0.397	0.350
4000	1.23	1.74	1.25	3.85	2.02	1.79	2.17	2.10	2.36	2.10	0.250	0.345	0.300	0.362	0.313
8000	1.20	1.93	1.63	2.04	1.79	1.37	2.14	2.21	2.24	2.04	0.228	0.320	0.290	0.340	0.29
Mean	1.29	2.08	1.97	2.94	2.07	2.12	2.62	2.65	2.91	2.58	0.28	0.37	0.32	0.41	0.35
$L.S.D_{0.01}$	S=0.679 F= 0.588 SXF=0.42					S=0.56 F= 0.485 SXF=0.280					S=0.155 F= 0.134 SXF=0.077				
	K%					Na %					Ca %				
400	2.39	2.53	2.41	3.37	2.68	0.48	0.38	0.44	0.34	0.41	0.40	0.73	0.51	0.76	0.60
1000	2.34	2.49	2.38	2.80	2.50	0.71	0.63	0.65	0.61	0.65	0.37	0.48	0.40	0.55	0.45
2000	2.16	2.39	2.28	2.60	2.36	1.30	1.13	1.21	1.12	1.19	0.30	0.42	0.36	0.44	0.38
4000	2.09	2.30	2.33	2.43	2.29	2.22	2.00	2.10	1.92	2.06	0.26	0.30	0.28	0.33	0.29
8000	1.27	1.30	1.50	2.30	1.59	3.88	3.59	3.70	3.51	3.67	0.20	0.24	0.22	0.26	0.23
Mean	2.05	2.20	2.18	2.70	2.28	1.71	1.54	1.62	1.50	1.59	0.30	0.43	0.35	0.46	0.39
$L.S.D_{0.01}$	S=0.332 F= 0.287 SXF=0.166					S=0.041 F= 0.036 SXF=0.031					S=0.022 F= 0.02 SXF=0.017				

The increase in P content reflects phosphate dissolution by the PDB which, convert insoluble phosphate into soluble form by secreting organic acids and consequently reduce the soil pH. These results are in harmony with those reported by El-Touky *et al.* (2002).

Na and Ca Concentrations of Soybean Plants

Data presented in Table 3 reveal that the Na concentration of soybean plants significantly increased with increasing salinity level of the applied irrigation water. This reflects the high Na content of the saline water. The mean Na concentration corresponding to waters of 400, 1000, 2000, 4000 and 8000 mgL^{-1} were 0.41, 0.65, 1.19, 2.06 and 3.67%, respectively. Regarding fertilization treatments, the RP + PDB was showed the lowest of Na concentration. These results agree with those obtained by El-Haddad (1989) and Amer *et al.* (1993).

The regression equation could be summarized as follows:

Na^{+}-concentration%= 0.272 + 0.000430 X (mgL^{-1}) (r = 0.993***)

Concerning the Ca concentration of soybean plants, data in Table 3 show a decrease as affected by salinity stress. Ca concentration was positively affected as the application of RP and/or PDB. The treatment of RP+PDB was superior to the others.

The effect of salinity (X) could be presented according to the following equation: current study:

Ca^{+} - concentration % = 0.517 - 0.000041 X (mgL^{-1}) (r = 0.71***)

Total Counts of Bacteria and Phosphate Dissolving Bacteria and Co_2 Evolved

From results presented in Table 4 it could be reported that total counts of bacteria, phosphate dissolving bacteria and evolved CO_2 increased with addition of RP and / or PDB. This increase indicates microbial activity enhancement because of introduction of PDB and presence of RP. These results are similar to those by Estefanous *et al.* (1997) and Luo and Sun (1994).

Results indicated that the treatment of RP+PDB caused higher activity as compared with other treatments microbial counts decreased by increasing salinity. This shows the unfavorable conditions of salinity on microbial activity. These results are in accordance with those obtained by El-Ghanam *et al.* (2000).

The high numbers of total bacteria, phosphate dissolving bacteria as well as evolved CO_2 were associated with treatments RP + PDB. The effect of salinity (X) could be summarized by the following equation:

T. Bacteria count = 53.6-0.00592 X (mgL^{-1}) (r= -0.754***)

Soil Available P

Data in Table 4 show that available P was decreased negatively with increasing salinity of irrigation.

The average values of available P for EC of irrigation water of 1000, 2000, 4000 and 8000 mgL^{-1} were 11.9, 10.8, 10.2 and 9.17mg/kg^{-1}soil, respectively. This decrease in available P reflects the negative effect on PDB activity and also a decrease in the mobility of P. The data also reveal that soil available P increased with addition of RP fertilizer, PDB inoculant or RP+PDB treatment. The highest available P was obtained with RP+PDB. These results are in agreement with those reported by Abd-El-Salam (2007) and Mehasen and El-Ghozoli (2003) who found that available –P increased with inoculation with phosphate dissolving bacteria and rock phosphate. The effect of salinity (X) could be presented according to the following equation:

available – P = 13.6 –0.000658 X (mgL^{-1}) (r = - 0.467**)

Nodulation and N_2-ase Activity

In general, data presented in Table 5 reveal that the number of nodules as well as their dry weight of along with N_2-ase activity increased with application of RP and/or PDB. The highest value of nodules number, dry weight of nodules and N_2-ase activity were obtained with RP+PDB. This indicates to a stimulatory effect of PDB and RP on nodule formation (Mehasen and El-Ghozoli, 2003). These was a decrease of nodules number, dry weight of nodules and N_2-ase when the salinity of irrigation water was increased.

This reflects the negative effect of salinity of irrigation water on microorganisms activity and nodule formation.

The regression equation and correlation coefficient are as follows:

No. of nodules= 16.0-0.00214 X (mgL^{-1}) (r= - 0.847***)

D.W of nodules = 204-0.026 X (mgL^{-1}) (r=0.871***)

N_2-ase = 71.8-0.00949 X (mgL^{-1}) (r= 0.912***)

Table 4. Effect of rock phosphate and phosphate dissolving bacteria on total counts of bacteria and phosphate dissolving bacteria, CO_2 evolved and available P under irrigation with different levels of saline water

Salinity mgL^{-1} (S)	Fertilization (F)									
	Total bacteria count (X 10^6)					CO_2 (µg/g dry soil/ hr.)				
	None	RP	PDB	RP+ PDB	mean	None	RP	PDB	RP+ PDB	mean
400	38.0	50.0	62.0	84.0	58.5	18.0	43.0	100	147.0	77.0
1000	32.0	48.0	54.0	70.0	51.0	14.0	32.0	93.3	121.0	65.0
2000	20.0	25.0	43.0	50.0	34.5	12.0	26.0	74.0	87.0	49.8
4000	10.0	21.0	28.0	32.0	22.8	9.0	17.0	25.0	42.0	23.3
8000	6.0	8.0	11.0	19.0	11.0	7.0	9.0	13.0	21.0	12.5
Mean	21.2	30.4	39.6	51.0	35.6	12.0	25.4	61.0	83.6	45.5
	$L.S.D_{0.01}$ S=9.89 F= 8.57 SXF=4.94					$L.S.D_{0.01}$ S=28.9 F= 23.7 SXF=19.4				
	Phosphate dissolving bacteria (X 10^4)					Available –P (mg Kg^{-1})				
400	266.3	292.4	310.7	318.6	297.0	10.2	18.3	12.4	23.0	16.0
1000	215.2	275.0	251.9	298.2	260.1	9.58	13.3	10.5	14.4	11.9
2000	202.6	225.8	270.2	321.4	255.0	9.33	11.3	9.76	12.8	10.8
4000	196.9	246.3	257.9	295.1	249.1	7.33	11.9	8.88	12.6	10.2
8000	185.3	243.2	254.8	291.4	243.6	7.0	10.0	7.99	11.7	9.17
Mean	213.2	256.5	269.1	304.9	260.9	8.68	13.0	9.91	14.9	11.6
	$L.S.D_{0.01}$ S=10.7 F= 9.28 SXF=5.36					$L.S.D_{0.01}$ S=2.31 F= 1.99 SXF=1.15				

Table 5. Effect of rock phosphate and phosphate dissolving bacteria on number, dry weight of nodules and N_2-ase. under salinity levels of irrigation water

Salinity mgL^{-1} (S)	Fertilization (F)				
	No. of nodules/plant				
	None	RP	PDB	RP+PDB	mean
400	11	14	15	19	14.8
1000	10	13	14	16	13.3
2000	7	10	9	14	10.0
4000	1	3	2	5	2.75
8000	0.0	0.0	0.0	2.0	0.5
Mean	5.8	8.0	8.0	11.2	8.30
$L.S.D_{0.01}$	**S=4.27**		**F= 3.70**	**SXF=2.13**	
	Dry weight of nodules (mg/plant)				
400	142.1	199.3	249.8	271.4	186.3
1000	133.0	155.2	202.4	254.6	136.4
2000	102.7	120.5	138.3	184.0	60.4
4000	21.1	60.9	53.6	106.1	7.10
8000	0.0	0.0	0.0	28.2	123.8
Mean	79.8	107.2	128.8	179.7	
$L.S.D_{0.01}$	**S=3.04**		**F= 2.63**	**SXF=1.51**	
	N_2-ase activity (n.moles C_2H_4/hr/g dry nodules)				
400	60.2	72.5	81.6	86.0	75.3
1000	52.3	57.2	70.0	78.5	64.5
2000	23.7	42.3	38.9	55.6	40.1
4000	13.6	19.7	18.1	29.9	20.3
8000	0.0	0.0	0.0	4.0	1.0
Mean	37.5	38.3	41.7	50.8	40.2
$L.S.D_{0.01}$	**S=6.16**		**F= 5.33**	**SXF=3.08**	

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استجابة نباتات فول الصويا لإضافة صخر الفوسفات مع البكتريا المذيبة للفوسفات تحت ظروف الإجهاد الملحى

محمد عبد المؤمن الغزولي

معهد بحوث الأراضي والمياه والبيئة- مركز البحوث الزراعية – الجيزة – مصر

أجريت تجربة أصص لدراسة تأثير التسميد بالصخر الفوسفاتي بمعدل ١٤,٥ ملليجرام فوسفور /كجم تربة والتلقيح بالبكتريا المذيبة للفوسفات تحت ظروف الري بمياه متدرجة الملوحة وهي ١٠٠٠، ٢٠٠٠، ٤٠٠٠ ، ٨٠٠٠ جزء في المليون علي تكوين العقد الجذرية لنباتات فول الصويا – نشاط إنزيم النيتروجينز – الفوسفور الميسر – العدد الكلي للبكتريا والبكتريا المذيبة للفوسفات – معدل انطلاق ثاني أكسيد الكربون في منطقة الريزوسفير – محتوي النبات من بعض العناصر الكبرى.

وقد أوضحت النتائج ما يلي:

- تأثر إنتاج المادة الجافة لنبات فول الصويا سلبياً ومعنويا بزيادة مستويات ملوحة مياه الري وكان النقص أقل وضوحاً في حالة التسميد بالصخر الفوسفاتي مع التلقيح بالبكتريا المذيبة للفوسفات.
- انخفض تركيز كل من النيتروجين والفوسفور والبوتاسيوم والكالسيوم في نباتات فول الصويا معنويا بزيادة مستوي ملوحة ماء الري بينما تحسن تركيز هذه العناصر بإضافة الصخر الفوسفاتي أو التلقيح بالبكتريا المذيبة للفوسفات أو كلاهما معاً.
- ازداد تركيز الصوديوم معنوياً في نباتات فول الصويا بزيادة مستوي ملوحة مياه الرى.
- أدي التلقيح بالبكتريا المذيبة للفوسفات مع الفوسفات الصخري إلي زيادة في العقد البكترية من حيث العدد والوزن الجاف ونشاط إنزيم النيتروجينز والفوسفور الميسر ومعدل انطلاق ثاني أكسيد الكربون في منطقة الريزوسفير لنباتات فول الصويا حتى مستوي ماء الري ٤٠٠٠ جزء في المليون.
- انخفض العدد الكلي البكتيري وعدد البكتريا المذيبة للفوسفات بزيادة ملوحة مياه الري وحصلنا علي أعلي قيمة لهم تحت نفس هذه الظروف بإضافة الفوسفات الصخري مع التلقيح بالبكتريا المذيبة للفوسفات.