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GROWTH PROMOTION OF SOME SOYBEAN CULTIVARS BY RHIZOBIUM AND PHOSPHATE-SOLUBILIZING BACTERIA

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ABSTRACT

A field experiment was carried out during 2001 at the Experimental Farm of the Faculty of Agriculture, Suez Canal University. The aim of the present study was to evaluate the response of four soybean (*Glycine max* (L) Merrill) cultivars, Giza 21, Giza 35, Crawford and Clark, grown on sandy soil inoculated with rhizobium and phosphate solubilizing bacteria (PSB). Also, it includes the effects of each and both bacterial inoculation on root, shoot and seed N, P, protein and oil contents.

Roots, straw dry weights, seed yield, 100-seed weight, nodule fresh weights and nodule numbers were siginificantly higher for both single and combined inoculation by rizobia and/or PSB than the uninoculated seeds.Giza 35 soybean cultivar exhibited the best results.

Roots, straw and seed N and P contents of soybean cultivars were greatly influenced by bacterial inoculation. The highest mean values for roots, straw and seed N and seed protein contents were obtained when soybean seeds were treated with the combined inoculation by rhizobium and PSB as compared with the single inoculation or uninoculated seeds. Meanwhile, the highest mean values for roots, straw and seed P content were obtained for soybean seeds inoculated with PSB alone as compared with the inoculated seeds with rhizobium alone or combined inoculation by rhizobium and PSB. Crawford soybean cultivar gave the highest root N and P contents while Giza 21 cultivar gave the highest straw and seed N and P contents. There were no significant differences in seed oil content as a result of bacreial inoculation. Slight differences in seed oil content were found regarding the variation in soybean cultivars.

keywords: sandy soil, soybean cultivars, Rhizobium, phosphate-solubilizing bacteria

INTRODUCTION

Soybean (*Glycine max* (L) Merrill) is the world's most important oil seed and grain legume crop. Special attention should be directed toward the propen choice of cultivars and management practices to increase both grain yield and oil production. Inoculation of soybean with superior N_2-fixing strains of rhizobia resulted in increasing nodulation in plant roots and improving N2 fixation. Several investigators found that seed inoculation increased fresh plant weight, seed yield and seed oil and protein contents Boonkerd et.al., (1978) and Badawy et. al., (1992).

The availability of phosphate anions in soil has been associated with the presence and activity of soil microorganisms;Chabot et.al.,(1996a). Many factors influence the distribution of microbial communities in the soil and the rhizosphere such as the roots morphology, the stage of plant growth, and the soil physical and chemical properties.

A considerable number of bacterial species, mostly those associated with the plant rhizosphere, are able to exert a beneficial effect upon plant growth. Therefore, their use as biofertilizers or control agents for agriculture improvement has been a focus of numerous researchers for a number of years; Lemanceau,(1992),Kloepper,(1994);and Glick,(1995). Phosphate-solubilizing bacteria have increased roots and shoot elongation in canola, lettuce, and tomato; Hall et. al., (1996) and Glick et. al.,(1997) as well as crop yields for potato, radishes, rice, sugar beet, tomato, lettuce, apple, citrus, beans, ornamental plants, and wheat; Lemanceau,(1992), Kloepper, (1994),and Kloepper et. al.,(1988).

It is well known that microbial biomass contains a large reservoir of P, which is potentially available to plants; Beever and Burns, (1980) as a result of mineralization process. However, insoluble inorganic P associated with the solid phase can be adsorbed to the surfaces of soil constituents or can be present in Ca, Fe, or Al minerals. Mineral P is further released and made available to plants mostly by the action of the phosphate-solubilizing microorganisms; Kucey and Legett (1989). An increase in P availability to plants through the inoculation of phosphate-solubilizing microorganisms has been reported in pot experiments; Kucey and Leggett,(1989) , Salih et. al.(1989) and under field conditions; Chabot et. al. (1996b) and Kucey and Leggett,(1989). Recently, the addition of rock phosphate significantly increased N, P, total plant biomass, infection by vascular arbuscular mycorrhizal fungi, and the number of nodules of legume species under field conditions; Vanlauwe et. al.(2000). Nevertheless, little is known about the colonization of the rhizosphere by the introduced mineral phosphate solubilizing microorganisms and their impact on the size of rhizospheric microbial communities when different phosphate sources are used in a plant–soil system.

In a soybean greenhouse experiment, Suwastika (1997) found that there is an interaction effect between rock phosphate application and phosphate solubizing bacteria on phosphorus uptake 45 days after planting. The single effect of rock phosphate application and phosphate solubilizing bacteria was significant on soluble phosphate.

The aim of the present study was to evaluate the response of four soybean (*Glycine max* (L) Merrill) cultivars (Giza 21, Giza 35, Crawford and Clark) grown on sandy soil to single or combined inoculation with rhizobium and phosphate solubilizing bacteria (PSB). Also, the study evalutes the effects of R and PSB on roots, shoot and seed contents of N and P.

MATERIALS AND METHODS

A field experiment was carried out during 2001 at the Experimental Farm of the Faculty of Agriculture, Suez Canal University. The aim of the present study was to evaluate the response of four soybean (*Glycine max* (L) Merrill) cultivars (Giza 21, Giza 35, Crawford and Clark) grown on sandy soil inoculated with rhizobium (R) and phosphate solubilizing bacteria (PSB). Also, it includes the effects of each and both bacterial inoculation on roots, shoot, seed N , P contents and oil content. Table (1) represent some characteristics of the sandy soil.

The soil was treated with superphosphate (15.5% P_2O_5) at rate of 50 kg ha^{-1} before sowing.

Table (1). Some characteristics of the used sandy soil

Chemical properties	
pH (in 1: 2.5 water suspension)	7.30
EC, dSm^{-1} (in saturated paste extract)	1.2
Total N, %	0.10
Total P, %	0.03
Total K, %	0.11
Organic matter, %	0.35
Physical properties	
Coarse sand, %	62.6
Fine sand, %	31.3
Silt, %	3.5
Clay, %	2.6
Texture class	Sandy

The plot area was 4m x 4m. Soybean seeds were sown on furrows 4 m long and 60 cm between furrows. The seeds were placed in holes 15 cm apart at one bank of the furrow. Plants were thinned to 2 plants per hole after germination.

The single inoculation with R or PSB was carried out by mixing cell suspension from each inoculum with soybean seeds. For the combined bacterial inoculation, Equal amounts of cell suspension for R and PSB were mixed and soybean seeds were treated with the mixture. After germination, nitrogen as $(NH_4)_2SO_4$ (20.5% N) and potassium as K_2SO_4 (48% K_2O) were added to the soil, 40 kg ha^{-1} each .The bio- and chemical fertilization treatments were as the follows:

1. Soil (control)
2. Soil + Rhizobium (R)
3. Soil + phosphate solubilizing bacteria (PSB)
4. Soil + R + PSB

At the end of pod filling stage, root samples were taken to determine nodule numbers and weights. Root samples were collected and washed from soil particles on 1 mm sieve holes for 24 h. Nodules were carefully removed and freshly weighed. At full maturity, yields of soybean shoots, pods and seeds were determined. Plant samples were oven dried at 70 °C, ground, digested and analyzed for roots, shoot and seed N and P contents according to Chapman and Pratt (1961). Seed oil content was determined by using Soxhlet apparatus according to AOAC (1975). Soil samples were prepared and analyzed according to Page *et.al.* (1982). Split-plot design with three replicates was used and Plabstat version 2D computer program was used for statistical analysis.

RESULTS AND DISCUSSION

The obtained results for roots and straw dry weights, as well as seed yield and 100-seed weight of four soybean cultivars grown on a sandy soil as affected by single or combined inocualtion by rhizobia and/or phosphate-solubilizing bacteria (PSB) are shown in Table (2). The forementioned 4 determined parameters for the investigated soybean cultivars siginificantly increased compared to the single or combined inoculation by rhizobia and/or PSB compared with the uninoculated plants. The highest mean values were obtained for inoculated soybean seeds with combined bacterial inoculation.

There are significant differences between soybean cultivars. However, roots and straw dry weights as well as seed yield and 100-seed weight varied between Giza 21 and Giza 35 cultivars between757.5 and 885.5, 3153.5 and 3686.0, 2607.3 and 3047.5 kg ha^{-1} and 16.6 and17.4 g, respectively.

Several investigators obtained similar results for the effect of rhizobium and PSB inoculation on the soybean yield and plant growth characters. El-Essawi et.al., (1986) found that the increase in seed yield for inoculated soybean with rhizobium was more pronounced at low nitrogen application rates beside P and K fertilizers were added. Also, Atmaja (1997) and Rahmani and Rastin (2001) reported that soybean shoot dry matter, seed yield and total N uptake increased as result of rhizobium inoculation.

Results obtained for the numbers of nodules and their weights for the four soybean cultivars increased as a result of rhizobium and/or phosphate solubilizing bacterial inoculation, Table (3). Similar trends were obtained for the 4 determined plant growth parameters. The highest mean values for the numbers and weights of nodules were o btained f or t reated s oybean s eeds with the combined rhizobium and PSB inoculants. Also, Giza 35 cultivar exhibited the highest response to bacterial inoculation than the other 3 soybean cultivars.

Table (2): Roots and straw dry weights as well as seed yield and 100-seed weight for four soybean cultivars affected by rhizobium and phosphate solubilizing bacterial inoculation

Treatments	Giza 21	Giza 35	Crawford	Clark	Mean
	Roots dry weight, kg ha^{-1}				
Control	589	651	573	539	588.0
" + R	806	992	887	934	904.8
" + PSB	713	841	725	667	761.4
" + R +PSB	922	1058	696	1025	1000.3
Mean	757.5	885.5	795.3	816.2	813.6
	Straw dry weight, kg ha^{-1}				
Control	2452	2711	2388	2243	2448.5
" + R	3355	4129	3694	3887	3766.3
" + PSB	2968	3500	3017	3194	3169.8
" + R +PSB	2839	4404	4146	4268	4164.3
Mean	3153.5	3686.0	3311.3	3398.0	3387.2
	Seed yield, kg ha^{-1}				
Control	2027	2241	1974	1854	2024.0
" + R	2774	3414	3054	3214	3114.0
" + PSB	2454	2894	2494	2641	2620.8
" + R +PSB	3174	3641	2428	3528	3442.8
Mean	2607.3	3047.5	2737.5	2809.3	2800.4
	100-seed weight, g				
Control	12.9	14.3	12.6	12.3	13.0
" + R	17.6	21.8	19.5	20.5	19.8
" + PSB	15.6	18.4	15.9	16.8	16.7
" + R +PSB	20.3	23.2	21.8	22.5	22.0
Mean	16.6	19.4	17.4	18.0	17.9

R= rhizobium **PSB= phosphate solubilizing bacteria**

$LSD_{0.05}$ for:	**Treatment**	**Cultivar**	**Treatment X cultivar**
Roots dry weight	**9.26**	**4.72**	**15.20**
Straw dry weight	**4.47**	**9.85**	**17.02**
Seed yield	**7.11**	**13.08**	**16.31**
100-seed weight	**0.86**	**0.79**	**0.80**

Table (3): Nodules numbers and weights for four soybean cultivars affected by rhizobium and phosphate solubilizing bacterial inoculation

Treatments	Giza 21	Giza 35	Crawford	Clark	Mean
	Nodules fresh weight, g/plant				
Control	1.15	1.27	1.11	1.01	1.14
" + R	1.64	2.01	1.86	1.82	1.83
" + PSB	1.47	1.71	1.57	1.54	1.57
" + R +PSB	1.87	2.13	2.09	2.07	2.04
Mean	1.53	1.78	1.66	1.61	1.65
	Nodules numbers per plant				
Control	30	32	28	26	29.0
" + R	41	50	47	46	46.0
" + PSB	37	43	40	39	39.8
" + R +PSB	48	54	53	52	51.8
Mean	39.0	44.8	42.0	40.8	41.7

R= rhizobium PSB= phosphate solubilizing bacteria

LSD $_{0.05}$ for:	Treatment	Cultivar	Treatment X cultivar
Nodules fresh weight	0.012	0.011	0.037
Nodules numbers	3.70	2.25	Ns

Data obtained for roots, straw and seed-N and P contents for four soybean cultivars affected by rhizobium and/or phosphate solubilizing bacterial inoculation are presented in Tables (4 and 5). The obtained results were greatly influenced by bacterial inoculation. The highest mean values for N contents were obtained for treated soybean seeds with combined rhizobium and PSB inoculants compared with the single inoculation or uninoculated seeds. Meanwhile, the highest mean values for P contents were obtained for inoculated soybean seeds with single PSB as compared with the single rhizobium inoculation or the combined rhizobium and PSB inoculation. Crawford soybean cultivar exhibited the highest roots N and P contents while Giza 21 cultivar exhibited the highest straw and seed N and P contents. Similar results were obtained by Badr El-Din et.al., (1986). Also, Suwastika (1997) found that single PSB inoculation significantly increased soybean P uptake.

Results obtained for seeds protein and oil contents for four soybean cultivars affected by rhizobium and/or phosphate solubilizing bacterial inoculation are presented in Table (6). The trend obtained for seed protein content was similar to that obtained for seed N content regarding bacterial inoculation and the response soybean cultivar. Seed oil content was not significantly affected by bacreial inoculation. Slight differences in seed oil content were found among soybean cultivars. Seeds oil content mean values were 26.9, 27.0, 27.6 and 27.6% for Giza 21, Crawford, Giza 35 and Clark, respectively. Badawi and El-Moursy (1997) and Ahmed et.al., (1997) found similar results. Their highest seed yield, seed oil and protein contents of peanut were obtained as a result of bacterial inoculation.

Co-inoculation studies with PSP and rhizobium have shown increased roots and shoot weight, N_2 fixation and seed yield of soybean, Dashti et.al., (1998). Bacterial inoculants must be effective nodulators as well as good nitrogen fixers, Parmar and Dadarwal, (1999). Plant growth promotion as a result of inoculation by PSB has been attributed to the increase in the mobilization of insoluble nutrients and subsequent enhancement of nutrient uptake by the plants, Lifshitz et.al., (1987).

Table (4): Roots, straw and seed-N contents for four soybean cultivars affected by rhizobium and phosphate solubilizing bacterial inoculation

Treatments	Giza 21	Giza 35	Crawford	Clark	Mean
			Roots N,%		
Control	1.14	0.82	1.06	0.89	0.98
" + R	1.28	1.07	1.31	1.21	1.22
" + PSB	1.17	0.96	1.17	0.92	1.06
" + R +PSB	1.30	1.58	1.64	1.40	1.48
Mean	1.22	1.11	1.30	1.11	1.19
			Straw N,%		
Control	1.55	0.98	1.15	0.94	1.16
" + R	1.84	2.21	1.73	1.18	
" + PSB	1.74	1.07	1.26	1.19	1.32
" + R +PSB	2.48	2.29	2.36	1.61	2.19
Mean	1.90	1.63	1.63	1.23	1.60
			Seed N,%		
Control	5.72	4.49	4.39	4.28	4.72
" + R	6.41	5.83	5.61	6.00	5.96
" + PSB	6.33	5.14	5.10	5.63	5.55
" + R +PSB	6.55	6.59	6.17	6.72	6.51
Mean	6.25	5.51	5.32	5.66	5.69

R= rhizobium **PSB= phosphate solubilizing bacteria**

LSD $_{0.05}$ for:	Treatment	Cultivar	Treatment X cultivar
Roots N	0.009	0.021	0.027
Straw N	0.043	0.013	0.052
Seed N	0.117	0.057	0.091

Table (5): Roots, straw and seed-P contents for four soybean cultivars affected by rhizobium and/or phosphate solubilizing bacterial inoculation

Treatments	Giza 21	Giza 35	Crawford	Clark	Mean
			Roots P,%		
Control	0.156	0.112	0.147	0.121	0.134
" + R	0.162	0.133	0.161	0.103	0.140
" + PSB	0.176	0.147	0.179	0.166	0.167
" + R +PSB	0.178	0.216	0.224	0.192	0.203
Mean	0.168	0.152	0.178	0.146	0.161
			Straw P,%		
Control	0.197	0.125	0.145	0.119	0.147
" + R	0.220	0.136	0.160	0.151	0.167
" + PSB	0.233	0.280	0.220	0.152	0.221
" + R +PSB	0.313	0.290	0.299	0.204	0.277
Mean	0.241	0.208	0.206	0.157	0.203
			Seed P,%		
Control	0.603	0.473	0.463	0.455	0.499
" + R	0.667	0.541	0.537	0.593	0.585
" + PSB	0.676	0.614	0.591	0.632	0.628
" + R +PSB	0.690	0.694	0.651	0.709	0.686
Mean	0.659	0.581	0.561	0.597	0.600

R= rhizobium **PSB= phosphate solubilizing bacteria**

LSD $_{0.05}$ for:	Treatment	Cultivar	Treatment X cultivar
Roots P	0.001	0.002	0.003
Straw P	0.003	0.002	0.005
Seed P	0.002	0.009	0.015

Table (6):Seed protein and oil contents for four soybean cultivars affected by rhizobium and phosphate solubilizing bacteria inoculation

Treatments	Giza 21	Giza 35	Crawford	Clark	Mean
			Seed protein, %		
Control	35.7	28.0	27.4	27.0	29.5
" + R	40.1	36.4	35.0	37.5	37.3
" + PSB	39.6	32.1	31.9	35.2	34.7
" + R +PSB	40.9	41.2	38.6	42.0	40.7
Mean	39.1	34.4	33.2	35.4	35.5
			Seed oil, %		
Control	26.2	27.4	26.2	27.1	26.7
" + R	27.3	27.5	27.3	27.5	27.4
" + PSB	26.4	27.3	26.9	27.4	27.0
" + R +PSB	27.8	28.3	27.5	28.4	28.0
Mean	26.9	27.6	27.0	27.6	27.3

R= rhizobium PSB= phosphate solubilizing bacteria

$LSD_{0.05}$ for:	Treatment	Cultivar	Treatment X cultivar
Seed protein	1.73	1.45	1.78
Seed oil	Ns	0.26	Ns

CONCLUSION

Inoculating the soil or soybean seeds with rhizobium and/or phosphate-solubilizng bacteria will lead to producing high number of bacterial nodules, which may fix more N_2 and release more P,thus increase dry matter production as well plant growth characters.

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تشجيع نمو بعض أصناف فول الصويا بالتلقيح بـــالريزبيوم والبكتيريـــا المذيبـــة للفوسفات

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أجريت تجربة حقلية خلال سنة ٢٠٠١ فى المزرعة التجريبية لجامعـــة قنـــاة الســـويس ، لدراسة تأثير التلقيح بالريزبيوم و/أوالبكتيريا المذيبة للفوسفات فى وجودالتســـميد المعـــدنى علـــى الصفات المحصوتيه لأربعة أصناف من فول الصويا (جيزة ٢١ – جيزة ٣٥ – كـــراو فـــورد – كلارك).

تم استخدام المعاملات التالية:

١- تسميد معدنى
٢- تسميد معدنى + تلقيح بالريزبيوم
٣- تسيمد معدنى + تلقيح بالبكتيريا المذيبة للفوسفات
٤- تسميد معدنى + تلقيح بالريزبيوم + تلقيح بالبكتيريا المذيبة للفوسفات

وقد تم الحصول على النتائج التالية:

١- زاد وزن كل من الجذور والقش ومحصول البذور ووزن ١٠٠ بـــذرة ووزن وعـــدد العقـــد الجذرية ومحتوى الجذور والقش والبذور من النتروجين والفوســـفور والبـــروتين باســـتخدام التسميد الحيوى مع التسميد المعدنى بالمقارنة بالتسميد المعدنى منفردا.
٢- تم الحصول على أعلى قيم لوزن الجذور والقش ومحصول البذور ووزن ١٠٠ بـــذرة ووزن وعدد العقد الجذرية ومحتوى الجذور والقش والبذور من النتـــروجين والبـــروتين باســـتخدام معاملة التلقيح المزدوج مع التسميد المعدنى.
٣- تم الحصول على أعلى قيم لمحتوى الجذور والقش والبذور من الفوسفور باســـتخدام معاملـــة التلقيح بالبكتيريا المذيبة للفوسفات مع التسميد المعدنى.
٤- أعطى صنف "جيزة ٣٥" أعلى استجابة لمعاملات التسميد المختلفة لصـــفات وزن كـــل مـــن الجذور والقش ومحصول البذور ووزن ١٠٠ بذرة ووزن وعدد العقد الجذرية ، بينما أعطى صنف "كراوفورد" أعلى استجابة لصفة محتوى الجذور من النتروجين والفوسفور ، وأعطى صنف "جيزة ٢١" أعلى استجابة لصفات محتوى القش والبذور من النتروجين والفوسفور.
٥- لم يتأثر محتوى البذور من الزيت معنويا بالمعاملات التسميدية المختلفة ، بينما كانت هنـــاك فروق بسيطة فى محتوى الزيت بين الأصناف المستخدمة فى الدراسة.