Egypt. J. Agric. Res. , 76 (3) 1998

INFLUENCE OF *RHIZOBIUM* AND PHOSPHATE-SOLUBILIZING BACTERIA ON NUTRIENT UPTAKE AND YIELD OF LENTIL IN THE NEW VALLEY

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(Manuscript received 3 July 1997)

Abstract

Combined inoculation of *Rhizobium* leguminosarum b.v. vicia (ARC 200 L) and "phosphate-solubilizing"; *Pseudomonas striata* or *Bacillus polymyxa* with and without added chemical fertilizer on lentil yield and nutrient content was studied under greenhouse conditions. The single inoculation of *Rhizohbium* increased the nodulation and nitrogenase activity and the "phosphate-solubilizers" increased the available phosphorus content of the soil. Combined inoculation of Rhizobia and *P.striata* or *B.polymyxa* increased the above parameters and also the dry matter content. Grain yield, and nitrogen and phosphorus uptake significantly increased over the uninoculated control. The inoculation effects were more pronounced in the presence of added fertilizers. The possibilities of saving half the dose of N and replacing superphosphate with rock-phosphate and inoculation with "phosphate-solubilizers" are discussed.

INTRODUCTION

Increased yield response of crop plants have been observed following seed inoculation with *Rhizobium* (Dorosinksy and Kadyrov 1975, Harnandez and Hill 1983, and Ernskine et al., 1993) and phosphate-solubilizing bacteria (Gaur et al 1980, Gaur 1985, and El-Sayed 1989). When inoculated, these organisms colonize the rhizosphere and enhance plant growth by providing it with nitrogen and phosphorus (Kundu and Gaur 1980, and Alagawadi and Gaur 1988). However, little work has been done on the effect of combined or dual inoculation of *Rhizobium* and phosphate-solubilizing bacteria on plant productivity (Gaind and Gaur 1991). It is of great practical importance to study the associative effect of these two groups of organisms, each having a specific role to play in plant growth and yield particularly in the present day of spiralling costs of fertilizers. The present greenhouse experiment was undertaken to examine the interaction of *Rhizobium* and phosphate-solubilizing bacteria and its effect on nodulation, nitrogen fixation, and yield of lentil (*Lens culinaris* L) variety Giza 370, with and without fertilizers.

MATERIALS AND METHODS

Cultures:

An efficient strain of *Rhizobium leguminosarum* b.v. vicia and the phosphate-solubilizing *Pseudomonas striata* and *Bacillus polymyxa* were obtained from the Agricultural Microbiology Res. Dept., Soil, Water, and Environment Res. Inst., Agric. Res. Centre, Ministry of Agriculture, Giza, Egypt.

Rhizobia strains were grown on yeast-extract mannitol broth (Vincent, 1970) for six days (cell counts : 5x108 CFU/ml) and *P.striata* and *B.polymxa* were grown in Pikovskaya's medium (Pikovskaya 1948) for 3 days at 28±2^{o}C (cell count of *P.striata* was 3.5x109 CFU/ml and the that of *B.polymyxa* 5.5x109 CFU/ml).

Sandy-loam soil (unsterilized) from the New Valley Governorate, Agriculture Research Center farm, having pH 7.8, organic carbon 0.38%, available nitrogen 60 kg/Feddan, and available P 120 kg/Feddan, was used. The soil was mixed thoroughly with farmyard manure with the rate of 5 tons/Feddan and packed in earthen pots of 15 Kg capacity (35 cm diameter). Rock-Phosphate (RP) or superphosphate (SP) at 100 kg P2O5/Feddan and urea at 15 or 25 kg N/Feddan were added as basal dose to the soil as per treatment schedule.

Experimental Procedure:

Lentil seeds were inoculated by soaking in a liquid culture with either *Rhozobium leguminosarum* b.v. vicia (R.I.V), *Pseudomonas striata* (P.S.), *Bacillus polymyxa* (B.P.) alone or mixture of the three strains.

Planting :

Twenty seeds were sown in each pot and thinned to ten plants per pot. The experiment was conducted using a completely randomized design with twelve replicates. Four pots at a time were used for observation at 60 days, 120 days, and to record the grain yield at harvest. The pots were placed in a greenhouse and watered regularly to maintain an optimum moisture level At 60 and 120 days, the plants were depotted and the soil was removed by washing gently under running tap water. The nodules per plant were then counted.

Sampling:

Plants were harvested after complete maturity. Grain and straw yield were recorded. The plant samples collected at 60 days, 120 days, and grain and straw were analysed for total nitrogen using Technicon autoanalyser (Technicon Monograph I, 1971) and total phosphorus by Jackson's method (Jackson 1958). The soil samples collected at the same intervals were analysed for available phosphorus (Olson et al., 1954).

Bacterial Counts:

Enumeration of phosphate-solubilizing bacteria in the rhizosphere of lentil was done at 60 and 120 days by dilution plate technique using Pikovskaya's medium (Pikovskaya 1948).

Data were statistically analysed by "F" test and the critical difference (CD) was calculated by student's "t" test at 0.05 P level of significance (Cochran and Cox 1957).

C2H2 reduction activity:

Acetylene reduction activity of root nodules was measured after 60 and 120 days using a gas chromatography (Shimadzu GC5A) fitted with a hydrogen flame ionization detector and poropak-R column of 80-100 mesh at a column temperature of 90oC and N2 flow rate of 60 ml/min. Four plants from each pot were uprooted gently and the nodules were detached from the roots and were placed in 25-ml serum vials. The vials were closed with serum stoppers. The gas phase in the vials was replaced with high-purity acetylene (10% V/V) with a gas tight hypodermic syringe and the vials were incubated for one hour at 28±2oC. From each vial, one ml sample was drowned and analysed for ethylene production.

RESULTS

Maximum grain yields were obtained by Rhizobium + $N_{25}SP_{100}$ (25 kg N+100 kg P_2O_5 as superphosphate) followed by *Rhizobium* + *P.striata* + $N_{15}RP_{100}$(15 kg N+100 $k_2P_2O_5$ as rock-phosphate) and Rhizobium + *B.polymyxa* + $N_{15}RP_{100}$. These three treatments showed significant ($P \leq 0.05$) increase in the grain yield over all other treatments (Table 1).

All inoculation treatments, except single inoculation of *P.striata* and *B.polymyxa* without $N_{15}RP_{100}$, showed significant ($P \leq 0.05$) increases in the grain and straw yield over that of the control. Combined inoculations gave higher yields than single inoculations.

The dry matter content at both 60 and 120 days of plant growth was maximum in *Rhizobium* + *P.striata* + $N_{15}RP_{100}$, followed by *Rhizobium* + N_{25} SP_{100} and and *Rhizobium* + *B.polymyxa* + $N_{15}RP_{100}$ (Table 1). Single inoculation of *P.striat* of *B.polymyxa* without $N_{15}RP_{100}$ did not show significant ($P \leq 0.05$) increase in plant dry matter content over that of the control at 60 days. However, at 120 days there was a significant increase in dry matter content of plants inoculated with *P.striata* or *B.polmyxa* with or without $N_{15}RP_{100}$ compared to control.

Table 1. Dry matter production, grain and straw yield of lentil as influenced by combined inoculation.

Treatment	Dry matter yield (g/plant)		Grain yield (g/plant)	Straw yield (g/plant)
	60 days	120 days		
Control	0.35	0.86	1.3	2.6
Rhizobium (R.L.V.)	0.47	1.35	1.9	3.1
P.striata (P.s.)	0.41	1.11	1.4	2.8
B.polymyxa (B.p.)	0.40	1.21	1.5	2.7
R.L.V.+P.s.	0.48	1.37	1.9	3.2
R.L.V.+P.s.	0.50	1.49	2.1	3.3
*$N15\ RP_{100}$**	0.39	1.16	1.6	3.4
*R.L.V.+$N_{15}RP_{100}$**	0.44	1.23	2.1	3.5
P.S. + $N15\ RP_{100}$	0.46	1.36	1.6	3.3
*B.P.+$N_{15}\ RP_{100}$ **	0.47	1.45	1.9	3.2
*R.L.V.+Ps+$N_{15}RP_{100}$ **	0.57	1.72	2.6	3.7
*R.L.V.+B.P.+$N_{15}\ RP_{100}$ **	0.44	1.53	2.6	3.6
*R.L.V.+$N_{25}\ SP_{100}$***	0.48	1.54	2.8	3.9
SE (m)±	0.024	0.073	0.12	0.16
C.D. (5%)	0.050	0.060	0.25	0.33

(*) (15 kg N + 100 kg P_2O_5 as rock-phosphate/Feddan)

(**) (25 kg N + 100 Kg P_2O_5 as superphosphate)

Nitrogen and phosphorus uptake in grain and straw showed a similar trend to grain and straw yields (Table 2). Inoculation of lentil plants with *Rhizobium*, *P.striata* or *B.polymyxa* significantly ($P \leq 0.05$) increased nitrogen and phosphorus uptake over the control. The uptake was further enhanced with the application of N_{15} RP_{100}. The highest N and P uptakes were recorded in *Rhizobium* + $N_{25}SP_{100}$.

Single inoculation of *Rhizobium* with and without $N_{15}RP_{100}$ and combined inoculation of *Rhizobium* and *P.striata* or *B.polymyxa* with $N_{15}RP_{100}$ showed significantly there nodules and nitrogenase activity than the control at 60 days (Table 3). However, at 120 days combined inoculation of *Rhizobium* and phosphate-solubilizing bacteria increased nodules number significantly over that of the control even without $N_{15}RP_{100}$. All inoculation treatments, except Rhizobium + $N_{15}SP_{100}$, recorded statistically significant higher dry weight of nodules at 60 days, whereas only combined inoculation treatments recorded significant increase in nodules dry weight at 120 days (Table 3). Inoculation of *Rhizobium* either singly or in combination with *P.striata* or *B.polymyxa* without $N_{15}RP_{100}$ and both single and combined inoculation with $N_{15}RP_{100}$ showed higher C2H2 reduction activity than that of the control at 60 days. At 120 days, all the inoculated treatments with and without $N_{15}RP_{100}$ showed a significant ($P \leq 0.05$) increase in C2H2 reduction activity.

Table 2. Nitrogen and phosphorus uptake in grains and straw of lentil as influenced by combined inoculation.

Treatment	N uptake (mg/plant)			P uptake (mg/plant)		
	Grains	Straw	Total	Grains	Straw	Total
Control	36.3	21.0	57.3	3.8	1.8	5.5
Rhizobium (R.L.V.)	60.1	26.3	86.4	5.5	2.1	7.6
P.striata (P.s.)	42.2	22.9	65.1	4.5	2.3	6.8
B.polymyxa (B.p.)	43.5	22.3	65.8	4.5	2.2	6.7
R.L.V.+P.s.	62.4	28.7	91.1	6.0	3.2	9.2
R.L.V.+P.s.	64.4	29.5	93.9	6.6	2.8	9.4
*N_{15} RP_{100}**	51.2	29.8	81.0	5.0	3.0	8.2
*R.L.V.+N_{15} RP_{100}**	69.1	31.1	100.2	6.3	3.3	9.6
P.S. + N_{15} RP_{100}	53.6	28.9	82.5	5.1	3.6	8.7
*B.P.+N_{15} RP_{100} **	60.1	28.3	88.4	5.9	3.4	9.3
*R.L.V.+Ps+N_{15} RP_{100} **	94.8	34.2	129.0	9.0	4.4	13.4
*R.L.V.+B.P.+N_{15} RP_{100} **	90.2	32.9	123.1	8.6	4.0	12.6
*R.L.V.+N_{25} SP_{100}***	98.1	36.3	134.4	9.5	4.5	14.0
SE (m)±	1.47	0.57		0.16	0.23	
C.D. (5%)	3.20	1.30		0.35	0.50	

(*) (15 kg N + 100 kg P_2O_5 as rock-phosphate/Feddan)

(**) (25 kg N + 100 Kg P_2O_5 as superphosphate)

The soil available P content increased following inoculation with *P. striata* or B.polymyxa (Table 4). With the addition of $N_{15}RP_{100}$ the available phosphorus content was further augmented. The increase was more prominent in combined inoculation treatments than in single inoculations. The available phosphorus content in soil receiving *Rhizobium* + $N_{25}SP_{100}$ treatment, was decreaed from 11.0 mg P/kg soil at 60 days to 8.5 mg P/kg soil after harvest.

Population counts of phosphate-solubilizing bacteria in the rhizosphere indicated that the phosphate-solubilizing bacteria population generally gave more counts after 60 days than after 120 days (Table 4). Wherever the plants were inoculated with *P.striata* or *B.polymyxa* or fertilized with the phosphate-solubilizing bacteria counts increased.

Table 3. Combined inoculation effect on nodulation and nodule itrogenase activity in lentil.

Treatment	Number of nodules/plant		Dry weight nodules (mg/plant)		Nodule nitrogenase activity (mC_2H_4 formed/ g.dry nodules/hr.)	
	60days	120days	60days	120days	60days	120days
Control	9	21	11.2	40.5	0.3	1.6
Rhizobium (R.L.V.)	15	46	17.4	99.6	3.0	16.9
P.striata (P.s.)	13	23	16.8	60.9	1.4	8.1
B.polymyxa (B.p.)	14	29	16.3	64.2	1.3	7.6
R.L.V.+P.s.	12	54	15.9	99.9	4.4	25.8
R.L.V.+P.s.	14	55	19.7	92.7	4.1	23.8
*N_{15} RP_{100}**	12	28	13.3	53.1	0.3	2.5
*R.L.V.+N_{15} RP_{100}**	16	46	12.8	71.1	2.8	17.7
P.S. + N_{15} RP_{100}	11	30	18.3	66.9	2.7	15.6
*B.P.+N_{15} RP_{100} **	14	31	15.4	74.1	1.9	11.2
*R.L.V.+Ps+N_{15} RP_{100} **	18	69	21.0	114.6	5.7	29.0
*R.L.V.+B.P.+N_{15} RP_{100} **	17	65	15.8	93.0	4.9	28.7
*R.L.V.+N_{25} SP_{100} ***	13	27	12.2	48.9	1.2	12.8
SE (m)±	2.77	4.72	1.58	23.97	0.56	1.79
C.D. (5%)	5.72	9.74	3.25	49.46	1.15	3.70

(*) (15 kg N + 100 kg P_2O_5 as rock-phosphate/Feddan)

(**) (25 kg N + 100 Kg P_2O_5 as superphosphate)

Table 4. Poplation counts of phosphate-solubilizing bacteria in lentil rhizosphere and available P in soil as influenced by combined inoculation.

Treatment	Population ($X10^5$/g soil) of phosphat-esolubilizing bacteria		Available P (mg/soil)		
	60 days	120 days	60 days	120 days	after harvest
Control	3	6	6.6	6.8	21
Rhizobium (R.L.V.)	3	6	7.0	6.8	46
P.striata (P.s.)	14	18	8.7	8.8	23
B.polymyxa (B.p.)	13	17	8.5	8.6	29
R.L.V.+P.s.	18	20	9.0	8.8	54
R.L.V.+P.s.	15	17	8.9	8.8	55
*N_{15} RP_{100}**	4	7	7.7	8.0	28
*R.L.V.+N_{15} RP_{100}**	4	8	7.5	8.0	46
P.S. + N_{15} RP_{100}	16	22	9.5	9.7	30
*B.P.+N_{15} RP_{100} **	16	19	9.2	9.5	31
*R.L.V.+Ps+N_{15} RP_{100} **	18	23	11.3	12.1	69
*R.L.V.+B.P.+N_{15} RP_{100} **	15	23	11.2	11.8	65
*R.L.V.+N_{25} SP_{100} ***	5	9	11.0	10.4	27
SE (m)±	0.83	1.08	0.46	0.67	4.72
C.D. (5%)	1.81	2.36	1.00	1.46	9.74

(*) (15 kg N + 100 kg P_2O_5 as rock-phosphate/Feddan)

(**) (25 kg N + 100 Kg P_2O_5 as superphosphate)

DISCUSSION

Inoculation of lentil with *Rhizobium* has been reported to increase the nodulation, nitrogen fixation, and the yield of crop (Dorosinksy and Kadyrov, 1975. Patil and Shinde, 1980; Zhang and Smith, 1996b; and Van Kessel, 1994). Seed or soil inoculation with phosphobacteria and simultaneous application of rock-phosphate to soil have been reported as a possible substitute for superphosphate application apparently without any reduction in the crop yield (Gaur, 1972, Gaur, et al., 1980; and El-Sayed, 1995a). The phosphate-solubilizing bacteria utilize organic compounds as carbon and energy source and produce organic acids thereby solubilizing insoluble inorganic phosphates (Pareek and Gaur, 1973; Gaur et al., 1979; and Gaind Gaur 1991). Apart from making P available to the crop, there are indications that these bacteria may produce growth promoting substances such as auxins, gibberellins and cytokinins (Brown, 1974, Barea et al., 1976; and Yahya and Al-Azawi, 1989). Such substances may improve plant growth and stimulate the microbial development (Lu et al., 1958; Sullia, 1968; Saber et al., 1981; Emskine et al., 1993; El-Sayed, 1995b; Huggins and Pan, 1993; and Dashti et al., 1997).

The two groups of organisms, *Rhizobium* and phosphate-solubilizing bacteria, when inoculated together on lentil, increased the yield, nutrient uptake, nodulation, and nitrogenase activity substantially over the control and single inoculation treatments. The available P content in soil was always higher in combined inoculation with $N_{15}RP_{100}$ where as the available P cantent in soil was decreased with plant growth in Rhizobimt N_{15} SP_{100} treatment. The observed benefits of lentil by combined inoculation of *Rhizobium* and phosphate-solubilizng bacteria may be due to the cumulative effects such as supply of N and P to the crop in addition to growth promoting substances produced by these organisms. Further, *Rhizobia* inoculation alone has given more yield than $N_{15}RP_{100}$; *Rhizobia* + *P. striata* + $N_{15}RP_{100}$ and *Rhizobia* + *B.polymyxa* + $N_{15}RP_{100}$ have given almost similar yields to that obtained in Rhizobium+$N_{15}SP_{100}$. These results indicate that by combined inoculation of *Rhizobium* and phosphate-solubilizing bacteria with simultaneous application of rock-phosphate. Therefore, there is a possibility of saving 10 kg N and replacing entire superphosphate with rock-phosphate and phosphate-solubilizing bacteria inoculation. However, more studies are needed to confirm the results under field conditions.

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تاثير كل من بكتيريا الريزوبيا والبكتيريا المذيبة للفوسفات على امتصاص العناصر الغذائية وانتاج محصول العدس بمحافظة الوادى الجديد

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أجريت هذه التجارب بغرض دراسة إستجابة محصول العدس للتلقيح بكل من البكتيريا المذيبة للفوسفات وبكتيريا الريزوبيا على إمتصاص العناصر الغذائية بمزرعة المحطة الإقليمية للبحوث الزراعية بمحافظة الوادى الجديد وقد تبين من نتائج التجارب أنه عند تلقيح بذور العدس (ببكتيريا الريزوبيا مع بكتيريا جنس *B. polymyxa* أدى إلى زيادة كل من الوزن الجاف ومحصول الحبوب للعدس والى زيادة امتصاص نبات العدس لعنصرى النيتروجين والفوسفور حيث بلغ ٩٣,٩ ملليجرام نيتروجين/نبات، ٩,٤ ملليجرام فوسفور/نبات .

تتواجد البكتيريا المذيبة للفوسفات بأعداد كبيرة فى منطقة ريزوسفير نبات العدس وكانت أعلى الأعداد فى حالة تلقيح بذور العدس بالريزوبيا مع جنس *Pseudomonas striata* وجنس *Bacillus Polymyxa* حيث تبلغ ٢ مليون بكتيريا ذائبة للفوسفات/جم أرض ، وهذا أدى الى زيادة إذابة الفوسفات المعدنية غير الذائبة فى التربة وتحويلها الى صورة ذائبة قابلة للإستفادة لنبات العدس حيث بلغ ٩ ملليجرام فوسفور/جرام تربة، وزيادة أعداد العقد والبكتيرية لكل نبات وزيادة التثبيت الحيوى للنيتروجين لمحصول العدس.

ويتضح من نتائج التجارب ان إضافة كل من الأسمدة النيتروجينية والفوسفاتية مع بكتيريا الريزوبيا أدت الى زيادة كل من الوزن الجاف بمقدار ٢,٨ جرام/نبات، وزيادة العناصر الغذائية المتصة لكل من عنصرى النيتروجين والفوسفور بمقدار ١٣٤,٤ ملليجرام نيتروجين / نبات، ١٤ ملليجرام فوسفور/نبات بينما أدت إضافة كل من الأسمدة النيتروجينية والفوسفاتية مع بكتيريا الريزوبيا وجنس *Pseudomonas striata* الى زيادة أعداد العقد البكتيرية لكل نبات (٦٩ عقدة بكتيرية/نبات) وزيادة التثبيت الحيوى للنيتروجين، وزيادة أعداد البكتريا المذيبة للفوسفات (٢,٣ X ٥١٠ بكتيريا/جرام أرض) وبالتالى زيادة الفوسفات الذائب الصالح للإمتصاص.

يتضح من نتائج هذه الدراسة إمكانية استخدام صخر الفوسفات بدلاً من السوبر فوسفات، وتوفير ١٠ كجم نيتروجين واقتراح إستمرار الدراسة فى الحقل.