DIAZOTROPH BIOFERTILIZATION IN RELATION TO PRODUCTIVITY OF WHEAT AND FABA BEAN

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(Manuscript received 25 December 2007)

Abstract

Two pot experiments were conducted to evaluate the response of wheat (Giza 168) and faba bean (Giza 717) grown in sandy soil to the inoculation with *Azotobacter* spp., *Bacillus* spp., *Azospirillum* spp. and *Rhizobium leguminosarium.* Results indicated that inoculation with different nitrogen fixing bacteria showed significant increases in nitrogenase activity in the rhizosphere of inoculated wheat plants and in faba bean nodules at half dose of nitrogen fertilizer (7.5 kg N/fed). Nitrogen fixing bacteria significantly increased the dehydrogenase enzyme activity (DHA) in the rhizosphere soil compared to un-inoculated control in both plants.

Wheat yield significantly increased with mixed inoculants + 120 kg N/fed as compared with the other treatments. Inoculated by *Bacillus* spp. + *Rhizobium leguminosarium* with 15 kg N/fed showed highly significant increase in grain yield of faba bean (42.5 g/plant).

INTRODUCTION

Biological nitrogen fixation plays a significant role in the nitrogen of soil in subtropical agriculture. The use of nitrogen fixing bacteria minimizes the use of chemical fertilizers, decrease environmental pollution and increase crop production that satisfies the demand of the growing population.

Different microorganisms have recently gained agricultural importance because of their dinitrogen utilizing capacity. In rhizosphere, *Azospirillum* inoculation generally increased the total yield of field grown plants from 10 to 30 % compared to uninoculated control (Omar *et al.*, 1991). Biological N_2-fixation by *Rhizobium* spp. in symbiosis with legumes is important element in agricultural productivity.

Some N_2-fixing bacteria produce extra cellular polysaccharides (EPS). These EPS located at outer surface of microorganisms, are an interface between the cells and their immediate surroundings (Chenu, 1995). The extra cellular polysaccharides occur in two major forms. A dense form which is intimately associated with the cell is termed EPSc (capsule). The other form is looser and can separate easily from the cell, and is known as EPSs (slime) .

The ability of microorganism to surround itself with a highly hydrated polysaccharides layer may provide it with protection against desiccation. Also, the

presence of polysaccharides layer around the cell may have significant effects on the diffusion properties, both into and from the cell (Whitfield, 1988). The EPS of *Rhizobium* strains are of interest because they are thought to play a role is identifying their host leguminous plant (Suresh *et al.*, 1981). Bashan *et al.* (1991) suggested that the nature of *Azospirillum* fibrils could be complex in composition and that there could be polysaccharides among their constituent biopolymers.

Omar *et al.* (2000) evaluated the effect of inoculation on faba bean cv. Giza-714 using nitrogen fixing bacteria (*Azotobacter chroococcum* or *Bacillus polymyxa* with Rhizobium *leguminosarium* bv. *viceae*). Data showed that significant increase in grain yield of faba bean with average value 33 % over the control.

El-Howeity *et al.* (2004) found that inoculation of wheat with diazotrophs (*Azotobacter chroococcum*, *Azospirillum brasilense*, *B. polymyxa*) under different level of nitrogen fertilizer significantly increased shoot, root dry weights and yield, compared to control.

The aim of this study is to evaluate the effect of inoculation with effective isolates of *Azotobacter* HA2, *Bacillus* HA32, *Azospirillum* HA23, *Rhizobium* HA52 in presence of two levels of nitrogen fertilizer on growth of wheat and faba bean in pot experiments.

MATERIALS AND METHODS

Materials :

Soils

The physico–chemical properties of soil were determined according to Black *et al.* (1965). Soil texture was sandy and its pH was 7.4. Soil organic matter was 0.23 % and total nitrogen was 0.019 %

Bacterial strains used

Isolates	Host	Source of reference strains
Azotobacter spp.	*Triticum aestivum* (wheat)	Soils, Water and Environ. Res . Inst. , ARC, Giza, Egypt
Bacillus spp.	*Triticum aestivum* (wheat)	
Azospirillum spp.	*Oryza sativum* (Rice)	
Rhizobium leguminosarum	*Vicia faba* (faba bean)	

Seeds

Seeds of wheat (*Triticum aestivum* cv.) variety Giza 168 and faba bean (*Vicia faba*) variety Giza 717 were used in the present study. Seeds were kindly supplied from Crop Research Institute, Agricultural Research Center, Giza, Egypt.

Mineral fertilizers used

Super phosphate (15.5 % P_2O_5) 150 kg P /fed, potassium sulphate (48 % K_2O) 100 kg K/fed. and ammonium nitrate (33.5 % N) 120 kg N/fed for wheat and 15 kg N/fed for faba bean were used.

Methods:

Nitrogenase activity assay

The ability of isolates to fix nitrogen was assayed by acetylene reduction technique according to Hardy *et al.* (1973) using Hewlett Packard chromatography model HP 6890 GC fitted with dual flam detector and 150 cm x 0,4 cm diameter stainless still column fitted with propak X R100-120 mesh. The results were expressed as μ mol C_2H_4/ml culture/hr.

Pot experiments

Pot experiments were carried out to evaluate the effect of biofertilization with diazotrophs plus inorganic nitrogen , on growth of wheat and faba bean . Pots were filled with sandy soil (10 kg/pot) and 10 seeds were planted in each pot. After complete germination (10 days after planting), pots were thinned to six plants. These pots were divided into 2 groups, the 1st group was supplemented with the full dose of N-fertilizer in the form of ammonium nitrate (120 Kg N/fed for wheat and 15 Kg N/fed for faba bean), the 2 nd group was supplemented with half dose of ammonium nitrate (60 Kg N/fed for wheat and 7.5 Kg N/fed for faba bean).

Wheat

The treatments were:

Pots cultivated with wheat were divided into 12 treatments as follows:

1- Control (supplemented with 60 kg N/fed

2- *Azotobacter* spp. + 60 Kg N/fed

3- *Bacillus* spp. + 60 Kg N/fed

4- *Azospirillum* spp. + 60 kg N/fed

5- *Rhizobium leguminosarium* + 60 Kg N/fed

6- Mixed culture of nitrogen fixing bacteria + 60 Kg N/fed

7- Control (supplemented with 120 kg N/fed)

8- *Azotobacter* spp. + 120 Kg N/fed

9- *Bacillus* spp. + 120 Kg N/fed

10- *Azospirillum* spp. + 120 Kg N/fed

11- *Rhizobium leguminosarium* + 120 Kg N/fed

12- Mixed culture of nitrogen fixing bacteria + 120 Kg N/fed

Faba bean

The treatments were:

1- Control (supplemented with 7.5 kg N/fed)

2- *Azotobacter* spp. + *Rhizobium leguminosarium* + 7.5 Kg N/fed.

3- *Bacillus* spp. + *Rhizobium leguminosarium* + 7.5 Kg N/fed.

4- *Azospirillum* spp. + *Rhizobium leguminosarium* + 7.5 Kg N/fed.

5- *Rhizobium leguminosarium* + 7.5 Kg N/fed.

6- Mixed culture of nitrogen fixing bacteria + 7.5 Kg N/fed.

7- Control (supplemented with15 kg N/fed.).

8- *Azotobacter* spp. + *Rhizobium leguminosarium* + 15 Kg N/fed.

9- *Bacillus* spp. + *Rhizobium leguminosarium* + 15 Kg N/fed.

10- *Azospirillum* spp. + *Rhizobium leguminosarium* + 15 Kg N/fed.

11- *Rhizobium leguminosarium* + 15 Kg N/fed.

12- Mixed culture of nitrogen fixing bacteria + 15 Kg N/fed.

Inoculum preparation:

Vermiculite based inocula was prepared by mixing cell culture suspensions of the strains with pre-sterilized vermiculite and peatmoss at the rate of 1:1. The prepared inocula was applied to the seeds after being mixed with a suitable amount of Arabic gum, then carefully mixed to ensure enough coating with the inoculum.

At harvest, plant height, spike length, number of spikes/plant, weight of grains per spike/plant, number of spikelets per spike/plant and weight of 1000 grains as well as total nitrogen in grains and straw, grain yield and straw yield were determined.

Microbiological analyses

Rhizosphere samples of wheat were collected at 30 and 60 days from sowing,while those of faba bean plants were collected at 50 and 80 days to determine dehydrogenase activity (DHA) according to Thalman (1967) and nitrogenase activity as described by Hardy *et al.* (1973) .

Total nitrogen

Total nitrogen percentages in both wheat and faba bean grains was determined according to micro – Kjeldahel method as described by Black *et al.* (1965).

Statistical analysis

Analysis of variance (ANOVA) and LSD test were carried out for the obtained results according to Steel and Torrie (1980).

RESULTS AND DISCUSSION

Response of wheat to inoculation with nitrogen fixing bacteria in presence of nitrogen fertilization in sandy soils

Nitrogenase activity (N_2-ase)

N_2-ase estimated in the rhizosphere soil and root area showed a different trend where increasing the dose of N-fertilizer drastically did negatively affect N_2-ase (Table 1).

N_2-ase in roots was higher than that in the rhizosphere soil. Inoculation with *Azotobacter* + half dose of nitrogen gave the highest significant value of N_2-ase in the rhizosphere soil and rhizoplane at 30 and 60 days after sowing of wheat seed values

being 60.65 μ mole C_2H_4/g soil/day rhizosphere soil and 182.12 μ mole C_2H_4 μ mole/ rhizoplane dry weight at 60 days.

In all cases, it was obvious that inoculation with a mixture culture + half dose of nitrogen fertilizer (60 kg N/fed) gave considerably higher N_2-ase levels representing 44.34 μ mole C_2H_4 in rhizosphere soil and 146.15 μ mole C_2H_4 in rhizoplane than the control.

Inoculation with *Rhizobium* gave a significant value of N_2-ase of 31.87 μ mole C_2H_4/g dry weight rhizosphere soil/day with N-fertilizer 60 kg N after 60 days. Data showed that *Azotobacter* was the best active isolate, where it gave 18.29 μ mole C_2H_4/g dry weight rhizosphere soil/day after 30 days after sowing with 60 kg N/fed

Generally, it is substantial to note that N_2-ase in all treatments increased to maximum activity after 60 days after sowing and then declined.

Dehydrogenase activity (DHA)

Data presented in Table 2 show the effect of inoculation with N_2-fixing bacteria with two levels of N-fertilizer on dehydrogenase activity in rhizosphere soil. Results indicated that bacterial inoculation significantly increased dehydrogenase activity. Mixed inoculants from the bacterial strain were highly efficient than single inoculum of *Bacillus*, *Azotobacter*, *Azospirillum* or *Rhizobium* at 90 days after sowing with 120kgN/fed .Values were 89.38, 74.69, 55.77, 74.11 and 42.54 μ TPF/g soil/day for mixed inoculants bacterial strains, *Bacillus* spp., *Azotobacter* spp., *Azospirillum* spp. and *Rhizobium leguminosarium*; respectively. Increasing N-fertilizer rate increased DHA. These results are in harmony with those obtained by Omar and Hamouda (1998).

Wheat yield components

Data presented in Table 3 show that inoculation with different diazotrophs significantly affected the wheat grain, straw yield and weight of 1000 grains compared to control (uninoculated) wheat plant.

Inoculation with mixture inoculants of bacterial strains showed highly significant increase in grain yield, straw and weight of 1000 grains compared to inoculation with single N_2-fixing isolates being 5.00, 4.78 and 52.01 (g/plant) at 120 kg N/fed.

Table 1. Effect of wheat inoculated with nitrogen fixing bacteria in presence of N-fertilizer on N_2-ase (as μ mole C_2H_4/g dry soil /day).

Bacterial Strains	Rhizosphere soil								Rhizoplane							
	60 Kg N/fed				120 Kg N/fed				60 Kg N/fed				120 Kg N/fed			
	Days after sowing															
	30	60	90	120	30	60	90	120	30	60	90	120	30	60	90	120
Control	2.47	6.88	7.14	2.47	2.12	6.48	2.87	0.91	34.09	36.15	17.34	6.25	0.93	15.64	14.30	1.69
Azotobacter spp.	18.29	60.65	43.17	7.13	15.07	51.04	36.51	6.31	90.69	182.12	62.64	42.33	50.41	139.6	38.79	9.92
Bacillus spp.	14.04	41.34	31.68	5.05	11.81	35.21	27.01	4.04	70.92	132.29	57.03	39.86	83.80	115.7	61.31	11.09
Azospirillum spp.	12.21	36.61	29.74	5.85	9.34	32.08	25.18	4.24	54.32	104.24	62.11	14.87	50.87	50.41	98.48	6.99
Rhizobium leguminosarum	12.01	31.87	22.04	5.05	10.77	31.17	18.38	3.88	87.79	109.85	55.14	18.64	46.47	92.21	37.08	3.67
Mixed inoculants of bacterial strains	14.08	44.34	32.91	5.20	11.81	35.34	27.54	4.81	87.79	146.15	76.40	18.50	74.50	117.1	55.54	35.53

LSD. (5%)	Rhizosphere soil	Rhizoplane
Biofertilizer strains (B)	1.088	5.472
Nitrogen fertilizer (N)	1.944	7.018
Interaction(BxN)	1.538	7.738

Table 2. Effect of wheat inoculated with nitrogen fixing bacteria in presence of N-fertilizer on dehydrogenase activity (as µg TPF/g dry weight soil /day).

Bacterial Strains	60 Kg N/fed					120 Kg N/fed				
	Days after sowing									
	30	60	90	120	Mean	30	60	90	120	Mean
Control	9.57	13.94	25.89	22.34	17.94	10.01	13.91	22.92	15.27	14.78
Azotobacter spp.	27.11	39.71	48.39	34.64	38.46	34.34	47.21	55.77	35.98	51.94
Bacillus spp.	29.85	40.65	68.75	46.65	46.48	36.18	51.68	74.69	46.01	52.14
Azospirillum spp.	25.11	36.37	46.59	29.54	34.40	30.17	41.51	74.11	34.08	44.97
Rhizobium leguminosarum	20.88	37.55	41.39	26.69	31.63	21.98	34.11	42.54	31.19	32.46
Mixed inoculants of bacterial strains	36.50	49.78	73.11	38.54	49.48	42.26	59.78	89.38	45.29	59.18
Mean	24.84	36.33	50.69	33.07	36.23	6 29.1	41.37	59.91	34.64	42.58

	Biofertilizer strains (B)	Nitrogen fertilizer (N)	Interaction (BxN)
LSD. (5%)	3.689	12.553	8.873

Table 3. Effect of wheat inoculated in presence of N-fertilizer on yield and nitrogen uptake (kg N/fed) in grain and straw.

Bacterial Strains	60 kg N/fed					120 kg N/fed				
	Grain (g/plant)	Straw (g/plant)	Weight of 1000 grains (g)	Grain nitrogen uptake	Straw nitrogen uptake	Grain (g/plant)	Straw (g/plant)	Weight of 1000 grains (g)	Grain nitrogen uptake	Straw nitrogen uptake
Control	0.72	0.63	24.75	1.06	0.06	1.20	0.95	29.00	2.45	0.25
Azotobacter spp.	4.32	3.53	39.05	11.75	1.98	4.52	3.91	45.67	16.14	3.13
Bacillus spp.	4.09	2.86	39.84	11.74	1.14	4.21	3.42	41.97	12.71	2.57
Azospirillum spp.	3.28	2.55	37.04	7.22	1.25	3.76	3.16	38.83	11.13	2.18
Rhizobium leguminosarum	2.87	2.04	34.02	5.57	0.71	2.97	2.86	37.51	6.56	1.63
Mixed inoculants of bacterial strains	4.78	3.90	42.99	18.45	2.61	5.00	4.78	52.01	18.0	4.64
LSD (5%)	0.199	0.257	3.410	-	-	0.282	0.364	4.882	-	-

LSD 5% nitrogen uptake in grains

(nitrogen = 0.3817) (Bio = 0.6612) nitrogen uptake in straw (nitrogen = 0.1199) (Bio = 0.2077)

Inoculation with *Rhizobium* gave significant increase in grain and straw yields and weight of 1000 grains being 2.97, 2.86 and 37.51 (g/plant) with 120 kg N/fed, but with 60 kg N/fed gave 2.87, 2.04 and 34.02 (g/plant) as compared with uninoculated treatment.

Full dose of nitrogen fertilizer significantly enhanced wheat grain, straw and weight of 1000 grains. The increase in yield with nitrogen supply was due to increase vegetative growth which could be considered as a criterion for the photosynthetic efficiency of the plant.

On the other hand, there were no significant differences in grain yield and weight of 1000 grains at 60 kg N/fed between *Azotobacter* and *Bacillus* inoculation, being 4.32 and 4.09 (g/plant) grain yield and 39.05 and 39.84 (g/plant) weights of 1000 grains.

Nitrogen uptake in grains and straw

Data in Table 3 show that total nitrogen increased in grain and straw of wheat as affected by inoculation of diazotrophs and with increasing N-dose of N-fertilizer comparing with control.

High significant of nitrogen uptake in grains and straw wheat were obtained with the inoculation of mixture of cultures with nitrogen fertilizer 120 kg N/fed being 18.0 and 4.64. Low significant in nitrogen uptake in grains and straw of wheat were obtained in inoculated treatment with *Rhizobium* being 6.56 and 1.63 with nitrogen fertilizer of 120 kg N/fed. compared to control

Generally, control (nitrogen fertilizer non-inoculated) gave a low nitrogen uptake in grains and straw wheat comparing to other treatments being 1.06 and 0.06 with 60 kg N/fed.

No significant differences were obtained between inoculation with *Azotobacter* and *Bacillus* in nitrogen uptake in grain with half nitrogen fertilizer of 60 kg N/fed being 11.75 and 11.74.

Table 4. Effect of inoculation with nitrogen fixing bacterial strains on N_2-ase in faba bean with two levels of nitrogen fertilizer in the greenhouse experiment (as nano mole C_2H_4/g dry weight nodules/h).

Bacterial Strains	Nitrogen fertilizer					
	7.5Kg N/fed			15Kg N/fed		
	Days after sowing					
	50	80	Mean	50	80	Mean
Control	3870	3180	3530	4750	1590	3170
Azotobacter spp. + *Rh. leguminosarium*	31350	7000	19180	8720	6690	7710
Bacillus spp. + *Rh. leguminosarium*	24820	9610	17220	8900	6720	7810
Azospirillum spp. + *Rh. leguminosarium*	41570	11170	26370	20040	9630	14860
Rhizobium leguminosarium	13570	8260	10920	8840	4090	6470
Mixed inoculants of bacterial strains	11270	8030	9650	8340	5680	7010
Mean	21080	7880	14480	9940	5730	7830

	Biofertilizer strains (B)	Nitrogen fertilizer (N)	Interaction (BxN)
LSD (5%)	1.881	2.269	2.661

Response of faba bean to inoculation by diazotrophs with nitrogen fertilization Nitrogenase activity (N_2-ase)

Data presented in Table 5 show the acetylene reduction activity (ARA) of nodulated roots supplemented with half and full doses of nitrogen fertilizer.

Inoculation with *Rhizobium* + *Azospirillum* gave the highest significant value of N_2-ase in inoculated treatments compared with control at 50 and 80 days after sowing being 41570 and 1170 nmoles C_2H_4 g/dry nodule hr/with half dose nitrogen fertilizer and 20050 and 9630 nmoles C_2H_4 g/dry nodule hr/with full dose of nitrogen fertilizer.

Inoculation with *Rhizobium* + *Azotobacter* gave high significant value of N_2-ase at the following stage (50 days) being 31350 nmoles C_2H_4 g/dry nodule hr/with half dose nitrogen fertilizer. These results are in agreement with Rodelas *et al.* (1999) who found that inoculated faba bean with *Azotobacter chroococcum* and *Rhizobium leguminosarum* led to significant increase in nitrogenase activity of nodulated root at the flowering stage.

Inoculation with *Rhizobium* + *Bacillus* gave a significant increase in acetylene reduction assays being 24820 and 4610 nmoles C_2H_4 g/dry nodule hr/during growth period of 50 and 80 days after sowing.

On the contrary, there were no significant differences in N_2-ase activity between inoculations with *Rhizobium* individually and mixed cultures, being 13570 and 11270 n mole C_2H_4 g /dry nodule hr; respectively, at 50 % N fertilizer.

Table 5. Effect of inoculation with diazotrophs on dehydrogenase activity in faba bean with two levels of nitrogen fertilizer (as µg TPF/g dry weight soil/day).

Bacterial Strains	Nitrogen fertilizer (kg N/fed)					
	7.5			15		
	Days after sowing					
	50	80	Mean	50	80	Mean
Control	1.85	11.86	6.86	3.67	14.08	8.88
Azotobacter spp. + *Rh. leguminosarium*	5.16	22.75	13.96	6.13	33.18	19.66
Bacillus spp. + *Rh. leguminosarium*	8.37	50.51	29.44	8.94	58.64	33.79
Azospirillum spp. + *Rh. leguminosarium*	6.25	43.82	25.04	7.82	49.37	28.59
Rhizobium leguminosarium	5.32	27.81	16.57	7.06	35.37	21.22
Mixed inoculants of bacterial strains	5.42	39.71	22.57	6.43	40.22	23.33
Mean	5.39	32.74	19.07	6.68	38.48	22.58

	Biofertilizer strains (B)	Nitrogen fertilizer (N)	Interaction (BxN)
LS D (5 %)	0.819	11.75	1.159

Table 6. Effect of inoculation with nitrogen fixing bacteria on nodule numbers and dry weights of faba bean with two levels of nitrogen fertilizer.

Bacterial strains	7.5 kg N/fed				15 kg N/fed			
	50 days after sowing		80 days after sowing		50 days after sowing		80 days after sowing	
	Nodules No. / plant	Nodules dry wt. (mg)/ plant	Nodules No./ plant	Nodules dry wt. (mg)/ plant	Nodules No./ plant	Nodules dry wt. (mg)/ plant	Nodules No./ plant	Nodules dry wt. (mg)/ plant
Control	21	84	34	115	16	79	19	113
Azotobacter spp. + *Rh. Leguminosarium*	50	112	69	212	26	89	50	231
Bacillus spp. + *Rh. Leguminosarium*	81	170	99	305	39	175	40	171
Azospirillum spp. + *Rh. Leguminosarium*	72	177	99	282	36	163	54	227
Rhizobium Leguminosarium	54	119	74	237	29	98	45	199
Mixed inoculant of bacterial strains	58	161	78	272	32	126	58	212
LSD (5 %)	5.924	14.39	6.217	22.68	8.378	20.34	8.791	21.41

Azotobacter + *Azospirillum* caused the highest increase in N_2-ase. It could also be noticed that addition of 100 % N decreased N_2-ase activity of nodulated roots for all treatments.

Dehydrogenase activity (DHA)

Inoculation with *Rhizobium* + *Azospirillum* gave highly significant DHA being 6.25, 43.82, 7.82 and 49.37 µg TPF/g soil/day; respectively, during growth period 50 and 80 days after sowing under two levels of nitrogen fertilizer 50 and 100 % (Table 5).

Mixed inoculants of bacterial strains gave significant increase in DHA comparing to un-inoculated control and other treatments being 5.42, 39.71, 6.43 and 40.22 µg TPF/g soil/day; respectively (Table 5), at 50 and 80 days after sowing under half and full dose 50 and 100 % N F.

Inoculation with *Rhizobium* individually gave the least significant value with 100 % N being 35.37 µg TPF/g soil/day, after 80 days from sowing.

The results indicated that dehydrogenase activity increased with increasing the plant age up to the 80 days after sowing.

Faba bean nodulation

The nodule numbers and dry weight varied depending upon nitrogen fertilizer and inoculation with nitrogen fixing bacteria Table 6. Generally, inoculation with *Rhizobium* + *Bacillus* combined with half dose of nitrogen fertilizer recorded significant increase in number and dry weight of nodule as compared to the other treatments and uninoculated control being 81 and 99 nodule/plant and 179 and 305 mg/plant; respectively, after 50 and 80 days of sowing. Increases of 39 and 58 nodules/plant and 175 and 231 mg/plant respectively, after 50 and 80 days after sowing, were recorded in case of inoculation with *Rhizobium* + *Bacillus* combined with full dose of nitrogen fertilizer.

Inoculated with *Rhizobium* + *Azospirillum* showed significant increase in number and dry weight of nodules being 72 and 99 nodules/plant and 177 and 282 mg/plant respectively with 50 % N. These data were 36 and 54 nodules/plant and 163 and 227 mg/plant; respectively, with 100 % N after 50 and 80 days of sowing.

Hamaoui *et al.* (2001) stated that inoculation of faba bean with *Rhizobium leguminosarum* and *Azospirillum brasilense* significantly increased nodulation.

Mixted culture inoculation gave a significant increase in nodule number and dry weight as compared to control *Rhizobium* inoculation (alone) and *Rhizobium* + *Azotobacter* gave similar results in number and dry weight of nodule with 50 % and 100 % N. Inoculation with *Rhizobium* + *Azotobacter* gave low significant results in nodule number and dry weight being 50 and 69 nodules/plant and 112 and 212

mg/plant with 50 % N and 26 and 40/plant and 89 and 171 mg/plant with 100 % N. *Azotobacter chroococcum* inoculation promoted plant growth and significantly increased dry matter accumulation in all plants parts including nodules (Rodelas *et al.*, 1999).

Considerable information are available regarding the ecological interactions of bacteria that are able to colonize the roots of legumes and non-legumes and subsequently fix nitrogen either in a symbiotic or as associative manner .

Nodule number and nodule dry weight increased with increasing plant age in all treatments. Full dose of N reduced both nodule number/ plant and nodule dry weight/plant. The nodule number and dry weight decreased with fertilizer application.

Plant height, seed and straw yield

Inoculation with *Rhizobium* + *Bacillus* gave the highest plant height of 86.00 and 92.67 cm with 50 % and 100 % nitrogen fertilizer (Table 7).

Inoculation with *Rhizobium* + *Azospirillum* gave high increase in plant height followed by inoculation with mixed inoculants of bacterial strains and *Rhizobium* + *Azotobacter* of 76.67, 76.67 and 74.33 cm with supplemented 50 % nitrogen fertilizer, where 100 % nitrogen fertilizer recorded 90.00, 83.33 and 80.67 cm.

Table 7. Effect of inoculation with nitrogen fixing bacteria on plant height, grain yield, straw yield and nitrogen uptake (kg N/fed) in straw and grain of faba bean with two levels of nitrogen fertilizer.

Bacterial strains			7.5 kg N/fed					15 Kg N/fed		
	Plant height (cm)	Grain yield (g) plant	Straw yield (g) plant	Nitrogen uptake grain	Nitrogen uptake straw	Plant height (cm)	Grain yield (g) plant	Straw yield (g) plant	Nitrogen uptake grain	Nitrogen uptake straw
Control	51.33	5.17	9.57	8.94	2.58	60.00	13.87	14.24	27.32	12.25
Azotobacter spp. + *Rh. Leguminosarum*	74.33	13.74	17.57	25.97	8.79	80.67	18.32	23.10	40.30	22.18
Bacillus spp. + *Rh. Leguminosarum*	86.00	22.52	38.19	47.29	38.95	92.67	29.78	42.46	109.59	59.01
Azospirillum spp. + *Rh. Leguminosarum*	76.67	19.68	34.37	37.39	29.90	90.00	23.92	33.98	78.46	46.89
Rhizobium Leguminosarum	67.33	14.98	21.03	23.67	13.67	72.67	14.54	19.51	35.91	19.31
Mixed inoculants of bacterial strains	76.67	14.98	31.98	35.35	47.97	83.33	19.44	26.47	78.34	41.29
LSD (5 %)	2.876	1.89	3.89	--	--	4.067	2.673	5.514	--	--

LSD 5%: nitrogen uptake in grains (nitrogen= 0.229) (bio = 0.398) nitrogen uptake in straw (nitrogen = 0.284) (bio = 0.493)

On the contrary inoculation with *Rhizobium* alone gave low height of 67.33 cm with 50 % nitrogen fertilizer and 72.7 cm with 100 % nitrogen fertilizer.

Rodelas *et al.* (1999) reported that inoculation of faba bean with *Rhizobium leguminosarum* and *Azotobacter chroococcum* or *Azotobacter vinelandii* produced significantly higher plants.

The treatments in Table 7 showed that there were significant differences in seed and straw yields between inoculated and uninoculated plants (control).Inoculation with *Rhizobium* + *Bacillus* plus full dose of nitrogen fertilizer recorded the highest seed yield of 29.78 g/plant, followed by *Rhizobium* + *Azospirillum*, mixed culture and *Rhizobium* + *Azotobacter* of 23.42, 19.44 and 18.32 g/plant respectively, over those plants inoculated with only rhizobia. Inoculation with diazotrophs and received half dose of nitrogen fertilizer recorded low results. The best results were obtained in treatments inoculated with *Rhizobium* and *Bacillus* being 22.52 g/plant.

While, faba bean plants inoculated with *Rhizobium* and *Azospirillum* plus 50 % nitrogen fertilizer recorded seed yield of 19.68 g/plant comparing with control, followed by mixed inoculants of bacterial strains and dual inoculation with *Rhizobium* + *Azotobacter* of 14.98 and 13.74 g/plant; respectively.

The straw yield Table 7 had the same trend of that obtained with seed yield. Inoculation with dual culture (*Rhizobium* + *Bacillus*) gave the best results with full dose of nitrogen fertilizer followed by inoculation with *Rhizobium* + *Azospirillum*, mixed inoculants of bacterial strains and *Rhizobium* + *Azotobacter* being 42.46, 33.98, 26.47 and 23.10 g/plant; respectively.

Concerning to the mineral nitrogen fertilizer, 100 % of nitrogen fertilizer gave slight increases in seed and straw yields than 50 %.

Nitrogen uptake in straw and seed yields

Data of faba bean nitrogen uptake in straw seed yields as affected by different nitrogen fixing bacteria under two levels of nitrogen are shown in Table 7. Regardless of mixture inoculants of bacterial strains inoculation with full dose of nitrogen fertilizer recorded higher significant values of straw and nitrogen uptake of seed yield compared to uninoculated control plants or to other treatments of 41.29 and 78.34; respectively. Inoculation with mixed inoculants of bacterial strains with half dose of nitrogen fertilizer recorded highly significantly increases of 47.97 and 35.35; respectively.

Regarding the influence of dual inoculation of *Rhizobium* + *Bacillus*, it was clear that inoculation of faba bean seeds led to an improvement of N-uptake of straw and seed yield with full dose of nitrogen fertilizer of 59.0 and 109.6 respectively. On the contrary the results recorded low values with half dose of nitrogen fertilizer of 38.9 and 47.3; respectively.

Inoculation with *Rhizobium* + *Azospirillum* gave high increase N-uptake of straw and seed yields with half dose being 29.9, 46.9 and 78.5; respectively.

Rhizobium + *Azotobacter* inoculation, showed an increase in N-uptake of straw and seed yields compared with uninoculated control of 8.8 and 25.9 with full dose of nitrogen fertilizer. However, decrease in N-uptake in both straw and straw was recorded when *Rhizobium* alone was applied in the presence of 50 % and 100 % nitrogen fertilizer.

Generally, increasing of faba bean yield as a result of using different nitrogen fixing bacteria inoculants could be attributed to the efficiency of associative nitrogen fixer strains to increase the roots surface area which reflect on efficient acceleration of nutrient uptake. Rodelas *et al.* (1999) repeated that inoculation faba bean with *Rhizobium leguminosarum* and *Azotobacter chroococcum* or *Azotobacter vinelandii*, increased total N content in all plant parts compared with control plants inoculated with *Rhizobium* alone.

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تقييم أثر إستخدام التلقيح البكتيري علي زياده انتاجيه نباتات القمح و الفول البلدي

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أقيمت تجربتي أصص في الصوبه بالجيزه لتقييم إستجابه نباتات القمح صنف جيزه ١٦٨ و الفول البلدي صنف جيزه ٧١٧ في تربه رمليه للتلقيح البكتيري بإستخدام بعض مثباتات أزوت الهواء الجوي مثل الأزوتوباكتر و الباسلس و الأزوسبيريللم والريزوبيا . تم اضافه اللقاح البكتيري سواء مفردا أو خليط من هذه الأنواع مع إضافه مستويين من التسميد الازوتي المعدني .

أوضحت النتائج المتحصل عليها ما يلى :

١. ان إستخدام التلقيح البكتيري أظهر معنويه في نشاط انزيم النيتروجينيز في نباتات القمح والفول البلدي مع نصف جرعه الأزوت المعدني.
٢. أظهر نشاط إنزيم الديهيدروجينيز في منطقه الجذور نشاطاً معنويا مقارنا بمعامله الكنترول في كلا المحصولين .
٣. أعطت معامله التلقيح البكتيري الخليط مع ١٢٠ كج ازوت معدني أعلي إنتاج لمحصول القمح بينما أدي إستخدام الباسلس و الريزوبيا مع ١٥ كج أزوت معدني في الفول البلدي أعلي انتاجيه.