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Ecophysiology of Nitrogen Fixation Ability on 3 cultivars Common Bean (*Phaseolus vulgaris* L.) with some types of Inoculants which contain different strains of *Rhizobium leguminosarum;* bv.phaseoli

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ABSTRACT

Nitrogen Fixation Ability on Common Bean (*Phaseolus vulgaris* L.) cultivars with Some types of Inoculants which contain different strains of *Rhizobium leguminosarum;* bv. *phaseoli* was evaluated in an experimental design. In this investigation three types of biological fertilizers such as Rb117, Rb123, Rb136 and a commercial inoculants (Rhizobean super plus) with two treatment N100 (use of 100 Kg nitrogen/ha) and control (without seeds inoculated and fertilizer) and three bean cultivars: COS16 (spotted bean), Derakhshan and Akhtar (red beans) used as factorial experiment in randomized complete block design in 2006 and 2007 in Zanjan province(Khoramdareh Region). Combined Analysis preformed with SAS9.2 of statistical program and graphs were provided by Excel and Σplot programs and means comparison were done with Duncan's Multiple Range Test. Rb117 strain was showed an 59 percent increasing in seed yield compared with non-inoculants treatment. Among of inoculants the effect of Rb123 strain, was at least (32%) in compared with others. The results showed that significant difference in seed yield, nitrogen percent per plant, seed protein percentage, number and weight of nodules (50 day after emergence),seed and dry matter yield were observed among seed inoculated and non-inoculated. Most protein percent were achieved from 100 kg nitrogen, and Rb117 treatment (About 24%) and highest protein yield (864 kg/ha) was gained from Rb117 and lowest protein yield was produced from control. Among of all inoculants (or strains of *Rhizobium leguminosarum;*bv.*phaseoli*) The Rb117 strain has more effectiveness on common bean, as compared with other strains. In three cultivars the COS16 line was successful in compared with other cultivars. High seed yield and many evaluated traits achieved from COS16 line (spotted bean), second year of experiment and Rb117 strain. This investigation showed that to achievement for satisfying yield and sustainable agriculture, all of industrial bio fertilizers must be used as complementary materials with mineral fertilizers.

Keywords: Common bean, Biological Nitrogen Fixation, Seed yield, *Rhizobium leguminosarum;*bv.*phaseoli*.

INTRODUCTION

One of the most important ways of taking the advantages of microorganism interactions and to maintain the diversity of agricultural ecosystems, is using of terrestrial microorganisms. Now the rhizosphers bacteria are using for biological fertilizers in many countries. Some researchers believe that nitrogen fixation is a hereditary trait and common bean varieties are genetically different in biological nitrogen fixation (Bliss and Miller., 1986, Graham and Rosas., 1997, Barron *et al*., 2000).

Nitrate Reductase enzyme always is active in the cell, even when the nitrate is not received by plants, but for increasing the activity of Nitrate reductase enzyme, it is required that the plant be exposed to nitrate (Taghavi, 2004., Nakamura *et al*., 1993). Yahya Abadi (2008) evaluated the potential of some rhizobium bacteria to nitrogen fixation and other nutrients uptake and concluded that some strains of native bacteria are effective. These results are consistent with reports that some researchers have presented (Biswas *et al*., 2000). There are many reports that shows the ability of different bacterial strains for the dissolution of insoluble inorganic phosphates (Goldstein., 1986).

Organic acids production by soil bacteria has been detected the main mechanism of dissolution of mineral phosphates. Organic acids cause decrease the rhizospher's pH, thus P element can be release by replacement of ions H^+ with calcium ions in the environment, among the organic acids, Gluconic acid seems to be the most frequent factor in the dissolution of mineral phosphates (Illmer and Schinner., 1995). The purpose of study was evaluation the reaction of different cultivars of common beans to application of some Inoculants which contain different strains of *Rhizobium leguminosarum;*bv.*phaseoli*.

MATERIAL AND METHODS

This project was conducted on as a factorial experiment based on randomized complete block design with three replications for two-year in 2006 and 2007 in Zanjan province (Khoramdareh Region). The altitude was 1547 meter above sea level. Levels of non-inoculated and inoculated with various bacteria strains, included six levels such as without inoculation and without fertilizer (control), inoculated with strain Rb117, inoculated with strain Rb123, inoculated with strain Rb136, inoculated with a commercial inoculants (Rhizobean Super Plus) and using 100 kg of urea with three indeterminate varieties (growth type I) including Pinto bean COS16 and two red bean Akhtar and derakhshan.

For providing strains in the laboratory we used medium Yeast Manitol Broth (YMB) (including Di potassium hydrogen phosphate0.5, magnesium sulfate 0.1, NaCl 0.1, manitol 10 and yeast extract 0.5 g in a liter of distilled water, and pH was 7) (Beck *et al*, 1993). White strains were appearance in incubator (28-25 ° C for one week).Population in all the packages were more than 4×10^8 cells per gram (10^8CFU/ml). About 20 kg nitrogen per hectare as a starter nitrogen was used in this study.

RESULTS AND DISCUSSION

There was a significant different in inoculation and fertilizer treatment on yield (Table1 Analysis of variance).

Table1: Complex analysis of variance of Yield, Protein%, Nitrate Reductase activities and P and K uptake

P Uptake	Nitrate Reductase activities	K Uptake	Protein%	Seed yield	df	S.O.V
98.25**	59053.2**	4.55*	16 n.s	592889**	1	(Y)
2.5**	2192.63 n.s	5.33 **	0.657 n.s	303392.7**	4	(R)YEAR
9.1**	245463**	38.71**	15.2**	3651492.2**	5	Inoculation A
1.6**	94006**	19.57 **	0.17 n.s	2737721**	2	Varieties B
0.87**	22920.4**	0.527 *	4.06**	437916.9**	10	A×B
1.89**	1355.92 n.s	0.135 n.s	0.111 n.s	50110 n.s	5	Y×A
2.55 **	4052.32 n.s	0.36 n.s	0.53 n.s	19034.3 n.s	2	Y×B
0.71 *	6603 n.s	0.327 n.s	0.44 n.s	13543.7 n.s	10	Y×A×B
0.32	4687.7	0.252	0.857	25971.7	68	E
9.26	7.37	2.9	3.9	5.3		CV(/.)

Generally seeds priming by various inoculation could increase about 43 percent of yield than the control (no inoculation and no fertilizers). RB117 inoculants (3557.7 kg/ha), had the highest effect (59 percent) on grain yield than the control (2230.4 kg/ha) and the effect of RB123 with 32 percent on grain yield, was the least.

Rhizobean and 100 kg nitrogen fertilizer had a same effective on the yield (44 percent) and RB136 had increased %37.5 than control treatment (Table 2).

Table 2: Mean comparison of seed yield (kg), K uptake (mg/kg), Nitrate Reductase activities nmol/gFW/h), Protein yield (kg) and P uptake (mg/kg).

P Uptake	Protein yield	Nitrate Reductase activities	K Uptake	Seed yield	
LSD= 0.22	LSD=21.4	LSD=26.29	LSD=0.19	LSD=61.89	Year
5.13b	706.7 b	910.35b	16.85 b	2973.54 b	2006
7.04a	740.8 a	957.12a	17.26a	3121.7 a	2007
LSD= 0.37	LSD=37.2	LSD= 45.5	LSD=0.33	L S D=107.19	Inoculation
4.97d	500.0 e	720.32c	15.2 c	2230.44 e	N0
6.31b	751.51 c	1099.18 a	17.4 b	3198.8 b	N100
6.90a	864.29 a	846.09bc	18.6 a	3557.71 a	Rb117
5.52c	695.39 d	900.63 b	15.3 c	2947.4 d	Rb123
6.22 b	742.0 c	887.03 b	17.4 b	3074.3 c	Rb136
6.58 ab	789.13 b	889.16 b	18.36 a	3277.9 b	Rhizobean
LSD=0.265	LSD=26.3	LSD= 32.3	LSD=0.232	LSD=57.8	Varieties
5.8 b	781.47 a	885.0 c	17.82a	3308a	COS16
6.3 a	663.7c	986.91a	17 b	2758.7 c	Akhtar
6.08 ab	726.0 b	929.30b	16.35c	3076 b	Derakhshan

Highest level of enzyme activity of nitrate Reductase was 1099.18 nmol/gFW/h, which related to fertilizer treatment (100 kg/ha nitrogen) and the lowest enzyme activity was in non inoculants treatment or control (720.3nmol/gFW/h). Other treatments were very close together, about 880 nmol/gFW/h in the same statistical class. Interaction of inoculation and varieties on Nitrate Reductase activity in common bean leaves showed in Fig. 1.

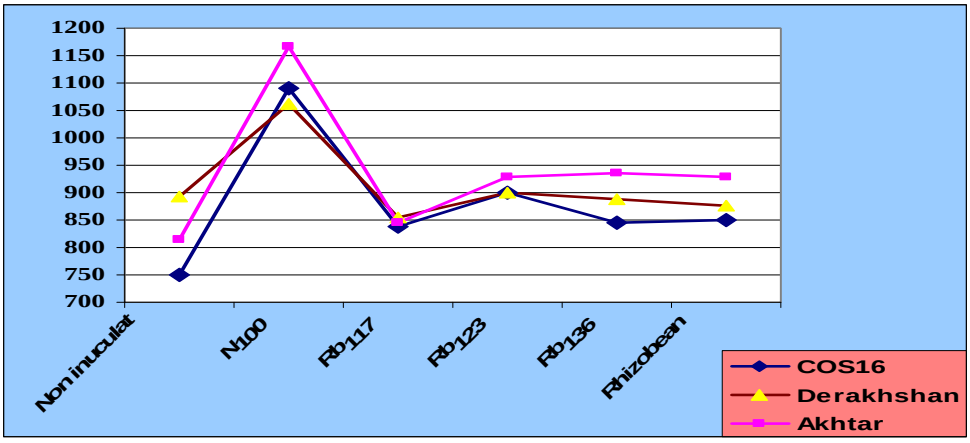

Fig. 1: Interaction of inoculation and varieties on Nitrate Reductase activity in common bean leaves.

The results of Table 2 show that the effect of inoculants and fertilizer treatments on average K uptake per kilogram of dry matter of seeds, has been significant and their class has statistically different. So that the highest means were obtained from inoculation treatments and the lowest was about control.

Results showed that the grain's percent of protein, nitrogen, K and P, in the inoculation treatments are higher than control. Most potassium accumulated were in grains of COS16 variety, with inoculation by Rb117 (mean 19.6g per kg of seed dry matter). That is 33% higher than the lowest mean values. Interaction of inoculation and varieties on potassium accumulation showed in Fig. 2.

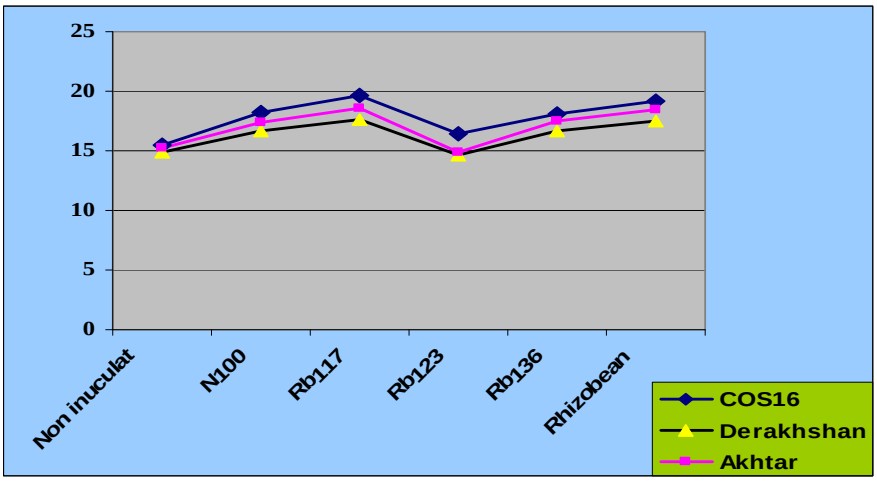

Fig. 2: Interaction of inoculation and varieties on grain potassium accumulation.

The mean comparison results show, that the effect of inoculants and fertilizer treatments has been significant on average K uptake in above ground dry matter. So that the highest means were obtained by inoculation treatments and the lowest mean related to control. Control with mean 17.48 and Rb117 inoculated with average 21.8 g potassium /kg dry matter. Effect of inoculation and fertilizer treatments on the mean phosphorus absorption of dry matter per kg of seed, has been significant and their class has statistically different.

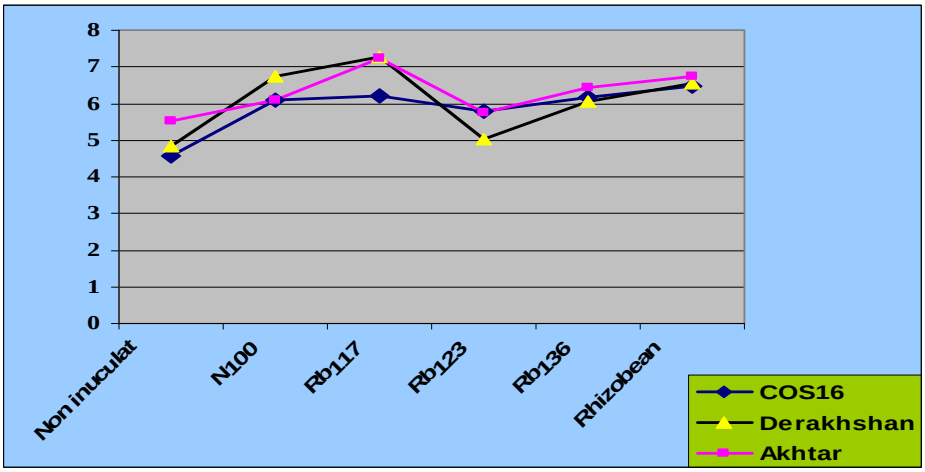

Fig. 3: Interaction of inoculation and varieties on grain phosphorus accumulation.

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