

Nitrogen fixation efficiency in native strains compared with non-native strains of *Rhizobium leguminosarum*

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Abstract— An experimental design was conducted in North West region of Iran. In this investigation three types of rhizobium strains such as Rb117 (a native strain) Rb123, Rb136 and a biological inoculants (Rhizobean), with two treatment N100 and control (without seeds inoculated and fertilizer) and three erect type (Type I) common bean cultivars used as factorial experiment in randomized complete block design in 2006 and 2007 in North West region of Iran. Combined analysis performed with SAS 9.1 and means comparison were done with Duncan's multiple range test. Rb117 strain was showed highest increasing in seed yield compared with other strains and non inoculants treatments. The results showed that significant difference in seed and protein yield, seed protein %, number and weight of nodules. Most protein% were achieved from N100 and Rb117. Among of all 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), and Rb117 strain.

Keywords- Common bean, Biological Nitrogen Fixation and Seed yield

I. INTRODUCTION

The nitrogen fertilizer efficiency is low, but it has high potential in creating polluted environments (Assadi-Rahmani et al., 2005). Many soils contain large number of indigenous rhizobial that are not powerful for nitrogen fixation, but have good compatibility with environment, and have highly competitive ability (Khavazi, 2005). Plant genotype plays an important role in establishing symbiosis between plants and beneficial bacteria and those have taken carbon compounds for nitrogen stabilizers are the most effective plants with symbiotic bacteria (May and Bohlool, 1983). Competition between new and indigenous strains of cowbean was studied and the results showed that the competitive ability of different strains is significant and native strains are so effective (Danso and Owirdu., 1998). With rhizobial inoculation yield, tannin and protein were significantly increased in bean seeds, and bean yellow mosaic virus infection also decreased (Babiker et al., 1995). Some native rhizobial strains that inoculated for common beans, increased the dry weight of shoot and nitrogen % in shoot, in addition increased absorption of nutrients as well be, and was determined that inoculants L-75 increased 100% in weight

nodules and 70% in nitrogen uptake has been compared to the control and inoculated treatments L-54 compared to the control treatment and N200 kg/ha Urea, increased 45% and 32% nitrogen uptake, respectively (Yahya-Abadi et al., 2008). There are many reports that showed the ability of different bacterial strains for the dissolution of insoluble inorganic phosphates (Goldstein, 1986). Response to inoculation remains highly site specific and depends on factors beyond the effectiveness and competitiveness of the strains used and host cultivars seeded. N₂ fixation in grain legumes has focused on selection of superior rhizobial strains, however, significant variation in rhizobial effectiveness has been observed (Kessel and Hartley., 2000). Many factors contribute to high quality legume inoculant products. The most important of these included high numbers of live rhizobia capable of nodulation and nitrogen fixation. When farmers realize that poor inoculant quality, results in limited inoculant response, their confidence in the technology wanes and both the manufacturers and consumers are ultimate losers (Lupway et al., 2000). 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).

The purpose of this investigation was evaluation the ability of different strains of *Rhizobium leguminosarum* on cultivars of common beans in onfarm conditions.

II. MATERIAL AND METHODS

In order to determine the best rhizobial strain(s) or inoculants (Indigenous and non-Indigenous) for N₂ fixation in different cultivars of common bean in North West of Iran an experiment was conducted based on factorial design.

The region's climate is Semi-Arid with 298 mm Annual precipitation mean. This study was conducted on as a factorial experiment based on randomized complete block design with three replications for two year in 2006 and 2007 in North West region of Iran. 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) and using 100 kg of Urea with three

indeterminate varieties (type I) including Pinto bean COS16, Akhtar and derakhshan (red beans).

For providing strains in the laboratory we used medium YMB (Beck et al., 1993). In all of the packages were more than 4×10^8 cells per gram (108CFU/ml). About 20 kg nitrogen per hectare as a starter nitrogen was used in this study. For estimating N₂ fixation, we used N balance, based on the difference in total N between a grain legume and a non-N₂-fixing reference crop.

$$N_2 \text{ fixed} = (N_{\text{leg}} - N_{\text{nonfix}}) + (\text{Soil } N_{\text{leg}} - \text{Soil } N_{\text{nonfix}})$$

Table1- Results of soil analysis (0-40cm)

K ppm	P ppm	Total N%	OC %	T.N.V %	pH	EC*1000	S.P %
404	13	0.076	1.18	3.4	7.1	1.15	44.5

III. RESULTS AND DISCUSSION

The highest average nitrogen fixation was obtained from inoculated treatment and lowest nitrogen fixation rates were obtained in the control and fertilizer treatments (Figure 1).

There was a significant different in inoculation and fertilizer treatment on yield (Table1 Analysis of variance). 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). Based on research on the formation of nodules by bacteria Bradyrhizobium japonicum strains in soybean in Iowa soils was found that the introduced strains have different competitive ability (Berg and Logenachan, 1988).

Compared with other cultivars, the highest mean nitrogen fixation was observed in Pinto bean COS16 (Figure 1). This suggests that a better symbiosis between varieties and nitrogen fixing bacteria exist. The results also show that the highest rate of nitrogen fixation in all varieties of beans, derived from strain Rb117 and the lowest nitrogen fixation derived from N100 treatment. In all three varieties, fertilizer treatments in conditions due to nitrate poisoning of mineral properties in nitrogen fixation, nodules and nitrogen fixation rate of production are reduced.

Results Comparison table (Table 2) shows that the highest number of nodes is obtained and Minimum number of nodes is obtained from N100 treatment. So that the inoculums Rb117 (mean 9 / 55 pieces), the largest number of nodes can be obtained. Minimum number of nodes related to the treatment N100 and control respectively mean 5 / 13 numbers and 6 / 26 numbers. Results of a field experiment in six regions of Victoria, Australia, showed that grain yield in

all inoculation treatments has increased (Carter et al., 1994). Inoculation caused a significant increase in the nodes number and weight compared to the control, so that the two strains L-51 and L-100 respectively, creating the highest number (respectively 5 / 62 numbers for the L-51 and 8 / 58 numbers for L-100) and node weights, the ultimate strains of the 10 strains collected from different regions were identified (Assadi-Rahmani et al., 1999).

Inoculation treatments on beans in the Khuzestan province, showed seed yield increasing between 35 to 69 percent compared to controls (Khosravi et al., 2001). Bacterial inoculum used in soybeans led twice increased in grain yield than the maximum fertilizer treatments and compared with the controls has increased 10 times (Duong et al., 1984). Review the various strains showed that bean varieties in terms of yield, seed number per pod, pods, 100 seed weight, biological yield, LAI and dry weight of pods per square meter achieved very significant difference, while the Grain harvest index was not significantly different result (Ghasemi-Pirbaloti et al., 2003). Based on two-year project, can be said that the use of native strains due to adapting to soil conditions, to be effective in nitrogen fixation in bean and the ability to better compete with other bacteria, is more preferable.

Results showed that the grain's percent of protein and nitrogen, in the inoculation treatments are higher than control.

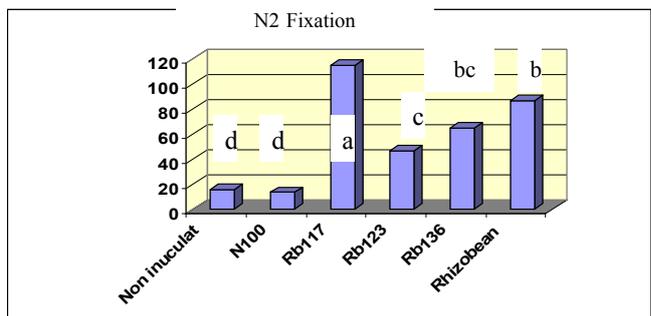


Figure1. Effect of different strains on nitrogen fixation.

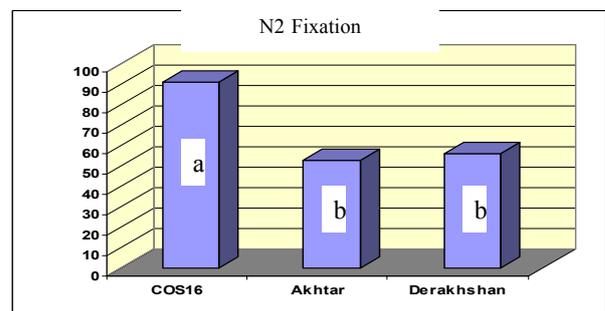


Figure 3. Effect of cultivars level on N2 fixation.

Table1: Complex analysis of variance of seed and protein Yield, Node number and dry weight and N fixation.

N fixation /ha	Nodules dry weight	Nodules numbers	Protein Yield	Seed yield	df	S.O.V
2086.2*	22594.0**	877.2*	31476.0**	592889**	1	(Y)
4784.1**	939.6**	283.05 ^{n.s}	13147.7	303392.7**	4	(R) YEAR
20892.5**	413101.8**	4063.97**	273534/0**	3651492.2**	5	Inoculation A
16374.4**	10415.1**	784.9**	12492.6**	2737721**	2	Varieties B
762.4*	1307.24**	74.1 ^{n.s}	18219**	437916.9**	10	A×B
84.5 ^{n.s}	1111.21**	56.04 ^{n.s}	2792 ^{n.s}	50110 ^{n.s}	5	Y×A
193.42 ^{n.s}	712.79*	22.420 ^{n.s}	17.57 ^{n.s}	19034.3 ^{n.s}	2	Y×B
6602.9 ^{n.s}	256.35 ^{n.s}	83.48 ^{n.s}	2467.0 ^{n.s}	13543.7 ^{n.s}	10	Y×A×B
275.7	187.15	126.7	3126.0	25971.7	68	E
12.17	13.3	30.08	7.7	5.3		(%) CV

Ns: Non-significant

* and **: Significant at 5% and 1% probability levels respectively.

Table 2- Mean comparison of seed yield (kg), Number and dry weight of nodules and Fixation (kg/ha).

N fixation /ha	Node number Per plant	Node dry weight	Node dry weight	Seed yield	
62.3 b	34.6 b	88.5b	88.5b	2973.54 b	Year
71.1 a	40.3 a	117.3a	117.3a	3121.7 a	2006
					2007
					Inoculation
15.8 e	26.65 c	56.8e	56.8e	2230.44 e	N0
13.7 e	13.5 d	39.6f	39.6f	3198.8 b	N100
115.8a	55.9 a	166.8 a	166.8 a	3557.71 a	Rb117
47.2 d	40.36 b	99.5d	99.5d	2947.4 d	Rb123
64.8 c	42.97 b	122.7c	122.7c	3074.3 c	Rb136
86.9 b	45.0 b	132.0 b	132.0 b	3277.9 b	Rhizobean Varieties
91.2 a	42.8 a	121.8 a	121.8 a	3308a	COS16
52.9b	34.7 b	88.9 c	88.9 c	2758.7 c	Akhtar
56.0b	34.8 b	98.0 b	98.0 b	3076 b	Derakhshan

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