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The Effect of *Rhizobium* spp. Inoculation on Seed Quality of Bean in Turkey

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Abstract: *Rhizobium* spp. (local isolate) was used to inoculate three bean (*Phaseolus vulgaris* L.) varieties; Akman 98, Göynük 98 and Şehirali 90, in a factorial field experiment. The objective of the experiment was to study effect of inoculation on seed yield, protein range, seed weight, non-soaker capacity and hydration index. *Rhizobium* spp. inoculation and nitrogen fertilizer significantly increased characters tested. This study revealed that inoculation with *Rhizobium* spp. improved seed quality.

Key words: Bean (*Phaseolus vulgaris* L.), *Rhizobium* spp., hydration index, non-soaker capacity

INTRODUCTION

In present rapidly developing world, a significant part of the population feeds on grains that lack enough protein. Among the sources of vegetable proteins, the highest amount of protein/seed is found in legumes, while among the grains the dry bean, which is rich in A, B and D vitamins, stands above the others with its protein content of between 17-35% (Graham *et al*., 2003). Alongside human nutrition, dry beans also play an important role due to its addition of nitrogen to the soil (Graham *et al*., 2003).

Despite over two hundred years of bean cultivation in Anatolia still more efforts need to overcome some problems of production and yield (Anonymous, 2000). One of the efforts contribute to increase the yield of beans was the inoculation with suitable *Rhizobium* bacteria (Slattery *et al*., 2004; Graham *et al*., 2003; Struz *et al*., 2000). However, it has been pointed out that Anatolia, not being origin of beans, does not have effective bacteria in its soils (Önder and Özkaynak, 1994). Bean is one of the major grain legume crops in Turkey (Anonymous, 2000) and many other parts of the world (Slattery *et al*., 2004; Graham *et al*., 2003). In the last seventy year period (1928-1998) growing area has reached to 171,000 hectares from 69,000 hectares (248% increase), production with an increase of 307.6% has reached 240,000 tons from 78,000 tons, while yield has reached 1415 from 724 kg ha^{-1} in this period (Küçük *et al.,* 2006). Among the legumes produced in Turkey, chickpeas, lentils and beans hold the most important place and account for 44, 35 and 18% respectively of the total legume production in Turkey (Anonymous, 2000).

Dry beans cover substantial farming areas in Turkey and are an essential part of the nutrition. Thus, this study was conducted with an aim to determine the effects of bacterial inoculation and application of nitrogen fertilizers on the yield of different varieties of dry beans and on seed quality of bean varieties under Central Anatolian conditions, Turkey.

MATERIALS AND METHODS

The three common bean varieties (Göynük 98, Akman 98, Şehirali 90) used in the research were obtained from the Anatolian Agricultural Research Institute, Turkey. As for the inoculating materials, *Rhizobium* spp. local isolate of the produced beans was obtained from the culture collection Department of the Biology, Faculty of Sciences, Anadolu University (Eskişehir, Turkey).

This research was conducted in Eskişehir, semiarid Central Anatolian Region of Turkey at a field during the growing seasons of 2002 and 2003. Eskişehir is located at 39° 48'N, 30° 31'E at an elevation of 789 m above the sea level. The soil texture was sandly loam with pH 7.75. The experimental design was factorial experimental with four replications. The total precipitation measured on the research area was 373.55 mm in 2002 and 324.4 mm in 2003 while the relative humidity measured was 61.7% in 2002 and 60% in 2003.

Rhizobium spp. incubated in Yeast Extract Mannitol Broth was grown and mixed in a ratio of 1: 1 with a sterilized carrier material (peat) and was then incubated at 28°C for four days (Daza *et al*., 2000). The number of live *Rhizobium* spp. in the inoculant was adjusted to 10^8. Seed inoculation was done at planting with *Rhizobium* spp.

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inoculant. Seeds were hand planted on 18 May 2002 and 14 May 2003 at a rate of 30 seeds/m of row. Plots were 5 m long and consisted of four rows 50 cm apart.

After planting the field was irrigated to ensure uniform germination. Irrigation was repeated during the growing season as necessary. Since no harm or diseases were seen on the plants in either of the two years of the experiment, no chemical treatment was applied.

The experiment 80 kg ha^{-1} P_2O_5 ha^{-1} as super phosphate (TSP) was broadcast applied and incorporated before planting. Two levels of the *Rhizobium* spp. [control (B_0) and inoculant (B_1)] and two levels of the nitrogen fertilizer [0 as control (N_0) and 40 kg ha^{-1} (N_1)]. Ammonium nitrate (33%) was used as the nitrogen source. The nitrogen-fertilized plots were set up as only with nitrogen fertilizer while others with nitrogen fertilizer and inoculated with bacterial culture.

At harvest, the and 1000 seed weight (g), protein range (%) and seed yield (kg da^{-1}) were estimated. Seed yield was determined by harvesting the four central rows, each 2 m long, from each plot.

For each treatment these seeds were selected at random and soaked in 150 mL distilled water for 16 h. The non-soakers capacities, 100 seed weight and hydration index of seeds were recorded according to Abdelgani *et al*. (1999).

In the experiment set up using the factorial design, the data acquired after the measuring, weighing, counting and analyzing was evaluated using the MSTAT-C computer program.

RESULTS AND DISCUSSION

Seed yield and 1000 seed weight were higher in inoculation with *Rhizobium* spp. than control (Table 1). In both years, inoculation increased seed yield and 1000 seed applicated at nitrogen level. Seed yield and 1000 seed weight were difference among varieties. Seed yield ranged from 150.7 to 420.5 kg da^{-1}. Göynük 98 bean variety produced more seed yield than other varieties and it was statistical significant (Table 2). Probably, this difference has the genetic backgrounds. Several investigators reported increased seed yield by using nitrogen fertilizers (Elsheikh and Ibrahim, 1999; Sturz *et al*., 2000; Shisanya, 2002). The lack of response to nitrogen application in this study (control) indicates that the initial nitrogen status of the soil used was adequate to meet the needs of the bean varieties. However, inoculation was necessary for maximum seed yield which were not realized by supplemental nitrogen only. Similar results were reported by Mostasso *et al*. (2002).

Rhizobium spp. significantly ($p = 0.01$) increased protein range (%) (Table 2) comparison the noninoculated treatment whereas Göynük 98, Şehirali 90 and Akman 98 bean varieties, increased the protein content over the control (Table 1). The protein range (%) increased from 15.07-25.82 (Table 1). There was a significant variety, *Rhizobium*, nitrogen, year x nitrogen, year x variety, variety x *Rhizobium*, variety x nitrogen, nitrogen x *Rhizobium* interaction and year x variety x nitrogen interaction ($p = 0.01$) (Table 2). Barran *et al*. (1999) reported that inoculation of *Rhizobium etli* increased protein and nitrogen content of bean. This could probably be attributed to be increase in the nitrogen fixing efficiency of inoculated plants where more nitrogen is fixed and translocated to the seeds. *Rhizobium* spp. inoculation has been reported to increase seed protein range of soybean (Koutroubas *et al*., 1998) faba bean (Cordovilla *et al*., 1999) and bean (Mostasso *et al*., 2002).

The inoculation of bean varieties with nitrogen fertilizer and *Rhizobium* spp. inoculation resulted in successful growth and a significant improvement in

Table 1: Effect of *Rhizobium* spp. inoculation and nitrogen fertilizer on 1000 seed weight (g), seed yield (kg da^{-1}) and protein range (%) of three bean varieties

	Characteristics					
	1000 seed weight (g)		Seed yield (kg da^{-1})		Protein range (%)	
Treatments	2002	2003	2002	2003	2002	2003
V_1 N_0 B_0	512.0	493.5	240.50	195.7	18.69	16.81
V_2 N_0 B_0	315.6	306.3	156.60	150.7	17.70	15.07
V_3 N_0 B_0	366.8	350.5	197.20	186.6	18.20	16.72
V_1 B_1 N_0	580.0	572.3	321.05	309.6	25.82	24.13
V_2 B_1 N_0	351.8	346.0	310.50	284.9	21.76	19.06
V_3 B_1 N_0	575.5	567.3	309.80	272.4	22.13	20.98
V_1 B_0 N_1	538.3	527.0	307.50	292.1	21.04	20.09
V_2 B_0 N_1	330.5	320.0	306.10	272.9	20.12	20.09
V_3 B_0 N_1	491.0	472.0	307.50	288.1	20.00	20.16
V_1 B_1 N_1	595.3	587.8	420.50	386.2	25.82	24.13
V_2 B_1 N_1	367.8	358.5	324.00	292.1	22.56	21.98
V_3 B_1 N_1	582.8	575.2	360.60	338.4	22.59	22.21

V: Variety (V_1: Göynük 98; V_2: Akman 98; V_3: Şehirali 90), N: Nitrogen fertilizer (N_0: nonfertilizer; N_1: Nitrogen fertilizer), B: *Rhizobium* spp. (B_0: uninoculation; B_1: Inoculation)

Table 2: Results of analysis of variance (mean squares)

Source of variation	Degrees of freedom	1000 seed weight	Seed yield	Protein range
Year (Y)	1	2857.98**	14215.53**	37.86338**
Replication	6	22.55	25.752	0.206
Variety (V)	2	396140.20**	17701.30**	44.50**
Nitrogen (N)	1	27818.85**	153664.015**	93.75330**
Rhizobium (R)	1	179072.65**	176250.62**	390.54768**
Y×V	2	33.69	33.635	1.70**
Y×N	1	0.09	71.76	10.91476**
Y×R	1	250.26**	189.28*	0.28930
V×N	2	5971.87**	306.11**	4.24**
V×R	2	29927.21**	1357.80**	19.10**
N×R	1	11245.01**	18509.26**	27.68128**
Y×V×N	2	20.53	271.53**	1.80**
Y×V×R	2	27.41	262.49**	0.028
Y×N×R	1	5.90	8.64	0.89128
V×N×R	2	7881.84**	8765.60**	0.68
Y×V×V×R	2	13.57	937.63**	0.035
Error	66	27.40	28.40	0.3060
Total	95			
LSD_{Year}		2.13	2.16	0.22
$LSD_{Variety}$		2.61	2.66	0.27
$LSD_{Nitrogen}$		2.13	2.16	0.22
$LSD_{Rhizobium}$		2.13	2.16	0.22
CV (%)		1.13	1.86	2.77

**significant at the 0.05 and 0.01 probability levels, respectively

Table 3: Effect of nitrogen fertilization and *Rhizobium* spp. inoculation on hydration index, nonsoakers capacity and 100 seed weight (two years)

	Treatments					
	Hydration index		Nonsoakers capacity		100 seed wet weight (g)	
Treatments	2002	2003	2002	2003	2002	2003
$V_1 N_0 B_0$	0.82±0.50	0.79±0.35	1.00±0.10	0.82±0.09	91.85±0.03	84.72±0.03
$V_2 N_0 B_0$	2.00±0.05	0.68±0.36	0.52±0.13	0.45±0.09	59.79±0.07	55.12±0.05
$V_3 N_0 B_0$	1.11±0.58	1.00±0.30	0.78±0.09	0.68±0.09	94.84±0.04	90.41±0.04
$V_1 B_1 N_0$	0.63±0.10	0.61±0.26	2.40±0.04	2.20±0.05	96.00±0.01	90.12±0.02
$V_2 B_1 N_0$	0.43±0.06	0.42±0.18	0.90±0.03	0.70±0.03	65.65±0.02	62.17±0.01
$V_3 B_1 N_0$	0.72±0.03	0.71±0.20	1.00±0.03	1.00±0.01	98.65±0.01	92.48±0.01
$V_1 B_0 N_1$	1.17±0.02	1.17±0.17	1.82±0.02	1.75±0.01	94.85±0.03	87.04±0.02
$V_2 B_0 N_1$	0.50±0.04	0.49±0.15	0.80±0.05	0.82±0.02	79.13±0.02	75.10±0.01
$V_3 B_0 N_1$	0.79±0.07	0.78±0.12	0.92±0.02	0.88±0.01	97.91±0.03	90.00±0.02
$V_1 B_1 N_1$	0.67±0.04	0.67±0.11	2.40±0.01	2.00±0.01	111.50±0.02	98.12±0.01
$V_2 B_1 N_1$	0.45±0.03	0.43±0.17	0.88±0.01	0.86±0.01	91.85±0.01	87.14±0.01
$V_3 B_1 N_1$	0.75±0.09	0.74±0.14	1.32±0.02	1.25±0.02	101.20±0.01	92.68±0.02

Values are means ±SD. V: Variety (V_1: Göynük 98; V_2: Akman 98; V_3: Þehirali 90). N: Nitrogen fertilizer (N_o: nonfertilizer; N_1: Nitrogen fertilizer) B: *Rhizobium* spp. (B_o: uninoculation; B_1: Inoculation)

100 seed wet weight (Table 3). *Rhizobium* spp. inoculation significantly increased 100 seed wet weight in Göynük 98 (V_1) bean variety compared to the control. The 100 seed wet weight was effected by inoculation and nitrogen fertilization in both of Þehirali 90 (V_3) and Akman 98 (V_2), respectively. The inoculated plants with *Rhizobium* spp. produced more seed weight than control (Table 3).

Similar results were obtained that Shisanya (2002) found a significant increase in inoculated bean with *Rhizobium* strains and inoculation was found to increase 100 seed wet weight of guar by Elsheikh and Ibrahim (1999). The area had not been cultivated with beans for 10 years. The selected *Rhizobium* spp. local strain resulted in statistically significant increases in 100 seed wet weight (Table 3). These results are similar to previous reports for different soils (Pacovsky *et al*., 1983; Hungria *et al*., 2000) in which inoculation of the bean crop can result in yield increases at low cost to the farmer.

Treatments had significantly effect on the non-soakers capacities of the three varieties (Table 3). The non-soaker capacity ranges from 0.52-0.92. In this investigation, high levels of non-soakers capacity were observed in the *Rhizobium* inoculation treatments (Table 3). Similar results were obtained with faba bean (Elsheikh and Elzidany, 1997; Abdelgani *et al*., 1999). Treatments were found to effect the hydration index of bean varieties significantly. In this investigation, high levels of hydration index were observed in nitrogen fertilizer and *Rhizobium* inoculation treatments (Table 3). The hydration index ranges from 0.10-0.82. Generally, hydration index is a very valuable quality factor for

consumers and a hydration index indicates that the bean varieties are capable of absorbing water very efficiently. Bean (*Phaseolus vulgaris* L.) is one of the major sources of protein in Turkey, efficient local isolates should be used to increase the seed yield and to improve seed protein range. Plant variety, fertilizer application, type of the irrigation, soil, sowing season and time of harvest influence effectively on the quality seed (Abdelgani *et al*., 1999).

In conclusion, to the best of our knowledge this is the first report for inoculation with *Rhizobium* spp. significantly affected the 100 seed weight, non-soaker capacity and hydration index on bean varieties in Turkey. Inoculation with local *Rhizobium* spp. strain significantly affected the 100 seed weight and seed quality of bean varieties.

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