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Inoculation with *Rhizobium* spp. in kidney bean (*Phaseolus vulgaris* L.) varieties

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Abstract

The current research was conducted to determine the effects of *Rhizobium* spp. inoculation and nitrogen applications (control, inoculation with *Rhizobium*, inoculation with *Rhizobium* + nitrogen fertilization, nitrogen fertilization) on bean (*Phaseolus vulgaris* L.) varieties ('Göynük 98', 'Akman 98', 'Şehirali 90') in the ecological conditions of Central Anatolian in Turkey. The research was arranged in a factorial design with four replications. The results from the two (2001–2002 and 2002–2003) years' study showed that inoculation with *Rhizobium* spp. and nitrogen treatment affected the grain yield and 1000 grain yield, the inoculation with *Rhizobium* spp. affected protein yield. As average of the two years, the protein yields from inoculated with *Rhizobium* spp. treatments were 80.1 kg (for 'Göynük 98'), 63.9 kg (for 'Şehirali 90') and 59.8 kg (for 'Akman 98'), respectively. The activity of leghemoglobin and glutamine synthetase in nodules differed between treatments and varieties.

Key words: bean, *Rhizobium*, yield, leghaemoglobin, glutamine synthetase.

Introduction

In our rapidly developing world, a significant part of the population feeds on grains that lack enough protein (Anonymous, 2008). Among the sources of vegetable proteins, the highest amount of protein per seed is found in legumes, while among the grains, in the kidney bean, which is rich in A, B and D vitamins, and protein content of between 17–35% (Piha, Munns, 1987). Alongside human nutrition, kidney beans also play an important role due to its addition of nitrogen to the soil (Pena-Cabriales et al., 1993; Graham, Ramalli, 1997; Graham et al., 2003).

Despite over two hundred years of bean cultivation in Anatolia, still more efforts are needed to overcome some problems of production and yield. One of the efforts contribute to increase the yield of beans was the inoculation with suitable *Rhizobium* bacteria (Graham, 1981; Somasegaran, Hoben, 1985; Sturz et al., 2000; Slattery et al., 2004). There are no effective bacteria in the soil because Anatolia is not the place of origin of the bean (Önder, Özkaynak, 1994). Over the eighty-one year period (1928–2009), the growing area has reached to 94928 hectares from 69000 hectares (137% increase), production with an increase of 361.5% has reached 282000 tons from 78000 tons, while yield has reached 1415 kg ha^{-1} from 724 kg ha^{-1} in this period (Şehirali et al., 2010).

In 2007, among the legumes produced in Turkey, chickpeas, lentils and beans hold the most important place and account for 41.4%, 43.8%, and 12.6% respectively of the total legume production in Turkey (Anonymous, 2008).

In legume root nodules, glutamine synthetase activity has a universal role in the nitrogen metabolism (Gordon, 1991). Billard and Boucaud (1980) and Gordon et al. (1999) observed that leghaemoglobin, glutamine synthetase and sucrose synthetase levels are independent of plant growth and environmental conditions.

Kidney beans cover substantial farming areas in Turkey and are an essential part of the nutrition. Thus, the current study was conducted with an aim of determining the effects of bacterial inoculation and application of nitrogen fertilizers on the yield of different varieties of kidney beans and on some yield components under Central Anatolian conditions (Eskişehir) in Turkey.

Materials and methods

Bean varieties and inoculants. The three bean varieties ('Göynük 98', 'Akman 98', 'Şehirali 90') used in the research were obtained from the Anatolian Agricultural Research Institute (Eskişehir, Turkey).

As for the inoculating materials, *Rhizobium* spp. was used from the culture collection of the Biology Department, Faculty of Sciences, Anadolu University (Eskişehir, Turkey). Local strain of *Rhizobium* used in this study was isolated from root nodules of bean plant (Küçük et al., 2006). *Rhizobium* spp. was grown in yeast mannitol extract broth and mixed in a ratio of 1:1 with a sterilized carrier material (perlite), and was then incubated at 28ºC for four days (Vincent, 1970; Daza et al., 2000). The number of live *Rhizobium* spp. in the inoculants was adjusted to 10^8 cell g^{-1}.

Study site. The research was carried out during 2002 and 2003 in Eskişehir (39º 48′ N, 30º 31′ E, 789 m elevation). Average temperatures of bean germination and growing periods were 19ºC in 2002 and 19.5ºC in 2003. Rainfall was differently distributed, in 2002 (141.8 mm) and 2003 (59.1 mm). The soil properties of the experimental area: low organic matter ratio, soil reaction slightly alkaline, low salt ratio, poor in lime, sandy, clay and loamy in composition (Table 1). The soils of the experimental sites were poor in organic matter, thus low in N supply to plants.

Table 1. Characteristics of the experimental area

Soil properties	Year	
	2002	2003
pH	7.75	7.60
$CaCO_3$	1.79	1.02
Soil texture	Sandy loam	Sandy loam
Organic mater %	0.76	2.07
P_2O_5 kg da^{-1}	26	18.50
K_2O kg da^{-1}	100.8	104.10
EC	0.037	0.064

Field experiment. The trials were set up in experimental fields of the Faculty of Agriculture, Eskişehir Osmangazi University, as 4 rows by 5 meter plots of four replications in accordance with the factorial experimental design (Düzgüneş et al., 1987). The characteristics of the soil are shown in Table 1.

The experimental area was first ploughed and the phosphorous requirements of plants were uniformly applied to all plots as triple super phosphate (TSP) (80 kg ha^{-1} P_2O_5). Nitrogen fertilization (0 or 40 kg ha^{-1}) after sowing was applied to plots as ammonium nitrate (33%). The nitrogen fertilized plots were set up as only with nitrogen fertilizer while others with nitrogen fertilizer and inoculated with *Rhizobium* spp. Before planting, beans for inoculation treatments were rinsed in 10% sucrose solution to help the inoculant carrier material to stick on the seeds (Daza et al., 2000).

The amount of seeds required for each plot (220 g $plot^{-1}$) was calculated. In both studies, the sowing was done in May (on 18th May in 2002 and 14th May in 2003) at correct soil temperature. Throughout the growing period in the experiment, the amount of water in the soil and the plants' water requirements were applied three times in 2002 and five times in 2003. Since no harm or diseases were seen on the plants in either of the two years of the experiment, no chemical treatment was applied. In each of the two years, the grain yield, 1000 grain weight, plant dry weight, protein yield and some morphological properties (plant height, pod number, number of seed per pod) were determined (Nelson, Sommers, 1973; Önder, Özkaynak, 1994; Barron et al., 1999).

Leghaemoglobin, glutamine synthetase and nodule protein. Nodules were homogenized in a mortar and pestle with 25 mM phosphate buffer (pH 7.0) and 1 mM $CaCI_2 \cdot 2H_2O$. The homogenate was centrifuged for 30 min at 20 000 rpm at 4ºC. Samples of the supernatant were immediately assayed for leghaemoglobin, glutamine synthetase and nodule protein (Gordon, 1991; Ceccatto et al., 1998). The glutamine synthetase for the reaction mixture contained 0.2 ml of 250 mM Tris HCl, pH 7.2; 0.2 ml of 30 mM ATP, pH 7.0; 0.2 ml of 500 mM $MgSO_4$; 0.2 ml 300 mM glutamate, pH 7.0; 0.25 ml supernatant. Reading was performed in a spectrophotometer at 540 nm (Ceccatto et al., 1998). The data were expressed in μM gama glutamyl hydroxamate mg $protein^{-1}$ h^{-1}. For leghaemoglobin levels, the supernatants samples were centrifuged at 20 000 rpm for 30 min to produce a 5 ml sample to give 5 ml of extraction in Drabkin's solution. The samples were mixed and centrifuged at 20 000 rpm for 30 min. The supernatant was read at 540 nm in a spectrophotometer (Ceccatto et al., 1998). The results are expressed in mg leghaemoglobin mg $protein^{-1}$. Total protein for samples, 5 μl supernatant; 100 mg of Coomassie Brillant Blue G 250 were dissolved in 50 ml of 95% ethanol; 100 ml of 85% phosphoric acid were added, and the solution was diluted with distilled water to make 1 litre. The mixture was read at 595 nm in a spectrophotometer (Ceccatto et al., 1998).

Statistical analysis. The data were subjected to analysis of variance using the MSTAT-C (version 1.4, USA) computer program. The Students-t test was used as the criterion in checking the importance of the applications and comparison of the averages.

Results and discussion

Alongside the bacteria inoculation, the nitrogen dose given also showed a difference in the yield. In the applications, differences were seen between the varieties. The highest average yield was obtained from 'Göynük 98' (4030 kg ha^{-1}). It was followed by 'Şehirali 90' and 'Akman 98', respectively (Table 2).

Table 2. Grain yield, 1000 grain weight, protein yield in the inoculated and nitrogen applied (N4)* common bean varieties

Treatments	Varieties			Mean
	'Göynük 98'	'Akman 98'	'Şehirali 90'	
1000 grain weight g				
Control	503 e	311 j	359 g	391 ± 0.02
Inoculation	576 bc	349 h	571 c	499 ± 0.01
Inoculation x N4	591.6 a	363 g	579 b	511 ± 0.01
N4	533 d	325 ı	482 f	446 ± 0.03
Grain yield kg ha^{-1}				
Control	2180 g	1540 ı	1920 h	1880 ± 0.03
Inoculation	3150 c	2980 e	2910 f	3010 ± 0.01
Inoculation x N4	4030 a	3080 d	3500 b	3540 ± 0.04
N4	3000 e	2890 f	2980 e	2960 ± 0.01
Protein yield kg ha^{-1}				
Control	51.6 d	42.6 e	43.2 e	45.8 ± 0.01
Inoculation	80.1 a	59.8 c	63.9 b	67.9 ± 0.01
Inoculation x N4	68.45c	46.65e	57.05d	57.3 ± 0.04
N4	49.38e	38.4f	41.25e	43.01 ± 0.03
Plant dry weight g $plant^{-1}$				
Control	6.13 h	5.47 h	5.02 h	5.53 ± 0.02
Inoculation	20.3 bc	18.9 cde	18.6 def	19.3 ± 0.01
Inoculationx N4	21.78 a	21.07 ab	20.88 cd	21.24 ± 0.01
N4	17.63 efg	17.14 fg	16.69 g	17.15 ± 0.01

Note. *N4 – 40 kg ha^{-1} ammonium nitrate (33%).

With respect to the combined results of the two years, the Student's-t test was done to determine the differences that resulted from inoculation with the *Rhizobium* spp. With respect to this, of all the characteristics investigated in the three bean varieties, the highest values were obtained from the inoculated 'Göynük 98'. Among the bean varieties investigated 'Göynük 98', which produced the highest grain yield in both experimental years was one of the most common bean varieties grown in the region.

During the experiment, the grain yield was significantly affected by variety, nitrogen, inoculation, variety x nitrogen, variety x inoculation, nitrogen x inoculation, variety x nitrogen x inoculation and year x variety x nitrogen x inoculation interactions and by the years (Table 3).

In each of the two experimental years of the experiment, the field trials without nitrogen and with inoculation were found to be effective for the grain yield. The difference between the highest and the lowest grain yield was 2490 kg ha^{-1}. Grain yield was significantly affected by the inoculation and nitrogen fertilization and by varieties. Similar findings were reported for bean (Sangakhara, Marambe 1989; Pinto et al., 2007; Nleya et al., 2009; Ramirez-Bahena et al., 2009). Popescu (1998) and Mnasri et al. (2007) determined that bacterial inoculation was not only important in nodulation but was also one of the most important factors in increasing seed yield. Also, the total dry matter weight was higher in 'Göynük 98' than in the other varieties (Table 2). Dry matter weight was affected ($P = 0.01$) by inoculation, inoculation and nitrogen, year x inoculation interaction, variety, year and nitrogen treatments (Table 3). The present data suggest that it is possible to select rhizobial inoculants with good field effectiveness and stability.

The values of the protein yield obtained from 'Akman 98', 'Göynük 98' and 'Şehirali 90' in the years 2002 and 2003 with *Rhizobium* inoculation and two nitrogen doses (N0 and N4) application are provided in Table 2. According to the com-

Table 3. Source of variation and mean squares for treatments of common bean varieties grown in Eskişehir, Turkey, during 2001–2002 and 2002–2003

Sources of variation	df	1000 grain weight	Grain yield	Plant dry weight	Protein yield
Year (y)	1	2857.9**	14215.53**	107.8**	11616**
Replication	6	22.55	25.75	1.9	2.47
Variety (v)	2	396140.2**	17701.3**	19.5**	1972.91**
Nitrogen (n)	1	27818.85**	153664.01**	1025.5**	3574.60**
Inoculation (i)	1	179072.65**	176250.62**	1786.6**	11757.30**
y x v	2	33.69	33.64	1.28	165.50**
y x n	1	0.09	71.76	6.05	306.02**
y x i	1	250.26**	189.28*	26.4**	475.26**
v x n	2	5971.87**	306.11**	1.13	18.96**
v x i	2	29927.21**	1357.8**	2.83	267.53**
n x i	1	11245.01**	18509.26**	628.9**	1641.76**
y x v x n	2	20.53	271.53**	2.59	0.802
y x v x i	2	27.41	262.49**	2.87	50.431**
y x n x i	1	5.90	8.64	0.74	591.03**
v x n x i	2	7881.84**	8765.6**	1.121	6.94
y x v x n x i	2	13.57	937.63**	2.38	7.79
Error	66	27.4	28.4	1.98	3.74
CV %		1.13	1.86	9	3.40
LSD (y)		2.13	2.16	0.57	0.78
LSD (v)		2.61	2.66	0.70	0.96
LSD (n)		2.13	2.16	0.57	0.78
LSD (i)		2.13	2.16	0.57	0.78

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

bined data of both years, 'Göynük 98' with a protein yield of 80.1 kg ha^{-1} topped the list while 'Akman 98' was at the bottom with a protein yield of 38.4 kg ha^{-1} (Table 2). In the year x variety x inoculation interaction, the effects of inoculation on protein yield in the three bean varieties in our study was determined to be effective. The highest protein yield in both years from the inoculated varieties was obtained from the plots of inoculated beans and nitrogen fertilizers used. Hungria and Neves (1987 a and b), Barron et al. (1999), Mostasso et al. (2002) and Elbanna et al. (2009) indicate that *Rhizobium* inoculation increased protein yield, however, application of nitrogen fertilizers on inoculated plots gave even better results. While Slattery et al. (2004) determined that lower content of the active *Rhizobium* bacteria in plant roots resulted in lower yield. The findings of both experimental years showed that the protein yields from the inoculated and nitrogen fertilized bean varieties were higher than those without inoculation, which agreed with our results. In the experimental years, the interactions of the year, varieties, nitrogen, inoculation, variety x nitrogen, variety x inoculation and variety x nitrogen x inoculation were different for the 1000 grain weight. Furthermore, the applications without nitrogen and with inoculation were found to be effective, the 1000 grain weight of 'Göynük 98' was the highest (576 g) and was followed by 'Şehirali 90' (571 g) and 'Akman 98' (349 g), respectively (Table 2).

From the combined data of the two years, it was determined that the tallest plants among the varieties were of 'Akman 98' with 64.9 cm, followed by 'Göynük 98' (63.8 cm) and 'Şehirali 90' (58.2 cm) (Table 4).

The bean varieties used in our experiment being dwarf, showed differences their heights in relation to genotype, applications and years. The highest average plant height in 2002 was observed in bacteria inoculated 'Göynük 98' and 'Akman 98', while in 2003 bacteria inoculated and nitrogen applied 'Göynük 98' topped the others. The lowest average plant height in both years was showed by 'Akman 98' from the control plots. Plant height differences were small between the years. This being related to ecological factors, the reason for the highest average could be associated with the higher amount of rainfall in 2002. The variance analysis results of the plant height of the bean varieties with inoculation and different nitrogen doses are presented in Table 5. In both experimental years, the different plant heights were significantly affected by the nitrogen, inoculation, variety x inoculation, variety x inoculation, nitrogen x inoculation and variety x nitrogen x inoculation interactions and the years.

Table 4. Plant height, pod number, seed number per pod in the inoculated and nitrogen applied (N4)* bean varieties

Characters	Varieties	Treatments			
		Control	Inoculation	Inoculation + N4	N4
Plant height cm	‘Göynük 98’	52.5 h	63.8 a	63.7 b	54 g
	‘Akman 98’	49.2 j	64.9 b	60.7 c	55.5 f
	‘Şehirali 90’	50.7 ı	58.2 d	58.9 d	56.8 e
	Mean	50.8 ± 0.03	62.3 ± 0.02	61.1 ± 0.01	55.4 ± 0.01
Pod number	‘Göynük 98’	14.1 h	20.1 a	22 bc	21 de
	‘Akman 98’	13.4 ı	21.5 cd	20.4 ef	18.8 g
	‘Şehirali 90’	11.4 j	22.8 f	22.5 ab	20.5 ef
	Mean	13±0.02	21.5 ± 0.03	21.6 ± 0.01	20.1 ± 0.01
Seed number of pod	‘Göynük 98’	4.15 cd	4.03 d	4.55 a	3.87 g
	‘Akman 98’	4.33 e	3.81 g	4.12 b	3.61 h
	‘Şehirali 90’	2.88 j	3.95 f	4.19 c	3.47 ı
	Mean	3.78 ± 0.03	3.93 ± 0.03	4.29 ± 0.01	3.65 ± 0.02

Note. *N4 – 40 kg ha^{-1} ammonium nitrate (33%).

The bacterial inoculation with its considerable positive effects showed increases in pod number per plant when compared to the control. In both experimental years, the number of pods per plant displayed differences depending on the nitrogen levels. The highest pod number per plant in the three varieties was showed by ‘Şehirali 90’ (22.8 pods) followed by ‘Göynük 98’ (20.1 pods) and ‘Akman 98’ (21.5 pods). The different groups made up from the averages are summarized in Table 3. According to the results obtained from variance analysis of nitrogen doses and inoculation, the year, variety, nitrogen, inoculation, variety x nitrogen, variety x inoculation, nitrogen x inoculation and variety x nitrogen x inoculation interactions showed significant differences in the number of pods per plant (Table 5). Year x variety and year x variety x nitrogen interactions were found to be 5% significant. In both experimental years, in terms of pod number per plant, applications with bacteria inoculation and without nitrogen were found to be effective (Table 5).

The highest seed number per pod was obtained from ‘Göynük 98’ (4.55) followed by ‘Akman 98’ (4.33) and ‘Şehirali 90’ (4.19) in both years.

Table 5. Source of variation and mean squares for treatments of common bean varieties

Sources of variation	df	Plant height cm	Pod number	Seed number per pod
Year (y)	1	35.16**	31.51**	5.68**
Replication	6	0.24	0.88	0.004
Variety (v)	2	44.88**	6.78**	2.30**
Nitrogen (n)	1	71.93**	319.01**	0.30**
Inoculation (i)	1	1765.6**	605.01**	3.68**
y x v	2	2.46	1.88*	0.18**
y x n	1	1.38	1.26	1.36**
y x i	1	0.86	1.76	0.01**
v x n	2	16.73**	13.88**	0.78**
v x i	2	87.02**	20.4**	1.70**
n x i	1	205.63**	209.51**	1.44**
y x v x n	2	3.41**	1.82*	0.06**
y x v x i	2	0.02	0.13	0.27**
y x n x i	1	6.15	0.09	0.37**
v x n x i	2	40.34**	9.88**	1.07
y x v x n x i	2	1.72**	1.15**	0.15
Error	66	1.04	0.52	0.004
CV %		1.79	3.78	1.66
LSD (y)		0.42	0.29	0.02
LSD (v)		0.50	0.36	0.032
LSD (n)		0.42	0.29	0.026
LSD (i)		0.42	0.29	0.026

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

According to the results got from the variance analysis of seed number per pod, the year, variety, nitrogen, inoculation, year x variety, year x nitrogen, variety x nitrogen, variety x inoculation, nitrogen x inoculation, year x variety x nitrogen, year x variety x inoculation, year x nitrogen x inoculation, variety x nitrogen x inoculation, year x variety x nitrogen x inoculation interactions and inoculation showed significant differences in number of seed per pod in both experimental years. The glutamine synthetase in the nodule extracts are shown in Table 6. In all inoculation and nitrogen treatments assayed, the glutamine synthetase activity was higher than that in the control treatments. Levels of glutamine synthetase activities were different between treatments (Table 7). The glutamine synthetase activity of variety 'Göynük 98' was higher than that of other varieties (Table 6). The level of glutamine synthetase activities was highest in inoculation and nitrogen (inoculation + N4) treatments. Similar results were obtained by Billard and Boucaud (1980) for bean plants with inoculation.

Table 6. Glutamine synthetase, leghaemoglobin, nodule protein in the inoculated and nitrogen applied (N4)* bean varieties

Characters	Varieties	Treatments			
		Control	Inoculation	Inoculation + N4	N4
Glutamine synthetase (μm gama glutamyl hydroxamate mg protein^{-1} h^{-1})	'Göynük 98'	0.0005 g	3.14 b	3.18 c	0.0001 g
	'Akman 98'	0.0001 g	2.90 e	3.08 c	0 h
	'Şehirali 90'	0.0001 g	2.83 f	3.03 d	0.0003 g
	Mean	0.0003 ± 0.001	2.95 ± 0.02	3.09 ± 0.01	0.0001 ± 0.01
Leghaemoglobin (mg leghaemoglobin mg protein^{-1})	'Göynük 98'	0 f	1.69 a	1.50 b	0 f
	'Akman 98'	0 f	1.48 a	1.35 c	0.0003 f
	'Şehirali 90'	0 f	1.22 c	1.56 e	0 f
	Mean	0 ± 0.00	1.46 ± 0.01	1.33 ± 0.02	0.00008 ± 0.01
Nodule protein content (mg^{-1} mg fresh nodule tissue^{-1})	'Göynük 98'	0.12 f	31.54 a	24.95 b	0.02 f
	'Akman 98'	0.15 f	13.64 e	13.40 e	0.004 f
	'Şehirali 90'	0.08 f	22.25 c	20.81 d	0.006 f
	Mean	0.12 ± 0.03	22.50 ± 0.03	19.70 ± 0.02	0.010 ± 0.02

Note. *N4: 40 kg ha^{-1} ammonium nitrate (33%).

Table 7. Source of variation and mean squares for enzyme activity, leghaemoglobin and nodule protein

Sources of variation	df	Glutamine synthetase	Leghaemoglobin	Nodule protein content
Year (y)	1	7.17**	0.069**	33.01**
Replication	6	0.006	0.0031	1.18
Variety (v)	2	0.110**	0.32**	434.3**
Nitrogen (n)	1	0.13**	0.09**	49.12**
Inoculation (i)	1	219.7**	46.12**	10619.1**
y x v	2	0.018**	0.009*	0.219**
y x n	1	0.0001	0.007	0.035**
y x i	1	7.17**	0.069**	30.73**
v x n	2	0.015**	0.009*	22.61
v x i	2	0.111**	0.32**	435.3**
n x i	1	0.130**	0.096**	42.04**
y x v x n	2	0.002**	0.003	1.62
y x v x i	2	0.018**	0.009*	0.26
y x n x i	1	0.0001	0.0068	0.001
v x n x i	2	0.017**	0.0085*	22.92**
y x v x n x i	2	0.0019**	0.003	1.63
Error	66	0.0016	0.002	1.510
CV %		2.64	6.37	11.60
LSD (y)		0.016	0.018	0.50
LSD (v)		0.020	0.022	0.61
LSD (n)		0.016	0.018	0.50
LSD (i)		0.016	0.018	0.50

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

'Göynük 98' variety showed higher activity (3.18 μM gama glutamyl hydroxamate mg protein^{-1} h^{-1}) than other varieties. Leghaemoglobin levels differed between varieties and treatments (Table 6–7).

'Göynük 98' showed a maximum activity (1.69 mg leghaemoglobin mg protein^{-1}). The higher nodule protein levels were obtained in 31.5 mg^{-1} mg fresh nodule tissue^{-1}, 24.95 mg^{-1} mg fresh nodule tissue^{-1}, 22.25 mg^{-1} mg fresh nodule tissue^{-1} and 20.81 mg^{-1} mg fresh nodule tissue^{-1}, respectively (Table 6), while the nitrogen treatment was less uniform. Concentration of leghaemoglobin was higher in the 'Göynük 98' variety than in the 'Şehirali 90' and 'Akman 98' (Table 6). Total protein content of nodules increased for inoculation treatments (Table 6). Leghaemoglobin and glutamine synthetase were most directly related to plant growth, inoculation and offered opportunity for selection of efficient nitrogen fixing symbioses. Similar observations were reported by other researchers (Gordon, 1991; Ceccatto et al., 1998).

To guarantee the effects of the inoculation, choosing an isolate suitable for the environment and bean variety and using the inoculant material prepared from it will increase the protein yield of the beans.

Conclusion

Since the *Rhizobium* spp., which is effective in the formation of nodules and fixation of nitrogen, show variation depending on the region, choosing a suitable isolate and using the inoculant material prepared from it will increase the yield. In both experimental years, only the plots inoculated with bacteria and applied with nitrogen fertilizer (40 kg ha^{-1}) showed similar results when compared to the control plots, while bacterial inoculant + 40 kg ha^{-1} N applications increased the yield. In order to obtain high seed and protein yield, approximately 40 kg ha^{-1} N with effective *Rhizobium* inoculant, depending on the soil state, could be recommended for similar ecologic conditions.

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Daržinės pupelės (*Phaseolus vulgaris* L.) veislių inokuliavimas *Rhizobium* spp.

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Santrauka

Tyrimai atlikti siekiant nustatyti inokuliavimo *Rhizobium* spp. ir tręšimo azotu (kontrolinis variantas, inokuliavimas *Rhizobium*, inokuliavimas *Rhizobium* + tręšimas azotu) poveikį daržinės pupelės (*Phaseolus vulgaris* L.) veislėms ʽGöynük 98', ʽAkman 98' bei ʽŞehirali 90' ekologinėmis sąlygomis Turkijoje, Centrinėje Anatolijoje. Buvo įrengti kelių veiksnių bandymai su keturiais pakartojimais. Dvejų (2001–2002 ir 2002–2003) metų tyrimų rezultatai parodė, kad inokuliavimas *Rhizobium* spp. ir tręšimas azotu turėjo įtakos grūdų derliui ir 1000-čio grūdų masei, o inokuliavimas *Rhizobium* spp. – baltymų derliui. Dvejų metų vidutiniais duomenimis, inokuliuotų *Rhizobium* spp. variantų baltymų derlius buvo 80,1 kg (veislės ʽGöynük 98'), 63,9 kg (veislės ʽŞehirali 90') ir 59,8 kg (veislės ʽAkman 98'). Leghemoglobino bei glutamino sintetazė gumbeliuose skyrėsi tarp variantų ir veislių.

Reikšminiai žodžiai: daržinė pupelė, *Rhizobium*, derlius, leghemoglobinas, glutamino sintetazė.