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Grain Yield and Quality Response of Common Bean Cultivar to Inoculation with Native Rhizobacteria

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ABSRACT

Field experiment was carried out to investigate the effect of native *Rhizobium* and CIAT899 inoculation on grain yield, protein content, cookability, nonsoaker capacity, hidration coefficient, seed weight and total defects of dry bean (*cv*. Yunus 90, the most popular cultivar in Turkey). The number of live *Rhizobium sp*. in the inoculant was adjusted to 10^8 CFU ml^{-1} and the seed inoculation was performed at sowing. Native *Rhizobium* inoculation significantly ($P<0.01$) increased the physical and cookability properties of the bean seed**.** Results also revealed that the inoculation with native *Rhizobium* increased the yield and protein content of beans compared to control (reference strain). Overall findings indicated that native *Rhizobium* inoculation, easy to handle, could be a promising fertilizer, improving the plant growth and seed quality. It is possible to conclude that the efficiency of native *Rhizobium* inoculation could further be improved with the additional use of biological, chemical or organic fertilizers.

1. Introduction

Grain legumes are widely recognised as an important source of food and feed proteins (Cristou 1997; Duranti and Guis 1997) and have become very important in human nutrition and as a feed for domestic animals (Egli 1998; Cummings et al. 2001). Bean plants, like other legumes with *Rhizobium* bacteria symbiotic with the roots of this feature and is capable of living through the air nodoziteler benefits from the free nitrogen was determined (Graham 1981; Martinez-Romero 2003).

Bean is one of the major grain legume crops cultivated in Turkey (Anonymous 2000) and many other parts of the world (Slattery et al. 2004; Graham et al. 2003). In human nutrition, dry beans also play an important role due to its addition of nitrogen to the soil (Graham et al. 2003).

Common bean is an interesting crop from the point of view of the consumer, farmer and processor. For the consumer, bean is important for its nutritional composition and its variable uses in different culinary forms (Elsheikh and Elzidany 1997).

Rhizobium sp. inoculation and nitrogen fertilizer significantly increase the plant growth characters. However, it has been revealed that inoculation with *Rhizobium sp*. improved seed quality (Küçük and Kıvanç 2008).

The effects of *Rhizobium* inoculation and nitrogen and molybdenum fertilization assessed on seed quality of fenugreek (*Trigonella foenumgraecum* L.) (Abdelgani et al. 1999). Molybdenum application significantly increased seed protein content and 1000 grain weight, while *Rhizobium* inoculation increased the protein content of seeds.

Rhizobium inoculation of legumes can increase N_2 fixation and plant yield and also improve the seed quality (Khan et al. 1997; Saini et al. 2004). However, little information is available about the effect of *Rhizobium* inoculation on the soil microbial communities of rhizosphere of intercropping plants. *Rhizobium* inoculation of faba beans was reported to increase yield and protein content (Babiker et al. 1995; Elshheikh and Osman 1995).

Enhancement of the N-fixing capacity of common bean is a major agronomic goal. Dry beans cover substantial farming areas in Turkey and are essential part of the nutrition (Küçük and Kıvanç 2008). Thus, this study was conducted with the aim to determine the effects of bacterial inoculation on the yield and seed quality of dry

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bean cultivated under Central Anatolian- Turkey ecological conditions.

2. Materials and Methods

Yunus 90 cultivar (developed by Anadolu Agricultural Research Institute, Eskisehir, Turkey) was used as the seed material. This genotype is one of the most popular and registered varieties currently grown in Central Anatolia, Turkey. *Rhizobium leguminosarum, vicea* strain, was used as inoculums (*R. tropici* CIAT899). The strain was maintained at +4 ºC on yeast extract mannitol agar (YMA) slopes.

Native *Rhizobium* 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 28ºC for seven days (Daza et al. 2000). The number of live *Rhizobium sp*. in the inoculant was adjusted to 10^8 CFU ml^{-1}. Seed inoculation was done at planting with *Rhizobium sp*. inoculant.

Field trial was conducted during the 2012 growing season in the Research Station of the Rural Services Research Institute in Konya, Turkey. A factorial design with three replicates was used. The experimental field is located in a plain in Konya (latitude 37º 55 N, longitude 32 47 E and altitude 1010 m above sea level) where the climate varies from arid to semi-arid. The weather is usually cool during the winter months and rainfall is low. According to long-term data (2006-2012), the annual average temperature, relative humidity and precipitation are 11.63ºC, 62.12 %, and 26.64 mm respectively (Anonymous 2012).

The pH and EC were measured in a aqueous extract (1/5 s/w) (Anonymous 1982); available P was extracted with sodium bicarbonate (Olsen and Sommers 1982) and determined by the method of available K (extracted with ammonium acetate) was measured by flame photometry (Knudsen et al. 1982). Total N was determined by the Kjeldahl method (Bremner 1965), $CaCO_3$ % (Hızalan and Ünal 1966), and organic matter (Jackson 1973) was extracted as described by texture was obtained by the hydrometer method (Bouyocous 1951). In addition, Fe, Cu, Mn, Zn contents of the soil was determined using the samples treated with the DTPA by ICP-AES (Soltanpour and Workman 1981). Some characteristics of the soil used in the experiments are shown in Table 2.

Table 1
Bacterial strains used in the experiment and their source of origin

Strain	Genetic name	Origin (County, Province, Country)
CIAT899	*Rhizobium tropici*	Ankara, Turkey
Native*Rhizobium* 1	Unknown	Derbent, Konya, Turkey
Native*Rhizobium* 2	Unknown	Beyşehir, Konya, Turkey
Native*Rhizobium* 3	Unknown	Beyşehir, Konya, Turkey
Native*Rhizobium* 4	Unknown	Çumra, Konya, Turkey
Native*Rhizobium* 5	Unknown	Erler village, Konya, Turkey
Native*Rhizobium* 6	Unknown	Ereğli, Konya, Turkey
Mix Native*Rhizobium*	Unknown	Turkey (mixture of all isolates)

Table 2
Some characteristics of the experiment area soils

Characteristics	Experiment Soil	Characteristics	Experiment Soil
Class	Loamy	P mg kg^{-1}	10.63
pH	7.55	K me/0.1 kg	0.99
EC(dSm^{-1})	0.300	Fe mg kg^{-1}	3.21
Organic matter (%)	1.16	Zn mg kg^{-1}	2.36
$CaCO_3$ %	22.42	Cu mg kg^{-1}	4.88
N mg kg^{-1}	123.11	Mn mg kg^{-1}	26.18

Trial field was prepared by deep ploughing, harrowing and leveling. The area was ridged and divided into 2.5 m x 2 m plots. The experiment was performed in accordance with a factorial experimental design of 10 x 3, 10 (6 *Rhizobium* strain + 1 mix (mixture of all isolates) + 1 *R. tropici*CIAT899 + 1 Control + 1 Nitrogen control) x 3 replications.

In the experiment, 60 kg ha^{-1} P_2O_5 as super phosphate (TSP), 50 kg ha^{-1} K_2O as potassium sulfate (K_2SO_4) and 40 kg ha^{-1} N as amonium sulfate $(NH_4)_2SO_4$ were broadcast applied and incorporated before planting. The seeds of the dry bean cultivar Yunus 90 were placed in a hole on top of the ridge with 20 cm spacing between holes and 70 cm between ridges. Plots were immediately irrigated after sowing and, subsequently, irrigated with 7–8 d intervals. At harvest, the seed yield (kg ha^{-1}), 100 seed weight (g), percentage content, percentage cookability, percentage nonsoaker, percentag ehydration coefficient and percentage total defect were estimated.

At maturity, seeds were collected and the yield of each treatment was expressed on a per hectare basis. The seeds were cleaned and freed from soil, stones and other extraneous grain and soil. From each sample, 100 seeds were counted randomly in triplicate and their weight was recorded (Elsheikh and Elzidany 1997).

Cookability test is an indicator of bean quality and nutritional value. Twenty grams of beans were processed in 200 ml of tap water in a Labconco apparatus at 110 ºC for 30 min. The sample was reweighed after processing. Cookability was calculated as follows (Elsheikh and Elzidany 1997).

For each treatment, 100 seeds were selected at random and soak tap water at a ratio of 1 part to 4 parts of water for 16 h. The percentage of non-soakers seeds in each sample was calculated as follows (Elsheikh and Elzidany 1997).

NS % = (WNS / IW) * 100 HC % = (WSB / IW) * 100

NS =Non soaker, HC = Hydration coefficient, WNS = Weight of non soakers, IW = Initial weight, WSB = Weight of soaked beans

Total defect is a term for seeds which have abnormalities such as being non-soakers, broken or physically damaged and being very small in size. The total defect percentage was calculated as follows (Elsheikh and Elzidany 1997).

TD % = OD % + NS %OD % = (WD / WBS) * 100

TD = Total defect, OD = Other defect, NS = Non soaker, WD = Weight of defect WBS = Weight before soaking

Statistical Analysis

All data collected were subjected to the analysis of variance appropriate to the design. Test of significance of the treatment differences was done on the basis of F-test. The significant differences between treatments were compared with the critical difference at 1 % level of probability by the Duncan's multiple range tests (Düzgüneş et al. 1987).

3. Results and Discussion

The effects of bacterial inoculation on yield, physicochemical and cookability properties of bean are given in table 3. I found significantly different effects induced by rhizobia on yield, physicochemical and cookability parameters of common bean.

Effect of treatments on yield and 100-seed weight: Inoculation of native*Rhizobium* significantly (P<0.01) increased dry bean yield compared to the control (Table 3, Figure 1). The highest dry bean yield was obtained from *Rhizobium* 3 inoculation (3214 kg ha^{-1}). Similar results were ontained with faba bean and dry bean(Elsheikh and Elzidany 1997; Uyanöz et al. 2007).On the other hand, *Rhizobium* 1 inoculation resulted in the highest 100 seed weight with the value of 51.9 g, although differences were in-significant (Table 3).

Effect of treatments on cookability: The cookability the beans of Yunus 90 variety was not significantly affected by the treatments. The highest cookability value was determined in mix *Rhizobium* inoculated beans followed by *Rhizobium* 6 inoculation (147.5 %) (Table 3, Figure 1). In this study, cookability increased significantly (P<0.01) by all inoculations.

Non-soakers: Usually, the incidence of nonsoakers (hard seeds) adversely affects the cooking quality and, therefore, the market value of the product(Salih and Elmubarak 1986).In the present study,this parameter has been significantly affected by the treatments (Table 3, Figure 1). All the isolates had significant affect on the nonsoaker capacities of Yunus 90 variety. The nonsoaker capacity ranged from 8.9 to 16.3 %. In this investigation, the highest level of nonsoakers capacity was observed in the *Rhizobium* 2 inoculation.

Total defects: The total defect percentage followed a similar pattern to that of the non-soakers. The total seed defect percentages varied between 15.2 and 21.9 % (Table 3, Figure 1). In general, the total defect was decreased by *Rhizobium* inoculation and also, the total defects of Mix *Rhizobium* inoculation were low. The variation in total defect was statistically significant (P<0.01)

Table 3

Effect of native*Rhizobium* inoculation on non-soaker, hydration coefficient, cookability, total defect, protein content, yield and 100 grain weight of seeds of bean (*Phaseolus vulgaris* L.)

Treatment	Yield kg ha^{-1}	Protein content %	Weight of 100 Seeds (g)	Cookability %	Nonsoaker %	Hydration coefficient %	Total defect %
Control	1700 ± 8.3	20.8 ± 0.3	47.8 ± 3.4	110.4 ± 1.8	8.9 ± 0.9	118.4 ± 13.7	16.2 ± 1.2
Nitrogen Control	2689 ± 161.2	21.2 ± 1.8	46.2 ± 2.3	132.5 ± 8.0	12.1 ± 1.0	135.9 ± 6.1	18.2 ± 1.1
Native*Rhizobium* 1	3010 ± 287.5	23.6 ± 3.1	51.9 ± 2.2	136.7 ± 10.4	14.6 ± 0.3	131.5 ± 10.4	19.4 ± 0.7
Native*Rhizobium* 2	3134 ± 21.7	26.2 ± 0.8	48.1 ± 1.4	136.7 ± 2.3	16.3 ± 0.5	135.2 ± 8.2	21.9 ± 0.2
Native*Rhizobium* 3	3214 ± 252.5	25.7 ± 0.8	49.8 ± 1.4	134.2 ± 2.6	14.7 ± 2.3	125.9 ± 7.7	21.8 ± 2.8
Native*Rhizobium* 4	2239 ± 271.6	22.3 ± 3.2	48.4 ± 2.9	139.5 ± 8.0	15.9 ± 1.5	149.3 ± 9.1	21.9 ± 1.3
Native*Rhizobium* 5	2729 ± 192.8	26.3 ± 1.2	47.7 ± 2.1	131.3 ± 8.6	13.9 ± 2.2	138.2 ± 14.8	20.7 ± 2.2
Native*Rhizobium* 6	2796 ± 202.4	25.3 ± 0.8	48.4 ± 0.9	147.5 ± 2.2	10.7 ± 1.2	139.4 ± 12.3	18.3 ± 1.6
R. tropici CIAT899	2688 ± 199.6	25.2 ± 0.5	48.1 ± 1.7	136.3 ± 10.3	15.2 ± 1.7	157.5 ± 9.5	20.5 ± 1.5
Mix *Rhizobium*	2829 ± 271.9	25.5 ± 0.8	49.9 ± 1.0	139.9 ± 10.6	9.9 ± 1.0	145.5 ± 2.0	15.2 ± 1.8
LSD P<0.01	243	3.9	ns	17.2	3.3	23.4	3.7

ns: no significant

mix *Rhizobium*: mixture of all native strain

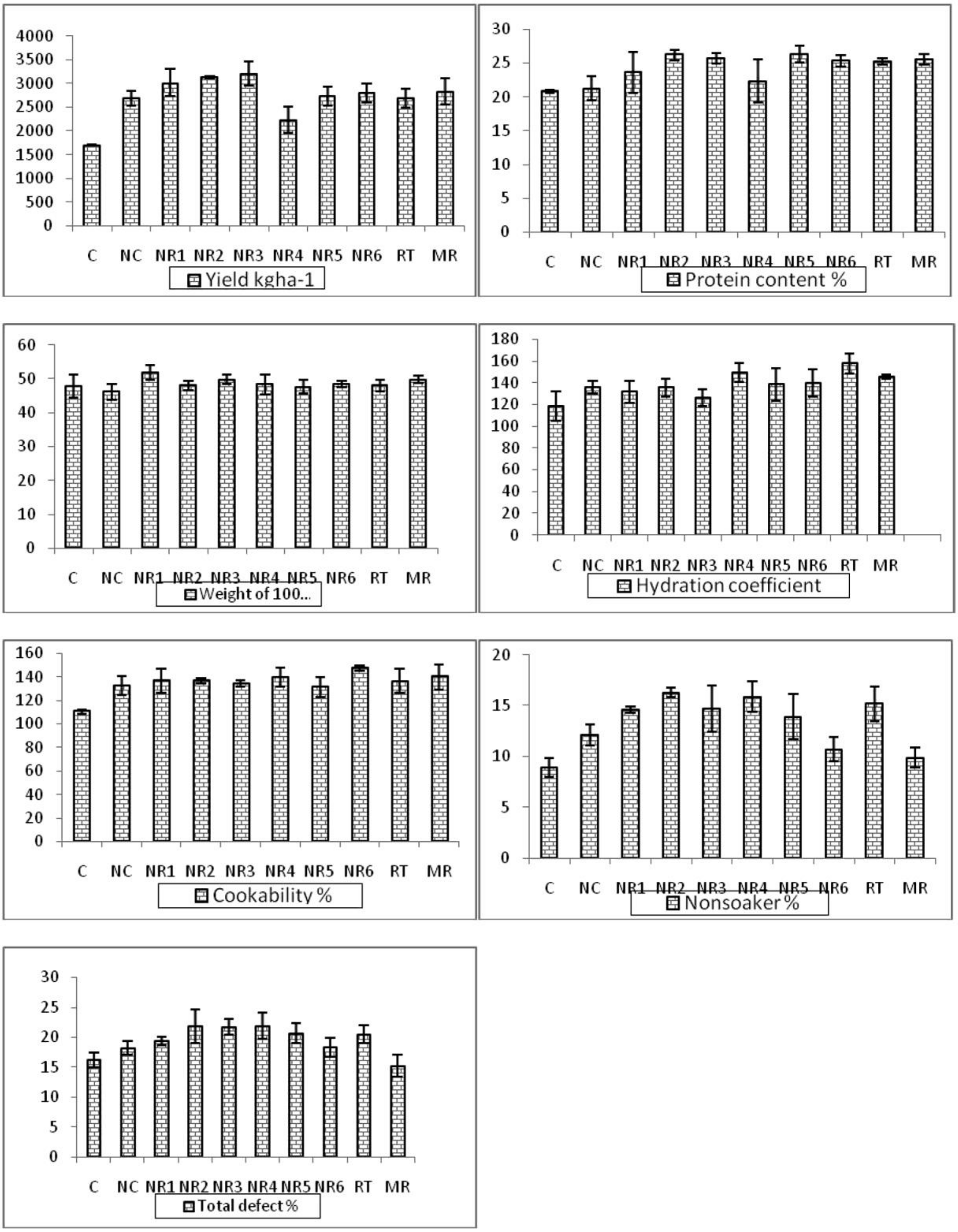

Figure 1

Effect of native *Rhizobium* inoculation on yield, protein content, hydration coefficient, 100-grain weight, cookability, nonsoaker and total defect of seeds of bean (*Phaseolus vulgaris* L.) (C=Control, NC=Nitrogen Control, NR=Native *Rhizobium*, RT= *R. Tropici* CIAT899, mix *Rhizobium*: mixture of all native strain)

Hydration coefficient (%): Hydration coefficient of the seeds was significantly affected by the *Rhizobium* inoculation (Table 3, Figure 1). The hydration coefficient ranged from 118.4 to 157.5 %. Legumes, in general, gave more than double initial weight after soaking (Ali et al., 1988). In this study, all treatments significantly increased the hydration coefficient. Hydration coefficient is a very valuable parameter for both consumer and processor. Low hydration coefficient indicates that the seeds are not capable of absorbing water efficiently (Ali et al., 1988).

Chemical results: The protein content of dry bean increased significantly with native*Rhizobium* inoculations. Protein content was the highest with *Rhizobium* 5 (26.3 %) and *Rhizobium* 2 (26.2 %) inoculations. Significant increase in the protein content of the seeds of *Rhizobium* inoculated plants could be attributed to the increase in nitrogen fixation efficiency in inoculated plants i.e. more nitrogen was fixed and translocated to the seeds.The isolates used in the experiment increased mostly the hydration coefficient and cookability (%) of the dry bean cultivar Yunus 90. On the other hand, they increased non-soaker and total defect percentages. Addition of N via *Rhizobium* inoculation and mineral fertilizers ($P < 0.05$) improved the yield and protein content of dry bean plants.

Inoculation of native *Rhizobium* significantly ($P<0.01$) increased dry bean yield compared to the control. Common bean is believed to be a poor nitrogen fixer due to the genetic characteristic of symbiotic partners as well as soil and environmental condition (Vessey 2003).Several investigators reported increased seed yield by using nitrogen fertilizers (Elsheikh and İbrahim 1999; Sturz et al. 2000; Shisanya 2002). However, inoculation was necessary for maximum seed yield which were not realized by supplemental nitrogen only. 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 İbrahim 1999).

Cookability is known to be affected by soaking time, type of water, time of cooking environmental factors, location and time of harvesting (Elmubarak 1988). Cooking time, taste texture, smell, appearance and aftertaste are considered to be the most important parameters that affect cooking quality. Permeability of the seed coat is an important factor in hard-seed-coatedness. This condition affects hydration of the seed and may double the cooking time (Williams and Nakkoul, 1983). It is noted that the organic and biological treatments had greater affects over the control. Most of the researchers recorded increased yield and 100-seed weight, on soaker hydration coefficient in the presence of biological inoculation (Elsheikh and Elzidany 1997a).

Hydration coefficient is a good indicator of seed quality because it plays a major role in defining the ability of seeds to absorb water and, hence, the seed becomes ready for the cooking process (Elsheikh and Elzidany 1997a). In general hydration coefficient was increased with *Rhizobium*. (Elsheikh and Elzidany 1997b); found that hydration coefficient of faba bean was significantly affected by the organic, biological and chemical fertilization treatments. Similar results were previously obtained with faba bean (Abdelgani et al. 1999).

Protein content of dry bean increased significantly with native *Rhizobium* inoculations. (El Tilib et al. 1994); reported that protein content increases with improved plant nutrition and that the application of manure results in a high exchangeable capacity, hence a considerable quantity of phosphorus is diverted to available form and thus increased protein. They found that *Rhizobium* inoculation significantly increased seed yield and total nitrogen content of faba bean (*Vicia faba)* (Elsheikh and Osman 1995).This inoculation increased the yield of dry bean. These results concur with those reported by they (Abdelhamit et al. 2004; Elsheikh and Elzidany 1997).

4. Conclusion

This experiment indicates that the *Rhizobium* inoculants are promising for legumes, since they improved the protein contents of dry bean. Furthermore, they improved the seed physical characteristics such as. The seed quality of legumes could be improved by selecting a good cultivar and competitive, infective and effective *Rhizobium* strains. 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.

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