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THE EFFECT OF BACTERIAL INOCULATION AND DIFFERENT NITROGEN DOSES ON YIELD AND YIELD COMPONENTS OF SOME DWARF DRY BEAN CULTIVARS (*Phaseolus vulgaris* L.)

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Abstract

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This study was carried out to determine the effects of bacterial inoculation and different nitrogen doses on yield and yield components of some dwarf dry beans varieties in Mustafakemalpaşa Vocational School Uludağ University, Bursa, Turkey, during 1998-2000 years. In the study, three dwarf dry bean cultivars (Sahin-90, Yalova-5 and Yalova-17) and five N doses (0, 30, 60, 90 and 120 kg ha^{-1}) as ammoniumnitrate and conditions with and without bacteria (*Rhizobium phaseoli*) on yield and yield components were investigated. The complete randomized blocks design in factorial arrangement with three replications was used and seed yield, plant height, number of branches per plant, number of pods per plant, number of seeds per plant and 1000 seed weight were observed in the study. Three years, it was found that effect of inoculation with bacteria was not on yield and yield components of dry beans. Increasing nitrogen doses, compared with 0 kg ha^{-1} N (control) increased the plant height, the number of branch per plant, and 1000 seed weight. But there were no significant differences between nitrogen doses in these characteristics. Yalova-5 gave the highest yield (1870.1 kg ha^{-1}) among these dry bean cultivars used.

Key words: Phaseolus vulgaris L., Nitrogen fertilizer, seed yield

Introduction

Marmara is a highly industrialized region with high population density in Turkey. Bursa as one of the most important city in the region has many technological and agricultural industries. Agriculture has retained its importance in Bursa from past till now; however, it is a known fact that agriculture has many crucial problems at present (Tumsavas et al., 2010). One of the problems in agriculture productions is unbalanced fertilization, also.

The nitrogen which is one of the reasons of green revolution is the main factor that the productivity of plants limits. It is given plants as a natural or artificial fertilizer. In the recent years, nitrogen provided by atmospheric happening and by organisms which absorbs nitrogen in the air in recent years because of the harms given to the

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environment and the food items by fertilizers. A lot of researches have been done intensively on the source of this nitrogen. The nitrogen gained by this way is a ready and economic source that both legumes and non legumes plants can consume (Karadogan et. al., 1999). Amounts of N_2 fixed per annum vary with crop, soil-type and husbandry, and are usually in the range of 30-300 kg per ha per annum (Beringer et al., 1988).

Legumes have played a crucial role in agricultural production throughout history. This is obviously due to their capacity to fix nitrogen association with rhizobia by using solar energy collected through plant photosynthesis. Cost escalation of fossil fuel required for the manufacture of fertilizer nitrogen has helped to increase awareness of the importance of the Rhizobium-legume symbiosis (Rupela and Saxena, 1984). Dwarf dry bean (*Phaseolus vulgaris* L.) is one of the major grain legumes cultivated in Turkey. It is produced by small holder farmer fields where the use of fertilizer is limited and inoculation of beans is not adopted by the majority of the farmers due to insufficient knowledge and doubts about the efficiency of inoculation on the yield increase**.**

To enhance legume nodulation and nitrogen fixation, the introduction in bacterial inoculants to agricultural fields has been a common practice approximately for over 100 years. Whenever the specific rhizobia are absent, inoculation readily enhances plant growth and yield (Singleton and Taveres, 1986; Streeter, 1994).

Material and Methods

The aim of this study is to determine the effects of bacterial inoculation and different nitrogen doses on yield and yield components of some dwarf dry bean varieties. The experiments was carried out at Mustafakemalpasa, Bursa located in Southern Marmara Region of Turkey, with average 713 mm rainfall and 14.4 °C mean monthly temperature, for long-term (Table 1).

Texture class of experimental soil is clay loam. It contains 0.1% total nitrogen, 0.164 mg/kg available phosphorus, 0.0079 meq/100 g soil exchangeable potassium and 3.0 % organic matter. The soils were slightly alkaline reactions (pH=7.3).

The average of monthly air temperature in the experimental years (1998, 1999 and 2000) is almost at the same as climatic data of long-term period (1928-1986).

Whereas, total amount of precipitation during the growing period in 1998 was much higher

Table 1

Mean air temperature and total monthly precipitation in 1999, 2000, 2001 years and in long-term (1928-1996)

Months	Temperature, ºC				Precipitation, mm			
	1998	1999	2000	Long -term	1998	1999	2000	Long -term
March	6	9	8	8	93.5	71	96	69
April	15.4	14	15	13	34.6	25	109	60
May	17.1	18	18	18	109	8	49	52
June	22.4	23	22	22	35.9	74	16	30
July	25.1	26	26	25	29.2	1	9	25
August	25.6	25	25	24	68.7	40	11	18
September	20.4	21	21	20	138.5	12	82	40
October	15.8	16	15	16	94	34	128	56
TOTAL					603.4	265	500	350

compared with the other experimental years (1999-2000) and long-term average. Also, total precipitation, during the growing period in 1999 was rather less than that in the other experimental years (1998 and 2000) and average of long-term period.

The experiments were designed in a randomized complete block design with three replications in each year. Plantings were made in 01 June 1988, 08 June 1999 and 18 June 2000.

The plot size was 8.0 m^2 (4.0 x 2.0 m) at the harvest time. Each plot had four rows. The inter- and intra-row spacing was 50 cm and 10 cm, respectively. The seeding rate was 33 seeds m^{-2}. Sahin-90, Yalova-5, Yalova-17 as dry bean cultivars; five nitrogen doses (0, 30, 60, 90 and 120 kg ha^{-1}) as ammonium nitrate and conditions with and without bacteria (*Rhizobium phaseoli*) were investigated as experimental factors. Bacteria were supplied from Soil and Fertilizer Research Institute in Ankara, Turkey. The *Rhizobium phaseoli* bacteria were inoculated surface of seeds (100 kg seed, 1 kg inoculate), just before sowing. Sixty kg of per hectare of phosphorus (as Triple Super Phosphate, 46% P_2O_5) were applied prior to sowing and the doses of ammonium nitrate were added to soil after emergency. The soil was irrigated by sprinkling for emergence after sowing in the each experimental year. Plants were irrigated three times at different growth periods such as vegetative stage, beginning of flowering and full pod. The plots were irrigated from deficit moisture content of 0-90 cm soil layer to field capacity at each growth stage. Weed control was done with hand after each irrigation. Plots were harvested by hand in October and threshed with hand to determine seed yield and other seed traits.

Agronomical traits such as plant height, number of branches per plant, number of pods per plant, number of seeds per plant and 1000 seed weight were measured on ten plants selected randomly from each plots. The seed yield was obtained from mature seed harvested and threshed from 4 m lengths of four rows and added 10 plant seed yield selected for measure.

The data were analyzed as a randomized complete block design. All the data were subjected to analyses of variance for each trait using MSTAT-C (version 2.1, Michigan State University, 1991). Also, results of ANOVA of single experiments were combined over years. The significance of the cultivar, N doses, bacteria inoculation and these interactions were determined at the 0.05 and 0.01 probability levels using appropriate F-values. For multiple corporations of means, the F-protected least significant difference (LSD) was calculated at the 0.05 probability level according to Steel and Torrie (1980). The relationships between yield components and nitrogen levels were determined using regression analysis.

Results and Discussion

The mean values of three years were given in Table 2. According to the results of research, bacteria inoculation had significant effect only on plant height at 5% probability level. Nitrogen doses had significant effect on plant height, branch number per plant and 1000 seed weight at 1% probability level. Moreover significant differences were found between cultivars at 1% level in terms of the plant height, branch number per plant, 1000 seed weight and seed yield. Effect of years was found significant at 1% probability level, for all characteristics.

The interactions of year x bacteria inoculation were found significant at 1% probability level for plant height and branch number per plant while the interaction of year x cultivar interactions were significant statistically plant height, branch number per plant, 1000 seed weight and seed yield per unit area and the year x N doses interaction was determined to be significant for of plant height, branch number per plant and 1000 seed weight.

Bacteria inoculation x cultivar interactions were found significantly for plant height, , branch number per plant; cultivars x N doses interactions were found significant regarding the plant height, branch number per plant and seed number per pod.

Table 2

The mean values of three years in terms of yield and yield components

Units	Factors	Plant height, cm	Branch number/ Plant	Pod number/ Plant	Seed number/ Plant	1000 seed weight, g	Seed yield, kg ha^{-1}
Bacteria	B1	54.5 b	10.66	16.4	40.1	449.9	1777.6
	B2	57.1 a	10.85	15.7	40.2	451.2	1798.8
L.S.D.		1.257	-	-	-	-	-
Cultivar	C1	58.1 a	9.6 c	15.5	39.3	447.1 b	1741.7 b
	C2	52.1 b	10.4 b	15.6	38.1	463.1 a	1870.1 a
	C3	57.4 a	12.3 a	16.5	42.9	441.6 b	1752.8 b
L.S.D.		**1.539**	**0.528**	**-**	**-**	**8.263**	**7.153**
Nitrogen	N_0	52.2 c	9.8 b	14.6	38.9	436.4 b	1731.5
	N_3	54.6 b	10.2 b	16.2	39.7	451.7 a	1772.2
	N_6	56.6 a	11.3 a	16.5	41.6	451.9 a	1798.2
	N_9	57.9 a	11.2 a	16.1	40.6	458.2 a	1864.3
	N_{12}	57.8 a	11.2 a	15.8	39.6	454.8 a	1774.6
L.S.D.		**1.987**	**0.6817**	**-**	**-**	**2.71**	**-**
Year	Y1	69.2 a	12,3 a	17.8 a	45.2 a	429.8 c	1869.4 b
	Y2	45.5 c	7.8 b	12.9 b	33.7 b	475.8 a	1176.4 c
	Y3	52.7 b	12.2 a	16.8 a	41.5 a	446.2 b	2318.7 a
L.S.D.		**1.539**	**0.5274**	**1.446**	**4.185**	**8.295**	**7.153**

N_0: 0 kg ha^{-1} N
N_3: 30 kg ha^{-1} N
N_6: 60 kg ha^{-1} N
N_9: 90 kg ha^{-1} N
N_{12} 120 kg ha^{-1} N

B1: Non Inoculated Bacteria
B2: Inoculated Bacteria

C1: Sahin-90
C2: Yalova-5
C3: Yalova-17

Y1: First year
Y2: Second year
Y3: Third year

Years x cultivar x bacteria application x N doses interaction was found significant at 1% probability level for the parameter 1000 seed weight**.**

In many studies on the legumes, optimum N rates were found as 20-50 kg ha^{-1} (Akçin, 1974, Önder and Özkaynak, 1994). Thus it is an expected status that linear and quadratic regressions between N rates and yield component were found as insignificant.

Average values and groupings belonging to characters investigated were presented in the Table 2, 3 and 4. Mean values were discussed and evaluated separately for each character.

Plant height

The effects of cultivars, applications of bacteria and nitrogen were statistically significant on the plant height. Maximum plant height was recorded at Sahin-90 (58.1 cm) and Yalova-17 (57.4 cm) varieties against minimum at Yalova-5 (52.1 cm) cultivar. Plant heights were positively affected by bacterial application (57.1 cm) compared to the plot with no bacteria application (54.5 cm).

Maximum plant heights were measured from

Table 3

The means values for Year x Cultivar interactions of agronomical characters

Cultivars	Plant height, cm			1000 seed weight, g			Seed yield, $kgha^{-1}$		
	Years			Years			Years		
	1998	1999	2000	1998	1999	2000	1998	1999	2000
Sahin-90	70.54	49.74	53.84	421,09 d	477,57 a	442,61 bc	1929.2 d	1129.3 f	2166.6c
Yalova-5	65.84	40.35	50	455.00 b	477.83 a	456.40 b	1925.4 d	1212.0 f	2472.8 a
Yalova-17	71.27	46.51	54.44	413.18 d	471.90 a	439.71 c	1753.7 e	1187.8 f	2316.9 b
LSD	ns	ns	ns	14.31	14.31	14.31	12.96	12.96	12.96

N_9, N_{12} and N_6 (57.9, 57.8 and 56.6 cm, respectively) doses whereas minimum (52.2 cm) at N_0 nitrogen dose (Table 2).

As a known, nitrogen increases the vegetative growth in plants (Çelik, 1998, Kaçar & Katkat, 2007).

Plant height is an important indicator of vegetative growth. As expected, therefore, plant height increases as N rates are increased.

The plant height was differently affected by the years (Table 2).

It was likely that plants were taller in 1998 compared with the other experimental years because of more rainfall. Similar results have been reported by Önder (1993), Karahan and Şehirali (1999) and Bozoglu et al. (1997).

The N rate x cultivar interaction was significant in plant height. Sahin-90 and Yalova-17 cultivars had higher plant height in high N-rates compared with No nitrogen dose whereas plant height of Yalova-5 cultivar was not affected by N-rates ranged from N_0 to N_6 dose but increased with higher nitrogen doses (Table 4).

Significant the year x bacteria application and year x nitrogen dose indicated that applications of bacteria and nitrogen affected differently the plant height according to the years.

Plant height and N rates relations for dry bean were obtained by plotting observed plant height on the Y-axis and the N rate on the X-axis, for the mean of 2-years (Figure1). Both linear and quadratic relationships were found between N rate and plant height at 1% and 5% of probability level, respectively).

The N rate for maximum plant height determined from quadratic regression models are illustrated in Figure 1 by using this equation, the nitrogen level which is responsible by the maximum plant height (57.93 cm) was found as 113.4 kg ha^{-1}. This research indicated that plant height was increased by N rates increasing up to 113.4 kg ha^{-1} but not increased in higher N rates.

Table 4

The means values for N rate x Cultivar interactions of some agronomical characters

Cultivars	Plant height, cm					Branch number / Plant				
	N_0	N_3	N_6	N_9	N_{12}	N_0	N_3	N_6	N_9	N_{12}
Sahin-90	54.7 c-e	56.9a-d	67.4 a	59.7 ab	57.4 a-d	9.6 cd	8.5 d	11.0 a-d	9.3 cd	9.6 cd
Yalova-5	49.1 f	48.9 f	51.2ef	55.8 b-d	55.9 cd	9.4 cd	9.6 cd	11.1 a-d	11.4 a-d	10.3 cd
Yalova-17	52.9 d-f	58.5a-c	57.2 a-d	58.4 a-c	60.0 ab	10.5a-d	12.3a-c	11.9 a-c	11.9a-c	13.7 a
LSD	4.574					3.316				

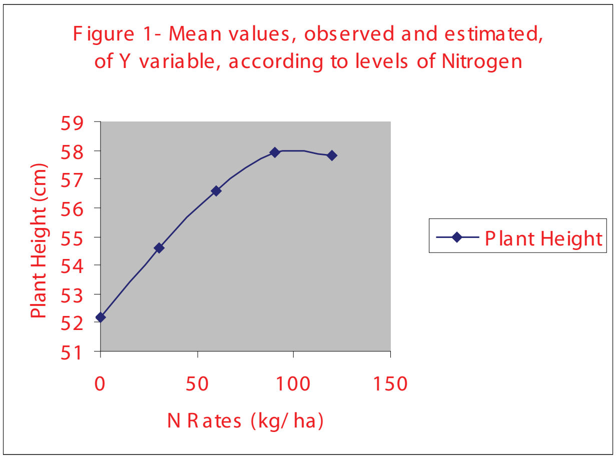

Fig. 1. Mean values, observed and estimated, of Y variable, according to levels of nitrogen

Branch number per plant

According to combined data of 3 years, effects of varieties and nitrogen applications were significant on the number of branches per plant ($p \leq 0.01$) although application of bacteria was no significant. Maximum the number of branches per plant was recorded on cultivar Yalova-17 (12.3 branch/plant) whereas the minimum values were measured on the varieties Yalova-5 and Sahin-90 (10.4 and 9.6 branch/plant, respectively). High nitrogen doses (N_6, N_9 and N_{12} kgha^{-1}) were increased the number of branches per plant (Table 2).

Effect of years occurred differently on the branch number, also. More branch number was obtained in 1998 and 2000 years (12.3 and 12.2 branch number, respectively) than 1999 year (7.8 branch number). Excessive drought conditions decreased the branch number in 1999.

The significant N-rate x cultivar interaction for this character indicated that number of branches per plant of Sahin-90 and Yalova-5 cultivars significantly increased as nitrogen doses increased from N_0 to N_6 dose, whereas Yalova-17 cultivar was not significantly influenced by increasing nitrogen doses (Table 4).

Significant year x cultivar interaction effect revealed that varieties were differently affected by years in number of branches per plant. Likewise, significant bacteria application x cultivar and nitrogen application x cultivar display that bacteria and nitrogen applications various reacted among varieties, also.

Pod number per plant

The effects of years only were found significantly on the number of pod per plant. The number of pod per plant was higher in 1998 and 2000 compared with 1999 experimental year (17.8, 16.8 and 12.9 pods/plant, respectively). In 1999, June month which to be the flowering period of plants had more rainfall. It was likely that more rainfall effected negatively the pollination and insemination, so it resulted in fewer the number of pods per plant and the number of seed per plant.

Some researchers reported that inoculation of bacteria and nitrogen applications were not affected the number of pod per plant (Bozoglu et al., 1997). However, Karahan and Şehirali (1999) explained that both inoculation of bacteria and application of nitrogen were increased the number of pod per plant.

Seed number per plant

The effect of varieties, bacteria the inoculation and nitrogen doses on number of seeds per plant were found insignificant.

The years affected differently the number of seeds per plant and mean values of 1998 and 2000 experimental years were higher than that of 1999. Kacar et al. (2004) explained that inoculation of bacteria didn't affect the number of seed per plant, although application of nitrogen significantly affected this character.

1000 seed weight

Varieties and nitrogen doses had significant effects on 1000 seed weight. Cultivar Yalova-5 was gave maximum value (463.1 g) while Yalova-17 and Sahin-90 varieties gave minimum values (441.6 g and 447.8 g, respectively) at the 1000 seed weight. Increasing nitrogen doses (N_3, N_6, N_9 and N_{12}) had similar effects on the 1000 seed weights

(458.2, 454.8, 451.9 and 451.7 g, respectively).

The significant year x cultivar and year x nitrogen dose interaction effects revealed that varieties and nitrogen applications differently affected the 1000 seed weight according to the years. Similar findings were explained by Karahan and Şehirali (1999) also. Bozoglu et al. (1997) and Kacar et al. (2004) reported that bacteria inoculation and nitrogen application didn't effect the 1000 seed weight.

The effects of years were found significantly 1% probability level on 1000 seed weight. The higher 1000 seed weight (475.8 g.) were obtained in 1999 compared with the 1998 and 2000 (429.8 and 446.2 g, respectively) years.

Seed yield per unit area

The effects of bacterial inoculation and nitrogen doses were found to be insignificant on seed yield, according to combined data over three years. Differences between cultivars were determined significantly at 1% probability level. The highest yield was obtained from the cultivar, Yalova-5 (1870.1 kg ha^{-1}), whereas Yalova-17 and Sahin-90 gave lower yields with 1752.8 and 1741.7 kg ha^{-1}, respectively.

Effect of the years on seed yield was found significantly at 1% probability level. The highest seed yield was obtained in 2000 (2318.7 kg ha^{-1}) and the lowest in 1999 (1176.4 kg ha^{-1}), whereas the yield in 1988 was 1869.4 kg ha^{-1}.

The significant year x cultivar indicated that the cultivars had different values accordingly to years in terms of seed yield. As can be seen in Table 3, among varieties the highest yield was obtained from Yalova-5 (2472.8 kg ha^{-1}) in 2000 while Sahin-90 gave the lowest yield (1129.3 kg ha^{-1}) in 1999.

In our study, the effects of bacteria inoculation and N doses on seed yield were found insignificant. Similarly, Amos et al. (2001) reported that Rhizobium inoculation affected none of the variables measured. In their 3 year study, Onder and Ozkaynak (1994) found that the effects of bacteria inoculation and N doses were no significant in the first experimental year; however these factors had significant effects in the second and third year. It found that the lowest yield obtained from the control plot (2783.9 kg ha^{-1}) and the highest yield from bacteria inoculation, N_5 treatment (3236.2 kg ha^{-1}). Yaman and Cinsoy (1997) reported that inoculated without N and non-inoculated with N treatments gave different yields in soybean and also inoculation +25 kg ha^{-1} N treatment gave similar yield as obtained with supplementary nitrogen application. Karahan and Şehirali (1999) determined in their study that higher seed yield was obtained from bacteria inoculated plots; the lowest seed yield was obtained from, N_0 treatment without bacteria inoculation (2079.9 kg ha^{-1}). In the study was determined that high yields were obtained from N_2, N_4, N_6 and N_6 + 4 treatments, however, differences in the yield caused by fertilizer doses were insignificant statistically (2598.2, 2726.3, 2762.0 and 2739.0 kg ha^{-1}). Similarly, Kacar et al. (2004) reported that lower yield (1149.0 kg ha^{-1}) was obtained from the bacteria inoculated plot compared with non-inoculated plots (1193.0 kg ha^{-1}).

Sing et al. (1993), Peres et al. (1994) reported that bacteria inoculation and bacteria inoculation plus N fertilizer treatments increased the seed yield.

Neuvel and Flood (1992), reported that, bacteria inoculation and 50 $kgha^{-1}$ N treatments gave the highest yield increase. In similar studies, Neuvel et al. (1994) reported that bacteria inoculation + 100 $kgha^{-1}$ N treatments gave the highest increase in yield.

Sali and Keya (1982) reported that, 20 kg ha^{-1} N treatment increased the seed yield by 1-13.4%; however, 100 kg ha^{-1} N treatment did not increase the seed yield, but decreased it by 12.6%. Bozoglu et al. (1997) reported that different N fertilizers and bacteria concentrations affected the yield. They found that the lowest seed yield (3270.0 kg ha^{-1}) was obtained from the control plot, whereas the type of N fertilizer did not affect the yield and the highest seed yield was obtained from the bacteria concentration of 1000-1250 g for 100 kg of seed with 1533.0 and 1467.0 kg ha^{-1}, respectively.

It is rather difficult to determine to which extend the inoculation material affected the nitrogen fixation when the inoculation treatments were carried out under field condition, (Cebel and Altintas, 1992) since nodule formation and nitrogen fixation are greatly affected by environmental factors (Azkan, 2002).

Environmental factors influence all aspects of nodulation and symbiotic N_2 fixation in some cases reducing rhizobial survival and diversity in soil; in others affecting nodulation or nitrogen fixation and even growth of host. Factors that are important include acidity, temperature, mineral nutrition, salinity and alkalinity (Graham, 1998).

In our study, the ineffectiveness of bacteria inoculation and nitrogen doses on yield may be dependent upon a lot of factors. Because seed yield is a quantitative character and is affected by genotype and environmental factors.

One of the major prerequisites for effective nodule formation is the selection of locally isolated bacteria strains (Bremer et al., 1990). A note worthy increase may be obtained in seed yield if Rhizobium strains which exhibit local superiority are isolated and used as inoculation material (Kantar et al., 1994).

However, there are not sufficient numbers of researches related to the extent of yield increase as affected by location in each of the legume species (Karuc, 1992). Mcloughlin and Dunican (1985) reported that the inoculated Rhizobium strains normally form nodules, however they compete with the native Rhizobium strains they meet and therefore no response could be obtained from Rhizobium inoculation.

High rates of soil nitrogen and the existence of native *Rhizobium phaseoli* population in soil decrease the efficiency of Rhizobium strains that are covered to the ambient via inoculation and therefore lead to an insufficient response to inoculation by bean (Sparrow and Ham, 1983). Furthermore, Esperanza (2003) reported that in some area of Latin America, inoculation, which normally promotes nodulation and nitrogen fixation, is hammered by the prevalence of native strains.

Heavy soil with poor aeration and sufficient nitrogen probably affected the nodule formation, since nodule formation is effected by physical components of soil as soil nutrients, light, soil reaction (pH), temperature, humidity and aeration (Azkan, 2002).

For this reason, effective *Rhizobium phaseoli* strains adapted to local climatically and edaphically conditions should be isolated and their effectiveness should tested in field experiments, and then the seeds should be inoculated by their effective strains. Applying 20-30 $kgha^{-1}$ N may be necessary in the case of nitrogen deficiency in the soil, whereas bacteria inoculation should directly be implemented if there is sufficient nitrogen in soil.

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