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Response of Field Pea (*Pisum sativum* sp. *Arvense* L.) to *Rhizobium* Inoculation and Nitrogen Application in Eastern Anotolia

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Abstract: A field experiment was conducted during 2005-2006 and 2006-2007 growing seasons to determine, the effects of *Rhizobium* inoculation and different levels of nitrogen on the yield and growth of field pea (*Pisum sativum* sp. *Arvense* L.). Nitrogen application had significant effect on the plant height, number of branches, root and shoot dry weight, number of nodules, seed yield, biomass yield, harvest index, number of pods, as well as crude protein rate of seeds in both years. Plant height, number of pods, harvest index, number of nodules, crude protein rate and root dry weight were higher with application of 20 kg N ha^{-1} while seed yield, shoot dry weight and number of branches were higher with application of 60 kg N ha^{-1}, in the 2 seasons. The lowest values related to these parameters were obtained from the control treatment. Inoculation treatment had also significant effect on the all parameters investigated in both years. The highest values regarding these parameters were obtained from inoculated plants, whereas the lowest values were obtained from the uninoculated plants. Interactions of nitrogen and inoculation had significant effects on the all parameters investigated, except for number of nodules in 2005-2006 and crude protein rate of seeds in both years. The highest seed yield was obtained under treatment 20 kg N ha^{-1} + inoculation with 1654.3 and 1625.0 kg ha^{-1} in 2005-06 and 2006-07, respectively.

Key words: *Rhizobium*, inoculation, nitrogen, field pea, Eastern Anotolia

INTRODUCTION

Nitrogen is major nutrient element for grain legumes. Although, nitrogen is abundant element in the atmosphere in gaseous form, plants cannot directly utilize it. Availability of nitrogen amount in soil is getting decreased by plant cultivation, increased oxidation in soil or it can also be easily washed away. Therefore, nitrogen becomes a limited factor in the plant cultivation. The main ways of the nitrogen application into soil are the mineral nitrogen fertilizer or *Rhizobium* inoculation of seeds or soil in order to obtain biological fixation of nitrogen. High cost and its dependence to moisture is the main disadvantageous of mineral fertilizer. Biological nitrogen fixation is an important nitrogen source due to the fact that it requires less energy and causes less environmental pollution.

Dry pea is a pulse crop and a member of the family Leguminacea. This crop like many other legumes is capable of fixing and utilizing atmospheric nitrogen through symbiotic relationship with Rhizobium. The crop thus, improves soil, economizes crop production reducing the requirement of added synthetic nitrogenous fertilizers. When properly inoculated with an appropriate Rhizobium inoculant, pea can derive up to 80% or more of its nitrogen through nitrogen fixation. The rest of the N must be provided from the soil or from fertilizer applications (Ali-Khan and Zimmer, 1989; Bowren *et al*., 1986). Seed inoculation is the most widely used methods of inoculant application. Many researchers (Rahman *et al.,* 1994; Solaiman and Rabbani, 2005) have reported the beneficial effects of inoculation of grain legumes.

Nodule formation and subsequent nitrogen fixation are very sensitive to external nitrogen sources, including fertilizer and available soil nitrogen. Nitrogen fertilizer applications generally inhibit biological N-fixation by *R. leguminosarum*. The inhibitory effect of N fertilizer on nodule formation results from the fertilizer's contribution to the soil N pool. Bowren *et al*. (1986) concluded that nodule formation became inhibited as soil N levels approached 40 kg ha^{-1} and were progressively inhibited as levels exceeded this amount. This suggests that N fertilizer applications will reduce biological N-fixation by *R. leguminosarum*, except where the amount of N applied as fertilizer plus that contained in the soil is <40 kg ha^{-1}. Small N fertilizer applications have stimulated nodule formation on pea roots in some low N environments and other pulse crops (Kauskik *et al*., 1995). It can take

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3-4 weeks after planting before nodules are fully functioning. Early plant growth may be poor in soils with low nitrogen levels and plants may appear yellow prior to the beginning of effective nitrogen fixation due to a nitrogen deficiency. This early deficiency can be overcome by adding low levels of starter nitrogen at seeding. Although, high levels of starter nitrogen may appear to help the crop overcome a nitrogen deficiency during early crop growth stages, final seed yields may not increase. Bowren *et al.* (1986) suggested that applications of 9-18 kg N ha^{-1} enhanced pea seedling growth prior to nodule development in low N soils. The soil of the region where the study was conducted has low nitrogen and organic matter content and their availabilities are low due to the various soil and other environmental factors. Therefore, it was aimed to determine the effects of nitrogen fertilizer and *Rhizobium* inoculation on the yield and growth parameters, as well as crude protein rate of seeds in field pea.

MATERIALS AND METHODS

The study was carried out at experimental field of the Yüzüncü Yil University, Van, Turkey, where legumes have not be grown for at least 10 years, in 2005-06 and 2006-07 growing seasons using field pea genotype with number 110121 adapted to the region. Seeds were inoculated using peat inoculant included a mixture of nodule-forming strains of *Rhizobium leguminosarum* bv viciae specific to pea. Peat inoculant was prepared as commercial peat cultures by Soil and Fertilizer Research Institute, Ankara, Turkey, according to the method of Somasegaran and Hoben (1994). Peat inoculant was kept in refrigerator at +4°C until use. It was separately provided from Soil and Fertilizer Research Institute in 2005-06 and 2006-07 growing seasons. Content and activity of peat inoculant was checked before trials of 2005-06 and 2006-07. Peat inoculant was used after cells count were adjusted to 1×10^8 *Rhizobium* cells g^{-1} so content of peat culture was standardized by diluting peat inoculant. The Most Probable Number (MPN) method was used for estimating viable cells of *Rhizobium* (Somesagaran and Hoben, 1994).

The soil of the experimental field was clay-loamy in texture, slightly alkaline (pH 7.8), poor in nitrogen (0.065%) and organic matter (1.43%) and had an average level of phosphorus (539.1 ppm). The soil received rainfall 391.8 mm and 363.1 mm in 2005-06 and 2006-07, respectively. Annual average temperature was 10.5°C in 2005-06 and 9.4°C in 2006-07.

The study was conducted in split-plot design with 3 replications. In order to eliminate the contamination, inoculation treatments (with and without) were randomly applied to main plots and nitrogen doses (0, 20, 40 and 60 kg N ha^{-1}) as ammonium sulphate were randomly applied to the subplots before sowing. Plot size was 5×1.2 m. The seed was sown by hand with 25 cm row spacing in late October in both years (18 October 2005 and 20 October 2006). The seeding rate was 45 seeds m^{-2}. A basal dose of 60 kg ha^{-1} triple super phosphate was given to each plot at the time of sowing. Seeds were inoculated with *R. leguminosarum* bv viciae bacteria at the recommended rate before sowing in all plots, except for uninoculated plots (Vincent, 1982). Application of the peat inoculant on the seeds was carried out by water, which contains 2% sugar. The experiment was carried out as rainfed. Plots were hand-weeded 2 times when needed in each season. Plants were harvested in late June in both years (24 June 2006 and 26 June 2007). At flowering, 10 random plants were removed from each plot. Numbers of nodules as well as dry weight of root and shoot/plant were recorded. Similarly, 10 plants from each plot were removed at the harvest to determine pods number per plant as well as plant height and branches number per plant. At harvest, 2 outer rows for each plot and 50 cm from each end of the plots were left as borders and the middle 4 m of the central rows were harvested. Biomass yield and seed yield were determined after harvest. In addition to these criteria, crude protein rate of seeds were determined using the methods proposed by Kacar and Inal (2008).

The influence of treatments on the field pea and differences among treatments were analysed using analysis of variance procedures for split-plot design in randomised blocks. Means of measured parameters were compared using Least Significance Difference (LSD) at $p<0.05$.

RESULTS AND DISCUSSION

The results indicated that the effects of nitrogen application and inoculation on the investigated parameters differed significantly between years. In general, compared to those in 2006-07, occurred significant increases in the values of parameters in 2005-06 when the soil received higher amount of rainfall (Table 1 and 2). Nitrogen application had significant effect on the plant height, number of branches, root and shoot dry weight, number of nodules, seed yield, biomass yield, harvest index, number of pods, as well as crude protein rate of seeds in both years. The lowest values related to these parameters were obtained from the control treatment. Plant height, number of pods, harvest index, number of nodules, crude protein rate and root dry weight

Table 1: Effect of inoculation and nitrogen on the seed yield, biomass yield, harvest index, number of pods and crude protein rate

	Seed yield (kg ha^{-1})		Biomass yield (kg ha^{-1})		Harvest Index (%)		No. pods (plant^{-1})		Crude protein rate (% seed^{-1})	
Treatments	2005-06	2006-07	2005-06	2006-07	2005-06	2006-07	2005-06	2006-07	2005-06	2006-07
Nitrogen (kg ha^{-1})										
0	963.3d	939.3d	2525.3c	2476.7c	37.40c	37.20c	3.80b	3.40c	16.70c	16.00c
20	1251.5b	1217.7b	2839.3b	2815.2b	43.50a	42.60a	5.00a	4.30a	19.60a	18.90a
40	1180.0c	1143.0c	3023.8a	2980.7a	40.80b	40.20b	4.80a	4.00b	18.10b	17.20b
60	1323.7a	1290.2a	3018.8a	2952.5a	42.1ab	41.7ab	4.70a	4.2ab	17.7bc	17.0bc
LSD (p = 0.05)	46.20	47.00	73.20	66.00	2.000	2.000	0.400	0.200	1.200	1.100
Inoculation										
With	1419.9a	1388.6a	3354.5a	3331.70a	42.20a	41.8ns	5.30a	4.20a	19.30a	18.50a
Without	939.3b	906.5b	2349.3b	2300.80b	39.60b	39.000	3.90b	3.70b	16.70b	16.10b
LSD (p = 0.05)	91.80	86.60	53.10	82.900	2.800	3.300	1.400	0.300	2.400	0.500
CV (%)	26.20	26.80	20.80	21.300	8.800	8.500	21.100	13.200	10.800	8.400

Table 2: Effect of inoculation and nitrogen on the root and shoot dry weight, number of nodules, plant height and number of branches

	Root dry weight (g plant^{-1})		Shoot dry weight (g plant^{-1})		Nodules number (plant^{-1})		Plant height (cm)		No. branches (plant^{-1})	
Treatments	2005-06	2006-07	2005-06	2006-07	2005-06	2006-07	2005-06	2006-07	2005-06	2006-07
Nitrogen (kg ha^{-1})										
0	0.295d	0.274d	1.490d	1.479c	13.7b	12.5c	35.50c	32.70c	1.50c	1.40c
20	0.387a	0.368a	2.084b	2.071a	20.7a	19.2a	44.70a	41.50a	2.3ab	2.2ab
40	0.339c	0.323c	1.859c	1.834b	15.3b	14.0b	41.80b	39.50b	2.10b	2.00b
60	0.362b	0.339b	2.120a	2.080a	11.5c	10.5d	43.5ab	40.8ab	2.40a	2.20a
LSD (p = 0.05)	0.0090	0.0040	0.0110	0.0240	1.90	1.20	2.300	1.400	0.200	0.200
Inoculation										
With	0.361a	0.327ns	1.991a	1.960a	17.8a	16.3a	44.20a	40.90a	2.30a	2.10a
Without	0.331b	0.32500	1.786b	1.772b	12.8b	11.8b	38.60b	36.30b	1.90b	1.80b
LSD (p = 0.05)	0.0110	0.01100	0.0150	0.0260	3.50	1.00	4.600	1.900	0.300	0.300
CV (%)	13.6000	13.30000	16.7000	16.7000	29.50	29.60	12.700	12.500	22.100	22.900

ns: Means in the same column are not significantly different; Means in the same column followed by the same letter are not significantly different at $p<0.05$

Table 3: Interaction effects of inoculation and nitrogen on the seed yield, biomass yield, harvest index and number of pods (2005-06)

	Inoculation							
	Seed yield (kg ha^{-1})		Biomass yield (kg ha^{-1})		Harvest Index (%)		No. pods (plant^{-1})	
Nitrogen (kg ha^{-1})	With	Without	With	Without	With	Without	With	Without
0	1267.0cd	659.7g	3121.3c	1929.3f	40.6cd	34.2e	4.3c	3.30d
20	1654.30a	848.7f	3623.0a	2056.7e	45.70a	41.3bcd	6.2a	3.9cd
40	1319.30c	1040.7e	3375.7b	2672.0d	42.7bc	39.0d	5.4b	4.10c
60	1439.00b	1208.3d	3298.3b	2739.3d	40.0cd	44.1ab	5.3b	4.20c
LSD (p = 0.05)	68.900	-	96.50	-	2.700	-	0.70	-
CV (%)	26.200	-	20.80	-	8.700	-	21.10	-

Means in the same parameter followed by the same letter are not significantly different at $p<0.05$

were higher with application of 20 kg N ha^{-1} while seed yield, shoot dry weight and number of branches were higher with application of 60 kg N ha^{-1}, in the 2 seasons. However, differences between 20 and 60 kg N ha^{-1} were not significant in terms of plant height, number of pods, harvest index and number of branches (Table 1 and 2). The number of nodules differed significantly among nitrogen treatments. However, it was observed that nodules formed also in the uninoculated plots. Availability of the nodules in the uninoculated treatment indicates that experimental soils contain native rhizobial population. Increases in these parameters by the application of 20 kg N ha^{-1} indicates the importance of starter nitrogen dose in the legumes for improving the efficiency of rhizobia for biological nitrogen fixation responsible for higher growth. These findings are close conformity to the findings of Igbasan *et al.* (1996) and Azad *et al.* (1992).

Inoculation treatment had also significant effect on the plant height, number of branches, number of nodules, root and shoot dry weight, number of pods, seed yield, biomass yield, harvest index and crude protein rate of seeds in both years (Table 1 and 2). The highest values regarding these parameters were obtained from inoculated treatment, whereas the lowest values were obtained from the uninoculated treatment. However, differences between inoculated and uninoculated treatments were not significant in terms of harvest index in both years and root

Table 4: Interaction effects of inoculation and nitrogen on plant height, number of branches, root and shoot dry weight (2005-06)

	Inoculation							
	Plant height (cm)		Number of branches ($plant^{-1}$)		Root dry weight ($g\ plant^{-1}$)		Shoot dry weight ($g plant^{-1}$)	
Nitrogen ($kg\ ha^{-1}$)	With	Without	With	Without	With	Without	With	Without
0	38.00e	33.00f	1.70e	1.40f	0.321d	0.268e	1.509f	1.471g
20	50.70a	38.7de	2.80a	1.8de	0.439a	0.334d	2.445a	1.723e
40	42.7bc	41.0cde	2.2bc	2.0cd	0.329d	0.348c	1.883c	1.834d
60	45.30b	41.7 cd	2.50b	2.40b	0.352c	0.372b	2.124b	2.115b
LSD (p = 0.05)	3.400	-	0.270	-	0.0120	-	0.0150	-
CV (%)	12.700	-	22.100	-	13.5000	-	16.7000	-

Table 5: Interaction effects of inoculation and nitrogen on the seed yield, biomass yield, harvest index and number of pods (2006-07)

	Inoculation							
	Seed yield ($kg\ ha^{-1}$)		Biomass yield ($kg\ ha^{-1}$)		Harvest index (%)		No. pods ($plant^{-1}$)	
Nitrogen ($kg\ ha^{-1}$)	With	Without	With	Without	With	Without	With	Without
0	1242.7c	636.0g	3086.7d	1866.7g	40.3bc	34.1d	3.6c	3.1d
20	1625.0a	810.3f	3616.7a	2013.7f	44.9a	40.3bc	4.8a	3.6c
40	1280.0c	1006.3e	3343.3b	2618.0e	42.1ab	38.4c	4.1b	4.0b
60	1406.7b	1173.7d	3200.0c	2705.0e	40.0bc	43.4a	4.3b	4.2b
LSD (p = 0.05)	68.80	-	90.60	-	2.900	-	0.30	-
CV (%)	26.80	-	21.30	-	8.500	-	13.20	-

Table 6: Interaction effects of inoculation and nitrogen on plant height, number of branches, root and shoot dry weight and nodules number (2006-07)

	Inoculation									
	Plant height (cm)		No. branches ($plant^{-1}$)		Root dry weight ($g\ plant^{-1}$)		Shoot dry weight ($g\ plant^{-1}$)		No. nodules ($plant^{-1}$)	
Nitrogen ($kg\ ha^{-1}$)	With	Without	With	Without	With	Without	With	Without	With	Without
0	35.00d	30.3e	1.50e	1.2f	0.285g	0.264h	1.497g	1.460h	15.0b	10.0d
20	47.00a	36.0d	2.60a	1.7de	0.406a	0.331d	2.430a	1.712f	22.3a	16.0b
40	40.0bc	39.0c	2.1bc	1.9cd	0.302f	0.343c	1.857d	1.811e	16.0b	12.0c
60	41.70b	40.0bc	2.30b	2.2b	0.315e	0.363b	2.057c	2.104b	12.0c	9.0d
LSD (p = 0.05)	1.900	-	0.280	-	0.0070	-	0.0330	-	1.50	-
CV (%)	12.500	-	22.900	-	13.3000	-	16.7000	-	29.60	-

Means in the same parameter followed by the same letter are not significantly different at $p<0.05$

dry weight in 2006-07. This positive relationship of yield and growth parameters with inoculation could be related to the nitrogen fixation ability of nodules, which consequently results in increased growth and yield. Similar results are found by Jha and Singh (1996) and Idris and Sandhu (1981).

Interactions of nitrogen and inoculation had significant effects on the seed yield, biomass yield and root and shoot dry weight, number of pods, plant height, number of branches and harvest index in both years, except for crude protein rate in both years and number of nodules in 2005-06 (Table 3-6).

While the application of 20 kg N ha^{-1} + inoculation gave the highest values related to all parameters, the lowest values were obtained from control without inoculation treatment. The highest seed yield was recorded in 20 kg N ha^{-1} + inoculation treatment with 1654.3 kg ha^{-1} in the 1st year and 1625.0 kg ha^{-1} in the 2nd year. These findings are in agreement with the results of Semu *et al*. (1982) and Kaya (2000).

CONCLUSION

It may be concluded that 20 kg N ha^{-1} + inoculation gave highest values related to yield and yield components of field pea. Thus, 20 kg N ha^{-1} + inoculation treatment could be preferred to grow in areas where amount of nitrogen are limiting biological nitrogen fixation in field pea production.

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