

Effect of Phosphorus Application and *Rhizobium* Inoculation on the Yield, Nodulation and Nutrient Uptake in Field Pea (*Pisum sativum* sp. *arvense* L.)

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Abstract: A field experiment was conducted during 2005-06 and 2006-07 growing seasons in Van, Turkey, to determine the effects of *Rhizobium* inoculation and different levels of phosphorus on the yield and nutrient uptake of field pea (*Pisum sativum* sp. *arvense* L.). Phosphorus application had significant effect on the plant height, number of branches, root and shoot dry weight, number of nodule, seed and biomass yields, number of pod, crude protein rate and phosphorus content of seed in both years. There was linear increase in the root dry weight, nodule number, crude protein rate and phosphorus content of seed up to 90 kg P₂O₅ ha⁻¹ application. The highest seed yield was obtained under treatment 60 kg P₂O₅ ha⁻¹+ inoculation with 2855.0 and 2828.3 kg ha⁻¹ in 2005-06 and 2006-07, respectively. Plant height, number of branches, shoot dry weight, number of pod, seed and biomass yields increased up to 60 kg P₂O₅ ha⁻¹ and then decreased at 90 kg P₂O₅ ha⁻¹. Inoculation treatment had also significant effect on the plant height, number of branches, root and shoot dry weight, number of nodules, seed and biomass yields, number of pod, crude protein rate and phosphorus content of seed in both years. The highest values regarding these parameters were obtained from inoculated plants, whereas the lowest values were obtained from the uninoculated plants.

Key words: *Rhizobium*, inoculation, phosphorus, field pea, nutrient uptake

INTRODUCTION

Phosphorus and nitrogen are major nutrient elements for grain legumes. Phosphorus has a key role in the energy metabolism of all plant cells and particularly for nitrogen fixation in legume crops. Pea has a relatively high requirement for phosphorus (Bowren *et al.*, 1986; Slinkard and Drew, 1988) and yield and seed quality can be enhanced by phosphorus fertilizer in soils testing low in phosphorus (Pulung, 1994). Phosphorus is needed to promote the development of extensive root systems and vigorous seedlings. Encouraging vigorous root growth is an important step in promoting good nodule development. Phosphorus also plays an important role in the nitrogen fixation process.

Pea inoculated with the appropriate strain of *Rhizobium* bacteria is able to fix a large portion of its nitrogen requirement from air in the soil. Field peas can be met their N needs between 30-80% through biological fixation (Ali-Khan and Zimmer, 1989; Bowren *et al.*, 1986). For this to occur, the seed or the soil surrounding the seed must be inoculated. The rhizobia enter the root hairs and induce nodule formation. The plant provides energy for the bacteria living inside the nodules and, in return, the rhizobia convert atmospheric nitrogen into plant-

useable forms. Biological nitrogen fixation is an important nitrogen source due to the fact that it requires less energy and causes less environmental pollution. Therefore, it is essential to establish and utilize the relation between the *Rhizobium* and the legumes in the production of these crops. The soil of the region where the study was conducted has low nitrogen and phosphorus content and their availabilities are low due to the various soil and other environmental factors. Therefore, it was aimed to determine the effects of phosphorus fertilizer and *Rhizobium* inoculation on the yield and growth parameters, as well as phosphorus content and crude protein rate of seed 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 been grown for at least 10 years, in 2005-06 and 2006-07 growing seasons using field pea genotype with number 110121-1, a well-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 Somesagaran 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 phosphorus doses (Control, 30, 60 and 90 kg P_2O_5 ha^{-1}) as triple super phosphate 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 (17 October 2005 and 18 October 2006). The seeding rate was 45 seeds m^{-2} . A basal dose of 30 kg ha^{-1} ammonium sulphate 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 (25 June 2006 and 28 June 2007). At flowering, 10 random plants were removed from each plot. Numbers of nodules as well as dry weight of root and shoot per 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, phosphorus content and crude protein rate of seed 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 phosphorus 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). Phosphorus application had significant effect on the plant height, number of branches, root and shoot dry weight, number of nodule, seed yield, biomass yield, number of pod, crude protein rate and phosphorus content of seed in both years. The lowest values were obtained from the control treatment. However, no significant differences between control and 30 kg P_2O_5 ha^{-1} application were observed in terms of crude protein rate in 2005-06, biomass yield, plant height and number of nodule in 2006-07, seed yield and number of pod in both years (Table 1 and 2). There was linear increase in the root dry weight, number of nodule, crude protein rate and phosphorus content of seed up to 90 kg P_2O_5 ha^{-1} application. Increases in these parameters by the application of phosphorus may be due to the fact that is positive correlation between phosphorus and these parameters. Because the soil of the region is poor for phosphorus, plants in this experiment also reacted positively to higher levels of phosphorus. These findings are close conformity to the findings of Rao and Reddy (1997) and Singh *et al.* (1981). Plant height, number of branches, shoot dry weight, number of pod, seed yield and biomass yield increased up to 60 kg P_2O_5 ha^{-1} and then decreased at 90 kg P_2O_5 ha^{-1} (Table 1 and 2). Phosphorus application above a threshold amount may cause decrease in these parameters (Shekhar and Sharma, 1991). The results are in agreements with the results of Gubbels (1992), Padmalatha and Rao (1993), Sarvaiya *et al.* (1993), Yadav and Chauhan (1997) and Verma *et al.* (1998).

Inoculation treatment had also significant effect on the plant height, number of branches, root and shoot dry weight, number of nodule, seed yield, biomass yield, number of pod, crude protein rate and phosphorus content of seed 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

Table 1: Effect of inoculation and phosphorus on the seed and biomass yield, plant height, number of branches and pods

Treatment	Seed yield (kg ha ⁻¹)		Biomass yield (kg ha ⁻¹)		Plant height (cm)		Number of branches (plant ⁻¹)		Number of pods (plant ⁻¹)	
	2005-06	2006-07	2005-06	2006-07	2005-06	2006-07	2005-06	2006-07	2005-06	2006-07
Phosphorus (kg ha⁻¹)										
0	2265.5c	2244.5c	5663.5c	5611.2d	43.3b	41.7b	1.4d	1.3d	3.3c	3.1b
30	2312.8c	2288.8c	5781.7c	5771.8c	45.2b	44.0b	1.9c	1.8c	3.6bc	3.4b
60	2681.5a	2659.8a	6703.5a	6649.3a	50.3a	48.3a	2.5a	2.3a	4.6a	4.3a
90	2446.8b	2431.0b	6117.0b	6077.5b	46.3ab	45.2ab	2.2b	2.1b	4.0b	3.8ab
LSD (p = 0.05)	77.6	49.04	193.9	110.09	4.36	3.38	0.19	0.16	0.48	0.68
Inoculation										
With	2583.7a	2566.6a	6458.9a	6416.3a	49.9a	48.3ns	2.2a	2.0a	4.4a	4.1ns
Without	2269.7b	2245.5b	5673.9b	5638.6b	42.7b	41.3	1.8b	1.7b	3.4b	3.2b
LSD (p = 0.05)	58.4	81.90	145.9	164.38	5.99	1.29	0.09	0.28	0.88	0.90
CV (%)	9.8	9.97	9.8	9.73	11.60	10.95	22.4	21.81	21.90	21.70

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 2: Effect of inoculation and phosphorus on the root and shoot dry weight, number of nodules, crude protein rate and phosphorus content

Treatment	Root dry weight (g plant ⁻¹)		Shoot dry weight (g plant ⁻¹)		Nodules number (plant ⁻¹)		Crude protein rate (seed ⁻¹ %)		Phosphorus content (seed ⁻¹ %)	
	2005-06	2006-07	2005-06	2006-07	2005-06	2006-07	2005-06	2006-07	2005-06	2006-07
Phosphorus (kg ha⁻¹)										
0	0.275d	0.268d	1.487d	1.480d	12.20c	11.2d	16.6c	16.0c	0.235d	0.229d
30	0.322c	0.311c	1.798c	1.793c	14.00bc	13.0c	17.4b	16.7bc	0.290c	0.280c
60	0.334b	0.327b	2.274a	2.263a	15.70b	14.7b	17.9b	17.3b	0.328b	0.310b
90	0.392a	0.381a	1.973b	1.962b	20.70a	19.8a	19.9a	18.9a	0.350a	0.336a
LSD (p = 0.05)	0.05	0.005	0.005	0.004	1.85	1.48	0.69	0.82	0.018	0.020
Inoculation										
With	0.333ns	0.326a	1.985a	1.974a	17.30a	15.9a	18.7a	17.9a	0.314a	0.299a
Without	0.329	0.317b	1.781b	1.775b	14.0b	13.4b	17.2b	16.6b	0.288b	0.278b
LSD (p = 0.05)	0.06	0.020	0.013	0.009	1.24	2.15	1.24	1.22	0.022	0.005
CV (%)	13.6	13.8	16.7	16.700	24.60	25.2	9.1	8.4	16.0	15.4

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 phosphorus on the seed and biomass yield, root and shoot dry weight (2005-06)

Phosphorus (kg ha ⁻¹)	Seed yield (kg ha ⁻¹)		Biomass yield (kg ha ⁻¹)		Root dry weight(g plant ⁻¹)		Shoot dry weight (g plant ⁻¹)	
	Inoculation		Inoculation		Inoculation		Inoculation	
	With	Without	With	Without	With	Without	With	Without
0	2486.0bc	2045.0f	6214.7bc	5112.3f	0.285g	0.265h	1.505f	1.468g
30	2440.7cd	2185.0e	6101.3cd	5462.0e	0.305f	0.337d	1.879c	1.718e
60	2855.0a	2508.0bc	7137.3a	6269.7bc	0.317e	0.351c	2.118b	1.827d
90	2553.0b	2340.7d	6382.3b	5851.7d	0.406a	0.377b	2.436a	2.112b
LSD (p = 0.05)	102.4		255.9		0.007		0.008	
CV (%)	9.8		9.9		13.6		16.7	

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

Table 4: Interaction effects of inoculation and phosphorus on the seed and biomass yields, root and shoot dry weight and crude protein rate (2006-07)

Phosphorus (kg ha ⁻¹)	Seed yield (kg ha ⁻¹)		Biomass yield (kg ha ⁻¹)		Root dry weight(g plant ⁻¹)		Shoot dry weight(g plant ⁻¹)		Crude protein rate (seed ⁻¹)	
	Inoculation		Inoculation		Inoculation		Inoculation		Inoculation	
	With	Without	With	Without	With	Without	With	Without	With	Without
0	2475.7bc	2013.3f	6189.0bc	5033.3f	0.273g	0.263h	1.499f	1.460g	16.2de	15.8e
30	2423.7c	2154.0e	6059.0c	5484.7e	0.290f	0.331d	1.871c	1.714e	17.3bcd	16.1de
60	2828.3a	2491.3bc	7070.7a	6228.0b	0.310e	0.344c	2.421a	2.105b	18.0b	16.7cde
90	2538.7b	2323.3d	6346.7b	5808.3d	0.395a	0.366b	2.105b	1.819d	20.2a	17.5bc
LSD (p = 0.05)	70.4		154.8		0.010		0.007		1.2	
CV (%)	10.0		9.7		13.8		16.7		8.4	

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

treatments were not significant in terms of plant height and number of pod in 2005-06 and root 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 many workers like Maiti *et al.* (1988), Bengtsson (1989) and Dravid (1991).

Results on interaction effects of phosphorus applications and rhizobial inoculation have been presented in Table 3 and 4. Interactions of phosphorus and inoculation had significant effects on the seed yield,

biomass yield, root and shoot dry weight in both years and on the crude protein rate in the second year. While the applications of 60 kg P₂O₅ ha⁻¹+ inoculation gave the highest seed yield, biomass yield and shoot dry weight, the application of 90 kg P₂O₅ ha⁻¹+ inoculation gave the highest crude protein rate and root dry weight. The lowest values were obtained from control without inoculation treatment. The highest seed yield was recorded in 60 kg P₂O₅ ha⁻¹+ inoculation treatment with 2828.3 kg ha⁻¹ in the first year and 2855.0 kg ha⁻¹ in the second year.

CONCLUSION

In conclusion, 60 kg P₂O₅ ha⁻¹ which produced higher seed yield could be preferred to grow in areas where amount of phosphorus are limiting biological nitrogen fixation in field pea production. Also, improvement in yield and yield attributes in inoculation treated plots suggests that this treatment could be utilized in order to increase number of nodules with rhizobial inoculation, which is important for atmospheric nitrogen fixation. Thus, it may be concluded that seed inoculation gave highest values related to yield and yield components of field pea with respect to the genotype of field pea tested and in the areas where the plots were sown.

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