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IS PLANT GROWTH PROMOTING RHIZOBACTERIA AN ALTERNATIVE TO MINERAL PHOSPHORUS FERTILIZER IN PEA SEED PRODUCTION?

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Abstract

The interest in the usage of biofertilizer as alternative to mineral fertilizer increase continuously due to increasing mineral fertilizer cost and heavy metal accumulation in the soil such as cadmium. The objective of this study was to assess the effects of four biofertilizer (N_2-fixing (NF), P-solibilizing (PS), N_2 fixing-P solubilizing (NF+PS), commercial biofertilizer (CB) with and without mineral phosphorus fertilizer on seed yield forage pea (Pisum sativum spp. arvense L.) The application of biofertilizer did not affect seed yield, biological yield, crude protein content and SPAD value. The use of mineral fertilizer only increased seed crude protein content. The effects of biofertilizer on pea seed yield, biological yield and crude protein content varied significantly depending on year. These results indicated that understanding of factors such as biofertilizer, mineral fertilizer and environment will enable us to use biofertilizer as an alternative to mineral fertilizer to optimize productivity and sustainability of pea production.

***Key words**: biofertilizer, mineral fertilizer, phosphorus, seed yield, pea.*

INTRODUCTION

Peas are cultivated widely as rotation or second crops for forage and pulse production in semi-arid environments. Both seeds and forages of pea are rich in protein and mineral content (Acikgoz et al., 1985). The productivity of peas like in the other legume crops are restricted by phosphorus deficiency. Thus, producers rely on mineral phosphorus fertilizer to achieve sustainable production. However, prices of chemical fertilizer increase continuously due to increasing energy cost which restricted their utilization economically. On the other hand, phosphorus fertilizers are not environmental friendly input in agriculture due to cadmium content (Al-Fayiz et al., 2007). Recently, there has been interest in more environmentally sustainable agricultural practices. A considerable numbers of bacteria species that are associated with the rhizosphere are able to exert a beneficial effect on plant growth (Rodriguez and Fraga, 1999). These microorganisms secrete different type organic acid (Illmer and Schinner, 1992*)* thus lowering the pH in the rhizosphere and consequently dissociate the bound form of phosphate (Rodriguez and Fraga, 1999). Phosphorus biofertilizers also help increase nitrogen fixation and availability of some microelements such as Fe, Zn etc. Generally, only 0.1% of total P in soil is available to plants (Scheffer and Schachtschabel, 1992). The way of increase to P available to plants is enzymatic decomposition or microbial inoculation (Illmer and Schinner, 1992). Hence, bacteria might be partially substitute chemical fertilizer or they are use together.

Plant growth promoting rhizobacteria (PGPR) are a group of bacteria that can actively colonize soils, plant rhizosphere, root or intercellular spaces of plants (Illmer and Schinner, 1992; Sahin et al., 2004; Cakmakci et al., 2007). PGPR promote plant growth either increasing nutrient intake or changes enzymatic or hormone synthesis, even some strains had pathogen control by having antibiotic effect (Xie et al., 1996; Stirk et al., 2002). PGPRs are changes chemical compounds of the applied plants. In general, PGPR application encourages an increase in crude protein content (Peix et al., 2001; Osman et al., 2010; Yolcu et al., 2012)

In general, there are currently no adequate knowledge on the effect of PGPR on the yield and chemical components of forage peas. The objective of this study were to determine the effects of phosphorus (with and without) and bacteria application on seed yield, biological yield, crude protein content and spat value of pea and possibility of phosphorus fertilizer or biofertilizer application in pea cultivation in semi-arid conditions.

MATERIALS AND METHODS

The field experiment was conducted at the experimental station of Faculty of Agriculture, University of Ataturk, Erzurum ($39^0 51^I$N and $41^0 61^I$E, 1850 m above sea level). The soil of experimental area was loamy with organic matter content of 1.92%, with lime 4.65% and pH of 7.24. Corresponding available P_2O_5 and K_2O contents were 27.3 kg ha^{-1} and 120.0 kg ha^{-1} in the first year, respectively. In the second year, it was loamy, with organic matter 1.85%, with lime 4.62%, pH of 7.80, available P_2O_5 88kg ha^{-1} and K_2O 181 kg ha^{-1}. In Erzurum, winters are long and extremely cold and summers are cool, short and arid. Long-term annual mean temperature is 5.0^oC, rainfall is 395.6 mm and relative humidity is 66.5% in the study area. Total annual precipitation and mean annual temperature were 437.8 mm and 5.8 ^0C in 2009 and 475.9 mm and 7.9 ^0C in 2010, respectively in the experiment years.

The experiment was arranged a randomized complete block design with three replications. Treatment consist of 0 or 50 kg P_2O_5 ha^{-1}, which suggested doses of phosphorus fertilizer in annual legumes cultivation in the region. Triple supper phosphate form of the phosphorus fertilizer were used and five different type biofertilizers were (a) control (C), (b) N_2-fixing (NF) (*Bacillus subtilis*), (c) P-solibilizing (PS) (*Bacillus megaterium*), (d) N_2 fixing-P solubilizing (NF+PS) (*Burkholderia cepacia* GC sup.B) and (e) commercial biofertilizer (CB) (Bio-one) was developed by Texas University which contain *Azotobacter vinelandi* living aerobic condition and *Clostridium pasteurianum* living anaerobic condition.

The biofertilizer were applied sterilized seeds before sowing and phosphorus fertilizer were broadcasted plots surface before sowing and it was incorporated the soil using hand harrow. Forage pea (*P. sativum* spp. *arvense* L. cv Taskent) was sown by hand with 100 seeds per m^2 in May 20th 2009 and May 15th 2010. The plot size was 1.5 m by 5 m = 7.5 m^2, consisting of 5 rows spaced 30 cm apart. Weed control was done by hand hoeing in the beginning of June. The plots were irrigated 3 times in 2009 and 2010 with flooding system when plant colour turns dark green due to lack of moisture in the soil during the growing season.

Harvesting was performed after taking out one row from each side of the plots and a 0.5 m area from beginning or end of each row. Seed yield was determined as harvesting the plant at seed maturity stage and samples were dried in the oven at 50^oC and then weighted to determine biological yield. Harvested and oven dried material were trashed by hand to separate seed. Seed yield was determined after cleaning the seeds. After weighting, hay and seed samples were grounded to pass through a 2 mm sieve and analysed for chemical characteristics. Total N content of the samples was determined by the Kjeldahl method and multiplied by 6.25 to give the crude protein content. Relative chlorophyll content (SPAD) was determined with a chlorophyll meter (SPAD-502, Minolta cam- era Co., Ltd., Japan) in characteristic development phases that beginning of development of fruits.

All data were subjected to analysis of variance based on General Linear Model for completely randomised design using the Statview package (SAS Institute, 1998). Means were separated using Duncan's multiple range tests.

RESULTS AND DISCUSSIONS

The seed yield was higher in the first year than in the second year (Table 1). Neither phosphorus fertilizer nor biofertilizer application had significant effect on seed yield of pea in the experiment. The plots received biofertilizer plus phosphorus fertilizer had similar or higher seed yield than control. The highest seed yield was obtained from NF+PS application among biofertilizer applications but it was not higher than alone phosphorus fertilizer application. As a result of these different responses, BF x P interaction was significant (Figure 1a). According to first year results, only phosphorus fertilizer application gave the best results with respect to seed production but in the second year PS and

NF+PS application without phosphorus fertilizer gave better result than the other treatments. Thus, BF x P x Y interaction was significant (Figure 1b). An average biological yield was 7.70 t ha^{-1}, it was higher in the first year than the second year. Neither main effects nor interaction effects were significant with respect to biological yield (Table 1).

An average CP content of seed was 27.00% and it changed depending on the years. The seeds had higher CP content in the second year than in the first year. While main effect of phosphorus fertilizer application was significant, biofertilizer applications were not significant. Phosphorus fertilizer application caused a decrease in seed CP content in the experiment. Seed CP content was higher in P_0 doses than P_{50} doses application (Table 1). Whereas, this differences were not recorded when adding biofertilizer to plots. Hence, BF x P interaction for seed CP was significant (Figure 2a). In the first year, there were no significant differences in CP content of seed with respect to phosphorus fertilizer application but higher seed CP content was recorded in P_{50} applications. Hence, P x Y interaction was significant (Figure 2b).

Biofertilizer, phosphorus and year effect was not significant on SPAD value (Table 1). SPAD value application of biofertilizer, phosphorus and year varied from 47.15 to 49.93, from 48.66 to 47.79 and from 47.93 to 48.33, respectively. Biofertilizer, phosphorus application, year and their interactions were not significant (Table 1).

Table 1. Analysis of variance results with main effects and interactions of biofertilizer and phosphorus fertilizer application on seed yield (SY), biological yield (BY) and crude protein content (CP) and SPAD value

Treatments	SY (t ha^{-1})	BY (t ha^{-1})	CP (%)	SPAD
C	1.65	7.56	26.46	47.75
NF	1.76	7.33	26.69	49.93
PS	1.74	7.85	26.67	47.89
NF+PS	1.84	7.86	27.10	47.93
CB	1.49	7.90	28.08	47.15
Average	**1.70**	**7.70**	**27.00**	**48.13**
P_0	1.59	7.51	27.56 A	48.56
P_{50}	1.80	7.89	26.44 B	47.69
Average	**1.70**	**7.70**	**27.00**	**48.13**
2009	1.87 A	8.61 A	25.05 B	47.93
2010	1.52 B	6.79 B	28.95 A	48.33
Average	**1.70**	**7.70**	**27.00**	**48.13**
BF	ns	ns	ns	Ns
P	ns	ns	***	Ns
Y	***	***	***	Ns
BF x P	*	ns	***	Ns
BF x Y	ns	ns	ns	Ns
P x Y	ns	ns	**	Ns
BF x P x Y	*	ns	ns	Ns

ns: non-significant, *: p<0.05, **: p<0.01, ***: p<0.001. Means in the same column with different letters are significant.

The differences in seed yield between years can be attributed to climatic differences because first year weather was cooler than the second year. Pea is a typically cool season plant as the first year prevailed cool weather extent the grain filling period, hence, seed yield was higher than the warmer second year. The yield increase in favourable soil and climate were also reported by various studies (Lambert and Linck, 1958; Egli and Wardlaw, 1980; Claphamet al., 2000). The highest seed yield was obtained by using alone phosphorus fertilizer, phosphorus fertilizer plus PS or NF+PS biofertilizer application (Figure 2a) but phosphorus fertilizer used in the combination of the other biofertilizer causes decreases in seed production. Phosphorus stimulate flowering and seed yield, hence, seed yield must increase with phosphorus fertilizer and PS biofertilizer application. But the decrease in the seed yield phosphorus fertilizer and the other biofertilizer application might be related to changes in rhizosphere microbial activity depending on new bacteria because biofertilizer changes root microbial activity and it can sometimes be harmful effect on plant growth (Rodriguez and Fraga, 1999). The different response related to years to phosphorus fertilizer and biofertilizer caused triple interaction in the experiment. The differences in soil microbial content might have caused this different response. Because, the effects of biofertilizer change depending on soil microbial content (Rodriguez and Fraga, 1999).

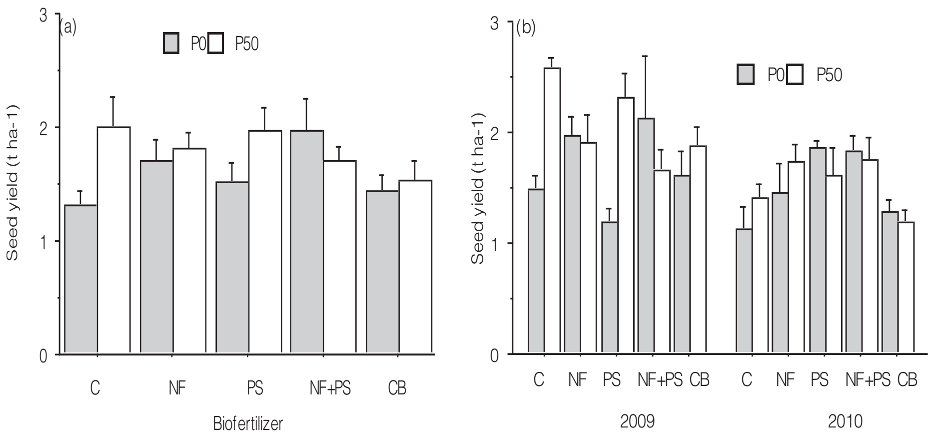

Figure 1. Seed yield of forage pea as affected by: (a) biofertilizer x phosphorus; (b) biofertilizer x phosphorus x year (Bars indicated ± s.e.)

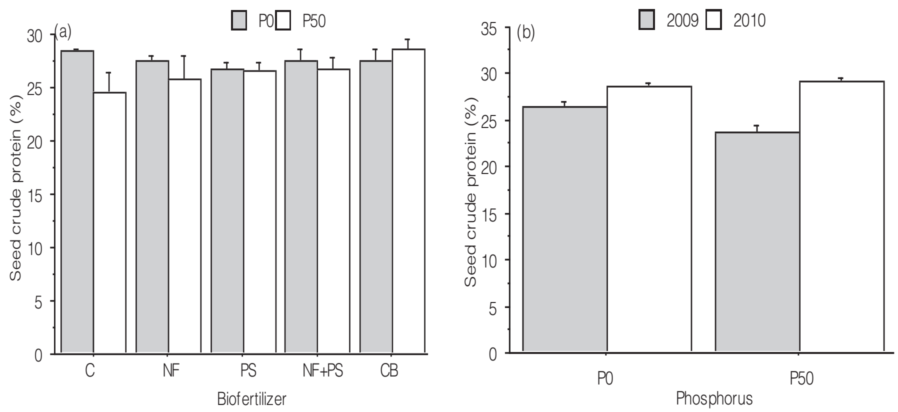

Figure 2. Seed crude protein content of forage pea as affected by: (a) biofertilizer x phosphorus; (b) phosphorus x year (Bars indicated ± s.e.)

As is in seed yield, biological yield showed significant differences between years. Biological yield was higher in the first year than second years because first year weather was cooler than the second year prevailed. Pea is well adapted cool climate (Lambert and Linck, 1958) hence first year biological yield was higher. Neither chemical nor biofertilizer had significant effect on biological yield and there also were not any interaction. These results implied that growing period which determined by climatic condition are more detrimental effect on pea production. Especially warm weather reduced final production in pea cultivation as happened in the second year in the experiment.

Chemical content of pea crops affected significantly by years. Higher crude protein content of both hay and seed were recorded second year in the experiment. The weather was warmer in the second year than in the first year. Since warmer weather restricted photosynthetic period, carbohydrate accumulation in vegetative tissue or grain decrease as a result of this effect crude protein content was higher in the first year. Because initially protein skeleton constituted in the cell thereafter carbohydrate accumulation occurred (Osman et al., 2010).

Phosphorus fertilizer or PS application causes significant changes in chemical content and it generally an increase in crude protein and mineral content (Peix et al.,2001). But crude protein content was not affected by phosphorus fertilizer or also slightly decreases in crude protein content of seeds in the experiment. However, PS bacteria causes significantly increase in crude protein content in both hay and seed. Crude protein content generally increases as phosphorus availability for plant. The decrease in crude protein content of seed might be related to the differences in environmental condition between years. P x Y interaction was significant for crude protein content of seed. Because phosphorus application causes an increases in crude protein content of seed in the first year, it cause a slight decreases in the second year. Environmental factors responsible for very wide changes in crude protein content are not fully understood (Reichert and MacKenzie, 1982). In our studies value of relative chlorophyll content (SPAD) was not differentiated by any treatment, year and their interactions. The value of SPAD ratio depends on the color of leaves, which informs not only of the nutritional status, but also is an inherited trait, as reported by Ambrose (2010). According to this author, genotypes of pea with dark-green bracts are characterized by higher SPAD values, > 60, and those of the bright green bracts have SPAD values < 30.

In conclusions, biofertilizer and inorganic phosphorus application had not positive effect on seed and biological yield in forage pea under Erzurum ecological condition. Conversely the effect of year was significant on seed yield, biological yield and crude protein content. But inorganic phosphorus application decreased crude protein content of seed.

CONCLUSIONS

In these studies, understanding of interaction between microbial fertilizer and soil microbial content will enable us to use microbial fertilizer as an alternative to mineral fertilizer. Because among biofertilizer, mineral fertilizer and year interaction is very common in the microbial fertilizer studies as it is in our study. These results indicated that biofertilizer or mineral fertilizer could be use in pea seed production.

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