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Effects of phosphorus fertilization and *Pseudomonas fluorescens* strain on the growth and yield of faba bean (*Vicia faba* L.)

Efecto de la aplicación de fósforo y cepas de Pseudomonas fluorescens *en el desarrollo y producción de haba (*Vicia faba *L.)*

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ABSTRACT

A field experiment was conducted in Lihijan, Iran in 2013 to evaluate the effect of phosphorus fertilization (P) and *Pseudomonas fluorescens* strain on the growth and yield of the faba bean. The experiment used a randomized complete block design with a factorial treatment arrangement and three replicates. The experimental factors were phosphorus application (0 and 25 kg ha^{-1}) and *P. fluorescens* strains (non-inoculated, strain 136, and strain 168). ANOVA showed a significant interaction effect of P and *P. fluorescens* strain on plant height, pod number per plant, 100 grain weight, pod length, and pod and grain yield. ANOVA also showed that grain number per pod was significantly affected by P application and *P. fluorescens* strain, while haulm yield was significantly affected only by *P. fluorescens* strain. In the absence of P, the grain yield of inoculated plants was not significantly different from that of non-inoculated plants. In the presence of P (25 kg ha^{-1}), strain 168 showed an increase in grain yield of 42% and 65% over strain 136 and non-inoculated plants, respectively. The results indicated that application of phosphorus (25 kg ha^{-1}) and seed inoculation with *P. fluorescens* strain 168 can be recommended to increase grain/pod yield significantly.

Key words: Faba bean, phosphorus, *Pseudomonas fluorescens,* yield.

RESUMEN

El experimento de campo fue llevado a cabo en Lihijan, Irán en 2013 con el objetivo de evaluar el efecto de la aplicación de fósforo (P) y Pseudomonas fluorescens *en el desarrollo y rendimiento de un cultivo de haba. El diseño experimental fue de bloques completos al azar con arreglo factorial 2x3, con tres repeticiones. Los factores experimentales fueron la aplicación de fósforo (0 y 25 kg ha*$^{-1}$*) y cepas de* P. fluorescens *cepas (no inoculados, cepa 136 y cepa 168). El ANOVA mostró un efecto interactivo significativo de P y cepa de* P. fluorescens *en altura de la planta, número de vainas por planta, peso del 100 granos, longitud de vaina y el rendimiento de vaina y grano. SE observó que el número de granos por vaina se vio afectado significativamente por la aplicación de fósforo y cepa de* P. fluorescens *cepa, mientras que el rendimiento del tallo sólo fue significativamente afectada por la cepa de* P. fluorescens. *En ausencia de P, el rendimiento de grano de plantas inoculadas fue igual que en plantas no inoculadas. En presencia de P (25 kg ha*$^{-1}$*), la cepa 168 mostró un aumento en el rendimiento de grano de 42% y 65%, en comparación a las plantas inoculadas con la cepa 136 y las plantas no inoculadas, respectivamente. Los resultados sugieren que la aplicación de fósforo (25 kg ha*$^{-1}$*) y la inoculación de semillas con* P. fluorescens *cepa 168 se puede recomendar para incrementar el rendimiento de vainas y grano en un cultivo de habas.*

Palabras clave: *haba, fósforo,* Pseudomonas fluorescentes*, rendimiento.*

Introduction

With protein contents of 12% to 32%, legume crops play an important role in human nutrition. Faba bean is an annual winter crop in temperate and subtropical regions; it was cultivated globally over an area of 7.3 million hectares in 2012 and produced a mean yield of 1174 kg ha^{-1}. In Iran 8000 ha are under cultivation with faba bean, with a mean yield of 2125 kg ha^{-1} (dry beans). Faba bean has four main functions in agro-ecosystems: (1) providing food and feed that is rich in protein; (2) supplying N to agro-ecosystems by symbiotic N_2 fixation with *Rhizobium* bacteria to increase soil fertility; (3) diversifying the crop system to reduce constraints on growth and yield by the other crops in the rotation; and (4) reducing fossil energy consumption for crop production. The faba bean is also grown for green manure and can

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significantly increase the yields of cereal and other crops (Wani *et al.*, 1994).

Adequately balanced amounts of nutrients are essential to achieve maximum productivity. Phosphorus (P) is an essential element for crops with greatly decreased uptake from fixation with mineral ions such as aluminum, iron, calcium and magnesium (Feng *et al.*, 2004). Plants can only absorb P as $H_2PO_4^{-1}$ or HPO_4^{-2}, which are mostly present in very low concentrations in the soil (Bhattacharyya and Jha, 2012). P plays a structural role in the nucleus and cell membrane (Raghothama and Karthikeyan, 2005). A large percentage of P from chemical phosphate fertilizers is not available to plants because at least 70–90% of P that enters the soil is fixed by Fe, Al, and Ca in soils (McBeath *et al.*, 2006). The lack or low rate of essential elements such as P and potassium in the soil negatively affect growth and the nitrogen fixation rate. Bolland *et al.* (2000) reported that P fertilizer increased crop yield 50% to 100% because of the increased number of pods per plant. Turk and Tawaha (2002) applied different rates of P (0, 17.5, 35 and 52.5 kg ha^{-1} of phosphorus oxide) to faba beans and obtained the highest yield at 52.5 kg ha^{-1}. They reported application of P to faba bean plants significantly increased 100 grain weight, grain number per pod and pod number per plant.

Bacteria of the genus *Pseudomonas* are widely distributed in soil and can colonize plant rhizospheres to produce different metabolites. Studies have found that the most important characteristic in promoting plant growth is phosphate-solubilizing bacteria (Rashid *et al.*, 2004). These bacteria are usually present around the roots and assist in nutrient uptake by the plants. The use of growth-promoting bacteria can increase the growth and yield of crops through direct and indirect mechanisms. It has been proven that these bacteria increase the qualitative/quantitative yields of most crops through biological fixation of nitrogen, biological control of plant pathogens (Saravanakumar *et al.*, 2007; Dey *et al.*, 2004), vitamin production, increased iron absorption by siderophore secretion (Shaharoona *et al.*, 2008; Braud *et al.*, 2009) and production of the plant hormones cytokinin, auxin and gibberellin (Dey *et al.*, 2004), decreasing levels of ethylene by production of Acc deaminase (Shaharoona *et al.*, 2008) and increasing phosphorus solubility. Previous studies have shown that co-inoculation of phosphate-solubilizing bacteria (PSB) with other growth-promoting rhizobacteria decreased phosphate fertilizer consumption by 50% without a significant reduction in grain yield (Jilani *et al.*, 2007; Yazdani *et al.*, 2009). The ability of *Pseudomonas* to solubilize P affects the yield, growth parameters, and P uptake of faba beans (Crowley, 2006). *Pseudomonas* species have been shown to increase the number and dry weight of the nodes, grain yield, yield components, and availability and uptake of elements in soybeans (Son *et al.*, 2006). Dey *et al.* (2004) reported that *Pseudomonas* strains increased pod yield by 18% to 26%. Saharan *et al.* (2010) reported a 46% increase in Vigna yield using *Pseudomonas* R81. In a pot experiment with mung bean, Sharma *et al.* (2003) reported that the *Pseudomonas* strain GRP3 increased the yield of shoots by 101%, roots by 39% and chlorophyll content by 40%.

To date few studies have examined the effect of simultaneous application of P and *Pseudomonas* strains on the growth and yield of faba bean. This experiment examined the effect of P rate and *Pseudomonas* strain on the growth and yield of the faba bean.

Materials and methods

Experimental site and design, crop management, and sampling

The present study was conducted during the 2013-2014 growing season in Lahijan in the Guilan province of Iran (longitude 50˚ 0E, latitude 37˚ 11N). The physical and chemical properties of the soil (0-30 cm) were 0.78% organic matter content, 15% clay, 49.5% silt, 35.5% sand, 7.7 pH, total N 0.07%, available phosphorous 10.5 mg kg^{-1}, available potassium 148.0 mg kg^{-1} and EC 0.43 ds m^{-1}. Weekly precipitation and mean temperature (maximum, minimum and average) are shown in Figures 1 and 2, respectively. The experiment was performed as a randomized complete block design with a factorial treatment arrangement with three replicates. The experimental factors were phosphate fertilizer application (0 and 25 kg ha^{-1}) and *Pseudomonas* strain (un-inoculated, *Pseudomonas fluorescens* strain 136, and *Pseudomonas fluorescens* strain 168). Phosphorus was applied as triple superphosphate fertilizer using the strip method. *Pseudomonas* bacteria were obtained from the Soil and Water Research Institute, Karaj, Iran and used as instructed. The field was prepared using two perpendicular disks and a leveling step. Nitrogen and potassium fertilizers were added before sowing based on the results of soil testing. Each plot was 4

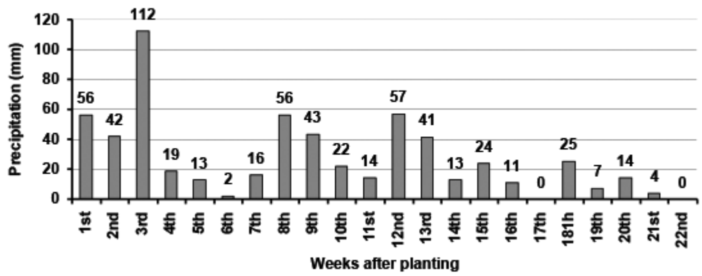

Figure 1. Weekly precipitation during faba bean growing period

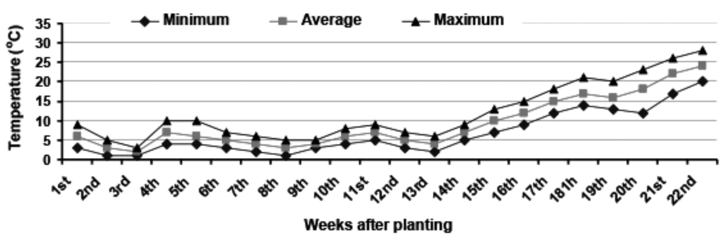

Figure 2. Weekly temperatures (maximum, minimum and mean) during the faba bean growing period

× 2 m with a planting distance of 20 × 50 cm. The two plots were 70 cm apart and the distance between repeats was 1 m. The bean seeds (variety Barkat) were cultivated at a depth of 5 cm. Crop protection (control of pests and diseases) was performed as needed during the crop growing period. Manual weeding was carried out in each plot. The border lines were excluded when measuring plant height; 5 plants were randomly selected and the distance from soil surface to end of main stem was measured and recorded in cm. The mean heights of 5 plants in a plot were reported as plant height. Similarly, pod number per plant, grain number per pod, dry weight of 100 grains, haulm yield and pod/grain yield were determined after excluding the border lines by randomly selecting 10 plants from each plot.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using SAS software (SAS, 2004) based on a factorial trial and randomized complete block design. The means were compared using LSD testing. The figures were drawn using Excel 2003.

Results and discussion

Plant height

ANOVA showed that the effect of P application on plant height was not significant, while the effect of *P. fluorescens* strain and the interaction of the two factors were significant (Table 1). In the absence

Table 1. Mean squares of ANOVA for plant height (H), pod number per plant (PN), grain number per pod (GN), hundred grain weight (HGW), pod length (PL), Haulm yield (HY), pod yield (PY), and grain yield (GY) as affected by phosphorus rate and *Pseudomonas fluorescens* strain

S.O.V	df	H	PN	GN	HGW	PL	HY	PY	GY
R	2	22^{ns}	1^{ns}	0.03^{ns}	78^{ns}	0.1^{ns}	38^{ns}	14937188^{ns}	7758794^{ns}
Phosphorus rate (P)	1	10^{ns}	34^{**}	0.16^{**}	122^{ns}	12^{**}	9614^{ns}	295787949^{**}	146952653^{**}
P.fluorescens strain (S)	2	144^{**}	68^{ns}	0.24^{**}	726^{**}	1.5^{**}	500619^{**}	200677752^{**}	67561897^{**}
P × S	2	353^{**}	44^{ns}	0.03^{ns}	689^{**}	5.1^{**}	5627^{ns}	95601942^{**}	79611150^{**}
Error	17	10	2	0.01	89	0.3	10881	6126719	2666268
CV (%)	–	2.2	5.4	3.8	8.1	4.8	7.6	5.4	7.0

*, ** represent significance at 0.05 and 0.01 probability level, respectively.
ns represents no significant difference.

of P, the seed inoculated plants with *P. fluorescens* strain168 and strain 136 showed greater plant heights than the un-inoculated plants (Figure 3). When P was applied, the plants inoculated with strain 168 recorded the greatest height; the lowest plant height was observed for un-inoculated plants (Figure 3). Phosphorus plays an important role in the initial establishment of a plant, meristematic activity, and cell division. Increased plant height by inoculation of *Pseudomonas* along with P_2O_5 may possibly be attributed to the inoculation-induced increase in indole acetic acid and gibberellic acid production, which subsequently increased the cell division and cell elongation (Afzal *et al.*, 2010). Moreover, it appears that *Pseudomonas* increases plant height by increasing phosphorus solubility and its availability to plants. These results are consistent with those of other researchers (Afzal *et al.*, 2010).

Pod number per plant

ANOVA showed that the number of pods per plant was significantly influenced by the effects of P application, *P. fluorescens* strain and their interaction (Table 1). In the absence of P, *Pseudomonas* 136 produced the largest number of pods per plant (30.4 ± 0.5). However, no significant difference in number of pods was found between plants inoculated with strain 168 and un-inoculated plants (Figure 4). In contrast, after application of P (25 kg ha^{-1}), plants inoculated with *P. fluorescens* strain 168 (32.8 ±0.6) and strain 136 (30.5 ±0.7) recorded the greatest number of pods per plant and the un-inoculated plants recorded the fewest pods per plant (26.3 ± 1.1). Figure 4 shows that the greatest number of pods per plant was obtained at a P application rate of 25 kg ha^{-1} and inoculation with *P. fluorescens*

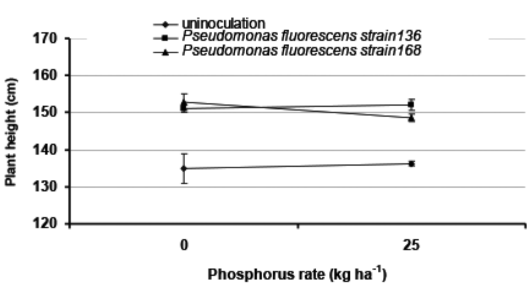

Figure 3. phosphorous rate × *Pseudomonas fluorescens* strain interaction effect on plant height. Vertical bars represent ± 1 SE of means.

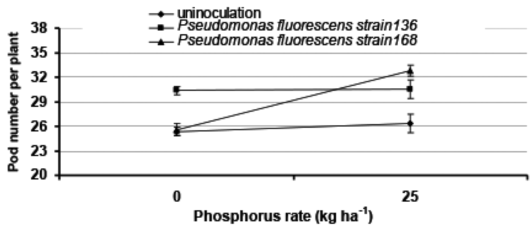

Figure 4. phosphorous rate × *Pseudomonas fluorescens* strain interaction effect on pod number per plant. Vertical bars represent ± 1 SE of means.

strain 168. Previous studies have suggested that P increases flowering and fruit setting; thus the number of pods per plant increased with the application of P. Sing *et al.* (2011) also found that the effect of P amount was significant at a probability level of 5% and that the number of pods per plant increased as the P application rate increased.

Grain number per pod

The effects of amount of P and *P. fluorescens* strain on the number of grains per pod were significant at the 1% probability level, but their interaction was not significant (Table 1). The application of P significantly increased the number of grains per pod by 8% (Table 2). Plants inoculated with *P. fluorescens* 168 recorded the largest number of grains per pod (2.8 grains pod^{-1}) and un-inoculated plants and plants inoculated with *P. fluorescens* stain136 recorded the fewest grains per pod (2.5 grains pod^{-1}) (Table 2). Phosphorus is essential for the cell nucleus during cell division as it is involved in meristem tissue development, particularly in the early rapid stage of growth. P also affects rooting,

Table 2. Grain number per pod (GP) and haulm yield (HY) as affected by phosphorus rate and *Pseudomonas fluorescens* strain

Traits / **Factors**	GP (No. pod^{-1})	HY (Kg ha^{-1})
Phosphorus rates (kg ha^{-1})		
0	2.5	1332
25	2.7	1378
LSD (0.05)	0.1	109
***Pseudomonas fluorescens* strains**		
Un-inoculation	2.5	1102
Pseudomonas fluorescens strain 136	2.5	1294
Pseudomonas fluorescens strain 168	2.8	1670
LSD (0.05)	0.1	134

photosynthesis, increases storage substances, transfer of carbohydrates, successful breeding and fruit set, color and coarseness and prematurity of fruits. The number of grains per pod has been shown to increase as the P applied increased (Turk and Tawaha (2002).

Hundred grain weight

ANOVA showed that the effect of P application on 100-grain weight was not significant. The effect of *P. fluorescens* strain and the interaction of the two factors were significant (Table 1). In the absence of P, the 100-grain weight was significantly lower for un-inoculated plants than for plants inoculated with both strains (Figure 5). As shown, the highest 100-grain weight was achieved using a P content of 25 kg ha^{-1} and seed inoculation with *P. fluorescens* strain 168 (Figure 5). Zeidan (2007) suggested that application of P at 0-60 kg ha^{-1} contributes to nutrient absorption (phosphorus, potassium, magnesium and zinc) caused by the increase in soluble phosphorus and assimilation of nutrients to the grain, resulting in larger grains. This could be the reason for the increased 100-grain weight. At low fertilizer treatments, a decrease in 100-grain weight resulted from the competition for nutrients and the decrease in carbohydrate stores. Increased soluble P content increased the amount of phytin stored in the seeds. Phyitin serves as the main source of stored P in most grains and is an important compound for germination and seed growth with a significant contribution to seed size and weight.

Pod length

ANOVA indicated that pod length was significantly influenced by P application and *P. fluorescens* strain and by their interactive effect (Table 1). In the absence of phosphorus *P. fluorescens* strain 136 produced the longest pod length (12.22 ± 0.17), and plants inoculated with *P. fluorescens* strain 168 (11.54 ± 0.38) and un-inoculated plants (11.35 ± 0.19) produced the shortest pod lengths (Figure 6). After application of P (25 kg ha^{-1}), plants inoculated with *P. fluorescens* strains 168 and 136 showed the longest pod lengths of 14.37 ± 0.19 and 13.95 ± 0.47, respectively. The shortest pod length (11.72 ± 0.28) was observed in un-inoculated plants (Figure 6). Kazemi-Poshtmasari *et al.* (2007) also concluded that application of P fertilizers increased the pod length of the faba bean.

Haulm yield

ANOVA indicated that only the effect of the *P. fluorescens* strain was significant for haulm yield (Table 1). The highest haulm yield (1670 kg ha) was obtained from *P. fluorescens* strain 168 and the lowest yield (1102 kg ha^{-1}) was recorded for the un-inoculated plants (Table 2). Dey *et al.* (2004) reported that *P. fluorescens* trains increased haulm yield in the peanut. Sharma *et al.* (2003) suggested that the increase in shoot weight of the mung bean resulted from application of bacteria.

Pod and grain yields

ANOVA showed that pod yield was significantly influenced by the effects of P application, *P. fluorescens* strain and their interaction (Table 1). In the absence of P, the highest pod yield was recorded for plants inoculated with *P. fluorescens* strain 168 and lowest was recorded for the un-inoculated plants (Figure 7). When P was applied (25 kg ha^{-1}), pod yield increased significantly for plants inoculated with *P. fluorescens* strain 168 (Figure 7). The greatest pod yield was obtained with a P application of 25 kg ha^{-1} for plants inoculated with *P. fluorescens* strain 168. ANOVA also showed that grain yield was significantly ($P \leq 0.01$) influenced

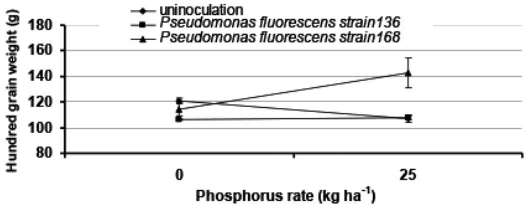

Figure 5. phosphorous rate × *Pseudomonas fluorescens* strain interaction effect on 100-grain weight. Vertical bars represent ± 1 SE of means.

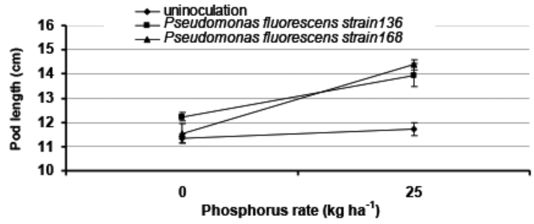

Figure 6. phosphorous rate × *Pseudomonas fluorescens* strain interaction effect on pod length. Vertical bars represent ± 1 SE of means.

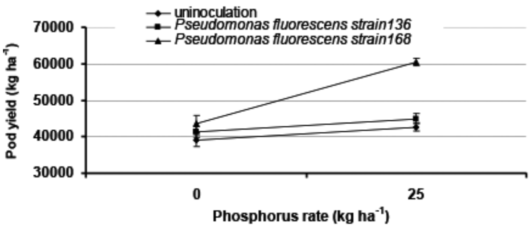

Figure 7. phosphorous application × *Pseudomonas fluorescens* strain interaction effect on pod yield. Vertical bars represent ± 1 SE of means.

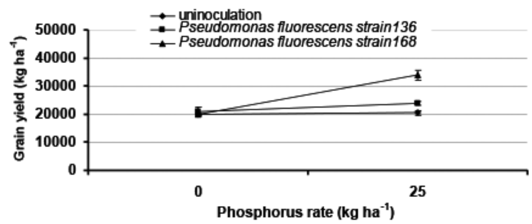

Figure 8. phosphorous application × *Pseudomonas fluorescens* strain interaction effect on grain yield. Vertical bars represent ± 1 SE of means.

by P application, *P. fluorescens* strain, and their interaction (Table 1). In the absence of phosphorus, grain yield with both *Pseudomonas* strains showed no significant difference from that of un-inoculated plants (Figure 8). With application of P fertilizer (25 kg ha^{-1}), grain yield for plants inoculated with *P. fluorescens* strain 168 increased by 42% over plants inoculated with *P. fluorescens* strain 136 and 65% over un-inoculated plants. Phosphorus is an essential macronutrient which promotes root growth and stimulates its lateral branching; this increases a plant's ability to absorb nutrients from the soil. Phosphorus also plays a vital role in flower formation and fruit set and in processes such as sugar and starch utilization, photosynthesis, cell division and nodule formation. Phosphorus increases the leaf area index by increasing leaf cell division, elongation and leaf number (Assuero *et al.*, 2004; Kavanova *et al.*, 2006); this increases light interception and photosynthesis assimilation, which increases plant biomass accumulation. Phosphorus also improves nitrogen metabolism in plants (Li and Zhao, 1990). Several groups of soil bacteria such as *Pseudomonas* exhibit phosphate-solubilizing ability through production of organic acids (Shaharoona *et al.*, 2008). By changing the environmental acidity and enzymatic processes, they release the insoluble P of soil in the form of organic phosphoric acid, which increases its mobility in the soil (Chung *et al.*, 2005; Gulati *et al.*, 2010). These bacteria can decrease soil pH during phosphate solubilization (Shaharoona *et al.*, 2008). Previous studies have found qualitative-quantitative improvements in the grain yield of crops from seed inoculation with phosphate-solubilizing bacteria (Afzal ea al., 2010; Saharan *et al.*, 2010; Crowley, 2006; Dey *et al.*, 2004).

Conclusions

The results of this study showed that the effect of phosphorus application and *P. fluorescens* strain was significant for grain/pod yield and most yield components of the faba bean. Seed inoculation with *P. fluorescens* strain 168 resulted in higher yield and yield components than *P. fluorescens* strain 136. The results of this experiment indicate that *P. fluorescens* strain 168 and application of phosphorus at a rate of 25 kg ha^{-1} are recommended to achieve maximum grain and pod yields of the faba bean.

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