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Effects of phosphorus fertilizer rate and *Pseudomonas fluorescens* strain on field pea (*Pisum sativum* subsp. *arvense* (L.) Asch.) growth and yield

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ABSTRACT

A field experiment was conducted at Rezvanshahr, Guilan province, Iran, to evaluate the effects of phosphorus fertilizer rate and *Pseudomonas fluorescens* strains on growth and yield of field pea (*Pisum sativum* L.). The experimental design was a randomized complete block in a factorial arrangement with three replicates. Factors were phosphorus fertilizer rates (0, 25, 50, 75, and 100 kg P_2O_5 ha^{-1} as triple superphosphate), and seed inoculation with *P. florescens* strains [control (non-inoculated), inoculated with strain R41, and strain R187). Analysis of variance showed that plant height, seed yield, pod number per m^2, 100-seed weight, biological yield, harvest index, and leaf P concentration were significantly influenced by phosphorus fertilizer rate and *P. florescens* strain. At the same time, phosphorus fertilizer rate × *P. fluorescens* strain interaction was significant only for 100-seed weight. On the other hand, seed number per pod was significantly affected neither by phosphorus fertilizer rate nor by pseudomonas strains. Result showed that seed yield was significantly increased from 1099 ± 67 to 1898 ± 118 kg ha^{-1} as P_2O_5 application rate increased from 0 to 75 kg ha^{-1}, and thereafter relatively remained constant. There was no significant difference in seed yield between plants raised from inoculated seeds with *P. fluorescens,* strain R187 (1664 ± 97 kg ha^{-1}) and those raised from inoculated seeds with *P. fluorescens,* strain R41 (1669 ± 104 kg ha^{-1}). At the same time, plants raised from inoculated seeds with *P. fluorescens* (both strains) produced greater grain yield compared to those raised from uninoculated seeds (1370 ± 80 kg ha^{-1}). Based on the results of this study, P_2O_5 application at the rate of 75 kg ha^{-1} and inoculation with pseudomonas bacteria are recommended for obtaining the greatest seed yield in field pea.

Key words: phosphorus, plant growth-promoting rhizobacteria, *Pisum sativum*

IZVLEČEK

UČINKI GNOJENJA S FOSFORJEM IN DODATKOV SEVOV BAKTERIJE *Pseudomonas fluorescens* NA RAST IN PRIDELEK POLJSKEGA GRAHA (*P. sativum* subsp. *arvense* (L.) Asch.)

Z namenom ovrednotenja vplivov gnojenja z različnimi odmerki fosfornih gnojil in dodatkov sevov bakterije *Pseudomonas fluorescens* na rast in pridelek poljskega graha (*P. sativum* subsp. *arvense* (L.) Asch.) je bil izveden poljski poskus v provinci Rezvanshahr, Guilan, Iran. Načrt poskusa je bil naključni bločni faktorski poskus s tremi ponovitvami. Faktorji v poskusu so bili gnojenje s fosforjem (0, 25, 50, 75 in 100 kg P_2O_5 ha^{-1}, kot trojni superfosfat) in inokulacija semen s sevi bakterije *P. florescens* (kontrola (ne inokulirano), inokulirano s sevom R41 in sevom R187). Analiza variance je pokazala, da so na parametre kot so višina rastlin, pridelek zrnja, število strokov na m^2, masa 100-semen, biološki pridelek, žetveni indeks in vsebnost P značilno vplivala gnojenja s fosforjem in inokulacija s sevi bakterije *P. florescens*, vendar je imelo hkratno gnojenje s fosforjem in inokulacija s sevi bakterije *P. fluorescens* značilen vpliv le na maso 100-semen. Po drugi strani se število semen na strok ni značilno spremenilo niti z različnimi odmerki fosforja niti z dodatki sevov bakterij. Rezultati so pokazali, da se je pridelek zrnja značilno povečal od 1099 ± 67 na 1898 ± 118 kg ha^{-1}, ko se je uporaba P_2O_5 povečala iz 0 na 75 kg ha^{-1}, in je potem ostal relativno konstanten. Med rastlinami, katerih semena so bila inokulirana s sevom bakterije *P. fluorescens,* R187 (1664 ± 97 kg ha^{-1}) in tistimi, katerih semena so bila inokulirana s sevom *P. fluorescens,* R41 (1669 ± 104 kg ha^{-1}) ni bilo značilnih razlik v pridelku zrnja, vendar je bil pridelek zrnja inokuliranih rastlin pri obeh sevih večji od neinokuliranih rastlin (1370 ± 80 kg ha^{-1}). Na osnovi izsledkov te raziskave lahko za doseganje večjih pridelkov poljskega graha priporočamo gnojenje s fosforjevimi gnojili v odmerku 75 kg, P_2O_5 ha^{-1} s hkratno inokulacijo s sevi zgoraj omenjenih bakterij.

Ključne besede: fosforjeva gnojila, rast-stimulirajoče rizobakterije, *Pisum sativum*

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1 INTRODUCTION

Pisum sativum subsp. *arvense* (L.) Asch., the field pea which is also known as the garden pea, is one of the most important pulse crop. World wide, green peas is produced on 2.25 million ha, with an estimated of total production of 18.5 million tons and dry peas is produced on 6.76 million ha, with an estimated of total production of 10.4 million tons in 2012 (FAO, 2012). Field pea is a cool-season crop which is usually cultivated in early-November and harvested in late-May in northern Iran. Field pea has a high nutritive value, and is high in fiber, protein, vitamins (folate and vitamin C), minerals (iron, magnesium, phosphorus and zinc), and lutein (Urbano et al., 2003).

Phosphorus is one of essential nutrients for plant growth and development. Phosphorus regulates protein synthesis in plants, because it is a component of the complex nucleic acid structure. Phosphorus is important in cell division and development of new tissue. Also, phosphorus plays a vital role in plant energy reactions, photosynthesis, respiration, genetic transfer, seed and fruit production, and nutrient transport in plants (Raghothama and Karthikeyan, 2005). Phosphorus is also a component of phytin, a major storage form of P in seeds, phospholipids, and ATP. Moreover, phosphorus promotes root growth and stimulates tillering and often hastens maturity. The responses of leguminous crops to P fertilizer are mainly determined by the soil P available, but are not related to soil organic matter, total N, total P, soil $CaCO_3$ contents, and soil N available (Li et al., 2011). Results show that, when the available P in the soil was less than 10 mg kg^{-1}, P fertilizer gave good effect and application of P fertilizer was required, while above 15 mg P kg^{-1} the application of P fertilizer alone had no consistent effect (Lin et al., 1964). Li and Li (1992) reported that leguminous crops had different response to P application rate compared to cereal crops. They reported that wheat yield was significantly increased from 2040 to 4491 kg ha^{-1} as P rate increased from 0 to 97.5 kg ha^{-1}. In contrast, the highest pea yield was at the P rate of 78 kg P ha^{-1}, and thereafter decreased. One of the reasons proposed for explaining the difference in responses to P fertilizer is the higher P demand of legumes than non-legumes.

Although P is abundant in soils in both organic and inorganic forms (Khan et al., 2009), the amount of available forms to plants is generally low, because the majority of soil P is found in insoluble forms, while the plants absorb it only in soluble ions ($H_2PO_4^-$ and HPO_4^{2-}) (Bhattacharyya and Jha, 2012). To overcome the P deficiency in soils, phosphorus fertilizers must be frequently added to agricultural fields, but regular application of phosphate fertilizers is not only costly but is also environmentally undesirable, as is possible cause of eutrophication. This has led to search for an economically viable and eco-friendly option for improving crop production in low P soils. Legume root is colonized by numerous rhizospheric microorganisms, and these organisms have definite influence on the survival and nodulation ability of seed inoculated rhizobia (Dashti et al., 1998; Davison, 1988). Phosphate solubilizing microorganisms could convert insoluble phosphates into available forms for plant via the process of acidification, chelation, exchange reactions, and production of gluconic acid (Chung et al., 2005; Gulati et al., 2010), and hence a viable substitute to chemical phosphorus fertilizers (Khan et al., 2006). Of the various phosphate solubilizing microorganisms, *Pseudomonas flurescens* is considered as one of the most significant phosphate solubilizing bacteria, which not only provide P to the plants, but also produce siderophore, antibiotic, and phytohormones such as indole-acetic acid (Leinhos and Nacek, 1994). Some PGPR strains such as pseudomonas (Grimes and Mount, 1984) enhance legume growth, nodulation, and nitrogen fixation when coinoculated with rhizobia. Fluorescent pseudomonads are also known to produce salicylic acid, which acts as local and systemic signal molecules in inducing resistance in plants (De Meyer and Hofte, 1997). Benhamou et al. (1996 a, b) found that *Pseudomonas fluorescens* induced accumulation of lignin in pea roots. *Pseudomonas spp*. can form gluconic acid through the oxidative glucose metabolism (Gyaneshwar et al., 2002). Sharma et al. (2003) point out that in 10 μM Fe–citrate along with *Pseudomonas* strain GRP3 treatment, chlorophyll a, chlorophyll b and total chlorophyll contents increased significantly by 34, 48 and 39 %, respectively, compared to the control. In a field experiment, van Elsas (1986) reported significant increase in wheat seedling growth after inoculation with pseudomonas and bacillus. Inoculation of plants with PGPR can enhance the drought tolerance (Figueiredo et al., 2008), which can be attributed to the production of IAA, cytokinins, antioxidants and ACC deaminase. Sgroy et al., (2009) declared that several PGPR strains such as *Bacillus subtilis*, *B. licheniformis*, *B. pumilus*, and *Pseudomonas putida* have crucial roles in cell elongation, increasing ACC deaminase activity, and plant growth promotion. Inoculation of *Pseudomonas fluorescens* significantly increased total root length, surface area and volume in tomato and cucumber (Saravanakumar et al., 2007).

Reduced application rates of chemical fertilizers through inoculation with plant growth-promoting rhizobacteria were proposed by researchers. Adesemoye et al. (2009) found that supplementing 75 % of the recommended fertilizer rate with inoculants produced plant growth, yield, and nutrient (nitrogen and phosphorus) uptake that were statistically equivalent to the full fertilizer rate without inoculants. At the same time, Shaharoona et al. (2008) reported that N use efficiency increased in response to inoculation with *Pseudomonas fluorescens* at all fertilizer levels in wheat, causing 115 %, 52 %, 26 %, and 27 % increase over the uninoculated (control) at N, P, and K application rates of 25 %, 50 %, 75 %, and 100 % recommended doses, respectively.

This experiment was conducted to evaluate the response of field pea growth and yield to different phosphorus fertilizer rate and *Pseudomonas fluorescens* strains.

2 MATERIALS AND METHODS

A field experiment was conducted at Rezvanshahr, Guilan province, Iran, from early-November 2013 to late-May 2014. Some soil properties of the experimental field were presented in table 1. The experimental design was a randomized complete block in a factorial arrangement with three replicates. Factors were phosphorus fertilizer rates (0, 25, 50, 75, and 100 kg P_2O_5 ha^{-1} as triple superphosphate which was applied as side placement), and *Pseudomonas florescens* strains [control (non-inoculated), seed inoculation with strain R41, and strain R187]. These strains were obtained from Soil and Water Research Institute, Karaj, Iran. Some properties of florescent Pseudomonads strains were presented in table 2. Before the inoculation, the Gum Arabic (10 %) was applied to the seeds. Then, the seeds of field pea (*P. sativum* subsp. *arvense* 'Utrillo') were inoculated with *P. florescen*s strain R41, or strain R187 in the proportion of 10 g of peat (10^8 cells/g peat) kg^{-1} seed according to Ferreira et al. (2010). The inoculated seeds were dried in sunshade for five hours and then were planted in seven 3-m rows spaced 30 cm apart at a density of 10 seeds m^{-2} (30 seeds in each row) on 5 November. N and K fertilizers were applied just before final land preparation as recommended doses i.e. 15 kg N ha^{-1} (as starter in the form of urea), and 50 kg K_2O ha^{-1} (as potassium sulphate). Weeds were controlled manually during the experiment. Plants were harvested on 28 May 2014.

Plant height was measured from the soil surface to the top of the main stem at harvest stage. Ten randomly selected plants were harvested from each plot at ground level for measuring number of pod per plant, number of seeds per pod, and 100-seed weight. Aboveground biomass from 1 m^2 of each plot was oven-dried at 70 $^{\circ}$C for 96 h, and weighted for biological yield determination. Seed yield was determined by hand-harvesting the crop plants from 2.5 m^2 per plot and was adjusted to 160 g kg^{-1} seed moisture content. In each plot, field pea leaves were oven-dried at 70 $^{\circ}$C for 48 h and grounded to pass through a 1-mm sieve and P concentration were measured using the spectrophotometric method of Lowry and Lopez (1946). Phosphorus concentration was expressed as the percent of leaf dry weight.

Analyses of variance were conducted using SAS procedures (SAS Institute, 2004) based on a factorial trial and randomized complete block design. For *Pseudomonas fluorescens* strains factor, the F-ratios were found to be significant for plant height , pod number per m^2, seed number per pod, biological yield and harvest index, so means separations were conducted using fisher's protected

LSD at the 5 % probability level. For P rate factor, the F-ratios were found to be significant for plant height, pod number per m^2, seed number per pod, biological yield and harvest index, so linear or quadratic regressions with standard error of the mean were used to describe the relationship between P application rate and these dependent variables. For traits such as 100-seed weight and leaf P concentration, the interaction between *Pseudomonas fluorescens* strain and P rate was significant. So, for each inoculation level, linear regressions with standard error of the means were used to describe the relationship between P application rate and these dependent variables.

Table 1: Some soil properties (0-30 cm) of experimental field prior to sowing

OC (%)	pH	Sand (%)	Silt (%)	Clay (%)	Texture	EC (ds m^{-1})	Total N (%)	Available P (mg kg^{-1})	Available K (mg kg^{-1})
3.25	7.1	54.4	17.2	28.4	sand loam	0.36	0.11	3.2	215

Table 2: Some bacteria properties using for this experiment (Soil and Water Research Institute, Karaj, Iran)

Bacteria	ACC-deaminase production	Phosphorus solubilizing activity	IAA production (mg l^{-1})	Siderophore production (halo diameter/colony diameter)
*P. **fluorescens*** strain R187	+	+	5.8	0.5
*P. **fluorescens*** strain R41	+	+	8.9	0.51

3 RESULTS AND DISCUSSION

3.1 Plant height

Analysis of variance showed that the main effects of phosphorus fertilizer rate and *Pseudomonas fluorescens* strain were significant for plant height, but the interaction between them was not significant (Table 3). For both *Pseudomonas fluorescens* strains and for the control, plant height was significantly increased from 72.9 ± 1.02 cm to 86.4 ± 1.71 cm as P_2O_5 rate increased from 0 to 75 kg ha^{-1}, and thereafter remained statistically constant (Figure 1). Phosphorus plays a vital role in cell division and elongation and, therefore, lower plant height in phosphorus-deficient plants may be due to the reduction in cell division and elongation (Kavanova´ et al., 2006). Averaged across P fertilizer rates, the plants raised from seed inoculated with *Pseudomonas fluorescens* (both strains) was significantly taller than uninoculated ones; however there was no significant difference in plant height between plants raised from seed inoculated with *Pseudomonas fluorescens* strain R41 and strain R187 (Table 4). Pseudomonas bacteria are considered as one of the most significant phosphate solubilizing bacteria which provide P to the plants. Promotion effect of high P level and pseudomonas bacteria on plant height was probably due to better development of root system and nutrient absorption (Hussain et al., 2006). In addition, Dey et al (2004) observed that inoculation of plant growth promoting rhizobacterial isolates *Pseudomonas fluorescens* PGPR1 and *Pseudomonas fluorescens* PGPR2 significantly enhanced the plant height of peanut (*Arachis hypogaea* L.).

Table 3: Mean squares of ANOVA for plant high (H), seed yield (Y), pod number per m^2 (PN), seed number per pod (SP), 100-seed weight (SW), biological yield (BY), harvest index (HI) and leaf P concentration (LPC) as affected by phosphorus rate and *Pseudomonas fluorescens* strain

S.O.V	df	H	Y	PN	SP	SW	BY	HI	LPC
R	2	7 ^{ns}	88109^{ns}	1125^{ns}	0.1^{ns}	0.2 ^{ns}	391806 ^{ns}	1 ^{ns}	0.00002^{ns}
Phosphorus rate (P)	4	471**	950549**	4123*	2.4^{ns}	37.9**	2480016**	114**	0.00321**
Pseudomonas fluorescens strains (Ps)	2	79*	440924**	4176*	1.7^{ns}	20.2**	2346986**	13 *	0.00462**
P × Ps	8	3 ^{ns}	30163^{ns}	431^{ns}	0.1^{ns}	1.6**	214903^{ns}	4 ^{ns}	0.00039**
Error	28	22	47994	1256	1.1	0.4	168554	4	0.00009
CV (%)	-	6	14	7	16	3	7	6	3

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

Table 4: Averages of plant high (H), seed yield (Y), pod number per m^2 (PN), seed number per pod (SP), biological yield (BY), and harvest index (HI) response to *Pseudomonas fluorescens* strains as average across P rates

Traits / **Pseudomonas strains**	H	Y	PN	SP	BY	HI
Control	79± 1.9	1370 ± 80.0	553± 9.6	6.0± 0.4	4714± 150.0	29.0± 0.7
Pseudomonas fluorescens Strain41	83± 2.1	1669± 104.0	553± 9.6	6.5± 0.3	5420± 176.0	30.8± 0.7
Pseudomonas fluorescens Strain187	83± 1.9	1664± 97.0	524± 9.3	6.7± 0.4	5375± 167.0	30.6± 0.8
LSD (0.05)	3	164	26	0.8	307	1.4

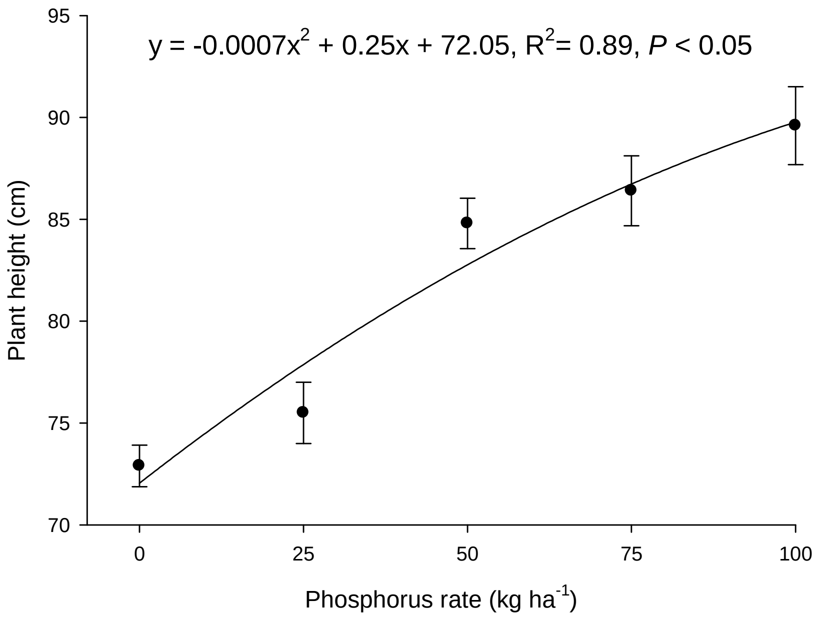

Figure 1: Effect of phosphorus fertilizer rate on plant height as average across *Pseudomonas fluorescens* strains. Vertical bars represent ± 1 SE of means

3.2 Seed yield

The main effects of phosphorus fertilizer rate and *Pseudomonas fluorescens* strain were significant for seed yield, while *Pseudomonas fluorescens* strain × phosphorus fertilizer rate interaction was not significant (Table 3). A quadratic equation ($Y = -0.049\ X^2 + 12.96\ X + 1103.77$, $R^2 = 0.98$) provided a good description of the relationship between seed yield and P application rate. Seed yield was significantly increased from 1099 ± 67 to 1898 ± 118 kg ha^{-1} as P_2O_5 application rate increased from 0 to 75 kg ha^{-1}, and thereafter relatively remained constant when averaged across *Pseudomonas fluorescens* strains (Figure 2). Regardless of P application rate, there was no significant difference in grain yield between plants raised from seed inoculated with *Pseudomonas fluorescens* strain R187 (1664 ± 97 kg ha^{-1}) and those raised from seed inoculated with *Pseudomonas fluorescens* strain R41 (1669 ± 104 kg ha^{-1}). At the same time, plants raised from seed inoculated with *Pseudomonas fluorescens* (both strains) produced greater grain yield compared to uninoculated (1370 ± 80 kg ha^{-1}) ones (Table 4). Pseudomonas bacteria not only could convert insoluble phosphates into available forms for plant via the process of acidification, chelation, exchange reactions, and production of gluconic acid (Chung et al., 2005; Gulati et al., 2010), but also could produce siderophore (Dey et al., 2004), ACC-deaminase enzyme (Dey et al., 2004; Shaharoona et al., 2008) and phytohormones such as indole-acetic acid (Leinhos and Nacek, 1994; Dey et al., 2004). At the same time, leaf growth depression under phosphorus deficiency is well documented (Assuero et al., 2004; Kavanova´ et al., 2006). Meanwhile, leaf number reduces by P deficiency. Therefore, the reduction in leaf surface area in phosphorus-deficient plants reduces light interception and photosynthesis assimilates, which in turn reduces plant dry matter. Also, the metabolism of N is inhibited with an inadequate supply of P, while the supply of N is necessary to allow crops to use P (Li and Zhao, 1990).

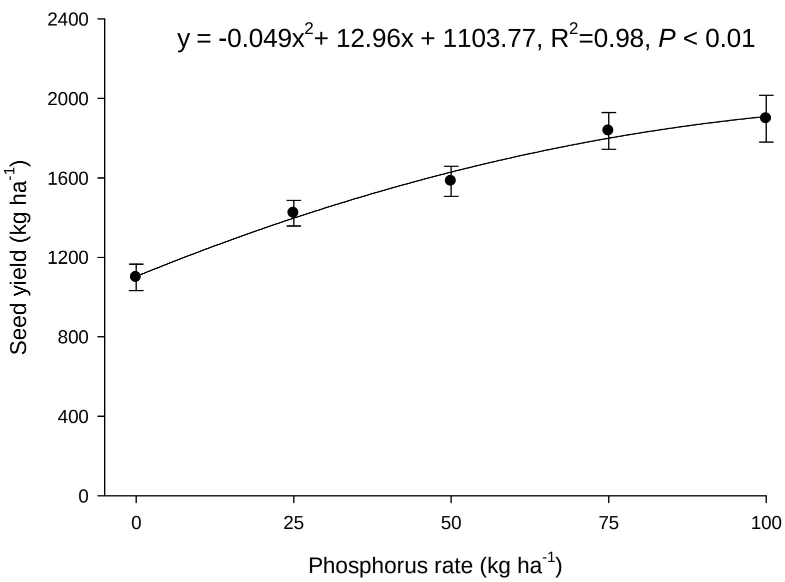

Figure 2: Effect of phosphorus fertilizer rate on seed yield as average across *Pseudomonas fluorescens* strains. Vertical bars represent ± 1 SE of means

3.3 Pod number per m^2

Analysis of variance showed that the main effects of phosphorus fertilizer rate and *Pseudomonas fluorescens* strain were significant for pod number per m^2, but the interaction between them was not significant (Table 3). Regardless of *Pseudomonas fluorescens* strain, pod number per m^2 was increased from 516 ± 12.0 to 574 ± 12.5 pod m^{-2} as P_2O_5 application rate increased from 0 to 100 kg ha^{-1} (Figure 3). Pod number per m^2 was significantly higher at inoculated plots (553 ± 9.6

pod m^{-2} for both strains) than uninoculated ones (524 ± 9.3 pod m^{-2}) as averaged across P application rates (Table 4). The increase in pod number per m^2 at high P level may be due to the positive P effects on increasing the flower formation and improving the fruit setting. Dey et al. (2004) reported that pod number in peanut (*Arachis hypogaea* L.) was significantly increased by inoculation with *Pseudomonas fluorescens* strain.

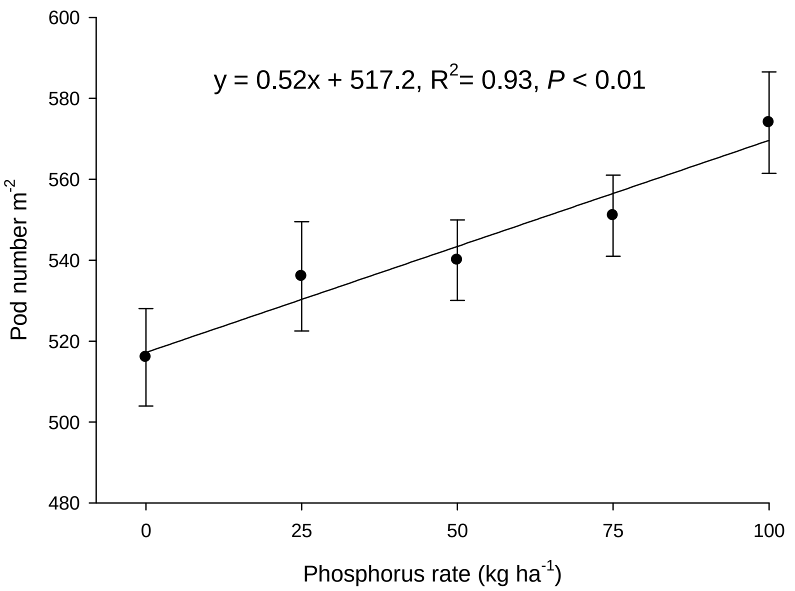

Figure 3: Effect of phosphorus fertilizer rate on pod number per m^2 as average across *Pseudomonas fluorescens* strains. Vertical bars represent ± 1 SE of means

3.4 Seed number per pod

Analysis of variance showed that seed number per pod was not significantly affected by phosphorus fertilizer rate and *Pseudomonas fluorescens* strains. At the same time, *Pseudomonas fluorescens* strains × phosphorus fertilizer rate interaction was not significant (Table 3 and 4).

3.5 100- Seed weight

Phosphorus fertilizer rate and *Pseudomonas fluorescens* strains had significant effect on 100-seed weight. Moreover, a significant phosphorus fertilizer rate × *Pseudomonas fluorescens* strain interaction (P < 0.01) was found for 100-seed weight (Table 3). For uninoculated treatment and strain R41, 100-seed weights were linearly increased as phosphorus fertilizer rate increased from 0 to 75 kg P_2O_5 ha^{-1}, and thereafter remained relatively constant (Figure 4). In contrast, for strain R187, 100-seed weight was linearly increased as phosphorus fertilizer rate increased from 0 to 100 kg P_2O_5 ha^{-1} (Figure 4). Better growth and development of crop plants due to phosphorus supply and nitrogen uptake might have increased the supply of assimilates to seed, which ultimately gained more weight (Ali et al., 2004). This finding is similar to the results of Ali et al. (2004), who reported that the highest 1000-seed weight for chickpea was obtained with seed inoculation and 90 kg ha^{-1} phosphorus application, while the lowest 1000-seed weight was obtained with uninoculation and zero applied phosphate. In addition, Dey et al. (2004) reported that, in most cases, seed inoculated plants produced significantly greater 100-seed mass over control plants.

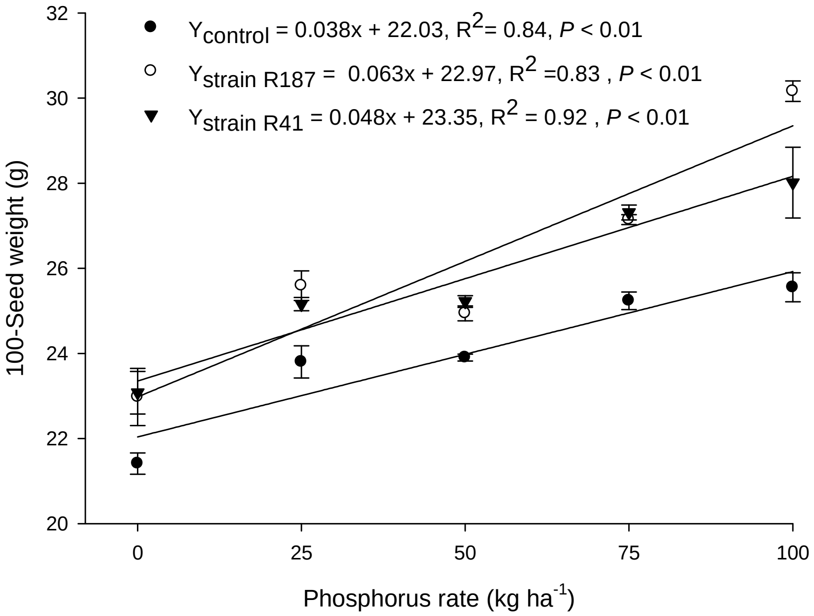

Figure 4: Phosphorus fertilizer rate × *Pseudomonas fluorescens* strains interaction effect on 100-seed weight. Vertical bars represent ± 1 SE of means

3.6 Biological yield

Main effects of *Pseudomonas fluorescens* and phosphorus fertilizer rate were significant for biological yield, while *Pseudomonas fluorescens* strain × phosphorus fertilizer rate interaction was not significant (Table 3). The relationship between P application rate and biological yield was well fitted by a quadratic equation ($y = -0.12x^2+25.16x+4438.28$, $R^2=0.96$). Regardless of *Pseudomonas fluorescens* strain, biological yield was significantly increased from 4483 ± 165 to 5726 ± 151 kg ha^{-1} as P_2O_5 application rate increased from 0 to 75 kg ha^{-1}, and thereafter statistically remained constant (Figure 5). This finding is similar to the result of Ali et al. (2004), who reported that the highest and the lowest biological yield for chickpea was obtained with 90 kg ha^{-1} and zero phosphorus application, respectively. Averaged across P fertilizer rates, biological yields were significantly increased by 15 and 14 % for plants raised from seed inoculated with *Pseudomonas fluorescens* strain R41 and those raised from seed inoculated *Pseudomonas fluorescens* strain R187, respectively, compared to plants raised from uninoculated seeds (Table 4). Consistent with this result, Ali et al. (2004) reported that seed inoculated chickpea plants produced greater biological yield than uninoculated ones. In addition, our findings also agree with those of Dey et al. (2004) who found greater haulm yield in seed inoculated plants of peanut (*Arachis hypogaea* L.) compared to control plants. Phosphorus is a component of ATP the "energy unit" of plants. ATP, forms during light reactions of photosynthesis, is then available as an energy source for dark reactions, which sugars are used as building blocks to produce other cell structural and storage components. At the same time, phosphorus increases leaf area index through increases in leaf number per plant, leaf cell division and elongation, which in turn increases radiation interception and plant photosynthesis rate and, therefore, increases plant biomass accumulation. Moreover, pseudomonas bacteria improve plant growth through increasing phosphorus availability to the plant as well as producing siderophore (Dey et al., 2004), ACC-deaminase enzyme (Dey et al., 2004; Shaharoona et al., 2008) and phytohormone such as indole-acetic acid (Leinhos and Nacek, 1994; Dey et al., 2004).

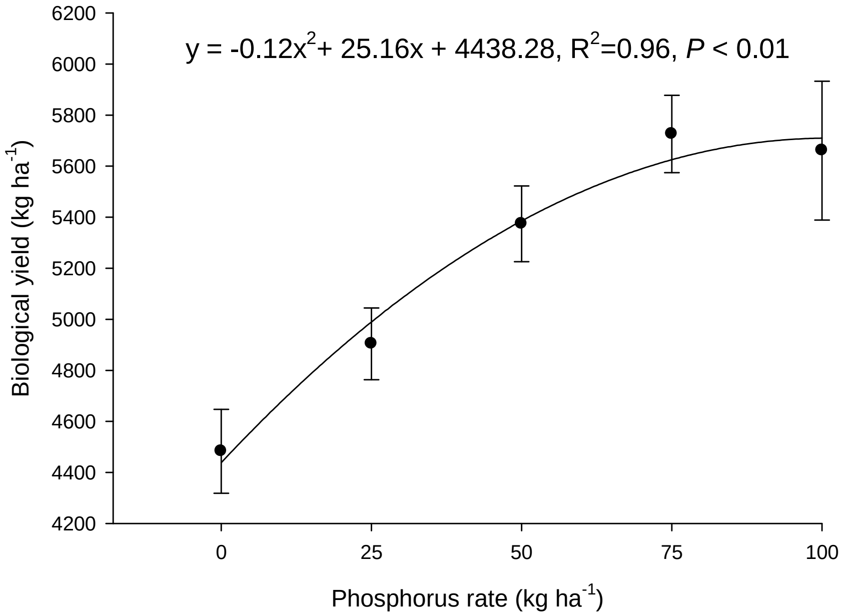

Figure 5: Effect of phosphorus fertilizer rate on biological yield as average across *Pseudomonas fluorescens* strains. Vertical bars represent ± 1 SE of means

3.7 Harvest index

Harvest index was significantly influenced by *Pseudomonas fluorescens* strain and phosphorus fertilizer rate (Table 3). However, the interaction between pseudomonas strain and phosphorus fertilizer rate was not significant (Table 3). Averaged across *Pseudomonas fluorescens* strains, harvest index was significantly increased from 24 ± 0.8 % to 32.5 ± 0.7 % as P_2O_5 application rate increased from 0 to 75 kg ha^{-1}, and thereafter slightly increased (Figure 6). Regardless of P application rate, plants raised from uninoculated seeds had lower HI than those raised from inoculated seeds, but no significant difference in HI was observed between those raised from inoculated seeds with *Pseudomonas fluorescens* strain R187 and strain R41 (Table 4). These indicate that plant produced higher biomass and advocated higher dry weight to seeds under high P application rate and seed inoculation with pseudomonas strains compared to low P application rate and uninoculated conditions. Similarly, Roy et al. (1995) concluded that HI for gram (*Cicer arietinum* L.) was increased by inoculation.

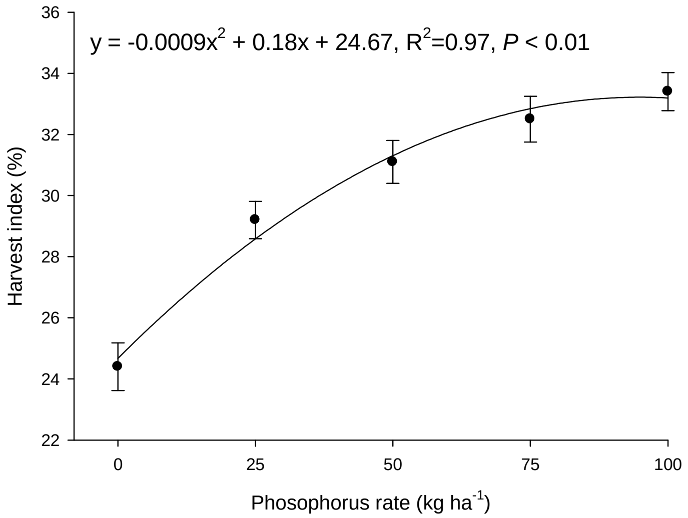

Figure 6: Effect of phosphorus fertilizer rate on harvest index as average across *Pseudomonas fluorescens* strains. Vertical bars represent ± 1 SE of means

3.8 Leaf P concentration

Phosphorus fertilizer rate, *Pseudomonas fluorescens*, and phosphorus fertilizer rate × *Pseudomonas fluorescens* strain had significant effect on leaf P concentration (Table 3). Leaf P concentration was more rapidly increased for seed inoculated plants with strains R187 and R41 than control plant when P_2O_5 application rate increased from 0 to 100 kg ha^{-1}. At the same time, the highest leaf P concentration was recorded for seed inoculated plants with strains R187 and plants received 100 kg ha^{-1} phosphorus fertilizer (Figure 7). This finding is similar to the result of Dey et al. (2004), who reported that seed inoculation with some *P. fluorescen* strains significantly enhanced the total phosphorus content in shoot of peanut (*Arachis hypogaea* L.) compared to control (uninoculated) plants.

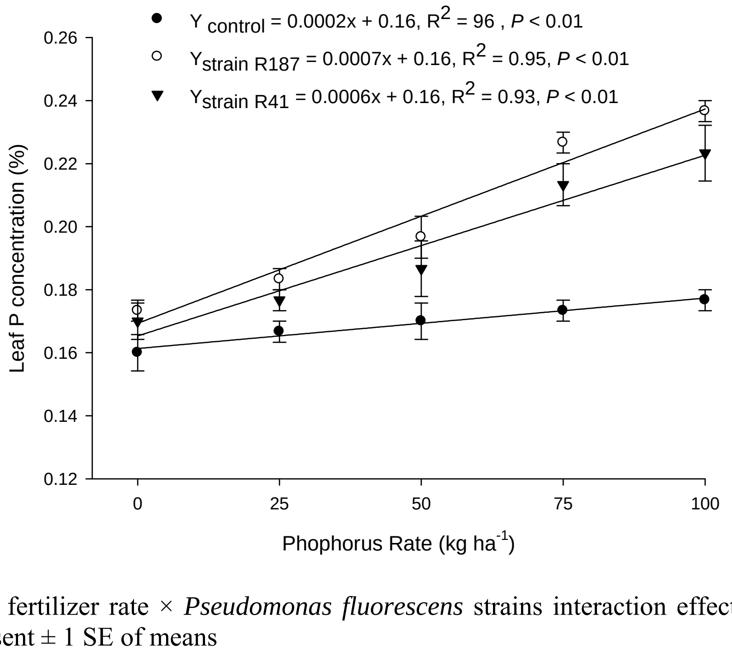

Figure 7: Phosphorus fertilizer rate × *Pseudomonas fluorescens* strains interaction effect on leaf P concentration. Vertical bars represent ± 1 SE of means

4 CONCLUSIONS

The experiment illustrated that seed yield was significantly increased from 1099 ± 67 to 1898 ± 118 kg ha^{-1} as P application rate increased from 0 to 75 kg ha^{-1}, and thereafter statistically remained constant. Plants raised from seeds inoculated with *Pseudomonas fluorescens* produced greater grain yield compared to those raised from uninoculated seeds. However, there was no significant difference in grain yield between plants raised from inoculated seeds with *Pseudomonas fluorescens* strain R187 and those raised from inoculated seeds with *Pseudomonas fluorescens* strain R41. Based on the results of this study, application of phosphorus at the rate of 75 kg ha^{-1} and seed inoculation with *Pseudomonas fluorescens* is recommended for obtaining the greatest seed yield in field pea.

5 REFERENCES

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