Effect of *Pseudomonas fluorescens* Inoculation on Yield and Yield Components of Rice (*Oryza sativa* L.) under Different Levels of Phosphorus Fertilizer

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Abstract

To evaluate the effect of phosphorus (P) fertilizer rate and *Pseudomonas fluorescens* inoculation on rice growth and grain yield, a factorial experiment was performed based on randomized complete blocks design with three replicates at Rice Research Station of Tonekabon, Iran, during 2013-2014 growing season. Studied factors include phosphorus fertilizer rates (0 (control), 25, 50, 75 and 100 kg ha^{-1} as triple superphosphate) and *Pseudomonas fluorescens* application (control (without inoculation) and seedling root tip inoculation). The studied traits were plant height, panicle number per m^2, filled grain number per panicle, 1000-grain weight, percentage of fertile panicle, grain yield, biological yield, and harvest index. Analysis of variance showed that the effect of phosphorus fertilizer rate was significant for panicle number per m^2, filled grain number per panicle, grain yield, and biological yield, while the effect of *P. fluorescens* inoculation was significant for filled grain number per panicle, biological and grain yield. Rice grain yield was significantly increased from 5873 to 7703 kg ha^{-1} as P application rate increased from 0 to 75 kg ha^{-1} and thereafter was slightly increased. Furthermore, grain yield was significantly higher for plants inoculated with *P. fluorescens* compared to un-inoculated plants. From the results of this experiment, application of phosphorus at the rate of 75 kg ha^{-1} and seed inoculation with *P. fluorescens* are recommended to obtain the highest grain yield in rice.

Keywords: grain yield, phosphate-solubilizing bacteria, rice (*Oryza sativa* L.), seedling root tip inoculation

Introduction

Phosphorus (P) is the second essential mineral nutrient for plants after nitrogen in terms of quantitative requirement. Enhancement of P acquisition used by plants is critical for economic, humanitarian and environmental reasons (Vance et al., 2003). This element plays essential roles in plants, such as energy generation, membrane synthesis and stability, nucleic acid synthesis, photosynthesis, respiration, enzyme activation/ inactivation, redox reactions, signaling, carbohydrate metabolism, and nitrogen (N_2) fixation in legumes (Schachtman et al., 1998). Although P is abundant in soils in both inorganic and organic forms, it is a major limiting factor for plant growth, as it exists in an almost unavailable form for plants. Inorganic P occurs in soil, mostly in insoluble mineral complexes, some of them appear after constant application of chemical fertilizers. These insoluble and precipitated forms cannot be absorbed by plants (Rengel and Marschner, 2005). Chemical fertilizers such as manufactured water-soluble phosphatic (WSP) fertilizers (superphosphates), play an important role in the green revolution and are commonly recommended to correct phosphorus deficiencies. Nevertheless, most developing countries import these fertilizers, which are often in limited supply and represent a major expense for resource-poor farmers. Since the indiscriminate and excessive application of chemical fertilizers has led to health and environmental hazards, agronomists are desperate to find alternative strategies that can ensure competitive yields while protecting the health of soils (Khan et al., 2007). Moreover, the use of inorganic fertilizer alone cannot solve the problem of low soil fertility status of most soils (Adediran et al., 2004).

The plant growth-promoting rhizobacteria (PGPR) are capable of improving plant growth through the supply of plant nutrients and may assist in sustaining environmental health and soil productivity (Esitken et al., 2005). The PGPRs increase the capacity of plants to absorb nutrients like nitrogen (N) and P, leading to stronger growth and higher crop yields (Kennedy et al., 2004). Specific P-mobilizing bacteria help directly by converting fixed P into plant available forms (Ahmed et al., 2008). Many studies on bacterial populations within the root environment of plants have revealed that the *P. fluorescens* constitutes a major group of rhizobacteria, which encourage the plant growth via its different mechanisms. It has been reported that seed inoculation with *Pseudomonas spp.* have increased grain yield in wheat (Hussain and Hasnain, 2011, Saharan et al., 2010), maize (Shaharoona et al. 2006), peanut (Dey et al., 2004) and rice (Mirza et al., 2006). Their beneficial effects are mainly due to the production of phytohormones such as indole acetic acid (IAA) and cytokinins, nutrient solubilization/uptake, production of siderophore, 1-Aminocyclopropane-1-carboxylate (ACC) excretion of deaminase enzyme and antagonism of deleterious soil bacteria and phytopathogenic fungi (Mitter et al., 2013).

This bio-control effect, in most cases, has been attributed to the production of antibiotics (Arora et al., 2008) and/or siderophores in the rhizosphere of the *Pseudomonas*-colonizedroots (Viswanathan and Samiyappan, 2007). Deshwal et al. (2011a) observed that 75% *Pseudomonas* strains produced Indole Acetic Acid (IAA) and increased plant growth in soybean. Deshwal et al. (2011b) observed that IAA producing *Pseudomonas aeruginosa*MR-9 improved maximum plant dry weight, plant height, nodule per plant, and nodule fresh weight in *Mucunapruriens* by 184, 124, 139 and 180% respectively, as compared to control. This present research was therefore designed in order to evaluate the effect of phosphorous fertilizer rate and *P. fluorescens* inoculation on growth and grain yield of rice.

Materials and Methods

Site Description, Soil properties and Experimental Design

This study was carried out at the Rice Research Station of Tonekabon, Northern Iran, (36° 51'N and 50° 46'E) in 2014. The physico-chemical conditions of the soil were 3.2% organic matter content, 30% clay, 50% silt, 20% sand, 7.6 pH, total N 0.338%, available phosphorous 6.1 mg kg^{-1}, available potassium 88.0 mg kg^{-1}, and EC 2.49 dS m^{-1}. The experimental region has a sub-Mediterranean climate. During the growing period, the mean maximum temperature was 16.5-30.2°C, while the mean minimum temperature was 10.5-24.5°C (Table 1). The monthly rainfall varied from 0.8 mm in August to 156.1 mm in September during rice growth period (Table 1). The experiment was carried out in a factorial arrangement based on randomized complete block design with three replications. Studied factors were chemical phosphorus fertilizer rates (0 (control), 25, 50, 75 and 100 kg ha^{-1} as triple superphosphate which was applied before transplanting) and *P. fluorescens* (control (without inoculation) and seedling root dip inoculation).

Table 1 Monthly precipitation and temperature from April to September in 2014 at Rice Research Station of Tonekabon

Month	Precipitation (mm)	Temperature (°C)		
		Maximum	Minimum	Average
April	43	16.5	10.5	13.5
May	17.3	24.6	17.4	21.0
June	25.5	28.3	21.1	24.5
July	11.0	30.2	24.5	26.8
August	0.8	31.7	24.1	27.9
September	156.1	28.5	22.3	25.4

Bacterial Inoculation

The phosphate-solubilizing bacteria (*P. fluorescens*) were obtained from the biological laboratory of Soil and Water Research Institute, Karaj, Iran. To clean the roots, the seedlings of Shiroudi were thoroughly washed in running tap water. Prior to transplanting, roots of seedlings were inoculated in a suspension containing the bacteria and one liter of water and sugar for at least 24 h to allow the bacteria to colonize the roots. After inoculating the seedlings, the seedlings were transplanted to the main land on 22 May, 2014. Each plot was 3 m wide and 4 m long at planting distance of 25×25 cm. Before final land preparation, the recommendation rates of nitrogen (50 kg ha^{-1}as urea) and potassium (100 kg ha^{-1} as KCl) were applied to the plots. Furthermore, 50 kg N ha^{-1} was applied as topdressing at 40 days after transplanting. In this experiment, no chemical herbicides were utilized for weed control and weeds were manually controlled at 20 and 40 days after transplanting.

Plant Sampling

Five hills per plot were randomly chosen and plant height was measured from the ground level to tip of the panicle at maturity stage. In addition, the tiller number from these five hills was counted and then expressed as number of panicle per m^2. Five panicles were randomly chosen from labeled hills and filled grain number, total grain number per panicle and 1000-grain weight were measured. Panicle fertility was calculated using the following formula:

$$\text{Panicle fertility} = \frac{\text{Number of filled grains}}{\text{Total number of grains per panicle}} \times 100$$

At maturity stage, 40 hills were harvested by hand-cutting from each plot and grain yield was adjusted to 14% moisture. Biological yield from each plot was placed in separate paper bags, dried at 72°C for 48 h, and weighed. Harvest index (HI) was calculated using the following formula:

$$\text{HI} = \frac{\text{Economical yield}}{\text{Biological yield}} \times 100$$

Statistical Analyses

The data of the various parameters were analyzed and subjected to ANOVA (Analysis of variance) based on the factorial design using SAS (SAS Institute, Inc, 2002) program. The various means were compared using Fisher's protected LSD test at α=0.05.

Results and Discussion

Plant Height

Plant height at maturity stage was significantly affected neither by P rate nor by *P. fluorescens* inoculation (Table 2). Furthermore, the interaction between P rate and *P. fluorescens* inoculation was not significant (Table 2).Contrary to this result, Mathivanan et al. (2006) reported that application of *P. fluorescens* significantly increased rice plant height. In addition, Naveed et al. (2008) reported that application of organic fertilizer and *Pseudomonas* strains significantly increased plant height (16%) of maize.

Grain Yield and Yield Components

ANOVA indicated that P rate had significant effect on grain yield ($P \leq 0.01$), panicle number ($P \leq 0.01$) and filled grain number per panicle ($P \leq 0.01$), while panicle fertility and 1000-grain weight were not significantly affected (Table 2). The relationship between grain yield and phosphorus rate was well expressed using a quadratic equation. Grain yield was significantly increased from 5873 to 7703 kg ha^{-1} as P application rate increased from 0 to 75 kg ha^{-1} and thereafter was slightly increased (Figure 1). Phosphorus plays a vital role in protein synthesis, photosynthesis, respiration, energy reactions, genetic transfer, cell division and development of new tissue, and nutrient transport in plants. It also increases seed and fruit production, promotes root growth and stimulates tillering (Raghothama and Karthikeyan, 2005).

A quadratic equation provided a good description of the relationship between panicle number and phosphorus rate. Panicle number was significantly increased from 308 to 368 panicles m^{-2} as P application rate increased from 0 to 50 kg ha^{-1}, and relatively remained constant at higher P rates (Figure 2). Matsuo et al. (1995) reported that it is

Table 2 Analysis of variance for yield and yield components of rice as affected by phosphorus fertilizer rate and *Pseudomonas fluorescens* inoculation.

Source of variation	df	plant height	Grain yield	Panicle (No. m^{-2})	Grain (No. $panicle^{-1}$)	1000-grain wt.	Panicle fertility	Biological yield	Harvest index
Rep.	2	37.9	170788	2254.3	1.1	0.59	12.4	897930	5.7
Phosphorus fertilizer (P)	4	6.1 ^{ns}	6800283**	6291.7 **	422.9 *	1.1 ^{ns}	26.9 ^{ns}	12364859**	38.4 ^{ns}
Pseudomonas Fluorescens (S)	1	8.5 ^{ns}	3472761*	1598.7 ^{ns}	1004.5 *	1.08 ^{ns}	43.9 ^{ns}	5956611**	20.8 ^{ns}
P×S	4	22.5 ^{ns}	8957 ^{ns}	493.6 ^{ns}	86.1 ^{ns}	0.21 ^{ns}	24.6 ^{ns}	867172 ^{ns}	13.5 ^{ns}
Error	18	13.7	744736	847.2	137.3	0.41	20.9	726253	33.0
CV (%)	-	3.6	12.5	8.3	11.9	2.29	6.2	6.3	10.6

*, ** significant at the 5% and 1% levels of probability, respectively; ns indicates none significant

important to apply much P fertilizers to assist rice plants inaccelerating the rate of phosphate absorption for increased tillering. It is clear that increase in the number of tillers would have resulted in increased assimilatory surface towards the interception of radiant energy thereby leading to better growth and yield.

The relationship between phosphorus application rate and filled grain number per panicle was well expressed via a linear equation. Regardless of *P. fluorescens* inoculation, filled grain number per panicle was significantly increased from 86.5 to 110.1 filled grains per panicle as P application rate increased from 0 to 100 kg ha^{-1} (Figure 3). This result is in line with the report of Sahar and Burbey (2003), who revealed that P application significantly increased grain number per panicle. ANOVA also showed that inoculation of *P. fluorescens* (S) had a significant effect on filled grain number per panicle and grain yield, while panicle number per m^2 and 1000-grain weight were not significantly affected. The P×S interaction was significant neither for grain yield nor for yield components.

Grain yield was significantly higher for plants inoculated with *P. fluorescens* compared toun-inoculated plants (Table 3). Similarly to this result, Mirza et al. (2006) also stated that grain yield of rice inoculated plant was significantly higher (up to 93%) compared to un-inoculated plant. Vessey (2003) observed that phosphate solubilizing bacteria promote plant growth and increase crops yield via the production of amino acids, vitamins and growth promoting substances. PSB also secrete organic acids and enzymes that act on insoluble phosphates in order to convert it into soluble form

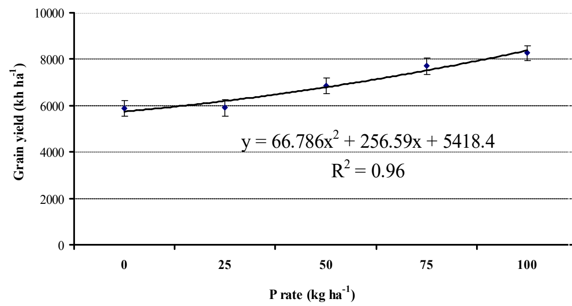

Figure 1 Effect of phosphorus fertilizer rate on grain yield, average across *Pseudomonas fluorescens* inoculations. Vertical bars represent ± 1 SE of means.

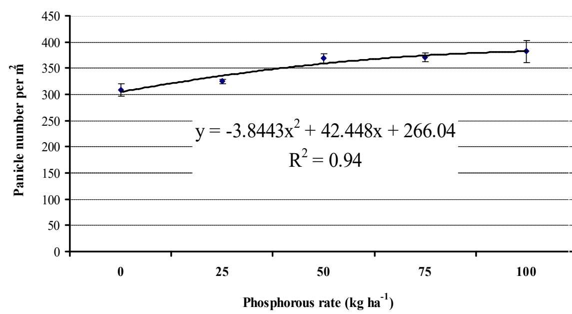

Figure 2 Effect of phosphorus fertilizer rate on panicle number per m^2, average across *Pseudomonas fluorescens* inoculations. Vertical bars represent ± 1 SE of means.

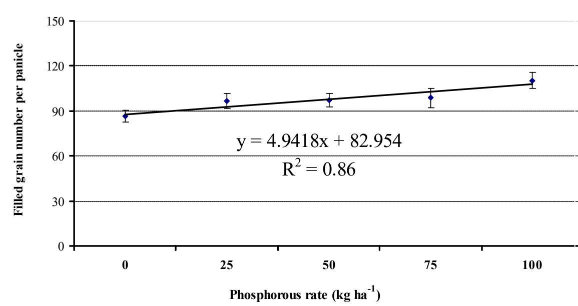

Figure 3 Effect of phosphorus fertilizer rate on filled grain number per panicle, average across Pseudomonas fluorescens inoculations. Vertical bars represent ± 1 SE of means.

Table 3 Yield and yield components of rice as affected by *Pseudomonas fluorescens* inoculation.

Factors	Plant height	Grain yield	Panicle (No. m^{-2})	Grain (No. $panicle^{-1}$)	1000-grain wt.	Panicle fertility	Biological yield	Harvest index
With inoculation	104.5	7263	358.4	103.5	28.1	75.0	13012	55.7
Without inoculation	103.4	6582	343.8	91.9	27.8	72.0	12121	54.0
LSD (0.05)	2.8	662	22.3	8.9	0.49	3.5	653	4.4

and provide phosphorus to plants. At the same time, Khalid et al. (2009), Singh et al. (2011) and Manivannan (2011) reported that PSB strains act as efficient bio-inoculants and so, increase yield and nutrient content of rice crop. Jha et al. (2009) observed significant increase in the grain yield (up to 26%) per plant in field trials when rice seedlings were inoculated with *Pseudomonas* sp. In maize, Naveed et al. (2008) reported that *P. fluorescens* strains significantly increased grain yield (39%) compared to non-inoculated control plants.

Grain number per panicle for inoculated plants with *P. fluorescens* was significantly higher compared to those for un-inoculated plants (Table 3). Similarly, Mathivanan et al. (2006) reported that the application of *P. fluorescens* significantly increased the growth and yield parameters like panicles $hill^{-1}$ and filled grain $panicle^{-1}$ in rice over control.

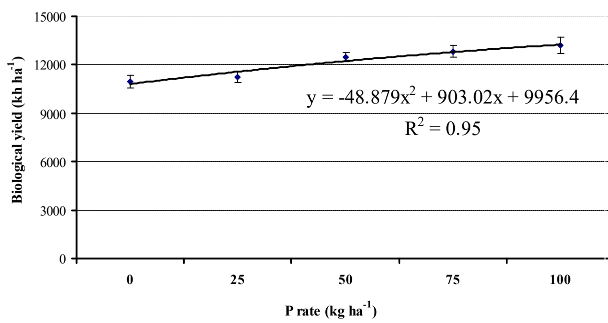

Figure 4 Effect of phosphorus fertilizer rate on biological yield, average across Pseudomonas fluorescens inoculations. Vertical bars represent ± 1 SE of means.

Biological Yield and Harvest Index

Biological yield was significantly affected by P rate and *P. fluorescens* inoculation, while the interaction effects of two factors was not significant on these two traits (Table 2). The relationship between phosphorus application rate and biological yield was well expressed via a quadratic equation. Regardless of *P. fluorescens* application, biological yield was significantly increased from 10945 to 13192 kg ha^{-1} as P application rate increased from 0 to 50 kg ha^{-1}, and relatively remained constant at higher P rates (Figure 4). Inoculation of rice seedling with phosphate-solubilizing microorganisms (*Pseudomonas*) revealed a significant increase (10.7%) in plant biomass compared with the control treatment (Table 3). Our findings were in line with the study of Adesemoye and Egamberdieva (2013), who reported that inoculation of wheat plants with *Pseudomonas extremorientalis* TSAU20 and *P. putida* TSAU1 increased biological yield by 28% compared to control plants. In a pot experiment, Mirza et al. (2006) observed that rice dry weight increased up to 60% as plants inoculated with *Pseudomonas* sp. strain K1. Hameedaet al. (2006) reported that two P-solubilizing bacteria species (*Serratiamarcescens* EB-67 and *Pseudomonas* spp. CDB-35 increased the biomass of maize by99 and 96% respectively, under greenhouse conditions. Increase in shoot dry mass has been reported in response to bacterial inoculationin maize (Gholami et al., 2009) and rice (Salamone et al., 2012). An increase in biological yield in inoculated plots over control may be as a result of the bacterial solubilization of insoluble phosphate in soil. These bacteria revealed an effective role in P uptake and growth promotion of plants by dissolution of inorganic insoluble phosphate (Narula et al., 2000). The main effects of P rate and *P. fluorescens* inoculation and their interaction were not significant for harvest index (Table 2). This is contrary to the findings of Salamone et al. (2012), who reported that combined inoculation of *Azospirillum* and *Pseudomonas* increased harvest index in rice by 16%.

Conclusion

This experiment showed that rice grain yield was significantly affected by the rate of phosphorous and *P. fluorescens* inoculation. Based on the result of this experiment, application of P at the rate of 75 kg ha^{-1} and seed inoculation with *P. fluorescens* are recommended to obtain the highest grain yield in rice.

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