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Response of yield and yield components of rice (*Oryza sativa* L. cv. Shiroodi) to different phosphate solubilizing microorganisms and mineral phosphorous

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Abstract

Plant growth stimulating rhizosphere bacteria are a heterogeneous group of rhizosphere bacteria which improve plant growth indices throughout diverse mechanisms. The present study aimed to evaluate the efficiency of phosphate solubilizing microorganisms (PSM) in terms of improving growth and yield of the rice plants (cv. Shiroodi). Experiment was conducted at Neka, Mazandaran province, Iran as split plot arrangement based randomized complete block design with four replicates in 2012. Main plots were phosphorous at three levels (0, 83 and 165 kg ha^{-1} from concentrated superphosphate triple source) and sub plots were phosphate solubilizing microorganisms (PSM) at four levels [control, inoculation with *Pseudomonas fluorescens* (PSF), *P. putida* (PSP), and coinoculation of PSF and PSP]. Results showed that there were a significant difference for mineral phosphorous and PSM in terms of number of stems, biological yield, straw yield, and paddy yield. Paddy yield varied from 457.8 gr m^{-2} in the control treatment to 625.1 and 630.2 gr m^{-2} in plots which received either 83 or 165 kg ha^{-1} mineral phosphorous, respectively. Inoculated rice plants with PSM had higher yield attributes. Paddy yield ranged from 498.9 gr m^{-2} in the control to 528.9, 561.5 and 561.6 gr m^{-2} in the PSF, PSF+PSP and PSP inoculated plants, respectively. Consequently, the maximum paddy yield of 699.9 gr m^{-2} (51.8% more than control) was recorded in plots which inoculated with PSP and received 83 kg ha^{-1} P.

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Introduction

Rice (*Oryza sativa* L.) is a cereal which forms an important part of the diet for many people worldwide including Iran. It is grown in some areas of northern Iran such as Mazandaran, Guilan, Golestan and in some parts of southern provinces including Fars and Khozestan (Bakhshandeh *et al.,* 2014). Phosphorus (P), the second most important nutrient after nitrogen, is one of the major macronutrients required for normal growth of plants. It has also a critical role in plant metabolism and other activities such as cell division, development, photosynthesis, nutrient uptake, plant disease resistance and regulation of metabolic pathways (Gupta *et al.,* 2012). The diverse forms of soil phosphate can be generally categorized as soil solution phosphate and insoluble organic and inorganic phosphate (Lavakush and Verma, 2012, Oliveira *et al.,* 2009). To overcome P deficiencies and ensure plant productivity in recent years, nearly 30 million tons of P fertilizer are applied worldwide every year, of which up to 80% is lost because it becomes immobile and unavailable for plant uptake due to absorption, precipitation or conversion to organic forms (Bakhshandeh *et al.,* 2014).

The rhizospheric soil in rice paddies contains a high diverse population of plant growth promoting rhizobacteria (PGPR). These microorganisms can influence plant growth and productivity in various ways (Taghavi *et al.,* 2009). Some of the underlying mechanisms are production of phytohormones (Cassán *et al.,* 2014), phosphate solubilization (Bakhshandeh *et al.,* 2014), nitrogen fixation and denitrification (Velasco *et al.,* 2013), and siderophore (Ahemad and Kibret, 2014). Meanwhile, microbial populations are key components of the soil plant continuum where they are immersed in a framework of interactions affecting plant development (Oliveira *et al.,* 2009). Generally, application of phosphate-solubilizing bacteria (PSB) increases the soil fertility due to their ability to convert insoluble P to soluble P by various mechanisms like acidification, chelation, exchange reactions, and also may be used as inoculants to increase P-availability to plants (Lavakush and Verma, 2012). This process not only compensates for the higher costs of manufacturing chemical fertilizers but also reduces the risk of environmental problems such as pollution through solubilization and mobilization of fertilizers (Hameeda *et al.,* 2008).

In recent years, several PSB belonging to the *Pseudomonas*, *Azospirillum*, *Burkholderia*, *Bacillus*, *Enterobacter*, *Rhizobium*, *Erwinia*, *Agrobacterium*, *Micrococcus*, *Serratia* and *Acinetobacter* genera, were applied in variety of crops (Lavakush and Verma, 2012, Ahemad and Kibret, 2014). For example, Sharma *et al.*, (2014) showed enhancement in rice growth and hence, increased rice yield in response to PGPR (P. putida, P. fluorescens, and A. lipoferum) application. Gopalakrishnan *et al.*,(2014) reported that in the rice field, the *actinomycetes* (different Streptomyces species) significantly enhanced tiller numbers, panicle numbers, filled grain numbers and weight, straw yield, grain yield, total dry matter, root length, volume and dry weight over the uninoculated control. Moreover, Cong *et al.*, (2011) evaluated the effects of different plant growth promoting rhizobacteria (PGPR) and concentration of phosphorus level on plant growth and yield of rice. They indicated that combinations of PGPR's increased the rice paddy grain yield more than control. Therefore, it can be concluded that application of PGPR strains is an important strategy to increase rice growth and development. Therefore, the aims of this study were to evaluate the efficiency of *Pseudomonas fluorescens* (PSF), *P. putida* (PSP), and combinations of PSF and PSP and various phosphorus fertilizer doses for promoting rice growth and yield related parameters.

Materials and methods

Field experiment

A field experiment was conducted during growing season of 2011-2012 in the city of Neka located at northern Iran. The experiment was laid out in split plot based randomized complete block design (RCBD) with four replicates. Main plots were phosphorous at the three levels (0, 83 and 165 kg ha^{-1} from concentrated superphosphate triple source) and sub plots were PSM at the four levels [control or non-inoculation, inoculation with *Pseudomonas fluorescens* (PSF), *P. putida* (PSP), and coinoculation of PSF and PSP]. PSM treatments were prepared in a slurry solution and applied by dipping the roots of the rice (*Oryza sativa* L. cv. Shiroodi) seedlings (into the slurry, 5 hour before transplanting in the field).

Plot sizes were 3 m long with row spacing 20 cm and included 6 rows. Plant population density was 25 hills per m^2 (with four seedlings per hill). The soil was silty loam with pH = 7.64. Based a soil test results (depth of 0-30 cm) different levels from P and 100 kg ha^{-1} of potassium sulfate was used at sowing time and 200 kg ha^{-1} urea was top dressed in three times as sowing time, beginning of tillering and beginning of anthesis. Weeds were hand-controlled and if was necessary appropriate chemicals were applied against pests and diseases, so the effect of diseases, pests and weeds were minimal. A water depth of 3–5 cm was applied at all plots from early tillering until two weeks before physiological maturity.

Measurement

In this study, grain yield (GY) and yield components were recorded on ten randomly selected plants at the time of harvest maturity. In this time all grain yields were about 14% moisture content. Grain harvest index was calculated using the following formula:

Grain harvest index = (Grain yield)/(Grain yield + straw yield).

Statistical analysis

Analysis of variance was conducted using MSTATC software and treatment means were compared by the Duncan's multiple-range test and were considered significant at $p \leq 0.05$.

Results and discussion

Effects of inoculation of PSB on yield and yield components

The results of this study indicated that among the yield attributes of rice (cv. Shiroodi), including, grain yield (gr m^{-2}), biological yield (gr m^{-2}), straw yield (gr m^{-2}), number of tiller per hill and 1000 grains weight (gr) were significantly influenced by the inoculation of PSB (Table 1). On the other hand, only harvest index (%) was not significantly influenced by the inoculation of PSB. The grain yield ranged from 499 gr m^{-2} in un-inoculated treatment to 562 gr m^{-2} in PSP inoculated plants while the biological and straw yields varied from 1447 and 948 gr m^{-2} in un-inoculated treatment to 1588 and 1027 gr m^{-2} in PSP treatment, respectively. The minimum number of tiller per hill at harvest time (15.7) recorded in un-inoculated plants while inoculation of PSP and coinoculation of PSF and PSP resulted in greater number of tiller per hill (26.3). The highest 1000 grain weight (23.5 gr) was obtained in PSF treatment followed by PSF (22.8 gr) and un-inoculated control (22.2 gr). Similarly, results of field experiments by researchers confirmed that inoculation of rice plants with P dissolving microorganism significantly increased some yield attributes (Alam *et al.,* 2008, Thakuria *et al.,* 2004).

Table 1. Variance analysis of the phosphate solubilizing bacteria and mineral phosphorous effects on yield attributes of rice.

Sources of change	Degree of freedom	grain yield (Y_G)	Biological yield (Y_B)	Straw yield (Y_S)	Harvest index (HI)	Number of tillers (N_T)	1000 grain Weight
Replication	3	17010.411	9676.303	3315.267	1.971	2.097	0.614
Mineral phosphorous	2	388455.229**	2411757.435**	864740.907**	39.611**	185.981**	0.184 ns
Error a	6	1857.108	10557.632	3613.110	2.084	0.773	0.248
Phosphate solubilizers	3	10871.450**	57364.240**	18375.168**	1.743 ns	26.531**	1.009**
Mutual effects	6	4682.988**	26737.151**	9111.012**	0.317 ns	1.352 ns	0.519**
Experimental Error	27	1222.543	6022.182	1858.202	2.031	0.724	0.135

The symbols ns, *, ** represent no significant differences, significant differences at the 1% and 5% level of probability, respectively.

Effects of different levels of P on yield attributes

The application of different levels of P alone exerted significant variation in grain yield (gr m^{-2}), biological yield (gr m^{-2}), straw yield (gr m^{-2}), number of tiller per hill and harvest index (%) while 1000 grain weight (gr) was not significantly influenced by the application of different P levels (Table 1). The grain, biological and straw yield increased with increase in P level up to 83 kg ha^{-1}. However, further increase in P level had no additional effect on these parameters. The lowest grain yield (358 gr m^{-2}), biological yield (1084 gr m^{-2}) and straw yield (727 gr m^{-2}) was observed in P level of 0 kg ha^{-1} treatment (control treatment). This finding is in agreement with Alam *et al.*,(2008) who reported that the yield of grain and straw of rice increased as the levels of P increased.

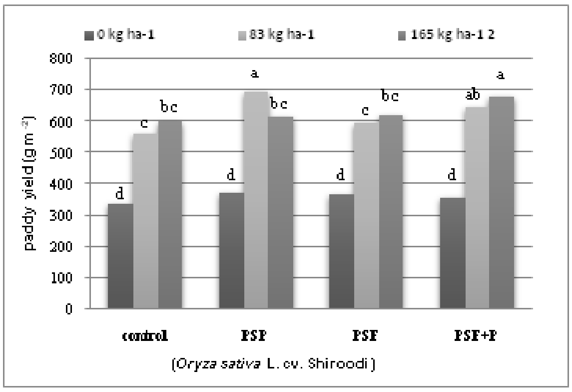

Fig. 1. Response of paddy yield to different phosphate solubilizing microorganisms (without the inoculation (control), inoculation with *Pseudomonas fluorescens* (PSF), *P. putida* (PSP), and coinoculation of PSF and PSP) and mineral phosphorous (0, 83 and 165 kg ha^{-1} from concentrated superphosphate triple (P) source).

The number of tiller per hill markedly increased due to the application of different P levels. Tiller number ranged from 17 in un-inoculated treatment to 24 in P level of 165 kg ha^{-1} treatment. The lowest harvest index (22.5%) was obtained from control plots while plots which received P both 83 and 165 kg ha^{-1} produced the maximum harvest index (about 27% more than the control). Similar results were recorded by Afzal *et al.*,(2005).

Interaction effects of PSB and different P levels on yield attributes

The interaction effect of PSB and different P levels significantly influenced the grain yield (gr m^{-2}), biological yield (gr m^{-2}), straw yield (gr m^{-2}) and 1000 grain weight (gr) of rice. Although, the number of tiller per hill and harvest index (%) increased than control, but there were not any significant difference between treatments (Table 1).

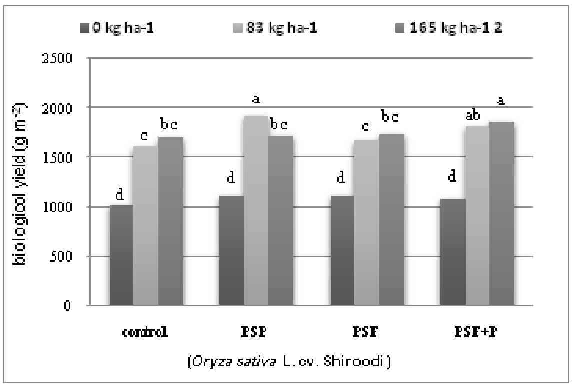

Fig. 2. Response of biological yield to different phosphate solubilizing microorganisms (without the inoculation (control), inoculation with *Pseudomonas fluorescens* (PSF), *P. putida* (PSP), and coinoculation of PSF and PSP) and mineral phosphorous (0, 83 and 165 kg ha^{-1} from concentrated superphosphate triple (P) source).

The treatment of PSP+83 kg ha^{-1} produced the maximum grain yield (696.9 gr m^{-2}) and that was minimum (335.5 gr m^{-2}) in un-inoculated and P level of 0 kg ha^{-1} treatment (Fig. 1). This finding is in agreement with Adesemoye and Egamberdieva, (2013).

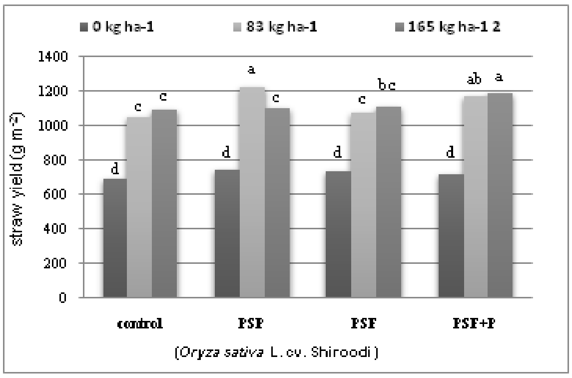

Fig. 3. Response of straw yield to different phosphate solubilizing microorganisms (without the inoculation (control), inoculation with *Pseudomonas fluorescens* (PSF), *P. putida* (PSP), and coinoculation of PSF and PSP) and mineral phosphorous (0, 83 and 165 kg ha^{-1} from concentrated superphosphate triple (P) source).

In this study, similar results observed in biological and straw yield (Fig. 2 and 3). However, the maximum straw yield of rice (1229.9 gr m^{-2}) was produced by treatments PSP+83 kg ha^{-1} of P and coinoculation of PSF and PSP+83 kg ha^{-1} of P and control treatment produced the minimum straw yield of rice (694.9 gr m^{-2}).

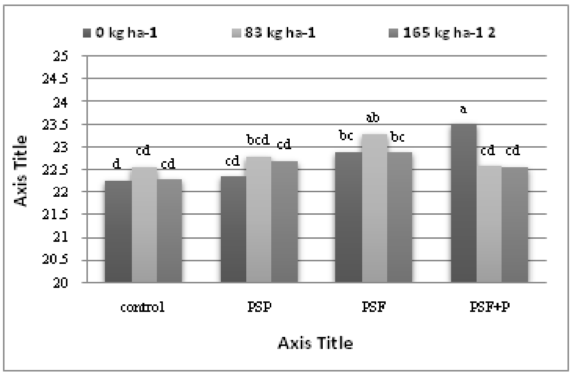

Fig. 4. Response of 1000 grain weight (gr) of rice to different phosphate solubilizing microorganisms (without the inoculation (control), inoculation with *Pseudomonas fluorescens* (PSF), *P. putida* (PSP), and coinoculation of PSF and PSP) and mineral phosphorous (0, 83 and 165 kg ha^{-1} from concentrated superphosphate triple (P) source).

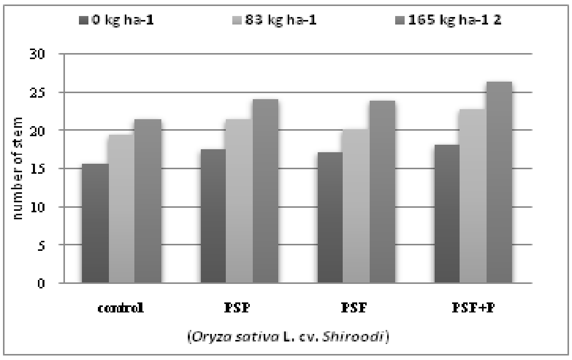

Fig. 5. Response of number of stem (per hill) to different phosphate solubilizing microorganisms (without the inoculation (control), inoculation with *Pseudomonas fluorescens* (PSF), *P. putida* (PSP), and coinoculation of PSF and PSP) and mineral phosphorous (0, 83 and 165 kg ha^{-1} from concentrated superphosphate triple (P) source).

The coinoculation of PSF and PS+P0 and PSF+P83 produced the maximum 1000 grain weight (23.5 gr) and (23.2 gr), respectively, while the minimum amount (22.2 gr) was recorded in the control plots (Fig. 4). The similar results were reported by Khorshidi *et al.*,(2011) who found more 1000 grain weight (27.18 gr) in rice plants which grown at 30-30-30 kg NPK ha^{-1} + *Sesbania rostrata* + phosphorus solubilizing bacteria. In conclusion, it is evident from the result that the PSB may improve the yield attributes of rice plants.

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