Pertanika J. Trop. Agric. Sci. 41 (2): 715 – 728 (2018)

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

Plant Growth Promoting Rhizobacteria (PGPR) Application with Different Nitrogen Fertilizer Levels in Rice (*Oryza sativa* L.)

Hamid Ghaffari[1]*, Abdollatif Gholizadeh[1], Abbas Biabani[1], Alireza Fallah[2] and Mohammad Mohammadian[3]

[1]*Faculty of Agriculture and Natural Resources, Gonbad-e Qabus University, Gonbad, Golestan, Iran*
[2]*The Research, Education and Agricultural Extension, Soil and Water Research Institute, Karaj, Alborz, Iran*
[3]*Rice Research Institute in Mazandaran, Amol, Mazandaran, Iran*

ABSTRACT

A factorial experiment was carried out through a completely randomised design with four replications in the Rice Research Institute of Iran, Deputy of Mazandaran (Amol), in 2012 in order to study the effect of Plant Growth Promoting Rhizobacteria (PGPR) with different levels of nitrogen fertilizer application on the yield and yield components of rice (*Oryza Sativa* L.).Treatments of this pot study included three factors; factor A - *Ppeudomonas* in four levels of control (no bacteria), *P. putida-1, P. putida-2* and *P. fluorescens* (0,3,3 and 3 g, respectively), Factor B - *azotobacter chroococcum* in two levels: no *aotobacter* and *azotobacter chroococcum* (0 and 3 g, respectively), and factor C: nitrogen fertiliser from urea in four levels of 0, 80, 140 and 200 mg N/kg soil in two stages. The experiment was conducted in pot culture and open air environment. Different parameters were studied that included fertile tillers number, shoot dry weight, harvest index, flag leaf chlorophyll content, grain yield and grain nitrogen concentration. According to the analysis results of data variance, all traits except chlorophyll content had significant difference at $P<0.01$ level in the interaction of three factors of *pseudomonas*, *azotobacter* and nitrogen fertiliser level. The highest mean of flag leaf chlorophyll content was observed in treatment of *pseudomonas putida-1* in 200 mg nitrogen fertiliser per kg soil of pot (43.48 SPAD number). The highest mean of grain yield was seen in treatment of *pseudomonas putida-1* and *azotobacter* in 200 mg nitrogen fertiliser per kg soil of pot (104.1 g/pot).

Keywords: Azotobacter and nitrogenous fertiliser, PGPR, *pseudomonas*, rice

ARTICLE INFO

Article history:
Received: 22 July 2017
Accepted: 4 January 2018

E-mail addresses:
h_ghaffari.info@yahoo.com (Hamid Ghaffari)
latif_gholizadeh@yahoo.com (Abdollatif Gholizadeh)
abs346@yahoo.com (Abbas Biabani)
rezafayah@yahoo.com (Alireza Fallah)
mohammadian953@yahoo.com (Mohammad Mohammadian)
* Corresponding author

ISSN: 1511-3701

INTRODUCTION

Chemical fertilisers are crucial elements in supply of plant nutrients. However, in recent years, excessive use of fertilisers in agricultural production has caused unpredicted environmental effects. Air and water pollution by pesticides, waste of soil and water resources and plant diseases are just a small part of environmental problems induced by chemical materials (Adesemoye, Torbert, & Kloepper, 2009; Koh & Song, 2007). Nitrogen nutrient management is a crucial strategy in regulating the rice growth and photosynthetic efficiency. In order to enhance production or yield, nitrogen fertiliser is increased which reduces farmer profits and deteriorates ecological environments (Long, Wan, Song, Jian, & Qin, 2013).

One way to reduce the negative impacts of chemical fertilisers on the environment is by inoculation with plant growth-promoting rhizobacteria (PGPR) (Velusamy, Immanuel, & Gnanamanickam, 2013). The recognition of plant growth-promoting rhizobacteria (PGPR), a group of beneficial plant bacteria, is potentially useful for stimulating plant growth and increasing crop yields PGPR has evolved over the past several years to where today researchers are able to repeatedly use it successfully in field experiments (Saharan & Nehra, 2011). PGPR is commonly used as inoculant for improving the growth and yield of agricultural crops and offers an attractive way to replace chemical fertilisers, pesticides, and supplements (Ashrafuzzaman et al., 2009).

Several chemical changes in soil are associated with PGPR, where some are able to establish themselves on the crop roots, especially if they are inoculated on the seed before planting. Also, most of the isolates have resulted in a significant increase in plant height, root length, and dry matter production of shoot and root of plants (Saharan & Nehra, 2011).

Various species of bacteria like *pseudomonas, azospirillum,* and *azotobacter* have been reported to enhance plant growth (Joseph, Ranjan Patra, & Lawrence, 2012; Kloepper, Lifshitz, & Zablotowicz, 2017). During the 1970s, *Pseudomonas fluorescens* and *P. putida* groups were investigated and *azospirillum* was found in order to enhance the growth of plants by affecting plant metabolism. In recent years, other bacterial genera, such as *bacillus, flavobacterium, acetobacter,* and several *azospirillum* have been evaluated (Nehra & Choudhary, 2015).

The study on the effect of *peudomonas* bacteria (*Pseudomonas fluorescens*) and *azospirillum* (*Azospirillum lipoferum*) with four levels of urea (25, 50, 75 and 100 kg /ha) Daylamani on Tarom rice showed that fertilization with *azospirillum* and *pseudomonas* bacteria has a significant difference at 1% (Khorshidi, Ardakani, Ramezanpour, Khavazi, & Zargari, 2011).

The study was conducted on the effects of several free-living rhizobacteria, including *azotobacter, bacillus, enterobacter* and *xanthobacter* in the accumulation of nitrogen, total dry matter yield and grain yield in rice plants (*Oryza sativa* L.)

indicated that the increase of grain yield was associated with the increase of root length, leaf area, and chlorophyll content (Alam, Cui, Yamagishi, & Ishii, 2001).

In the study done on isolation and characterisation of effective plant growth promoting rhizobacteria from Rice Rhizosphere of Indian Soil, eight efficient PGPR isolates were selected and identified as *Pseudomonas aeruginosa*, *Pseudomonas putida*, *P. aeruginosa*, *Pseudomonas* sp., *P. aeruginosa*, *P. putida*, *P. aeruginosa* and *P. aeruginosa*. Among all the strains, *Pseudomonas putida* was found to be the best in phosphorus solubilisation and was effective for rice production under Indo-Gangetic plain of Eastern Uttar Pradesh (Lavakush & Verma, 2012).

Evaluation of the effect of *azosprillum, azobacter* and chemical nitrogen fertiliser on rice (*Oryza sativa* L. var. Hashemi) has indicated that biofertiliser significantly affects the number of tillers. Nitrogen fertilisers significantly affect all measured traits and the interaction of biofertiliser × N fertiliser significantly affects flag leaf area, number of total tillers and biomass (Shakouri, Vajargah, Gavabar, Mafakheri, & Zargar, 2012).

This pot study investigates the effect of PGPR with different levels of nitrogen fertiliser application on the characteristics of flag leaves and yield components of rice in order to study the possibility of reducing the consumption of urea and the environmental degradation effects caused by the use of chemical fertilisers in agricultural products.

MATERIAL AND METHODS

The pot experiment was carried out in a completely randomised factorial design with four replications in the Rice Research Institute of Iran, Deputy of Mazandaran (Amol) during a growing season in one year and open-air environment in 2012. The experiment was conducted on Shiroodi high yielding rice variety. It was performed in the pot to control bacteria in the environment in order to ensure the preservation of bacterial populations and to avoid any interference with the treatment combinations of design.

The treatments consisted of three factors: factor A - *pseudomonas* in four levels of control (no bacteria), *P. putida-1*, *P. putida-2* and *P. fluorescens* (0,3,3 and 3 g, respectively), factor B - *azotobacter chroococcum* in two levels control (no *azotobacter*) and *azotobacter* application (0 and 3 g, respectively), and factor C: nitrogen fertiliser from urea in four levels of 1- control (without nitrogen fertiliser), 2-80 mg nitrogen fertiliser per kilogramme of soil (in two stages of 40 mg), 3-140 mg of nitrogen fertiliser per kilogramme of soil (in two stages of 70 mg), and 4-200 mg of nitrogen fertiliser per kilogramme of soil (in two stages of 100 mg), respectively. The bacteria were prepared from the Iranian Soil and Water Research Institute, which were isolated from wheat fields.

In order to achieve the maximum utilisation of bacterial activity, the soil with low organic matter (0.67%) , total content of nitrogen 0.03%, and loam sandy texture was selected (Table 1).

Table 1
Soil characteristics

Sand	*Silt*	*Clay*	*Soil type*	*Organic carbon*	*Organic matter*	*The electrical conductivity*	*Total nitrogen (N)*	*Absorbable phosphorus (P)*	*Absorbable potassium (K)*
Percent	Percent	Percent	Loamy sand	Percent	Percent	DS.m	Percent	Mg/kg	Mg/kg
86	9	5		0.39	0.67	0.1	0.03	3.72	5.65

In order to prepare the soil for the pots, it was sieved and powdered with a 2 mm sieve in a chopper machine to obtain homogeneous soil. The pots were filled with 12 kg of soil, which was mixed with 2 g potassium sulfate fertiliser and 2 g of triple superphosphate fertiliser. After preparing the pots, 3 g of bacteria were measured for each treatment based on McFarland formula (3×10^8 cfu/ml) (El-Fattah, Eweda, Zayed, & Hassanein, 2013; Maheshwari, Saraf, & Aeron, 2013). Perlite was used as a carrier in the formulation of the inoculum to support the survival of bacteria (Nehra & Choudhary, 2015). Then, inoculum was placed under germinated seeds and a thin layer of soil was sprayed onto each pot to cover the seeds and bacteria (0.5 kg per pot in order to place the seeds at equal depth). After planting, slight irrigation was given and flooding of the pots was avoided until suitable seedling growth time. After the plant height reached 5 cm, depending on the age and development stage of the plant, 2 to 5 cm of water was maintained at soil surface of each pot. Six seeds were planted in each pot and after the beginning of tillering, three plants were kept for further evaluation. In the tillering stage, first installment of nitrogen was distributed and in the booting stage (swelling of the flag leaf sheath), the second installment of nitrogen was weighed and distributed based on the amounts specified for each treatment.

During the growing season until harvest, the plant parameters were measured, and harvesting was performed at physiological maturity. Flag leaf chlorophyll content was measured by SPAD. Data was analysed with MSTAT-C statistical software and mean comparison was done by LSD[1] test at 5% probability level.

RESULTS AND DISCUSSION

Table 2 reports the analysis of variance for the number of fertile tillers, shoot dry weight, harvest index, flag leaf chlorophyll content, grain yield and grain nitrogen concentration. Table 3 indicates the mean comparison for simple effect of *pseudomonas*, *azotobacter* and different levels of nitrogen fertilizer. Table 4 shows the mean comparison of interactions among *pseudomonas*, *azotobacter* and different levels of nitrogen fertiliser.

[1] Least significant difference test

Table 2
Analysis of variance of measured traits

		Mean Square					
SOV	df	Fertile tillers number (per pot)	Shoot dry weight (g/ pot)	Flag leaf chlorophyll content (SPAD number)	Harvest index	Grain yield (g/pot)	Grain nitrogen content (Percent)
Pse	3	170.362**	701.064**	1.775ns	2.090**	735.49**	0.039**
Az	1	43.945ns	306.498**	0.538ns	1.223*	274.89*	0.12**
Pse × Az	3	96.966**	498.07**	3.333**	1.627**	518.35**	0.036**
N	3	3266.008**	8947.586**	534.406**	52.884**	10494.01**	10.768**
Pse × N	9	35.918**	98.172*	3.972**	0.234ns	101.65*	0.036**
Az × N	3	42.487*	102.892ns	5.75**	0.388ns	99.31ns	0.162**
Pse × Az × N	9	57.508**	267.631**	1.296ns	0.861**	271.24**	0.034**
Error	96	13.721	41.549	0.806	0.235	44.49	0.002
CV%	-	9.56	8.75	2.36	1.04	10.24	3.47

Note. ns: non-significant, **: significant at 0.01%, *: significant at .05%. Az: *azotobacter chroococcum*, Pse: *psedumonas* L., N: Nitrogen fertiliser. Data are the mean values for four replicates.

Fertile Tillers

Based on the results of the analysis of variance for fertile tillers trait (Table 2), different levels of *pseudomonas* bacteria showed significant difference ($p<0.01$). However, levels of *azotobacter* bacteria had no significant difference. Interactions between *pseudomonas*, *azotobacter* and nitrogen fertiliser levels showed significant differences at $p<0.01$. The interactions of *pseudomonas* and nitrogen fertiliser at 0.01 level of probability, and the interaction of *azotobacter* and nitrogen fertiliser levels at $p<0.01$ showed significant differences. Accordingly, interactions (three-way) *pseudomonas*, *azotobacter* and different levels of nitrogen fertiliser showed significant differences at $p<0.01$ (Table 4).

Based on mean comparison by LSD method at 0.05 level of probability in the study of interactions, the treatment of *pseudomonas putida-1*, *azotobacter* and 200 mg nitrogen fertiliser per kg of soil in pots with 61.25 fertile tillers had the highest average, in comparison to the control group which showed increase of 102.43% (Table 4).

Khorshidi et al. (2011) studied the effect of bacterial strain of *pseudomonas* based on morphophysiology traits and nutrient uptake of rice. They evaluated positive effect of PGPR on the increase of the number of tillers, because it attracts more macronutrients and also produces more hormone in the plant. Khorshidi et al. (2011) examined the effects of nitrogen levels on grain yield of rice and reported the grain yield to have increased because of nitrogen effect on the increasing number of fertile tillers. Abbasi, Esfahani, Rabiei and Kavousi (2007) also reported the effect of the nitrogen uptake on tiller numbers similarly.

Shoot Dry Weight

Based on the results of the analysis of variance for shoot dry weight trait (Table 2), interactions between *pseudomonas* and nitrogen fertiliser levels showed significant differences ($p<0.01$). The interaction of different levels of nitrogen fertiliser and *azotobacter* had no statistically significant difference. Other factors showed significant differences at 0.01 level of probability.

Based on the mean comparison by LSD method at 0.05 level of probability in the study of simple effect (Table 3), the treatment of *Pseudomonas putida-1* with 78.70 mg had the highest average. In the study of interactions, the treatment of *Pseudomonas putida-1* and 200 mg nitrogen fertiliser per kg of soil in pots without *azotobacter* application had the highest average.

Gardner, Pearce and Mitchell (2003) demonstrated that nitrogen is effective on the increase of biomass. Rajabi Agra, Bahmanyar, Ramezanpoor and Khavazi (2011) reported that application of the *pseudomonas* bacteria increases shoot dry weight of plants that could be attributed to the increased absorption of nutrients and improvement of the root systems and plant growth (Cakmakçi, Dönmez, Aydın, & Şahin, 2006). It seems that simultaneous use of both bacteria at higher levels of nitrogen or increasing nitrogen absorption contributed to the growth of shoots, while consumption of bacteria in terms of lack of nitrogen in the soil suffered from nutrients bacteria did not have a successful performance that could be attributed to carbon and nitrogen deficiency or competition between bacteria in food shortages (Sylvia, Fuhrmann, Hartel, & Zuberer, 2005).

Harvest Index

Based on the results of the analysis of variance for harvest index trait (Table 2), levels of *pseudomonas* bacteria and the nitrogen fertiliser levels at 0.01 level of probability showed significant differences. The interactions of *pseudomonas* bacteria, *azotobacter* and nitrogen fertiliser levels showed significant differences at 0.01 level of probability.

Based on mean comparison by LSD method at 0.05 level of probability in the study of interactions, treatments of *Pseudomonas putida-1* combined with *azotobacter* and consumption of 200 mg nitrogen fertiliser level per kg of soil with 48.67% showed the highest mean (Table 4).

Khorshidi et al. (2011) stated that harvest index is reduced by increase in the amount of nitrogen in rice production due to increased body weight, growth and biomass production. Also, they acknowledged that harvest index is decreased in the control treatment due to the extreme decrease of grain and straw yield.

Zargari, Khorshidi and Ardakani (2014) illustrated that the interaction between nitrogen, bacteria *pseudomonas fluorescens* and *azospirillum lipoferum* had a significant effect ($p<0.01$) on harvest index. Rahmani, Maleki, Mirzaeiheydari, and Naseri (2014) showed that bio-fertiliser has a significant effect on harvest index and the highest harvest index obtained from nitroxin treatment.

Flag Leaf Chlorophyll Content

Nitrogen rate with the effect on flag leaf chlorophyll content has a direct effect on photosynthetic response, the amount of photosynthesis per unit leaf area, plant growth and yield (Maheshwari et al., 2013). According to the results of the analysis of variance for flag leaf chlorophyll content (Table 2), different levels of nitrogen, interactions between *azaotobacter* and *pseudomonas, pseudomonas* and *azotobacter,* interactions between *pseudomonas* and nitrogen fertiliser application showed significant difference ($p<0.01$). No significant differences were observed in the remaining factors (Table 4).

Based on the mean comparison by LSD method at 0.05 level of probability, the treatment of 200 mg of nitrogen fertiliser and the treatment of no consumption of nitrogen fertilisers in the comparison of the simple effects (Table 3), had the highest (SAPD 42.69) and lowest (SAPD 33.09) mean, respectively. Comparison of the interactions between *pseudomonas* bacteria and *azotobacter* showed that all treatments except the treatment of non-use were placed in Class A, indicating higher values. Comparison of interactions between *pseudomonas* and nitrogen fertiliser, treatment of *Pseudomonas putida-1* at level of 200 mg nitrogen fertiliser per kg soil of pot demonstrated the highest mean and treatment of *pseudomonas putida-1* without nitrogen fertiliser showed the lowest mean (Figure 1-3) .

It seems that the use of bacteria is useful when it is used along with enough nitrogen fertiliser and non-use of nitrogen fertiliser in this soil with the condition of the food poverty showed content less than control treatment. Faraji, Esfahani, Kavoosi, Nahvi and Rabiee (2011) stated that there is a significant linear relationship between the concentration of chlorophyll and nitrogen concentrations. Although the levels of *azotobacter* and *pseudomonas* had no significant difference in the change of chlorophyll, the difference was statistically significant at levels of nitrogen fertiliser alone. The interactions between *pseudomonas* bacteria and nitrogen as well as interactions between nitrogen and *azotobacter* indicated the importance of this element in determining the concentration of chlorophyll and photosynthesis in plants, thereby increasing the plant performance (Mahanta, Jha, & Rajkhowa, 2012; Maheshwari,et al. 2013; Okon & Labandera-Gonzalez, 1994). Alam, Cui, Yamagishi, and Ishii (2001) also illustrated that bacterial inoculation led to the increase in chlorophyll content, particularly in the last stage of the grain filling.

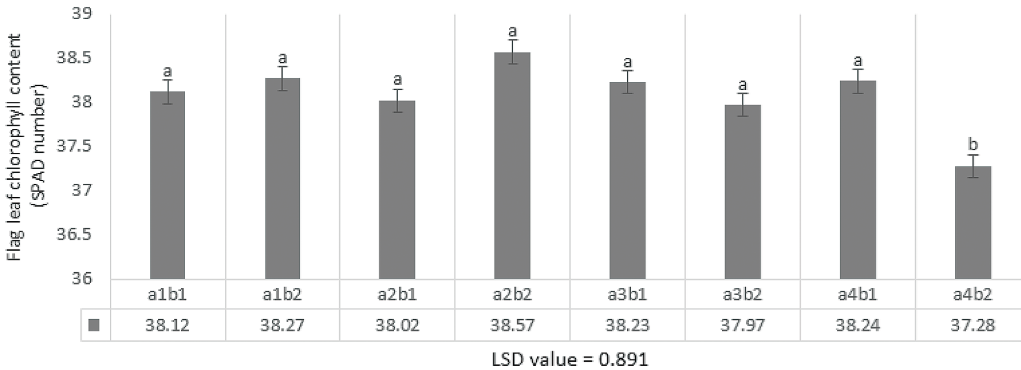

Figure 1. Interactions between *pseudomonas* and *azotobacter* in rice (*Oryza Sativa*) Var Shiroodi. a1: *P. putida-1*, a2: *P. putida-2*, a3: *P. fluorescens*, and a4: no *pseudomonas*. b1: *azotobacter*, b2: no a*azotobacter*. Data are the mean values of four replicates. LSD: least significant difference

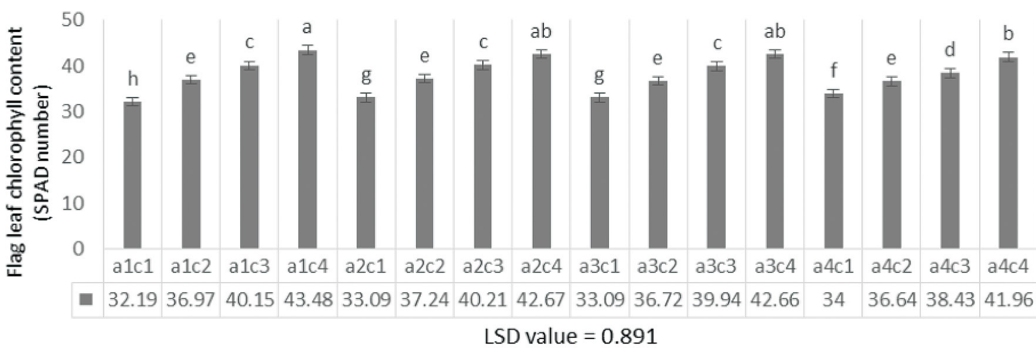

Figure 2. Interactions between *pseudomonas* and different levels of nitrogen in rice (*Oryza Sativa*) Var Shiroodi. a1: *P. putida-1*, a2: *P. putida-2*, a3: *P. fluorescens* , and a4: no *pseudomonas*. c1: no nitrogen, c2: 80 mg nitrogen fertiliser per kg of soil, c3: 140 mg nitrogen fertiliser per kg of soil, c4: 200 mg nitrogen fertiliser per kg of soil. Data are the mean values of four replicates. LSD: least significant difference

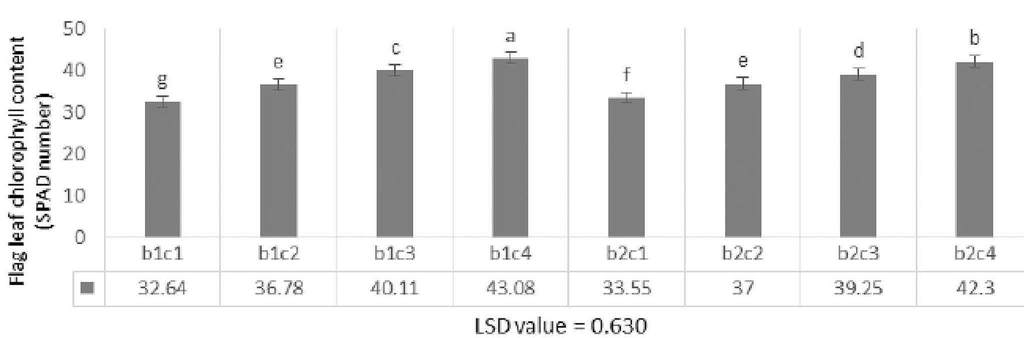

Figure 3. Interactions between *azotobacter* and different levels of nitrogen in rice (*Oryza Sativa*) Var. Shiroodi. b1: *azotobacter*, b2: no *azotobacter*, c1: no nitrogen, c2: 80 mg nitrogen fertiliser per kg of soil, c3: 140 mg nitrogen fertiliser per kg of soil, c4: 200 mg nitrogen fertiliser per kg of soil. Data are the mean values of 4 replicates. LSD: least significant difference

Grain Yield

According to the variance analysis results of the grain yield trait (Table 2), different levels of *azotobacter,* interaction between *pseudomonas* and nitrogen fertiliser at statistical level of 0.05, different levels of *pseudomonas,* interactions among *pseudomonas*, *azotobacter* and different levels of nitrogen fertiliser had significant differences at 0.01. However, interactions between *azotobacter* and different levels of nitrogen fertiliser did not have significant differences.

According to the mean comparison by LSD method at 0.05 of probability and comparison of interactions mean (Table 4), treatment of *P. putida-1, azotobacter* and 200 mg nitrogen fertiliser per kg of soil with 104.1 g in pot had the highest mean score, which showed a remarkable growth (163.27%) toward control treatment (39.54 g), and treatments of *P. putida-2,* no *azotobacter* and without nitrogen fertiliser had the minimum mean (Table 4).

Zargari et al. (2014) showed that simple effects of nitrogen and *pseudomonas* bacteria had a significant effect ($p<0.05$ and $p<0.01$ respectively) on grain yield and in the interaction between treatments. Alam et al. (2001) reported that grain yield was increased by inoculation of a mixture of several free-living rhizobacteria: *azotobacter, bacillus, enterobacter* and *xanthobacter.* Rahmani et al. (2014) also showed that bio-fertiliser has a significant effect on grain yield and the highest grain yield obtained from application of *azotobacter* treatment.

Grain Nitrogen Concentration

Based on the results of the analysis of variance for grain nitrogen concentration trait (Table 2), all levels of treatment combinations including *pseudomonas*, *azotobacter*, nitrogen fertiliser and their interactions showed significant difference ($P<0.01$).

Based on the mean comparison by LSD method at 0.05 level of probability in the study of interactions (Table 4), the treatment of *pseudomonas fluorescens* with concurrent use of *azotobacter* and 200 mg nitrogen fertiliser per kg of soil in the pots with 2.016% had the highest average of grain nitrogen concentration.

Bani Hashim, Rezaii, and Ramezanpoor (2010) reported that the amount of nitrogen in rice significantly increases, following inoculation of rice seedlings. Biswas, Ladha and Dazzo (2000) stated that bacterial inoculation increases the number of roots due to roots communication with a greater volume of soil and thus the plants absorb more food. Alam et al. (2001) indicated that the inoculation of free-living rhizobacteria to rice plants leads to increase of nitrogen concentration in plants.

Table 3
The mean comparison for simple effect of pseudomonas, azotobacter and different levels of nitrogen fertiliser in rice (Oryza sativa) *var. Shiroodi*

Fertile tillers number (per pot)	Shoot dry weight (g/pot)	Grain yield (g/pot)	Harvest index	Flag leaf chlorophyll content (SPAD number)	Grain nitrogen content (Percent)	Factors
41.34 a	78.70 a	124.1 a	46.85 a	38.20 ab	1.23 a	P. putida-1
37.16 b	70.05 b	118.6 b	46.38 b	38.30 a	1.22 a	P. putida-2
40.03 a	76.68 a	122.6 a	46.78 a	38.10 ab	1.21 a	P. fluorescens
36.50 b	69.40 b	119.0 b	46.37 b	37.76 b	1.15 b	Control
38.17 a	72.16 b	120.56 a	46.50 b	38.15 a	1.23 a	Azotobacter
39.34 a	75.25 a	121.59 a	46.69 a	38.02 a	1.17 b	Control
28.25 d	56.92 d	107.7 d	45.24 d	33.09 d	0.51 d	Control
33.88 c	65.33 c	115.0 c	45.92 c	36.89 c	0.99 c	Nitrogen 80
41.28 b	77.06 b	124.3 b	47.06 b	39.68 b	1.44 b	Nitrogen 140
51.63 a	95.51 a	137.3 a	48.16 a	42.69 a	1.86 a	Nitrogen 200
1.838	3.199	3.310	0.240	0.445	0.022	LSD ($P \leq 0.05$)

Note. According to the mean comparison by LSD method at level 0.05 of probability, the means in the same columns are not statistically different at 0.05 level by similar letters. Data are the mean values of four replicates.

Table 4
The mean *comparison of interactions among pseudomonas, azotobacter and different levels of nitrogen fertiliser in rice* (Oryza sativa) *var. Shiroodi*

Fertile tillers number (per pot)	Shoot dry weight (g/pot)	Grain yield (g/pot)	Harvest index	Grain nitrogen content (Percent)	Treatment
24.25 no	48.22 kl	39.06 m	44.72 m	0.44 o	a1b1c1
31.75 k-m	61.40 h-j	51.58 j-l	45.62 j-l	0.94 j	a1b1c2
44.25 de	87.35 cd	79.15 c-e	47.51 c-e	1.57 e	a1b1c3
61.25 a	109.8 a	104.1 a	48.67 a	2.01 a	a1b1c4
35.00 h-k	68.54 f-h	59.08 h-j	46.14 h-j	0.54 mn	a1b2c1
39.25 e-i	77.45 ef	67.91 f-h	46.71 f-h	1.08 i	a1b2c2
40.50 e-g	75.61 e-g	67.24 f-i	46.97 e-g	1.41 fg	a1b2c3
54.50 b	101.2 ab	95.24 ab	48.47 ab	1.81 bc	a1b2c4
22.25 o	45.61 l	36.67 m	44.56 m	0.45 o	a2b1c1
29.25 l-n	55.24 i-k	45.64 lm	45.22 lm	0.92 j	a2b1c2
37.25 g-j	67.84 gh	58.96 h-j	46.47 g-i	1.57 e	a2b1c3
50.25 bc	93.79 bc	86.93 bc	48.09 a-c	1.97 a	a2b1c4
30.50 k-m	61.42 h-j	51.37 j-l	45.46 kl	0.49 no	a2b2c1
33.00 j-l	62.06 h-j	52.58 j-l	45.77 j-l	1.12 i	a2b2c2
43.00 d-f	79.69 de	71.61 d-f	47.30 d-f	1.45 f	a2b2c3
51.75 bc	94.73 bc	88.23 bc	48.21 ab	1.77 c	a2b2c4
31.50 k-m	64.05 hi	53.83 j-l	45.58 j-l	0.43 o	a3b1c1
38.75 f-i	77.56 ef	67.96 f-h	46.68 f-h	0.92 j	a3b1c2
43.50 d-f	82.25 de	74.18 d-f	47.31 d-f	1.55 e	a3b1c3
53.50 b	99.51 b	93.44 b	48.42 ab	2.01 a	a3b1c4
31.75 k-m	63.71 h-j	53.54 j-l	45.61 jkl	0.53 mn	a3b2c1
32.75 j-l	62.30 h-j	52.66 j-l	45.79 i-l	1.10 i	a3b2c2
41.25 e-g	77.07 ef	68.53 fg	47.05 e-g	1.37 g	a3b2c3
47.25 cd	87.00 cd	79.80 cd	47.83 b-d	1.77 c	a3b2c4
27.25 m-o	54.84 jk	45.46 lm	45.22 lm	0.56 lm	a4b1c1
31.50 k-m	58.61 ij	49.06 kl	45.54 j-l	1.11 i	a4b1c2
40.00 e-h	67.82 gh	59.93 g-j	46.90 e-g	1.39 fg	a4b1c3
44.25 de	80.68 de	73.06 d-f	47.50 c-e	1.85 b	a4b1c4
23.50 o	48.97 kl	39.54 m	44.65 m	0.62 l	a4b2c1
34.75 i-k	67.99 gh	58.11 i-k	46.03 h-k	0.77 k	a4b2c2
40.50 e-g	78.88 de	70.09 ef	47.01 e-g	1.23 h	a4b2c3
50.25 bc	97.40 b	90.35 b	48.11 a-c	1.65 d	a4b2c4
5.20	9.04	9.36	0.68	0.06	LSD ($P \leq 0.05$)

According to the mean comparison by LSD method at level 0.05 of probability, the means in the same column are not statistically different at 0.05 level by similar letters. a1: *P. putida-1,* a2: *P. putida-2,* a3: *P. fluorescens ,* and a4: no *pseudomonas.* b1: *azotobacter,* b2: no *azotobacter,* c1: no nitrogen, c2: 80 mg nitrogen fertiliser per kg of soil, c3: 140 mg nitrogen fertiliser per kg of soil, c4: 200 mg nitrogen fertiliser per kg of soil. Data are the mean values of four replicates. LSD: least significant difference.

According to the mean comparison by LSD method at level 0.05 of probability, the means in the same column are not statistically different at 0.05 level by similar letters. a1: *P. putida-1,* a2: *P. putida-2,* a3: *P. fluorescens ,* and a4: no *pseudomonas.* b1: *azotobacter,* b2: no *azotobacter,* c1: no nitrogen, c2: 80 mg nitrogen fertiliser per kg of soil, c3: 140 mg nitrogen fertiliser per kg of soil, c4: 200 mg nitrogen fertiliser per kg of soil. Data are the mean values of four replicates. LSD: least significant difference.

CONCLUSIONS

The results show a large positive effect on uptake of nitrogen by plant and nitrogen content of grains. Flag leaf chlorophyll content is increased by usage of bacteria along with nitrogen fertiliser which has a significant effect on seed filling and yield enhancement. Increasing of grain yield along with harvest index and other yield components are promising results. Although fertilisers play a significant role in the increase of plant yield, PGPR bacteria can be used to increase the effect of nitrogen fertilisers in rice paddy. *Pseudomonas putida-1* has the best performance among *pseudomonas* bacteria in all traits. The combination of *P. putida-1, azotobacter* and 200 mg nitrogen fertiliser per kg of soil (the highest amount of fertiliser) is evaluated as the best treatment combination. Based on the results, the use of growth promoting bacteria with nitrogen fertiliser plays a useful and effective role in the increase of rice yield, especially when enough nitrogenous fertilisers are used, soil has little natural food sources and the high yielding varieties of rice are used (which require more fertilisers and food). On the other hand, consumption of PGPR bacteria is not useful without fertiliser and has a negative effect on most traits. The use of soils with a variety of tissue, porosity, fertility and other rice varieties can be investigated in future research.

REFERENCES

Abbasi, H. A., Esfahani, M., Rabiei, B., & Kavousi, M. (2007). Effect of nitrogen fertilizing management on rice (cv. Khazar) yield and its components in a paddy soil of Guilan Province. *JWSS-Isfahan University of Technology*, *10*(4), 293-307.

Adesemoye, A. O., Torbert, H. A., & Kloepper, J. W. (2009). Plant growth-promoting rhizobacteria allow reduced application rates of chemical fertilizers. *Microbial Ecology*, *58*(4), 921-929.

Alam, S., Cui, Z. J., Yamagishi, T., & Ishii, R. (2001). Grain yield and related physiological characteristics of rice plants (*oryza sativa* L.) inoculated with free-living rhizobacteria. *Plant Production Science*, *4*(2), 126-130.

Ashrafuzzaman, M., Hossen, F. A., Ismail, M. R., Hoque, A., Islam, M. Z., Shahidullah, S. M., & Meon, S. (2009). Efficiency of plant growth-promoting rhizobacteria (PGPR) for the enhancement of rice growth. *African Journal of Biotechnology*, *8*(7), 1247-1252.

Bani Hashim, F., Rezaii, M. A., & Ramezanpoor, M. R. (2010). The effect of bacteria on growth and nutrient concentration Pseudomonas with auxin production ability in rice (oryza sativa L. var Neda*). Journal of Plant Research, 3*(19), 63-56.

Biswas, J., Ladha, J., & Dazzo, F. (2000). Rhizobia inoculation improves nutrient uptake and growth of lowland rice. *Soil Science Society of America Journal*, *64*(5), 1644-1650.

Cakmakçi, R., Dönmez, F., Aydın, A., & Şahin, F. (2006). Growth promotion of plants by plant growth-promoting rhizobacteria under greenhouse and two different field soil conditions. *Soil Biology and Biochemistry*, *38*(6), 1482-1487.

Egamberdiyeva, D. (2005). Plant-growth-promoting rhizobacteria isolated from a Calcisol in a semi-arid region of Uzbekistan: Biochemical characterization and effectiveness. *Journal of Plant Nutrition and Soil Science*, *168*(1), 94-99.

El-Fattah, D. A. A., Eweda, W. E., Zayed, M. S., & Hassanein, M. K. (2013). Effect of carrier materials, sterilization method, and storage temperature on survival and biological activities of *Azotobacter chroococcum* inoculant. *Annals of Agricultural Sciences*, *58*(2), 111-118.

Faraji, F., Esfahani, M., Kavoosi, M., Nahvi, M., & Rabiee, B. (2011). Effect of nitrogen fertilizer on rice yield and conversion efficiency. *Iranian Journal of Crop Sciences*, *13*(1), 77-61.

Gardner, F. P., Pearce, R. B., & Mitchell, R. L. (2003). *Physiology of crop plants*. Jodhpur, India: Scientific Publishers

Joseph, B., Ranjan Patra, R., & Lawrence, R. (2012). Characterization of plant growth promoting rhizobacteria associated with chickpea (*Cicer arietinum* L.). *International Journal of Plant Production*, *1*(2), 141-152.

Khorshidi, Y. R., Ardakani, M. R., Ramezanpour, M. R., Khavazi, K., & Zargari, K. (2011). Response of yield and yield components of rice (oryza sativa L.) to *Pseudomonas flouresence* and *Azospirillum lipoferum* under different nitrogen levels. *American-Eurasian Journal of Agricultural & Environmental Sciences*, *10*(3), 387-95.

Kloepper, J. W., Lifshitz, R., & Zablotowicz, R. M. (2017). Free-living bacterial inocula for enhancing crop productivity. *Trends in Biotechnology*, *7*(2), 39-44.

Koh, R. H., & Song, H. G. (2007). Effects of application of Rhodopseudomonas sp. on seed germination and growth of tomato under axenic conditions. *Journal of microbiology and biotechnology*, *17*(11), 1805-1810.

Lavakush, J. Y., & Verma, J. P. (2012). Isolation and characterization of effective plant growth promoting rhizobacteria from rice rhizosphere of Indian soil. *Asian Journal Biological Science*, *5*, 294-303.

Long, J. R., Wan, Y. Z., Song, C. F., Jian, S. U. N., & Qin, R. J. (2013). Effects of nitrogen fertilizer level on chlorophyll fluorescens characteristics in flag leaf of super hybrid rice at late growth stage. *Rice Science*, *20*(3), 220-228.

Mahanta, K., Jha, D. K., Rajkhowa, D. J., & Manoj-Kumar. (2012). Microbial enrichment of vermicompost prepared from different plant biomasses and their effect on rice (*Oryza sativa* L.) growth and soil fertility. *Biological Agriculture & Horticulture*, *28*(4), 241-250.

Maheshwari, D. K., Saraf, M., & Aeron, A. (Eds.). (2013). *Bacteria in agrobiology: Crop productivity*. City: Springer Science & Business Media.

Nehra, V., & Choudhary, M. (2015). A review on plant growth promoting rhizobacteria acting as bioinoculants and their biological approach towards the production of sustainable agriculture. *Journal of Applied and Natural Science*, *7*(1), 540-556.

Okon, Y., & Labandera-Gonzalez, CA. (1994). Agronomic applications of azospirillum in improving plan productivity with rhizosphere bacteria. In M. H. Ryder, P. M. Stephens, & G. D. Brown (Eds.). *Commonwealth Scientific and Industrial Research Organization* (pp. 274-278). Adelaide: Publisher.

Rahmani, A., Maleki, A., Mirzaeiheydari, M., & Naseri, R. (2014). Effect of plant growth promoting rhizobacteria (PGPR) and planting pattern on yield and its components of rice (oryza sativa L.) in Ilam Province, Iran. *World Academy of Science, Engineering and Technology, International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering*, *8*(8), 912-918.

Rajabi Agra, S., Bahmanyar, M. A., Ramezanpoor, M. R., & Khavazi, K. (2011). The role of pseudomonas bacteria in reducing injuries from the use of saline water in rice (oryza sativa L.). *Journal of Water in Agriculture, 25*(1), 46-37.

Saharan, B. S., & Nehra, V. (2011). Plant growth promoting rhizobacteria: a critical review. *Life Sciences and Medicine Research*, *21*(1), 30.

Shakouri, M. J., Vajargah, A. V., Gavabar, M. G., Mafakheri, S., & Zargar, M. (2012). Rice vegetative response to different biological and chemical fertilizers. *Advances in Environmental Biology, 6*, 859-863.

Sylvia, D. M., Fuhrmann, J. J., Hartel, P. G., & Zuberer, D. A. (Eds.). (2005). *Principles and applications of soil microbiology.* Upper Saddle River, NJ: Pearson Prentice Hall.

Velusamy, P., Immanuel, J. E., & Gnanamanickam, S. S. (2013). Rhizosphere bacteria for biocontrol of bacterial blight and growth promotion of rice. *Rice Science*, *20*(5), 356-362.

Zargari, K., Khorshidi, Y. R., & Ardakani, M. R. (2014). Growth stimulant bacteria and nitrogen fertilizer effects plant nutrient uptake in rice (*Oryza sativa* L.). *International Journal of Biosciences (IJB)*, *4*(9), 218-226.