

Growth promotion of the rice genotypes by pgprs isolated from rice rhizosphere

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Abstract

Plant growth promoting rhizobacteria (PGPRs) improve growth of the host plants in a variety of ways. For this reason five bacterial strains isolated from the rice rhizospheric soil (B 15, B 17, B 19, BN 17 and BN 30) and three standard PGPR strains (viz. *Pseudomonas putida*, *Pseudomonas fluorescens* and *Azospirillum lipoferum*) were tested for plant growth promotion when applied to the rice plants as seedling treatments. The experiment was conducted for two rainy seasons of the years 2010 and 2011. Rice plants inoculated with the bacterial isolates recorded an improved plant growth and higher photosynthetic capacity signified by the higher chlorophyll content. Root and shoot dry mass was also found to be increased in the inoculated plants. Besides these iron and zinc content of the treated rice plants was also found to be higher in comparison with the uninoculated control plants. Hence, it can be concluded that application of PGPR has immense potential to be used as agricultural crop inoculants as they promote plant growth as well as improve the health and yield of the plants.

Keywords: PGPR, IAA product

1. Introduction

Bacteria that colonize the plant roots and surrounding rhizosphere, enhance plant growth by a variety of different mechanisms are referred to as plant growth-promoting rhizobacteria (PGPR). PGPR have been applied to various crops to enhance growth, seed emergence and crop yield, and some have also been commercialized (Herman *et al.*, 2008; Minorsky, 2008). *Pseudomonas fluorescens* B16 isolated from the roots of graminaceous plants has been shown to colonize the roots of various plants, and to increase the height, flower number, fruit number and total fruit

weight of tomato plants (Minorsky, 2008). PGPRs isolated from rice fields were shown to increase plant height, root length, and dry matter production of shoot and root of rice seedlings. The properties of PGPRs like growth enhancement, IAA production and phosphorus solubilization has extended the use of these bacteria as inoculant biofertilizers for many different host plants (Ashrafuzzaman *et al.*, 2009). With rice, *Azospirillum brasilense* (James *et al.*, 2000), *Herbaspirillum seropedicae* (Baldani *et al.*, 2000), *Burkholderia vietnamsensis* (Baldani *et al.*,

2000) and *Rhizobium leguminosarum* bv. *trifolii* (Yanni and El-Fattah, 1999) have all been proved to be effective in improving rice growth and showed an increase in rice yield in field studies. Plant growth promoting rhizobacteria exert their effect on plants in a variety of ways i.e. they can act as biofertilizers like *Rhizobium* and *Bradyrhizobium* (Van Rijn and Vanderlayden, 1995); *Azospirillum* (Okon et al., 1998) and phosphorus solubilizing bacteria (Vassilev et al., 2006). PGPR can also be used as rhizoremediators (Kuiper et al., 2001); phyto-stimulators (Lugtenberg and Kamilova, 2009) and stress controllers (Glick et al., 2007).

Various reports have been published on the growth promotion by the PGPR strains in a variety of crops. IAA production and P solubilization resulting in an increase in the root and shoot length, fresh weight, dry weight, nutrient uptake has been reported in lentil (Zafar et al., 2012) and cowpea (Minaxi et al., 2012). However, in case of rice increased root length, shoot length, aerial biomass and increased nutrient uptake has also been reported in response to the PGPR application (Salamone et al., 2012). These changes in the plant morphology and nutrient content result because root associated bacteria change the chemical and physical properties of the soil surrounding the roots of the host plants so as to facilitate the host plant to acquire better nourishment at the expense of minimum input. With these facts in mind an experiment was performed with the following objectives: 1) to evaluate the bacteria associated with rice roots for growth promoting characteristics in field trials conducted over two rainy seasons of 2010 and 2011; and 2) to study the growth promotion and micronutrient uptake by the rice plants to obtain consolidating proofs for the action of plant growth promoting rhizobacteria.

2. Materials and Methods

2.1. Bacteria isolates

Three standard PGPR strains viz. *Pseudomonas putida* MTCC 102, *Pseudomonas fluorescens* MTCC 103 and *Azospirillum lipoferum* MTCC 2694 were obtained

from Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh, India. Besides these, many bacteria were isolated from rice fields by enrichment culture technique. Soil was collected from the rhizospheric zone of rice plants from different locations in N E Borlaug Crop Research Centre, Pantnagar, from these soils different bacteria were isolated and purified to single species and assayed in vitro for Fe solubilisation on chrome azurol-S agar (CAS) media as described by Schwyn and Neilands (1987) and for zinc solubilization on Bunt and Rovira media with ZnO as insoluble zinc source (Bunt and Rovira, 1955). Of all the strains, five of the better Fe and Zn solubilising strains were selected and tested in the field for Fe and Zn solubilising activity *in situ* and they were proved to increase Fe/Zn uptake by the plants. Also the sequence analysis and alignment of the 16S rRNA gene of the bacterial isolates show that the isolates belong to the *Enterobacter* species.

2.2. Plant material and treatment

Seeds of three rice genotypes Jaya, PA6444, and Pusa basmati-1, were obtained from the Department of Genetics and Plant Breeding, College of Agriculture, G.B. Pant University of Agriculture and Technology, Pantnagar, India. The seeds were surface sterilized by 1% sodium hypochlorite solution for 2-3 min and 90% ethanol for 3-4 min, then washed with distilled water and sown for nursery raising. The bacterial isolates were grown as liquid cultures in Luria broth. After obtaining sufficient population density of around 10^6 to 10^7 cfu/ml, they were applied to plants; 21-day old seedlings were uprooted and dipped in the bacterial culture for sufficient time to allow the bacteria to colonize the roots, later analysis showed that roots had a population of almost 10^5 CFU/g of fresh roots. Then the seedlings were transplanted in the field in a strip plot design for two kharif seasons 2010 and 2011. The soil of the transplantation site belonged to Typic Haplo-doll. It had gentle to moderate slope and was fine, loamy and mixed hyperthermic type. The physico-chemical conditions of the soils include a pH of 7.4 having an electrical conductivity of 0.266 dS/m and organic

carbon content of 10.5 g/kg soil. Also the nitrogen content of the soil was 31.8 mg/kg and phosphorus content was 10.12 mg/kg. DTPA extractable Zn was 0.47 mg/kg, Fe was 25.3 mg/kg, Cu 1.24 mg/kg and Mn 5.97 mg/kg. Geographically, the site lies in the tarai plains about 30 km southwards of foothills of Shivalik range of Himalayas at 29°N latitude, 79°29'E longitude and at an altitude 243.8 meter above the sea level and experiences humid subtropical climate with hot dry summers and cool winters. Winter season extends from November to March. The monsoon sets during second or third week of June and continues till September end. Fertilizer doses of nitrogen (100 kg/ha), phosphorus (60 kg/ha), potassium (45 kg/ha) and irrigation were applied as per recommendations.

30 days after transplanting, 3 plants from each plot were selected and the observations like plant height, tiller number, leaf number, root dry matter, shoot dry matter, chlorophyll content, iron and zinc content in roots and shoots were recorded in 3 replications each. Plant height, tiller number and leaf number were measured in the field itself, whereas for root and shoot dry mass three plants from each plot were carefully uprooted. Chlorophyll content of the freshly harvested leaves was measured by the protocol given by Hiscox and Israelstam, 1979. 50 mg finely chopped leaves were dipped in 10 ml DMSO (Dimethyl Sulfoxide) and kept at 60 °C for 3 hours. Then OD was recorded at 663 and 645 nm with a UV-VIS spectrophotometer (UV-VIS Spectrophotometer 108; Systronics) and chlorophyll content was calculated by using following formulae:

$$chl\ a = \frac{[(12.7 \times OD_{663} - 2.63 \times OD_{645}) \times V]}{Wg \times 1000}$$

$$chl\ b = \frac{[(22.9 \times OD_{645} - 4.48 \times OD_{663}) \times V]}{Wg \times 1000}$$

Where,

Chlorophyll content = mg/g fresh weight. V = volume of DMSO used. Wt. = Weight of tissue used in gram

Thereafter, root and shoot portions were washed successively with tap water, 0.1 mol/l HCl and then distilled water to remove contamination. Then the samples were oven-dried and weighed separately and were also used for the estimation of Fe and Zn contents. 1 g dried samples from different plant parts were taken for Fe and Zn estimation by atomic absorption spectroscopy, according to the method described by Pirzadeh *et al.* (2010).

2.3. Statistical analysis

The data of the various parameters was analyzed in triplicates and subjected to ANOVA (Analysis of variance) in accordance with the experimental design (strip plot design) using SPSS-16 statistical package to quantify and evaluate the source of variation. The treatment means were compared at a significance level of 0.05 and the ranking of treatments denoted by alphabets. The treatments denoted by different letters in the each column of tables and figures represent significantly different values among the treatments.

3. Results

3.1. Vegetative plant growth

Various growth parameters like plant height, number of tillers, number of leaves, root dry matter and shoot dry matter were recorded to study the effect of bacterial application on the growth of inoculated rice plants in comparison to the uninoculated control plants. A significant increase in plant height of the rice plants treated with different bacterial isolates was observed in comparison to the untreated control plants (Table 1). Among different treatments, *P. Putida*, *P. fluorescens*, *A. lipoferum* and B 15 were effective in increasing the plant height in all the genotypes under study in comparison with uninoculated control. Plant height of the treated rice plants was higher for the treatments *P. fluorescens* with an increase of 25.2 % in Jaya and 25% in PA6444 and *A. lipoferum* in Pusa basmati-1 with an increase of

18.8% during 2010 in comparison to the control, while during 2011, treatments BN 30 in Jaya (9.8% increase), B 15 in PA6444 (18.8% increase) and *P. fluorescens* in Pusa basmati-1 (16.3% increase) were most effective in increasing the plant height. However, statistically *P. putida* proved to be most effective in influencing the plant height of the rice plants.

Besides plant height; tiller and leaf number are also important parameters in terms of the vegetative growth of the plants and were also found to be increased in response to bacterial treatments (Table 1). Maximum number of tillers was recorded for the treatments *P. fluorescens* in Jaya (48% higher than control), *A. lipoferum* in PA6444 (36.3% higher) and *P. fluorescens* in Pusa basmati-1 (29.4% higher) during 2010; while during 2011, treatment *P. fluorencens* was most effective in increasing the tiller number of treated rice plants with an increment of 24.4% in Jaya and PA6444 each and 29% in Pusa basmati-1 in comparison to the control. Similarly leaf number was also found higher for the treatments *P. fluorescens* in Jaya (55.4% increase), *P. putida* in PA6444 (42.7% increase) and BN 17 in Pusa basmati-1 (50.2% increase) during 2010 in comparison to the uninoculated control, and during 2011, treatments B 15 in Jaya (22.5% increase), BN 30 in PA6444 (27.6% increase) and *P. fluorescens* in Pusa basmati-1 (21.3% increase) proved most effective in increasing the leaf number of the treated plants in comparison with control.

Root and shoot dry matter was also recorded (Table 2) to study the effect of PGPR application on dry matter accumulation by the plants. Root dry matter was found to be higher for all the treatments in all the genotypes when compared with control over both the years. During 2010, the treatments B 15 in Jaya (41.2% increase), *P. fluorescens* and B 19 in PA6444 (each with 33.4% increase) and B 17 in Pusa basmati-1 (36.8% increase) proved to be most effective in increasing the root dry matter; whereas during 2011, maximum increase in the root dry matter was observed for the treatments B 19 in Jaya (49.7% increase) and *P. putida* in PA6444 and Pusa basmati-1 (44.3% and 31.1% increase respectively). Similarly, shoot dry matter was also found to be higher

for the treated rice plants when compared with the uninoculated control plants during both the years. During 2010, the treatments *P. putida* through B 19 showed a clear increase in the shoot dry mass in all the genotypes. The treatments B 15 in Jaya (33.2% increase), B 19 in PA6444 (28.9% increase) and B 17 in Pusa basmati-1 (36.8% increase) were found most effective in increasing the shoot dry mass of the rice plants in comparison with control, while during 2011, highest shoot dry mass was found for the treatments B 19 in Jaya (34.8% increase), B 17 in PA6444 (43% increase) and *P. fluorescens* in Pusa basmati-1 (32.8% increase) when compared with uninoculated control plants. Upon overall analysis of the data on vegetative growth of the rice plants, the treatments *P. putida*, *P. fluorescens*, B 17 and B 19 were found most effective in influencing the vegetative growth of the inoculated rice plants.

3.2. Chlorophyll content

Chlorophyll a (Figure 1) and chlorophyll b (Figure 2) content was also estimated to predict plant's health and photosynthetic capacity in response to the bacterial inoculation. Chlorophyll content was found higher for the treated plants in comparison to the uninoculated control plants. The treatment *A. lipoferum* showed maximum increase in the chlorophyll a as well as chlorophyll b content in all the three genotypes during both the years. In response to the bacterial treatments, an increase of 52.3% in Jaya, 58.8% in PA6444 and 57.4% in Pusa Basmati-1 was observed in chlorophyll a content and an increase of 58.2% in Jaya, 60% in PA6444 was recorded for chlorophyll b content in response to the treatment by *A. lipoferum*, however, Pusa basmati-1 showed an increase in chlorophyll b content in response to the treatment by bacterial isolate BN 30 showing an increase of 64% during 2010 while, during 2011 an increase of 44.4% in Jaya, 55% in PA6444 and 50.9% in Pusa basmati-1 in chlorophyll a content and 45.8% in Jaya, 51.9% in PA6444 and 51.2% in Pusa basmati-1 in chlorophyll b content in response to the treatment by *A. lipoferum* was recorded in the treated plants in comparison to the control.

Table 1. Effect of seedling treatment with different plant growth promoting rhizobacteria on plant height, tiller number, leaf number in three genotypes of rice 30 days after transplanting.

Genotypes	Treatments	Plant height (cm)		Tiller number		Leaf number	
		2010	2011	2010	2011	2010	2011
Jaya	<i>P. putida</i>	63.5±3.04abc	64.2±0.28 b-g	10.7±1.55b	11.8±0.76b-g	42.0±3.54ab	45.5±3.75ab
	<i>P. fluorescens</i>	64.2±6.71ab	60.5±4.77e-h	13.8±2.75a	12.2±1.25b-f	44.8±2.96a	38.8±1.89ab
	<i>A. lipoferum</i>	58.0±6.38a-e	64.2±3.78b-g	10.0±1.73b-e	11.5±0.50b-g	28.0±3.76cde	39.0±3.54ab
	B 15	58.3±7.34a-e	64.7±2.46b-g	10.5±1.04bc	10.8±0.28b-g	32.3±1.79a-d	47.3±7.28a
	B 17	52.8±2.51def	60.2±5.83e-h	10.3±1.04bcd	10.8±0.28b-g	26.3±0.76cde	40.3±2.84ab
	B 19	51.5±6.08ef	62.8±3.01c-h	9.0±0.50b-f	9.3±1.04efg	31.3±2.02a-e	38.0±5.22ab
	BN 17	51.3±6.11ef	60.7±2.02e-h	8.2±0.78b-f	10.2±0.36c-g	32.7±2.98a-d	38.2±4.16ab
	BN 30	50.8±0.76ef	64.8±2.93a-g	8.7±0.28b-f	11.2±0.52b-g	25.2±4.07cde	43.2±3.13ab
	Control	48.0±4.77f	58.5±1.51gh	7.2±0.57ef	9.2±0.57fg	20.0±1.50de	36.7±3.05ab
PA6444	<i>P. putida</i>	59.5±6.25a-e	65.8±2.08a-f	10.0±1.64b-e	12.8±0.76abc	33.2±4.60a-d	39.7±6.28ab
	<i>P. fluorescens</i>	67.3±6.35a	67.7±4.48a-d	10.0±1.51b-e	12.8±0.72abc	31.3±2.00a-e	44.0±3.50ab
	<i>A. lipoferum</i>	56.0±2.29b-f	61.3±1.89d-h	10.7±0.76b	12.7±0.76a-d	26.3±2.08cde	34.7±2.08ab
	B 15	59.3±7.34a-e	70.2±4.80ab	8.0±1.00b-f	9.0±0.80g	22.5±2.26cde	37.7±5.53ab
	B 17	52.7±4.02def	62.0±2.64d-h	9.5±1.32b-f	12.3±0.57b-e	25.3±2.12cde	44.8±4.30ab
	B 19	55.3±7.11b-f	59.2±5.83fgh	10.5±0.50bc	9.0±0.55g	26.4±1.63cde	42.5±6.53ab
	BN 17	52.3±3.68def	59.8±1.89fgh	7.0±0.50ef	10.8±0.28b-g	31.0±3.60b-e	43.5±3.07ab
	BN 30	51.5±1.70ef	61.7±4.53d-h	8.3±0.57b-f	9.3±0.60efg	23.2±3.64cde	45.3±2.21ab
	Control	50.5±3.69ef	57.0±4.77h	6.8±0.76f	9.7±0.52d-g	19.0±2.78de	32.8±3.01b
Pusa Basmati-1	<i>P. putida</i>	65.8±4.25a	68.8±5.00abc	10.0±1.80b-e	12.2±0.46b-f	34.3±5.10abc	38.7±3.75ab
	<i>P. fluorescens</i>	55.0±5.29b-f	71.3±2.51a	10.3±0.28bcd	15.5±0.86a	26.3±4.07cde	43.8±6.51ab
	<i>A. lipoferum</i>	66.8±5.08a	67.8±2.02a-d	9.2±1.52b-f	13.8±0.28ab	25.3±1.60cde	40.3±3.65ab
	B 15	61.3±2.36a-d	60.3±0.76e-h	8.2±0.57b-f	12.2±1.25b-f	22.8±1.60cde	43.7±4.51ab
	B 17	61.8±1.25a-d	64.7±0.76b-g	8.3±1.25b-f	11.3±1.01b-g	25.0±0.92cde	35.8±6.03ab
	B 19	60.2±4.61a-e	67.3±1.52a-d	7.5±0.50c-f	9.8±1.25c-g	25.6±5.18cde	36.8±4.07ab
	BN 17	58.7±4.75a-e	66.8±2.75a-e	6.8±1.52f	11.2±0.76b-g	35.6±2.60abc	37.2±2.46ab
	BN 30	56.2±2.45b-f	68.8±2.08abc	9.5±0.50b-f	9.7±0.60d-g	24.4±3.48cde	42.8±6.31ab
	Control	54.3±4.19c-f	59.7±4.16fgh	7.3±1.04def	11.0±0.79b-g	17.7±1.84e	34.5±3.46ab

Means followed by a common letter in the columns are not significantly different.

3.3. Iron and zinc content in plants

Iron and zinc content in roots and shoots of the treated rice plants was estimated to observe whether PGPR application had any effect on the micronutrient content of the plants. Root Fe content was found to be higher in all the treatments and in all the genotypes (Table 3a, b). During 2010, highest root Fe content was observed for the treatments *P. putida* in Jaya (36% higher) and PA6444 (33% higher) in comparison to the control and *P. fluorescens* in Pusa basmati-1 (33.5% higher) however, during 2011 the treatments B 17 in Jaya (27.6% increase), *P. putida* in (27.6% increase), *P. putida* in PA6444 (22.6% increase) and *P. fluorescens* in Pusa basmati-1 (33.3% increase) proved to be most effective in increasing the root Fe content in treated plants. Whereas, in case of shoots (Table 3a, b), the treatments B 19 in Jaya (54% increase), *P. fluorescens* in PA6444 (53.3% increase) and Pusa basmati-1

(37% increase) during 2010 and B 17 in Jaya (60.3% (37% increase) during 2010 and B 17 in Jaya (60.3% increase), *P. fluorescens* in PA6444 (49.5% increase) and *P. putida* in Pusa basmati-1 (48.9% increase) during 2011 proved to be most effective in increasing the shoot Fe content of the treated plants when compared with the control plants.

Measurement of the zinc content (Table 3a, b) revealed that the treatments *P. fluorescens* in Jaya (36.8% increase), *P. putida* in PA6444 (36.8% increase) and BN 30 in Pusa basmati-1 (26.5% increase) during 2010 and B 15 in Jaya (47.9% increase), BN 17 in PA6444 (74.4% increase) and *P. putida* in Pusa basmati-1 (63.6% increase) during 2011 were most effective in increasing the root Zn content of the treated rice plants in comparison to the uninoculated control plants. Whereas the treatment B 17 was most effective in increasing the shoot Zn content of the treated rice plants

during both the years. An increment of 45.1% in Jaya, during both the years. An increment of 45.1% in Jaya, 51.6% in PA6444 and 37% in Pusa basmati-1 during 2010 and 62.8% in Jaya, 60.9% in PA6444 and 62.2% in Pusa basmati-1 during 2011 was recorded in shoot Zn content in the rice plants treated with the isolate B 17 in comparison to the control.

3.4. Yield attributes

Harvesting of plants was done after grain maturity during both the years and the data on number of filled grains is presented in Figure 3. Data on grain number showed an enhancing effect of the bacterial treatments in increasing grain number. During 2010 maximum number of grains were reported for the treatments B 19 in Jaya (50.8% higher than the control), BN 30 in PA6444 (36.7% higher) and BN 17 in Pusa basmati-1 (43.5% higher). Whereas, during 2011 treatments B 19 in Jaya (40.3% increase), B 17 in PA6444 (39.1% increase) and *P. fluorescens* in Pusa basmati-1 (42.1% increase over the control) were most responsive in enhancing the filled grain number. Accordingly, grain yield showed an increase for the isolates BN 17 during 2010 and BN 30 during 2011 in Jaya (23.3% and 30.4% respectively), BN 30 during 2010 and B 17 and *P. putida* during 2011 in PA6444 (29.8% and 35.2% respectively) and B 15 during 2010 and *P. fluorescens* during 2011 in Pusa basmati – 1 (29.6% and 40.7% respectively) in comparison to the control (Figure 4).

4. Discussion

Vegetative growth phase is most important growth phase of any crop as it decides the amount of biomass generated and particularly in rice, it is important for the development of tillers. A vigorous vegetative crop growth represents a higher plant height, larger number of leaves and tillers and correspondingly higher root and shoot dry mass. In our investigation, plant height, leaf number, tiller number, root dry mass and shoot dry mass were all found to be higher in response to

the bacterial inoculation. Similarly a 20% increase in the plant height of 30 day old rice plants was observed in response to PGPR application (Ashraffuzzaman *et al.*, 2009). Besides plant height, increase in the number of tillers (Kumar *et al.*, 2012; Salamone *et al.*, 2012) and number of leaves (Ashraffuzzaman *et al.*, 2009) was also reported in the rice plants treated with the plant growth promoting rhizobacteria. Moreover, root and shoot dry matter was also found to be higher for the treated plants in comparison to the uninoculated control. The increase in the root dry mass directly correlates with the increased plant vigour, plant growth promotion and improvement in the micronutrient status of the plants. In similar experiments an increase in the root dry mass in response to treatment by *P. putida*, *P. fluorescence* and *A. lipoferum* was reported by Gholami *et al.* (2009) in maize; besides almost 30% higher root dry mass in response to bacterial isolates was reported by Souza *et al.* (2012) in rice; Rana *et al.* (2012), also reported higher root dry mass in wheat plants in response to PGPR treatment. Also, an increase in the shoot dry matter directly correlates to the plant's productivity. In our investigation, bacterial treatments increased plant vigour, number of leaves and tillers thereby signifying that the amount of photosynthates produced may have been higher in the treated plants in comparison to the uninoculated control plants, thereby, resulting in an increase in the above ground biomass of the rice plants. Increase in shoot dry mass has been reported in response to bacterial inoculation in maize (Gholami *et al.*, 2009) and rice (Salamone *et al.*, 2012).

Chlorophyll a content is an important parameter directly related to the amount of photosynthesis in the plants and hence in turn to the final production. In our investigation it was observed that the chlorophyll content was found higher in comparison to the untreated control plants in response to the bacterial treatment because inoculated bacteria improved the mineral nutrition, and in general, health of the plants thereby leading to an increase in the chlorophyll content. A linear relationship exists between

concentration, in this regard Arif *et al.* (2012) reported an increase of 43% in chlorophyll content in response to

Zn fertilization; further PGPR application increased the chlorophyll content by almost 50% (Bal *et al.*, 2012).

Table 2. Effect of seedling treatment with different plant growth promoting rhizobacteria on root and shoot dry matter in three genotypes of rice 30 days after transplanting.

Genotypes	Treatments	Root dry matter (g/plant)		Shoot dry matter (g/plant)	
		2010	2011	2010	2011
Jaya	<i>P. putida</i>	3.55±0.77abc	5.07±0.75a-d	8.88±1.83ab	12.23±1.25abc
	<i>P. fluorescens</i>	3.15±0.64abc	5.80±0.69abc	9.47±1.22ab	11.50±1.24abc
	<i>A. lipoferum</i>	3.26±0.33abc	5.27±0.34a-d	8.13±1.72ab	12.23±0.54abc
	B 15	4.47±0.39a	5.83±0.40abc	11.57±1.05a	13.33±0.80abc
	B 17	4.24±0.67ab	5.03±0.55a-d	10.30±0.65ab	13.67±0.71abc
	B 19	3.72±0.53abc	6.37±0.68ab	10.27±0.65ab	18.00±1.64a
	BN 17	3.40±0.29abc	3.80±0.43cd	10.03±0.68ab	11.80±1.61abc
	BN 30	3.02±0.55abc	4.73±0.72a-d	10.03±1.18ab	12.50±0.43abc
	Control	2.63±0.25bc	3.20±0.57d	7.73±0.55b	11.73±0.32abc
PA6444	<i>P. putida</i>	3.68±0.78abc	6.47±0.16a	9.30±1.18ab	14.57±0.97abc
	<i>P. fluorescens</i>	4.11±0.33abc	4.83±0.57a-d	10.27±1.41ab	13.37±0.82abc
	<i>A. lipoferum</i>	3.35±0.42abc	5.67±0.35abc	9.17±1.12ab	11.67±1.09abc
	B 15	3.08±0.64abc	4.37±0.35a-d	9.53±1.27ab	11.60±0.42abc
	B 17	3.89±0.65abc	5.73±0.32abc	9.77±1.12ab	17.20±0.83ab
	B 19	4.12±0.31abc	4.33±0.62a-d	11.07±0.58ab	11.93±0.75abc
	BN 17	3.29±0.92abc	5.60±0.27abc	9.97±0.67ab	15.20±1.75abc
	BN 30	3.71±0.15abc	4.07±0.46bcd	10.20±1.03ab	9.87±0.16c
	Control	2.74±0.16bc	3.60±0.50cd	7.87±0.83b	9.80±0.23c
Pusa Basmati-1	<i>P. putida</i>	3.96±0.71abc	5.27±1.41a-d	9.63±1.52ab	10.50±1.49bc
	<i>P. fluorescens</i>	2.84±0.12bc	4.37±0.86a-d	9.03±1.53ab	15.33±1.49abc
	<i>A. lipoferum</i>	4.36±0.75a	4.70±0.85a-d	10.10±1.22ab	13.63±1.45abc
	B 15	3.67±0.35abc	5.20±0.60a-d	9.37±1.20ab	13.30±1.75abc
	B 17	3.29±0.63abc	5.10±0.62a-d	11.87±0.52a	14.37±1.35abc
	B 19	4.16±0.32abc	4.00±0.58cd	8.67±1.24ab	10.67±1.05bc
	BN 17	3.74±0.70abc	4.87±0.42a-d	9.17±0.58ab	13.03±0.82abc
	BN 30	3.96±0.31abc	4.50±0.44a-d	9.87±1.07ab	14.07±0.36abc
	Control	2.61±0.17c	3.63±0.50cd	7.50±0.67b	10.30±0.15bc

Means followed by a common letter in the columns are not significantly different.

Iron and zinc content of roots is defined by the soil conditions, fertilization and activity of the roots and related micro organisms. We observed an increase in root iron and zinc content in response to the bacterial inoculation. The increased root uptake and accumulation of micronutrients was due to the increased root activity and also the solubilization of iron and zinc compounds present in the soil. In similar experiments Mishra *et al.*

(2011) has reported an increase in Fe and Zn content in lentil. In rice, Purakayastha and Chhonkar (2001) has shown similar results in response to mycorrhizal fungi. Besides increasing the Fe and Zn content in roots bacterial inoculation also improved the root activity thereby leading to an increase in the translocation of Fe and Zn from root to shoots, as a result shoot Fe and Zn content was also found to be increased.

In experiments based on similar approach Rana *et al.* (2012) reported an increase in Fe and Zn content of wheat shoots and Shabayev (2012) has reported an increase of around 30% in Fe and 41% in Zn in wheat in response to PGPR treatment; we have also reported an increase in the root and shoot Fe content in rice in response to PGPR treatment (Sharma *et al.*, 2013).

Grain number defines the amount of yield that can be obtained from any crop plant. Higher grain number results from increased translocation of photosynthates from the leaves to the developing spikelets. Hence, increase in grain number signifies an increased amount of photosynthesis which leads to disturbance of source-sink ratio shifting it towards sink, resulting in increased translocation of photosynthates from leaves to the grains thereby resulting in an increased number of grains per panicle and ultimately increased yield. However, this increase in grain yield might also be due to the high Zn solubilization potential of these isolates. All isolates viz. B 15 through BN 30 are strong Zn solubilizers, hence the assumption that increase in the Zn uptake by the plant results in a greater yield holds true. As was observed in our study, a large body of evidence shows that PGPR enhance the growth, seed emergence and crop yield, and contribute to the nutrient enrichment of plants (Dey *et al.*, 2004; Kloepper *et al.*, 2004; Herman *et al.*, 2008; Minorsky, 2008). Salamone *et al.* (2012) reported that PGPR inoculation reduced the chaffy grain percentage in rice plants; a 10% increase in grain number in wheat in response to PGPR treatment was reported by Rana *et al.* (2012); also higher number of grains in lentil plants were reported by Zafar *et al.* (2012) in response to bacterial inoculation; in another experiment increased grain number was reported in cowpea plants in response to PGPR treatment by Minaxi *et al.* (2012).

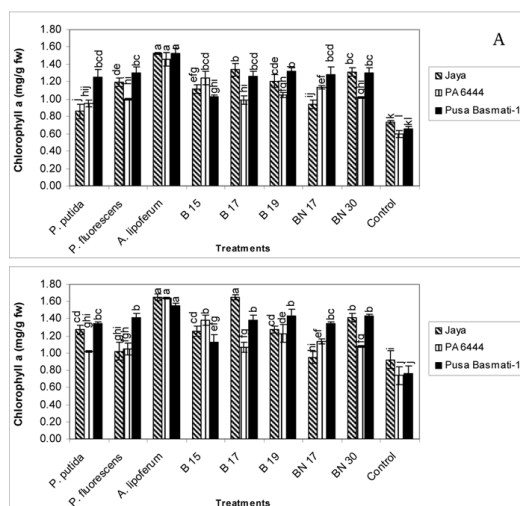


Figure 1. Chlorophyll a content (mg g⁻¹ FW) of three rice genotypes under different bacterial treatments 30 days after transplanting during the planting seasons 2010 (A) and 2011 (B). Means followed by a common letter in the columns are not significantly different.

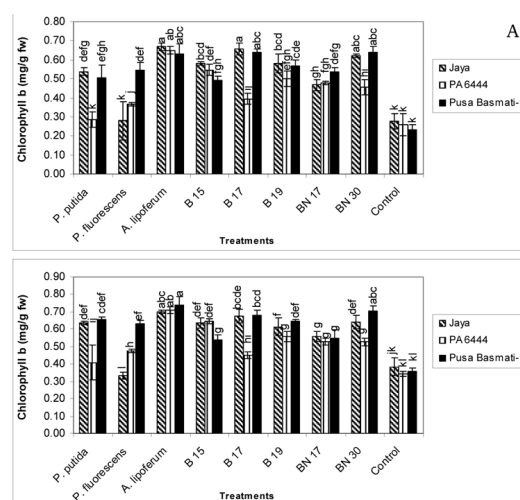


Figure 2. Chlorophyll b content (mg g⁻¹ FW) of three rice genotypes under different bacterial treatments 30 days after transplanting during the planting seasons 2010 (A) and 2011 (B). Means followed by a common letter in the columns are not significantly different.

Table 3a. Effect of seedling treatment with different plant growth promoting rhizobacteria on zinc and iron content in root and shoot portions of three genotypes of rice 30 days after transplanting.

Genotypes	Treatments	Root zinc content		Shoot zinc content	
		2010	2011	2010	2011
Jaya	<i>P. putida</i>	5.97±0.58c-f	14.70±1.50bcd	13.0±2.59e	19.3±1.72a-e
	<i>P. fluorescens</i>	8.10±1.05ab	12.98±1.81bcd	14.7±2.10de	19.7±2.11a-e
	<i>A. lipoferum</i>	7.75±0.87a-d	11.03±0.83bcd	15.5±1.99cde	17.2±0.57a-e
	B 15	7.23±0.32a-e	15.50±1.12bcd	18.4±2.55a-e	18.7±1.15a-e
	B 17	6.97±0.96a-f	9.78±1.79bcd	22.6±1.03a-e	28.5±143ab
	B 19	6.42±0.50b-f	10.30±0.57bcd	18.1±0.97a-e	21.1±2.42a-e
	BN 17	7.45±0.87a-e	9.57±0.72bcd	18.9±1.01a-e	21.6±3.44a-e
	BN 30	5.72±0.67ef	12.10±1.43bcd	20.4±1.22a-e	20.0±1.98a-e
	Control	5.12±0.47f	8.07±1.13bcd	12.4±0.97e	10.6±0.83de
PA6444	<i>P. putida</i>	8.67±0.83a	15.95±1.81bc	17.5±2.60a-e	22.4±0.38a-e
	<i>P. fluorescens</i>	7.13±1.05a-e	11.82±1.63bcd	12.6±0.99e	19.0±2.23a-e
	<i>A. lipoferum</i>	7.37±0.88a-e	10.38±0.61bcd	14.7±1.76de	19.0±1.51a-e
	B 15	6.32±0.94b-f	11.90±1.71bcd	13.9±2.22e	17.3±1.59a-e
	B 17	6.05±0.44c-f	11.37±0.84bcd	26.5±1.94ab	25.8±2.15a-e
	B 19	7.20±0.64a-e	9.32±1.32bcd	19.4±1.75a-e	17.8±1.75a-e
	BN 17	7.45±0.75a-e	26.67±0.61a	21.3±2.91a-e	11.6±0.48b-e
	BN 30	6.28±0.65b-f	8.28±1.15bcd	24.9±2.21a-d	11.8±1.65b-e
	Control	5.48±0.38ef	6.82±0.57cd	12.8±0.96e	10.1±0.95e
Pusa Basmati-1	<i>P. putida</i>	7.13±1.05a-e	17.02±1.54b	19.1±2.87a-e	28.3±1.43abc
	<i>P. fluorescens</i>	6.20±0.57b-f	12.98±1.75bcd	17.2±1.15a-e	16.6±1.07a-e
	<i>A. lipoferum</i>	7.07±0.78a-f	9.55±1.04bcd	17.8±1.09a-e	24.0±2.05a-e
	B 15	6.23±0.64b-f	14.15±1.40bcd	12.5±1.17e	14.4±1.10a-e
	B 17	6.38±0.07b-f	8.30±0.98bcd	26.7±1.91a	29.6±2.33a
	B 19	6.52±0.55b-f	14.13±0.54bcd	17.3±2.76a-e	27.4±2.15a-d
	BN 17	5.98±0.91c-f	10.15±0.66bcd	16.2±2.19b-e	13.7±1.13a-e
	BN 30	7.93±0.27abc	10.80±0.85bcd	25.4±1.96abc	12.0±0.97b-e
	Control	5.83±0.98def	6.20±0.72cd	16.8±1.09a-e	11.2±0.75cde

Means followed by a common letter in the columns are not significantly different.

Table 3b. Effect of seedling treatment with different plant growth promoting rhizobacteria on zinc and iron content in root and shoot portions of three genotypes of rice 30 days after transplanting.

Genotypes	Treatments	Root iron content		Shoot iron content	
		2010	2011	2010	2011
Jaya	<i>P. putida</i>	454.6±7.73a	448.6±10.9a	154.2±16.7def	201.5±30.8abc
	<i>P. fluorescens</i>	397.2±11.9a-f	444.2±16.3a	142.4±10.1e-h	183.7±22.6bcd
	<i>A. lipoferum</i>	409.2±29.6a-e	425.4±18.5ab	134.3±12.2e-i	172.9±13.1b-e
	B 15	357.8±27.5c-h	384.0±21.3abc	145.2±19.5d-h	107.4±16.1de
	B 17	366.8±27.1b-h	463.3±18.1a	203.9±17.9bc	245.4±13.5a
	B 19	304.1±26.1gh	386.9±18.3abc	222.2±9.77ab	234.1±18.3ab
	BN 17	303.0±15.8gh	419.5±21.6ab	123.0±16.6f-i	138.6±15.1b-e
	BN 30	356.3±21.2c-h	412.5±22.1ab	206.2±14.2bc	238.4±13.6ab
	Control	290.8±14.2h	335.3±15.8cd	102.2±3.50i	97.4±10.1e
PA6444	<i>P. putida</i>	455.1±7.46a	461.5±13.3a	199.1±28.4bc	200.1±24.1abc
	<i>P. fluorescens</i>	452.4±10.2ab	446.8±4.66a	245.2±24.9a	240.9±24.4a
	<i>A. lipoferum</i>	380.1±21.5a-g	449.8±15.1a	146.6±9.95d-h	182.3±15.2b-e
	B 15	384.3±12.3a-g	382.7±17.8abc	158.1±15.9def	145.1±18.6b-e
	B 17	343.2±30.6d-h	454.4±12.6a	140.0±10.5e-h	169.9±14.6b-e
	B 19	287.9±33.0h	401.4±26.4abc	133.1±11.3e-i	148.6±13.5b-e
	BN 17	287.7±23.5h	411.5±18.3abc	147.3±19.6d-g	149.9±13.9b-e
	BN 30	314.8±26.8fgh	431.2±12.2ab	133.6±8.49e-i	157.0±19.4b-e
	Control	304.9±22.4gh	357.2±21.4bcd	114.5±9.86ghi	121.6±11.5cde
Pusa Basmati-1	<i>P. putida</i>	421.9±23.9a-d	434.9±16.0ab	166.0±13.6de	209.6±21.5abc
	<i>P. fluorescens</i>	435.9±10.3abc	454.0±9.07a	179.5±29.3cd	158.9±24.2b-e
	<i>A. lipoferum</i>	327.8±30.4e-h	400.0±11.8abc	139.0±10.2e-h	125.7±14.6cde
	B 15	399.0±13.8a-f	396.1±18.9abc	155.0±13.4def	130.8±22.0cde
	B 17	311.9±24.3fgh	435.8±11.2ab	135.3±10.2e-i	106.8±20.8de
	B 19	357.6±29.8c-h	451.0±13.5a	154.4±14.2def	145.9±12.2b-e
	BN 17	303.6±21.5gh	427.3±21.5ab	111.3±11.4hi	134.2±18.2cde
	BN 30	305.0±28.1gh	422.4±25.8ab	127.8±7.38f-i	135.7±16.5b-e
	Control	289.9±12.8h	302.8±23.1d	113.0±10.5ghi	107.2±9.51de

Means followed by a common letter in the columns are not significantly different.

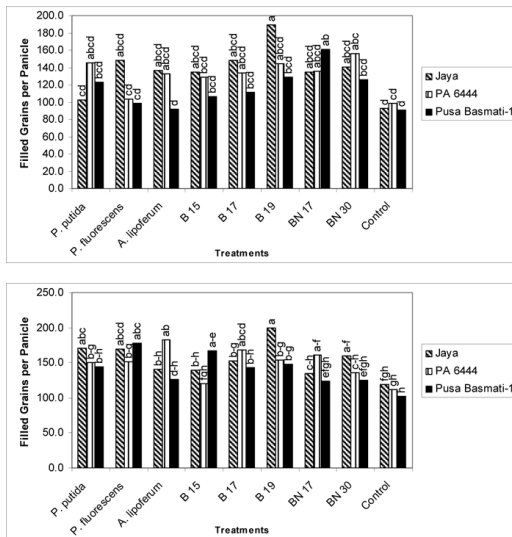


Figure 3. Filled grains per panicle of three rice genotypes under different bacterial treatments at harvesting during the planting seasons 2010 (A) and 2011 (B). Means followed by a common letter in the columns are not significantly different.

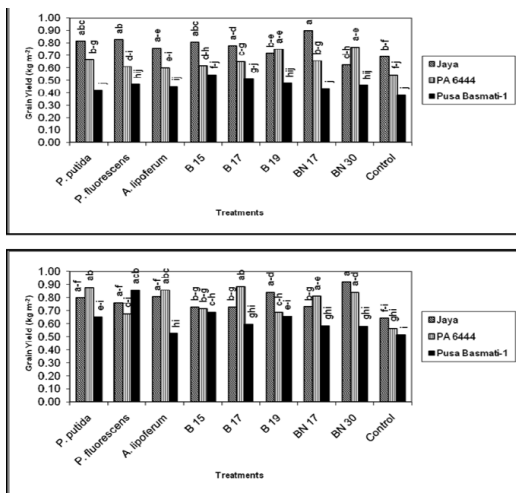


Figure 4. Grain Yield of three rice genotypes under different bacterial treatments at harvesting during the planting seasons 2010 (A) and 2011 (B). Means followed by a common letter in the columns are not significantly different.

5. Conclusion

The application of plant growth promoting rhizobacteria resulted in improving the vegetative growth of the rice plants and also helped in increasing the dry matter accumulation of the plants. Besides, improving growth of the plants, inoculation with PGPR also resulted in improving the zinc and iron status of the rice genotypes and an overall increase in the yield of the rice genotypes was observed. Hence it can be concluded that the application of PGPRs in rice not only improve the health of the plant but also increase the vegetative growth, photosynthetic capacity and micronutrient status of the crop. Therefore, judicious application of plant growth promoting rhizobacteria to rice holds immense potential that is still to be explored and it can also provide an answer to the global demand for the development of a sustainable strategy for biofortification.

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