Effect of nitrogen-fixing plant growth-promoting bacteria on germination, seedling vigour and growth enhancement of rice cultivars

Short running title:

Effect of nitrogen-fixing bacteria on germination indices and growth promotion of rice

Abstract

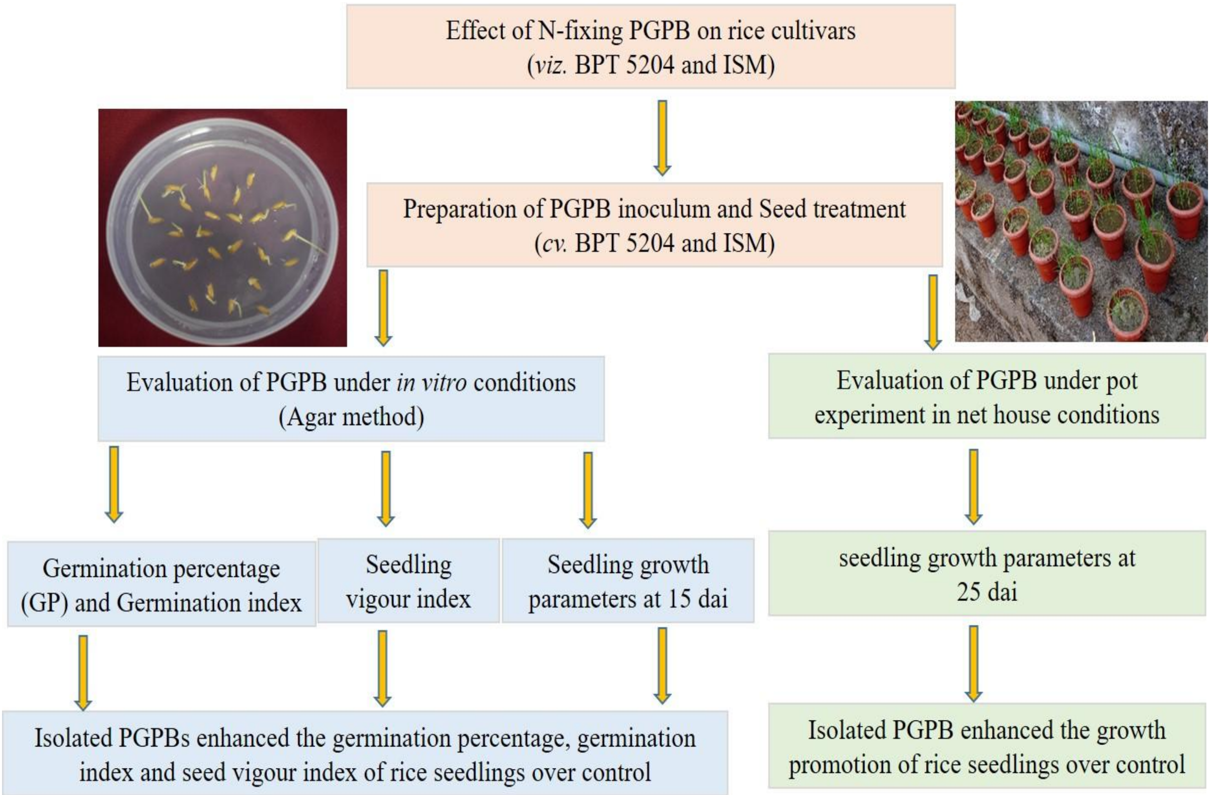
Aim: To evaluate the effect of isolated nitrogen fixing plant growth-promoting bacteria (PGPB) on seed germination and growth promotion of rice cultivars (cv. BPT 5204 and Improved Samba Mahsuri).

Methodology: Eight promising N-fixing PGPB along with two standard cultures (*viz. B. japonicum* and *G. diazotrophicus*) were inoculated as seed treatment to rice genotypes and the effect on seed germination, seed vigour index and plant growth promotion of rice cultivars was assessed under *in vitro* (agar method) and *in vivo* (pot experiment) net house conditions.

Results: PGPB (*viz., Paenibacillus sonchi IIRRBNF1, Paenibacillus sp. IIRRNF2, Ochrobactrum sp. IIRRNF3, Burkholderia cepacia IIRRNF4, Burkholderia sp. IIRRNF5, Stenotrophomonas sp. IIRRNF6, Rhizobium sp. IIRRNF7, Xanthomonas sacchari IIRRNF8)* were enhanced seed germination, seed vigour index, seedling growth and dry matter accumulation (root and shoot dry matter) of rice cultivars under *in vitro* as well as *in vivo* conditions. Among all PGPB, *Paenibacillus sonchi IIRRBNF1* exhibited the highest ability to stimulate plant growth promotion under both the conditions.

Interpretation: The eight PGPB isolates exhibited positive influence on seed germination indices as well as growth promotion traits of rice cultivars at seedling stage and can be further evaluated at different growth stages under pot and field experiment.

Graphical Abtract



Keywords: Growth promotion, Nitrogen fixation, Plant Growth Promoting Bacteria (PGPB), Rice, Seed germination.

Introduction

Nitrogen fixing plant growth-promoting bacteria (PGPB) provide a wide range of benefits to the plants and also act as a potential source of nitrogen for sustainable crop production as well as maintaining soil fertility (Rogers and Oldroyd, 2014; Singh *et al.*, 2017). Nitrogen-fixing PGPB transform inert atmospheric nitrogen ($N_{2)}$ to ammonia (Bakulin *et al.*, 2007) and are grouped into free-living bacteria (*Azotobacter* and *Azospirillium*) and symbionts such as *Rhizobium*, *Frankia* and *Azolla* (Gupta, 2004). Along with nitrogen-fixation, many soil micro-organisms have been reported to promote plant growth, suppress pathogens and improve abiotic stress tolerance (Paungfoo-Lonhienne *et al.*, 2014).

Diazotrophic free-living bacteria are known to contribute up to 20 kilograms per hectare per year in cereals, and in cereals rotation cropping system about 30-50% of the total nitrogen needs (Vadakattu and Paterson, 2006). Several groups of soil and root-associated nitrogen-fixing microorganisms such as *Azotobacter vinelandii* (Sahoo *et al.*, 2014), *Azospirillum brasilense*, *Azospirillum zeae* and *Pseudomonas stutzeri* (Venieraki *et al.*, 2011), *Acetobacter diazotrophicus* have been known to fix the nitrogen in different crops and stimulate plant growth (Boddey *et al.*, 1995).

Rice (*Oryza sativa* L.) is one of the most important staple foods for more than half of the world's population (Hegde and Hegde, 2013). India holds first position in area under rice cultivation (44.2 M ha) and second position in rice production after China (140.8 million tonnes) in the world. In India, rice production has increased by five-fold from 20.51 million tonnes during 1950 -1951 to more than 108.86 million tonnes in 2016-

17. Nitrogen (N) is one of the main limiting nutrients for crop productivity, including rice (Ladha and Reddy, 2003) and only one-third of the N applied as chemical fertilizer is used by rice plants (Araujo *et al.*, 2013).

The aim of present study was to evaluate the effect of nitrogen fixing PGPBs on seed germination, germination index, seedling vigour index and plant growth promoting activity of rice cultivars under *in vitro* and *in vivo* conditions.

Materials and methods

Bacterial isolates and Plant material

Eight promising PGPB *viz., Paenibacillus sonchi IIRRBNF1, Paenibacillus sp. IIRRNF2, Ochrobactrum sp. IIRRNF3, Burkholderia cepacia IIRRNF4, Burkholderia sp. IIRRNF5, Stenotrophomonas sp. IIRRNF6, Rhizobium sp. IIRRNF7, Xanthomonas sacchari IIRRNF8* isolates (Bandeppa *et al*., 2019) along with two standard cultures (*viz. B. japonicum* and *G. diazotrophicus*) were used as seed treatments to examine the effect of their inoculation on seed germination, seedling vigour index and plant growth promotion on two rice cultivars (BPT 5204 and Improved Samba Mahsuri i.e. ISM).

Seed treatment

Rice seeds (*cv.* BPT 5204 and ISM) were surfaced sterilized with 70% ethanol for 1 min followed by 0.2% $HgCl_2$ solution for 2 min and rinsed three times with sterile distilled water. The actively growing bacterial cultures on N-free Rennie's broth were pelleted, washed and suspended in phosphate-buffered saline (PBS) buffer to obatain a final cell concentration of 1×10^8 cells/ ml. The seeds were soaked overnight in the PBS buffer containing bacterial inoculum. The seeds soaked in the PBS buffer without culture was served as uninoculated control.

***In vitro* seed germination**

Seeds soaked in bacterial inoculum were placed in petri plates containing water agar (0.8 %, w/v) and incubated at 28 ± 2°C. Plates were assessed for seed germination (3rd day), germination index i.e., speed of germination (from 0 days to 3rd day), seedling vigour index and seedling growth parameters (15 dai, days after inoculation). The number of germinated seeds was counted every day for up to 3 days and the sum of daily counts was referred to as the final germination percentage (Pieper, 1952). The speed of germination was calculated by counting the number of germinated seeds every day of the experiment according to Gupta (1993): Speed of germination = Number of seeds germinated each day/ Total number of days. Seedling vigour index was calculated using the formula (Abdul-Baki and Anderson, 1973): Percent germination × Seedling height (i.e. shoot length + root length). Three replication per treatment were maintained and the experiment was repeated twice.

The seedling growth parameters *viz.*, root length (cm), shoot length (cm), seedling height (cm), root fresh weight (gm), shoot fresh weight (gm), seedling fresh weight (gm), root dry weight (gm), shoot dry weight (gm) and seedling dry weight (gm) were recorded at 15 dai in three replications and the experiment repeated twice.

Pot experiment in net house (*In vivo* condition)

The inoculated seeds with bacterial cultures were sown in small plastic pots (15 seeds/pot) for germination. Seedlings were thinned (5 seedlings/ pot) and maintained under flooded condition. The plants grown in the pots were harvested and washed thoroughly in running water without disturbing roots and growth parameters recorded at 25 dai in three replications and the experiment was repeated twice.

Statistical analysis

All recorded data were analysed by using a statistical package (Statistix 8.1 v2.0.1) by performing Analysis of Variance (ANOVA) and differences between the treatment means were compared by least significant differences (LSD) test at 5 % probability level ($p \leq 0.05$).

Results and Discussion

***In vitro* seed germination in response to PGPB**

Significantly higher germination percentage was recorded due to seed treatment with bacteria. The germination percent ranged from 100% to 92% for BPT 5204 and 100% to 92% for ISM when compared to untreated control (80% and 72% respectively). Among the bacterial cultures, *Paenibacillus sonchi IIRRBNF1* inoculation resulted in the highest germination percentage over control in both the cultivars (Table 1). Germination index was significantly higher in treated seeds of BPT 5204 (20 to 10.7) and ISM (16.3 to 12.2) over control (9.8 and 9.5 respectively) (Table 1). Seed treatment with *Paenibacillus sonchi IIRRBNF1, Paenibacillus sp. IIRRNF2* and *G. diazotrophicus* lead to a higher germination index in BPT 5204 cultivar. Whereas, *Stenotrophomonas sp. IIRRNF6* and *Paenibacillus sonchi IIRRBNF1* were showed the highest germination index in ISM cultivar. Seed vigour index was also significantly enhanced in treated seeds of BPT 5204 (1671 to 1071.5) and ISM (1590 to 1090) over control (BPT 5204, 305.50 and ISM, 331.5). Seeds (cv. BPT5204) inoculated with *Paenibacillus sonchi IIRRBNF1* was exhibited higher seed vigour index between the treatments (Figure 1). Whereas, ISM seeds treated with *Paenibacillus sonchi IIRRBNF1* and *Rhizobium sp. IIRRNF7* exhibited higher seed vigour index. Overall, all PGPBs treated seeds were enhanced the seed germination rate, vigour index and germination index compared to control in both the cultivars.

Table 1. Effect of PGPBs on the seed germination rate and germination index of rice cultivars (cv. BPT 5204 and ISM)

Treatment	BPT 5204		ISM	
	Germination (%)	Germination index (seeds/day)	Germination (%)	Germination index (seeds/day)
Uninoculated (Control)	80^{b}	9.8^{e}	72^{c}	9.5^{e}
Paenibacillus sonchi IIRRBNF1	100^{a}	17.0^{b}	100^{a}	16.2^{a}
Paenibacillus sp. IIRRNF2	98^{a}	16.7^{b}	100^{a}	15.1^{ab}
Ochrobactrum sp. IIRRNF3	100^{a}	16.0^{bc}	96^{ab}	14.8^{ab}
Burkholderia cepacia IIRRNF4	96^{a}	14.7^{cd}	92^{b}	11.6^{d}
Burkholderia sp. IIRRNF5	98^{a}	14.6^{cd}	98^{ab}	12.2^{d}
Stenotrophomonas sp. IIRRNF6	94^{a}	15.5^{bcd}	100^{a}	16.3^{a}
Rhizobium sp. IIRRNF7	94^{a}	13.7^{d}	100^{a}	12.2^{d}
Xanthomonas sacchari IIRRNF8	92^{a}	10.7^{e}	96^{ab}	12.5^{cd}
B. japonicum	98^{a}	16.5^{bc}	100^{a}	14.5^{abc}
G. diazotrophicus	100^{a}	20.0^{a}	98^{ab}	13.2^{bcd}
LSD ($P \leq 0.05$)	9.4	1.9	6.2	2.1
CV (%)	4.5	5.6	3.0	7.0

The mean values followed by different letters indicate significant differences (LSD, $P \leq 0.05$)

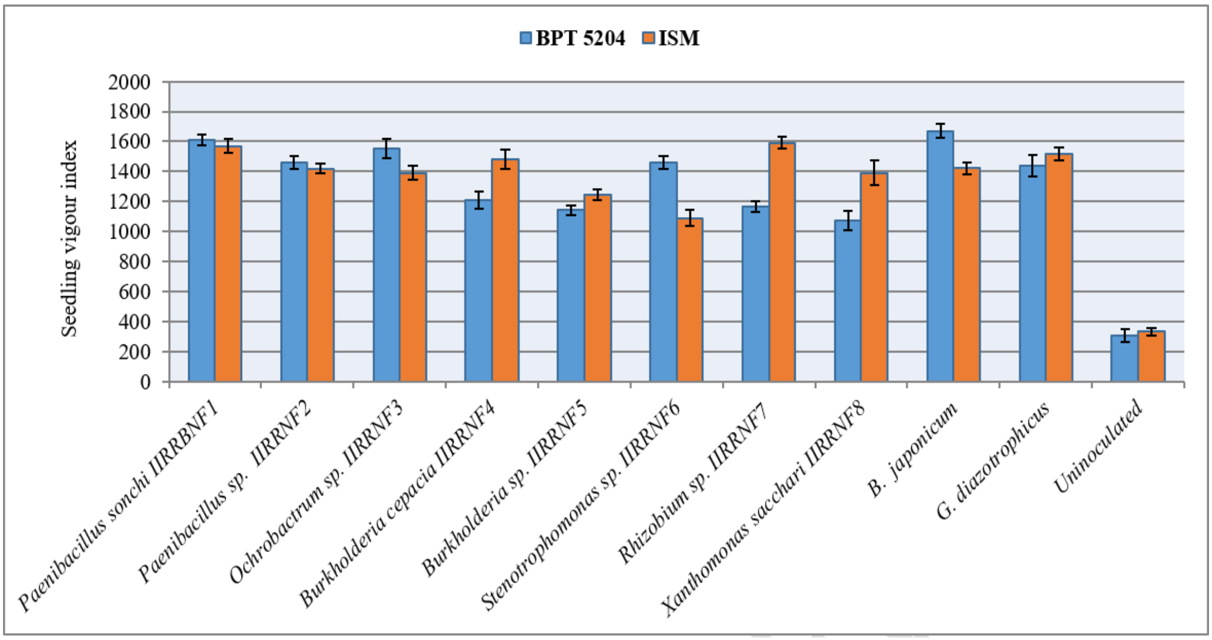


Figure 1. Effect of PGPBs on the seedling vigour index of rice cultivars (BPT 5204 and ISM). The error bar on the top indicates the standard deviation of biological replicates.

The results of germination percentage, germination index and vigour index obtained in the present investigation agree with an earlier report on rice, maize and soybean treated with PGPB. Bal *et al.* (2013) successfully demonstrated that *Paenibacillus sp.* culture enhanced the seed germination of rice (cv. Naveen) over control. It has been reported that germination percentage and seedling vigour index of rice seeds (*cv.* IR42) was significantly enhanced in response to *Paenibacillus sp. ANR-ACC3* over control (Bal and Adhya, 2021). Whereas in other crops, *Paenibacillus sp. s37* isolate increased the seed germination of Christmas tree species *Abies nordmanniana* (Garcia-Lemos *et al.*, 2020). Our findings of *Ochrobactrum sp.* are in agreement with Singh *et al.* (2018), who demonstrated that *Ochrobactrum intermedium AcRz3* treated seeds of black rice had enhanced seed germination over control. Vidhyasri *et al.* (2019) reported that improvement in the germination percentage as well as vigour index of rice seedlings in response to *Ochrobactrum sp.* (*MH685438*).

Similar to this study, Gholamalizadeh *et al.* (2014) also reported that *Stenotrophomonas maltophilia* inoculated rice (*cv.* Hashemi) exhibited improved the seed germination and higher vigour index compared to un-inoculated control. Similarly, Nevita *et al.* (2018) demonstrated that rice seeds (*cv.* Boro) had significantly enhanced germination percentage and vigour indices in response to *Stenotrophomonas maltophilia* RSD6. Maize, a non-legume crop was displayed better germination and seedling vigour in response to *Bradyrhizobium japonicum* treatment. (Cassan *et al.*, 2009).

In vitro (agar method) growth promotion of rice cultivars in response to PGPB

In the present study, inoculation with *Paenibacillus sonchi IIRRBNF1 and B. japonicum* resulted in higher seedling height, seedling fresh weight and seedling dry weight in BPT 5204 cultivar at 15 dai (Table 2). Whereas in ISM cultivar, higher seedling height, seedling fresh weight and seedling dry weight at 15 dai better were observed in treatments with *Paenibacillus sonchi IIRRBNF1*, *Rhizobium sp.* and *G. diazotrophicus* (Table 3).

Table 2. Effect of plant growth-promoting bacteria on the rice cultivar, BPT 5204 (Samba Mahsuri)

Treatment	Root length (cm)	Shoot length (cm)	Seedling height (cm)	Root fresh weight (g)	Shoot fresh weight (g)	Seedling fresh weight (g)	Root dry weight (g)	Shoot dry weight (g)	Seedling dry weight (g)
Paenibacillus sonchi IIRRBNF1	10.4^{ab}	5.7^{ab}	16.10^{ab}	0.017^{a}	0.017^{bcd}	0.035^{a}	0.0020^{ab}	0.0032^{a}	0.0052^{a}
Paenibacillus sp. IIRRNF2	9.1^{cd}	5.8^{a}	14.88^{bc}	0.017^{a}	0.019^{abc}	0.036^{a}	0.0016^{a}	0.0027^{ab}	0.0042^{abc}
Ochrobactrum sp. IIRRNF3	10.3^{bc}	5.3^{bcd}	15.53^{bc}	0.016^{ab}	0.018^{abc}	0.034^{ab}	0.0015^{ab}	0.0023^{bc}	0.0038^{bc}
Stenotrophomonas sp. IIRRNF6	10.3^{bc}	5.3^{abcd}	15.53^{bc}	0.016^{ab}	0.017^{bcd}	0.033^{abc}	0.0013^{b}	0.0025^{abc}	0.0038^{bc}
Burkholderia cepacia IIRRNF4	7.6^{ef}	5.0^{cd}	12.59^{d}	0.012^{c}	0.015^{bcd}	0.026^{c}	0.0012^{b}	0.0026^{abc}	0.0038^{bc}
Burkholderia sp. IIRRNF5	6.8^{f}	4.9^{d}	11.65^{d}	0.012^{bc}	0.014^{cd}	0.026^{c}	0.0016^{ab}	0.0019^{c}	0.0034^{c}
Rhizobium sp. IIRRNF7	7.1^{f}	5.4^{abcd}	12.40^{d}	0.011^{c}	0.018^{abc}	0.030^{abc}	0.0012^{b}	0.0027^{ab}	0.0038^{bc}
Xanthomonas sacchari IIRRNF8	6.4^{f}	5.3^{abcd}	11.65^{d}	0.009^{cd}	0.017^{abcd}	0.027^{bc}	0.0015^{b}	0.0031^{a}	0.0046^{ab}
B. japonicum	11.6^{a}	5.5^{abc}	17.05^{a}	0.012^{c}	0.019^{ab}	0.031^{abc}	0.0012^{b}	0.0027^{ab}	0.0039^{bc}
G. diazotrophicus	8.8^{de}	5.6^{ab}	14.36^{c}	0.009^{cd}	0.023^{a}	0.032^{abc}	0.0015^{b}	0.0028^{ab}	0.0043^{abc}
Uninoculated (Control)	0.2^{g}	3.6^{e}	3.82^{e}	0.006^{d}	0.012^{d}	0.018^{d}	0.0004^{c}	0.0018^{c}	0.0022^{d}
LSD ($P \leq 0.05$)	1.3	0.5	1.39	0.004	0.006	0.007	0.0005	0.0008	0.0011
CV (%)	10.9	7.2	7.3	22.2	22.7	17.5	26.6	21.1	19.2

The mean values followed by different letters indicate significant differences (LSD, $P \leq 0.05$)

Table 3. Effect of plant growth-promoting bacteria on the rice cultivar, Improved Samba Mahsuri

Treatment	Root length (cm)	Shoot length (cm)	Seedling height (cm)	Root fresh weight (g)	Shoot fresh weight (g)	Seedling fresh weight (g)	Root dry weight (g)	Shoot dry weight (g)	Seedling dry weight (g)
Paenibacillus sonchi IIRRBNF1	10.0^{ab}	5.7^{bc}	15.7^{a}	0.010^{bc}	0.014^{bc}	0.027^{cde}	0.0014^{abcd}	0.0021^{abc}	0.0036^{bc}
Paenibacillus sp. IIRRNF2	8.9^{abc}	5.2^{bcde}	14.2^{ab}	0.015^{abc}	0.013^{cd}	0.024^{de}	0.0015^{abcd}	0.0024^{ab}	0.0039^{ab}
Ochrobactrum sp. IIRRNF3	8.8^{abc}	5.7^{bc}	14.5^{ab}	0.020^{a}	0.017^{a}	0.037^{a}	0.0013^{bcd}	0.0020^{abc}	0.0034^{bcd}
Stenotrophomonas sp. IIRRNF6	5.8^{d}	5.1^{cde}	10.9^{c}	0.013^{abc}	0.012^{d}	0.025^{de}	0.0016^{abc}	0.0019^{bc}	0.0035^{bc}
Burkholderia cepacia IIRRNF4	8.0^{c}	8.0^{a}	16.1^{a}	0.018^{ab}	0.017^{a}	0.034^{ab}	0.0011^{d}	0.0020^{bc}	0.0031^{cd}
Burkholderia sp. IIRRNF5	7.9^{c}	4.8^{de}	12.7^{bc}	0.010^{bc}	0.013^{cd}	0.023^{de}	0.0014^{abcd}	0.0016^{c}	0.0031^{cd}
Rhizobium sp. IIRRNF7	10.1^{a}	5.7^{bc}	15.9^{a}	0.020^{a}	0.016^{a}	0.034^{ab}	0.0018^{a}	0.0026^{a}	0.0044^{a}
Xanthomonas sacchari IIRRNF8	8.4^{bc}	6.0^{b}	14.5^{ab}	0.015^{abc}	0.018^{a}	0.032^{abc}	0.0017^{ab}	0.0021^{abc}	0.0038^{abc}
B. japonicum	8.9^{abc}	5.5^{bcd}	14.5^{ab}	0.013^{abc}	0.016^{ab}	0.029^{bcd}	0.0012^{cd}	0.0021^{abc}	0.0033^{bcd}

G. diazotrophicus	9.7^{ab}	5.8^{bc}	15.5^{a}	0.015^{abc}	0.017^{a}	0.032^{abc}	0.0015^{abc}	0.0021^{abc}	0.0036^{bc}
Uninoculated (Control)	0.2^{e}	4.4^{e}	4.6^{d}	0.008^{c}	0.014^{cd}	0.022^{e}	0.0004^{e}	0.0022^{ab}	0.0026^{d}
LSD ($P \leq 0.05$)	1.6	0.8	2.2	0.008	0.002	0.007	0.0004	0.0006	0.0008
CV (%)	14.4	10.4	11.5	38.6	9.0	16.8	20.1	19.5	15.4

The mean values followed by different letters indicate significant differences (LSD, $P \leq 0.05$)

Overall, under *in vitro* conditions, seedling growth parameters *viz.* root length, shoot length, seedling height, root fresh weight, shoot fresh weight, seedling fresh weight, root dry weight, shoot dry weight and seedling dry weight were improved in response to PGPB over control in both cultivars.

In *vivo* growth promotion of the rice cultivars in response to PGPBs

The bacterial inoculants *viz. Paenibacillus sonchi IIRRBNF1, Paenibacillus sp. IIRRNF2, Ochrobactrum sp. IIRRNF3, Stenotrophomonas sp. IIRRNF6, Rhizobium sp. IIRRNF7, Xanthomonas sacchari IIRRNF8*, *B. japonicum* and *G. diazotrophicus* significantly and effectively enhanced the root length, shoot length, seedling height, root fresh weight, shoot fresh weight and seedling fresh weight in BPT 5204 cultivar over the control at 25 dai in pot experiment (Table 4; Figure 2).

Table 4. Effect of plant growth-promoting bacteria on rice cultivar, BPT 5204 under net house condition

Treatment	**Root length (cm)**	**Shoot length (cm)**	**Seedling height (cm)**	**Root fresh weight (g)**	**Shoot fresh weight (g)**	**Seedling fresh weight (g)**	**Root dry weight (g)**	**Shoot dry weight (g)**	**Seedling dry weight (g)**
Uninoculated (Control)	5.7^{d}	23.1^{cd}	28.77^{c}	0.034^{d}	0.076^{d}	0.110^{c}	0.008^{bcd}	0.022^{d}	0.030^{d}
Paenibacillus sonchi IIRRBNF1	9.2^{bcd}	20.4^{de}	29.67^{bc}	0.064^{abcd}	0.109^{d}	0.173^{bc}	0.008^{bcd}	0.031^{cd}	0.040^{cd}
Paenibacillus sp. IIRRNF2	9.2^{bcd}	27.6^{ab}	36.75^{a}	0.089^{ab}	0.183^{bcd}	0.272^{ab}	0.009^{bcd}	0.052^{ab}	0.062^{ab}
Ochrobactrum sp. IIRRNF3	13.7^{a}	28.7^{a}	42.33^{a}	0.085^{abc}	0.207^{a}	0.292^{a}	0.010^{bc}	0.063^{a}	0.073^{a}
Burkholderia cepacia IIRRNF4	7.6^{cd}	18.0^{ef}	25.60^{c}	0.048^{cd}	0.087^{d}	0.135^{c}	0.006^{d}	0.030^{cd}	0.036^{cd}
Burkholderia sp. IIRRNF5	10.6^{abc}	29.3^{a}	39.83^{a}	0.089^{ab}	0.206^{a}	0.294^{a}	0.007^{bcd}	0.060^{a}	0.067^{ab}
Stenotrophomonas sp. IIRRNF6.	12.1^{ab}	24.4^{bcd}	36.50^{ab}	0.051^{bcd}	0.159^{abc}	0.211^{abc}	0.008^{bcd}	0.044^{bc}	0.052^{bc}
Rhizobium sp. IIRRNF7	12.8^{ab}	15.2^{f}	28.03^{c}	0.040^{d}	0.083^{d}	0.123^{c}	0.006^{d}	0.026^{d}	0.031^{d}
Xanthomonas sacchari IIRRNF8	9.2^{bcd}	18.8^{ef}	28.03^{c}	0.038^{d}	0.092^{cd}	0.130^{c}	0.006^{cd}	0.030^{cd}	0.036^{cd}
B. japonicum	13.6^{a}	27.6^{ab}	41.13^{a}	0.085^{abc}	0.209^{a}	0.294^{a}	0.011^{b}	0.055^{ab}	0.066^{ab}
G. diazotrophicus	12.2^{ab}	25.4^{abc}	37.60^{a}	0.094^{a}	0.177^{ab}	0.271^{ab}	0.016^{a}	0.049^{ab}	0.065^{ab}
LSD ($P \leq 0.05$)	3.9	4.2	7.035	0.038	0.071	0.103	0.004	0.015	0.016
CV (%)	21.96	10.45	12.21	34.41	29.19	29.00	28.16	20.76	18.92

The mean values followed by different letters indicate significant differences (LSD, $P \leq 0.05$)

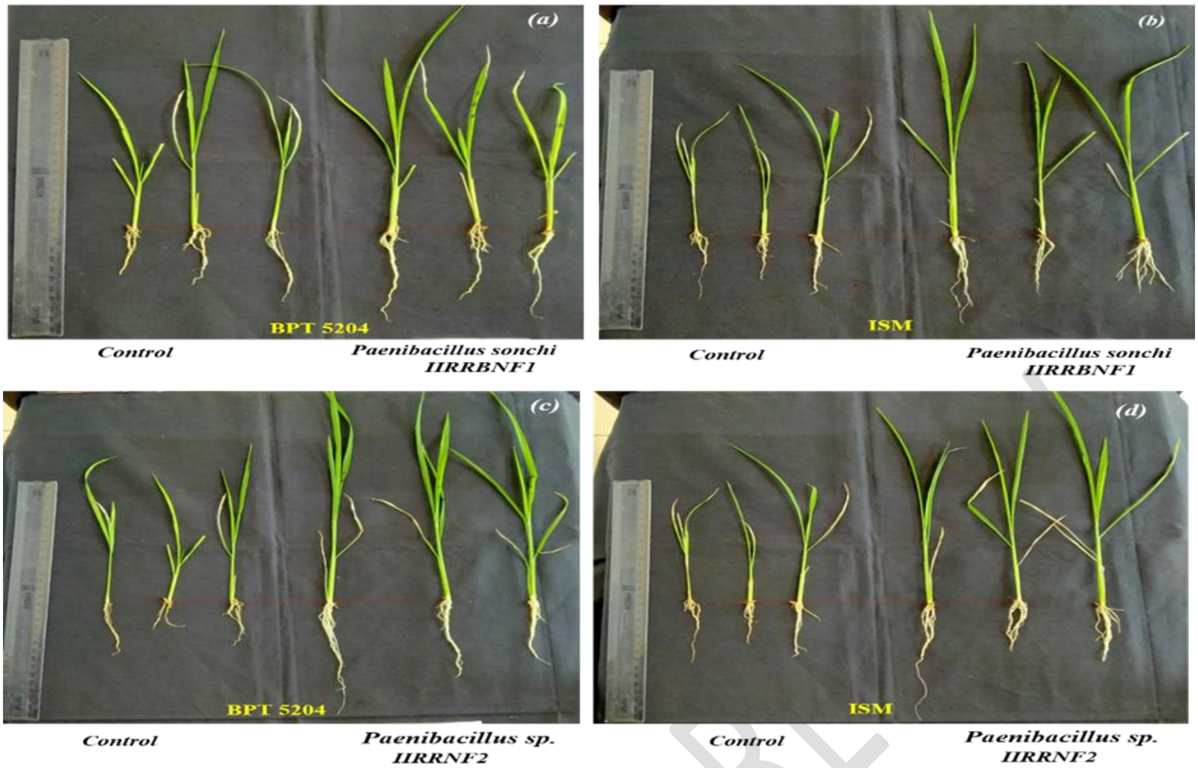


Figure 2. Growth promotion of rice cultivars in response to *Paenibacillus sonchi IIRRBNF1* and *Paenibacillus sp. IIRRNF2.*

Root and shoot dry matter were also recorded to understand the effect of nitrogen-fixing PGPB application on dry matter accumulation by the plants. Among the N-fixing PGPB, significant enhancement in shoot dry weight, root dry weight and seedling dry weight were observed in response to *Paenibacillus sonchi IIRRBNF1*, *Paenibacillus sp. IIRRNF2*, *Ochrobactrum sp. IIRRNF3*, *Stenotrophomonas sp. IIRRNF6.*, *B. japonicum* and *G. diazotrophicus* in comparison with control in BPT 5204 (Table 4, Figure 3).

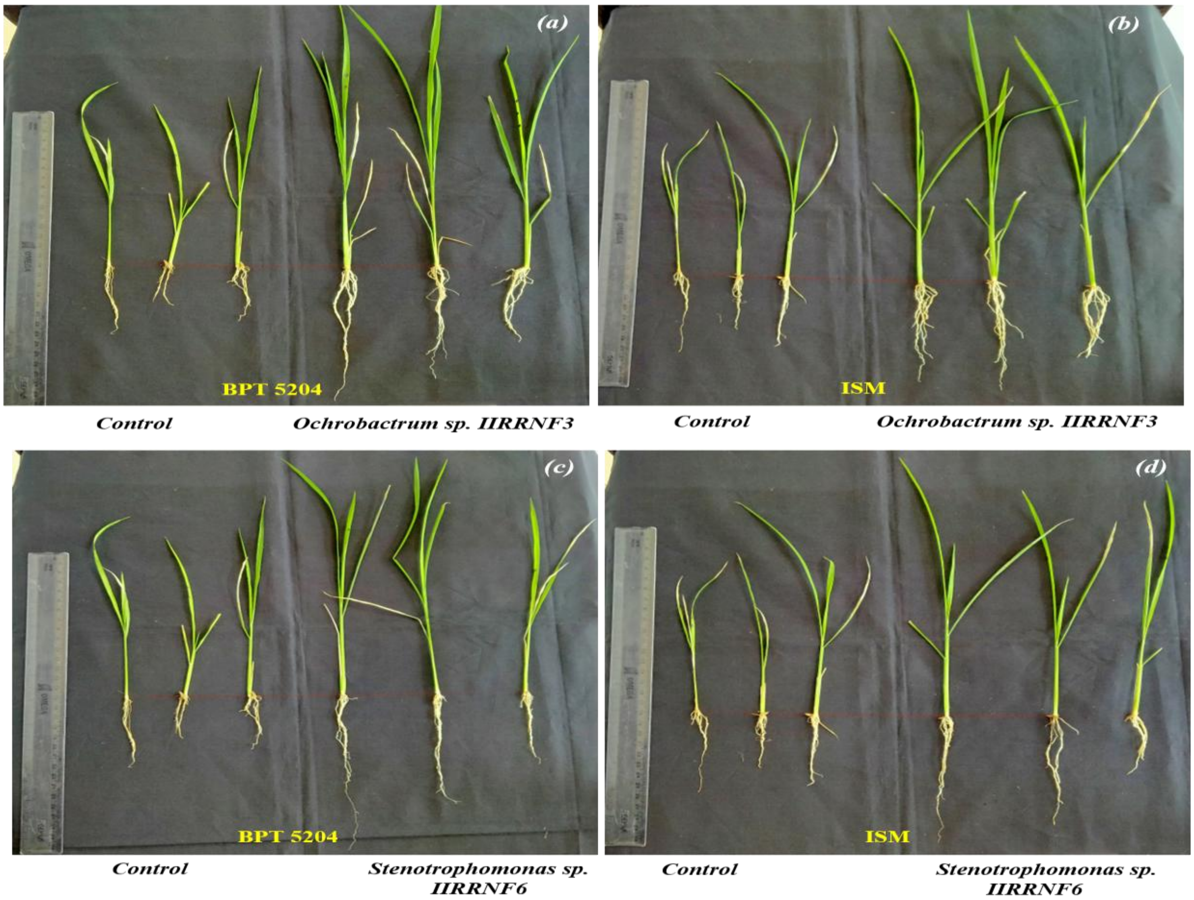


Figure 3. Growth promotion of rice cultivars in response to *Ochrobactrum sp. IIRRNF3* and *Stenotrophomonas sp. IIRRNF6*

In ISM cultivar, enhanced the root length, shoot length, seedling height, root fresh weight, shoot fresh weight and seedling fresh weight were observed in response to bacterial cultures *viz. Paenibacillus sonchi IIRRBNF1, Paenibacillus sp. IIRRNF2, Stenotrophomonas sp. IIRRNF6, Ochrobactrum sp. IIRRNF3, B. japonicum* and *G. diazotrophicus* over control at 25 dai (Table 5; Figure 2 and Figure 3). Also, increases in plant biomass (shoot dry weight, root dry weight and seedling dry weight) over control were observed in response to *Paenibacillus sp. IIRRNF2, Stenotrophomonas sp. IIRRNF6., Ochrobactrum sp., B. japonicum* and *G. diazotrophicus* (Table 5).

Table 5. Effect of plant growth-promoting bacteria on rice cultivar, Improved Samba Mahsuri under net house condition

Treatment	Root length (cm)	Shoot length (cm)	Seedling height (cm)	Root fresh weight (g)	Shoot fresh weight (g)	Seedling fresh weight (g)	Root dry weight (g)	Shoot dry weight (g)	Seedling dry weight (g)
Uninoculated (Control)	7.1^{bc}	15.7^{bc}	22.83^{d}	0.033^{bcd}	0.102^{cde}	0.135^{de}	0.009^{bcd}	0.039^{bcd}	0.048^{cde}
Paenibacillus sonchi IIRRBNF1	7.4^{bc}	24.9^{a}	32.23^{bc}	0.047^{abcd}	0.141^{abcd}	0.188^{abcd}	0.008^{bcd}	0.041^{abcd}	0.049^{bcde}
Paenibacillus sp. IIRRNF2	9.5^{abc}	25.7^{a}	35.17^{ab}	0.060^{ab}	0.159^{abc}	0.219^{abc}	0.011^{ab}	0.049^{abc}	0.060^{abcd}
Ochrobactrum	8.2^{abc}	27.0^{a}	35.20^{ab}	0.056^{abc}	0.167^{ab}	0.223^{abc}	0.013^{a}	0.054^{ab}	0.067^{ab}

sp. IIRRNF3									
Burkholderia cepacia IIRRNF4	9.1^{abc}	16.2^{bc}	25.33^{cd}	0.019^{d}	0.061^{e}	0.080^{e}	0.005^{e}	0.019^{e}	0.024^{f}
Burkholderia sp. IIRRNF5	5.9^{c}	18.8^{bc}	24.70^{d}	0.068^{a}	0.161^{abc}	0.229^{ab}	0.006^{de}	0.038^{bcd}	0.044^{cde}
Stenotrophomonas sp. IIRRNF6	9.2^{abc}	23.5^{a}	32.67^{ab}	0.043^{abcd}	0.112^{bcde}	0.155^{bcde}	0.007^{cde}	0.036^{cde}	0.043^{def}
Rhizobium sp. IIRRNF7	10.2^{ab}	14.1^{c}	24.30^{d}	0.049^{abc}	0.094^{de}	0.143^{cde}	0.007^{cde}	0.027^{de}	0.034^{ef}
Xanthomonas sacchari IIRRNF8	12.2^{a}	27.0^{a}	39.23^{a}	0.030^{cd}	0.086^{de}	0.116^{de}	0.009^{bcd}	0.029^{de}	0.038^{ef}
B. japonicum	10.1^{ab}	27.3^{a}	37.40^{ab}	0.070^{a}	0.189^{a}	0.259^{a}	0.012^{a}	0.058^{a}	0.070^{a}
G. diazotrophicus	8.1^{bc}	27.4^{a}	35.47^{ab}	0.059^{ab}	0.193^{a}	0.252^{a}	0.010^{abc}	0.053^{abc}	0.062^{abc}
LSD ($P \leq 0.05$)	4.1	4.4	6.93	0.028	0.059	0.082	0.003	0.017	0.019
CV (%)	27.4	11.7	13.07	34.08	26.35	26.65	21.88	25.32	23.16

The mean values followed by different letters indicate significant differences (LSD, $P \leq 0.05$)

Thus among all PGPBs, four *viz. Paenibacillus sonchi IIRRBNF1*, *Paenibacillus sp. IIRRNF2*, *Stenotrophomonas sp. IIRRNF6* and *Ochrobactrum sp. IIRRNF3* exhibited the ability for vegetative growth promotion and also increased the total dry matter accumulation (root and shoot dry matter) under net house conditions. Overall, *Paenibacillus sonchi IIRRBNF1*, *Paenibacillus sp. IIRRNF2*, *Ochrobactrum sp. IIRRNF3* and *Stenotrophomonas sp. IIRRNF6* has the highest ability to stimulate seedling height and dry matter accumulation *in vitro* as well as *in vivo* conditions.

It has been reported that, *Paenibacillus sp. ANR-ACC3* significantly enhanced the growth parameters like root and shoot length over control of rice (Bal and Adhya, 2021). Similarly, *Paenibacillus sp.* also enhanced the seedling growth of rice due to their ability to produce IAA and ammonia (Bal *et al.*, 2013). Our findings on *Paenibacillus sp.* is in accordance with earlier reports on other crops. Zhao *et al.* (2015) reported that *Paenibacillus sp.* which possessed a positive influence on phosphorous solubilization, siderophore, IAA production and ACC deaminase activity and lead to enhancement in growth and chlorophyll content of wheat plants under pot conditions. Similarly, *Paenibacillus sp. s37* increased the plant root growth, especially by inducing secondary root formation of christmas tree species *Abies nordmanniana* under in greenhouse conditions (Garcia-Lemos *et al.*, 2020). Singh *et al.* (2018) successfully demonstrated that *Ochrobactrum intermedium AcRz3* significantly increased the seedling growth (root and shoot length and number of leaves) of black rice over control under net house conditions. However, *Ochrobactrum sp.* (*MH685438*) improved plant growth and mitigate the drought stress of rice (Vidhyasri *et al.*, 2019). Gholamalizadeh *et al.* (2014) showed the enhancement of root length, stem length and weight of rice seedlings in response to *Stenotrophomonas maltophilia* in a pot experiment. Similarly, rice (*cv.* Boro) plants exhibited a significant increase in shoot length, root length and biomass in response to *Stenotrophomonas maltophilia RSD6* over control (Nevita *et al.*, 2018). It has been demonstrated that *Rhizobium sp.* treatment significantly enhanced the root elongation, root dry weight, shoot elongation and shoot dry weight in wheat (Zahir *et al.* 2004).

There are a few reports of *G. diazotrophicus* bacteria, which endophytically colonizing and enhancing the growth parameters *viz.* plant height, number of tillers, biomass and nitrogen content of rice (Muthukumarasamy *et al.*, 2005; Govindarajan *et al.*, 2008). Silva *et al.* (2020) observed that improvements in plant growth in response to *G. diazotrophicus* over control in rice. Our investigation of *B. japonicum* and *G. diazotrophicus* in accordance with earlier reports on soybean, maize and sugarcane crop. Cassan *et al.* (2009) observed that *Bradyrhizobium japonicum* enhanced the early growth promotion of seedlings in soybean and maize. However, sugarcane exhibited enhancement in stem diameter and dry matter in response to *G. diazotrophicus* (Schultz *et al*., 2017). Our findings on enhanced growth parameters of rice seedlings may be linked with the production of plant growth hormones or unknown metabolites and their interaction with rice root by PGPB (Dal Cortivo *et al.*, 2017).

In the present investigation, seed germination indices and growth promotion of rice cultivars might be due to various mechanisms by which PGPBs stimulate the plant growth involve the availability uptake of nutrients devising from genetic processes *viz.* phosphate solubilization and biological nitrogen fixation, stress alleviation, production of phytohormones and siderophores, among various others (De Souza *et al.*, 2015). Thus, our findings showed isolated PGPB inoculants enhanced growth parameters of rice at the seedling stage and there is a need to further evaluate the isolate for their effect on rice at different growth stages and yield under field conditions so that the best among these PGPBs can be deployed for preparing safe and effective biofertilizers that can act as alternative to chemical fertilizers for sustainable rice production.

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