Effect of nitrogen-fixing bacteria ongermination, seedling vigour and growth of two rice (*Oryza sativa* L.) cultivars

Abstract

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).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.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)* wereenhanced 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.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.

Keywords: PlantGrowth promoting bacteria, Nitrogen fixation,Rice Seed germination,and Rice Seedling growth

Introduction

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).

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) andthey 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 pathogen effect and improve the tolerance toabiotic stress (Paungfoo-Lonhienne *et al.*, 2014).

Diazotrophic free-living bacteria contribute up to 20 kilograms per hectare per year in cereal crop yields, and cereals rotational cropping systems with 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).

The aim of present study was to evaluate the effect of nitrogen fixing PGPBs on seed germination, germination index, seedlingvigour index and plant growth 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) andalong 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, seedlingvigour index and plant growth oftwo rice cultivars (BPT 5204 and Improved Samba Mahsuri i.e. ISM).

Seed treatment

The surface of the cultivar of rice seeds (*cv.* BPT 5204 and ISM) were 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 containingthe bacterial inoculum. Seeds soaked in the PBS buffer withoutany culturewas the control.

Seed germinationtraits*In vitro*condition

Seeds soaked in bacterial inoculum were placed inpetri plates containing water agar (0.8 %, w/v) and incubated at 28± 2°C.Every petri dishes were assessed for seed germination (3rd day), germination index i.e., speed of germination (from 0 to 3rd day), seedlingvigour index and seedling growth traits(15 dai, days after inoculation).

The germinated seeds wasdaily counted for 3 days and the sum of daily counts was the final germination percentage (Pieper, 1952). The rate of germination was calculated by counting the number of germinated seeds every day of the experiment according to Gupta (1993): Rate ofseed germination = Number of seeds germinated each day/ Total number of days. Seedlingvigour 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 traits*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 replicationsand the experiment repeated twice.

***In vivo* conditionunderpot experiment inthe net house**

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 data wereanalysed 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**

Significant higher germination percentage was recorded because of the seed treatment with bacteria.The germination ranged from 100% to 92% for BPT 5204and from100% to 92% for ISM when compared to untreated control (80% and 72% respectively). Among the bacterial cultures, *Paenibacillus sonchi IIRRBNF1*theinoculationresulted in the highest germination percentage than thecontrol 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 toa 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). In contrast, 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 onpercentages of seed germination rate and germination index of rice cultivars (cv. BPT 5204 andcv. 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, $P \leq 0.05$)

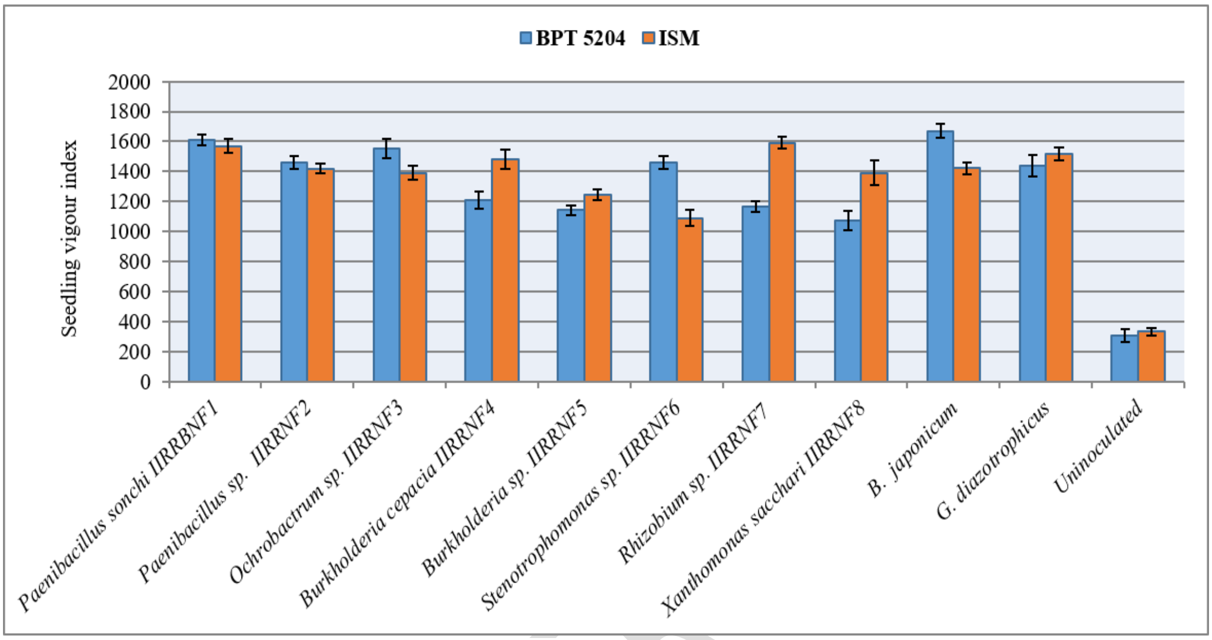

Figure 1. Effect of PGPBs on the seedling vigour index of rice cultivars (BPT 5204 and ISM). The error bar indicates the standard deviation.

The germination percentage, germination index and vigour index obtained in investigation agree with an earlier report about 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. We reported that germination percentage and seedling vigour index of rice seeds (*cv.* IR42) was significantlybetter 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 with *Ochrobactrum sp.* are in agreement with Singh *et al.* (2018), who demonstrated that *Ochrobactrum intermedium AcRz3* treated seeds of black rice had higher 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 tothe 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 had better germination and seedling vigour in response to *Bradyrhizobium japonicum* treatment. (Cassan *et al.*, 2009).

In vitro **(agar method) seedlinggrowth from rice cultivars in response to PGPB**

In the current study,inoculation with*Paenibacillus sonchi IIRRBNF1 andB. japonicum*resulted in higherseedling height, seedling fresh weight and seedling dry weight in the cultivar BPT 5204 evaluated at 15 dai (Table 2).In contrast the cultivarISM cultivar, higherseedling 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

In the columns, 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 smallletters 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 improvedin response to PGPB over control from 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 daiin 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 differentsmallletters indicate significant differences (LSD, $P \leq 0.05$)

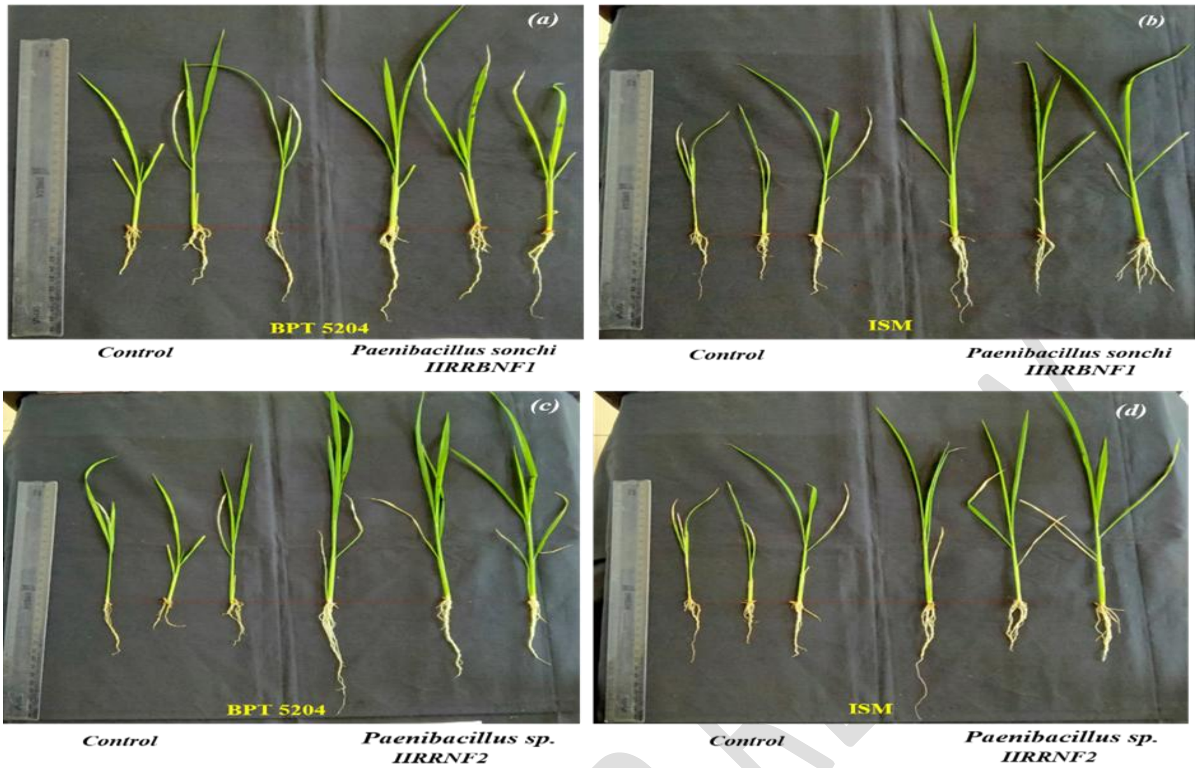

Figure 2. Growth promotion of rice cultivars in response to *Paenibacillus sonchi IIRRBNF1* and *Paenibacillus sp. IIRRNF2.*

Root and shoot dry biomass was also recorded to understand the effect of nitrogen-fixing PGPBs application on dry biomass accumulation by the plants. Among the N-fixing PGPBs, significant shoot, root and seedling dry biomass 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 the cv, BPT 5204 (Table 4, Figure 3).

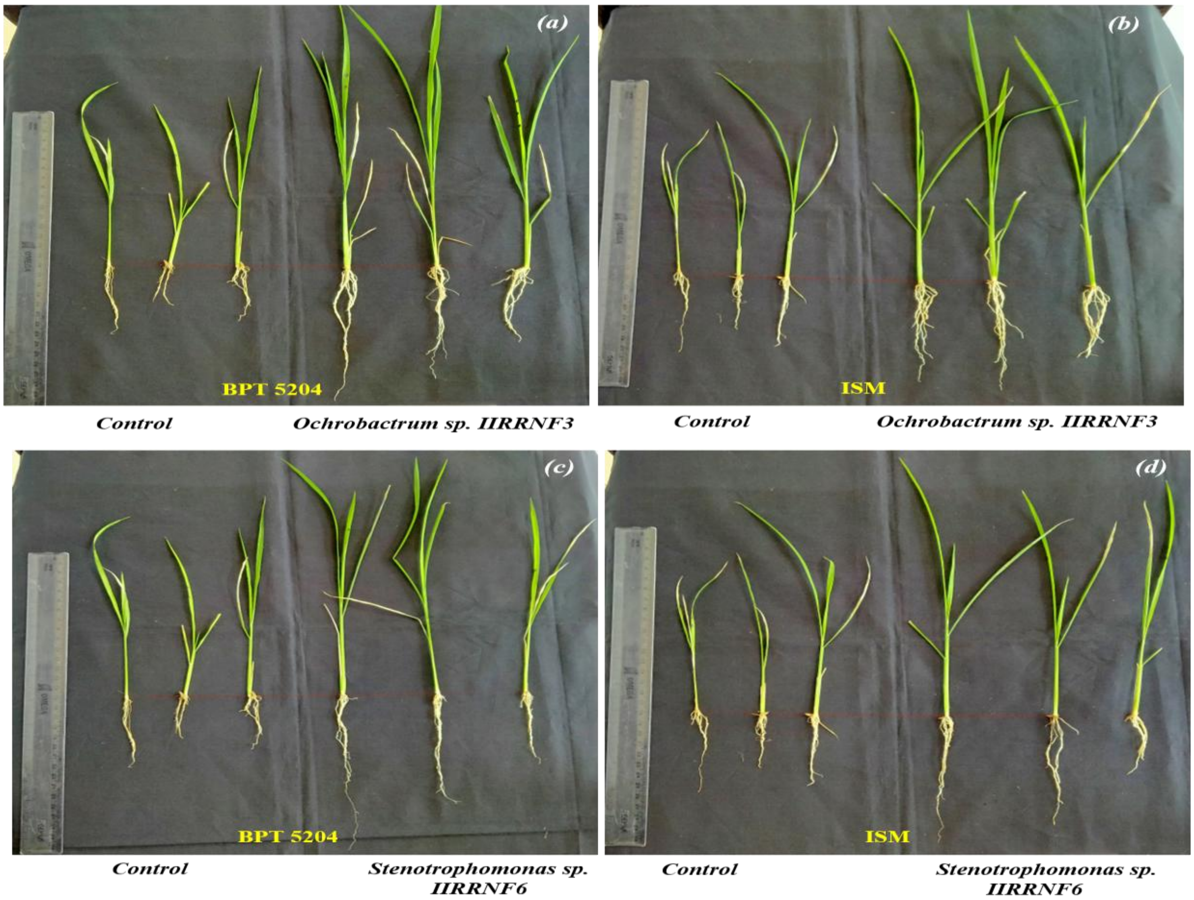

Figure 3. Growth of rice cultivars in response to *Ochrobactrum sp. IIRRNF3* and *Stenotrophomonas sp. IIRRNF6*

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). Furthermore, increases in plant biomass (shoot, root 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 the 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)
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	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}

IIRRBNF1									
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 sp. IIRRNF3	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}
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 small 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 from other crops. Zhao *et al.* (2015) reported that *Paenibacillus sp.*which possessed a positive influence on phosphorous solubilization, siderophore, IAA production and ACC deaminase activityand lead to increase growth and chlorophyll content of wheat plants under pot conditions. Similarly, *Paenibacillus sp. s37* increased the plant root growth, because of 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 and development (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 beendemonstrated 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 with *B. japonicum* and*G. diazotrophicus* are 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 isolatedPGPB inoculantsenhanced growth parameters of rice at the seedling stage and there is a need to further evaluate the isolate for their effect on riceat different growth stages and yield under field conditions so that the best among these PGPBs can be deployed for preparing safety and effective bio-fertilizers for sustainable rice production as an alternative to the application of chemical fertilizers.

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