International Journal of Plant & Soil Science

34(16): 94-106, 2022; Article no.IJPSS.84872
ISSN: 2320-7035

Effect of Nitrogen-fixing Bacteria on Germination, Seedling Vigour and Growth of Two Rice (*Oryza sativa* L.) Cultivars

Sonth Bandeppa [a], Amol S. Phule [a], G. Rajani [a], K. V. Prasad Babu [a], Kalyani M. Barbadikar [a], M. B. B. Prasad Babu [a], P. K. Mandal [b], R. M. Sundaram [a] and P. C. Latha [a*]

[a] *ICAR- Indian Institute of Rice Research, Telangana, India.*
[b] *ICAR-National Institute for Plant Biotechnology, India.*

Authors' contributions

This work was carried out in collaboration among all authors. Authors SB and ASP contributed equally to this work. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPSS/2022/v34i1631028

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/84872

Original Research Article

Received 04 February 2022
Accepted 08 April 2022
Published 27 April 2022

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. PGPBs (*viz., Paenibacillus sonchi IIRRBNF1, Paenibacillus sp. IIRRNF2, Ochrobactrum sp. IIRRNF3, Burkholderia cepacia IIRRNF4, Burkholderia sp. IIRRNF5, Stenotrophomonas sp. IIRRNF6, Rhizobium sp. IIRRNF7* and *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. 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.

**Corresponding author: E-mail: lathapc@gmail.com;*

Keywords: Plant growth promoting bacteria; nitrogen fixation; rice seed germination; rice seedling growth.

1. INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important staple foods for more than half of the world's population [1]. 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 [2] and only one-third of the N applied as chemical fertilizer is used by rice plants [3].

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 [4,5]. Nitrogen-fixing PGPB transform inert atmospheric nitrogen ($N_{2)}$ to ammonia [6] andthey are grouped into free-living bacteria (*Azotobacter* and *Azospirillium*) and symbionts such as *Rhizobium, Frankia* and *Azolla* [7]. Along with nitrogen-fixation, many soil micro-organisms have been reported to promote plant growth, suppress pathogen effect and improve the tolerance toabiotic stress [8].

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 [9]. Several groups of soil and root-associated nitrogen-fixing microorganisms such as *Azotobacter vinelandii* [10], *Azospirillum brasilense, Azospirillum zeae* and *Pseudomonas stutzeri* [11], *Acetobacter diazotrophicus* have been known to fix the nitrogen in different crops and stimulate plant growth [12].

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.

2. MATERIALS AND METHODS

2.1 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 [13] and 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 of two rice cultivars (BPT 5204 and Improved Samba Mahsuri i.e. ISM).

2.2 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 containing the bacterial inoculum. Seeds soaked in the PBS buffer without any culture was the control.

2.3 Seed Germination Traits *In vitro* Condition

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

The germinated seeds was daily counted for 3 days and the sum of daily counts was the final germination percentage [14]. The rate of germination was calculated by counting the number of germinated seeds every day of the experiment according to Gupta [15]: Rate of seed germination = Number of seeds germinated each day/ Total number of days. Seedling vigour index was calculated using the formula [16]: 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 replications and the experiment repeated twice.

2.4 *In vivo* Condition under Pot Experiment in the 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.

2.5 Statistical Analysis

All 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 ($p \leq 0.05$).

3. RESULTS AND DISCUSSION

3.1 *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 5204 and from 100% to 92% for ISM when compared to untreated control (80% and 72% respectively). Among the bacterial cultures, *Paenibacillus sonchi IIRRBNF1* the inoculation resulted in the highest germination percentage than the 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 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 (Fig. 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.

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. [17] 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 significantly better in response to *Paenibacillus sp. ANR-ACC3* over control [18]. Whereas in other crops, *Paenibacillus sp. s37* isolate increased the seed germination of Christmas tree species *Abies nordmanniana* [19]. Our findings with *Ochrobactrum sp.* are in agreement with Singh et al. [20], who demonstrated that *Ochrobactrum intermedium AcRz3* treated seeds of black rice had higher seed germination over control. Vidhyasri et al. [21] 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. [22] also reported that *Stenotrophomonas maltophilia* inoculated rice (*cv.* Hashemi) exhibited improved the seed germination and higher vigour index compared to the control. Similarly, Nevita et al. [23] 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 [24].

3.2 *In vitro* (Agar Method) Seedling Growth from Rice Cultivars in Response to PGPB

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

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 from both cultivars.

Table 1. Effect of PGPBs on percentages of seed germination rate and germination index of rice cultivars (cv. BPT 5204 and cv. 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 ($P \le 0.05$)	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, $P \le 0.05$)

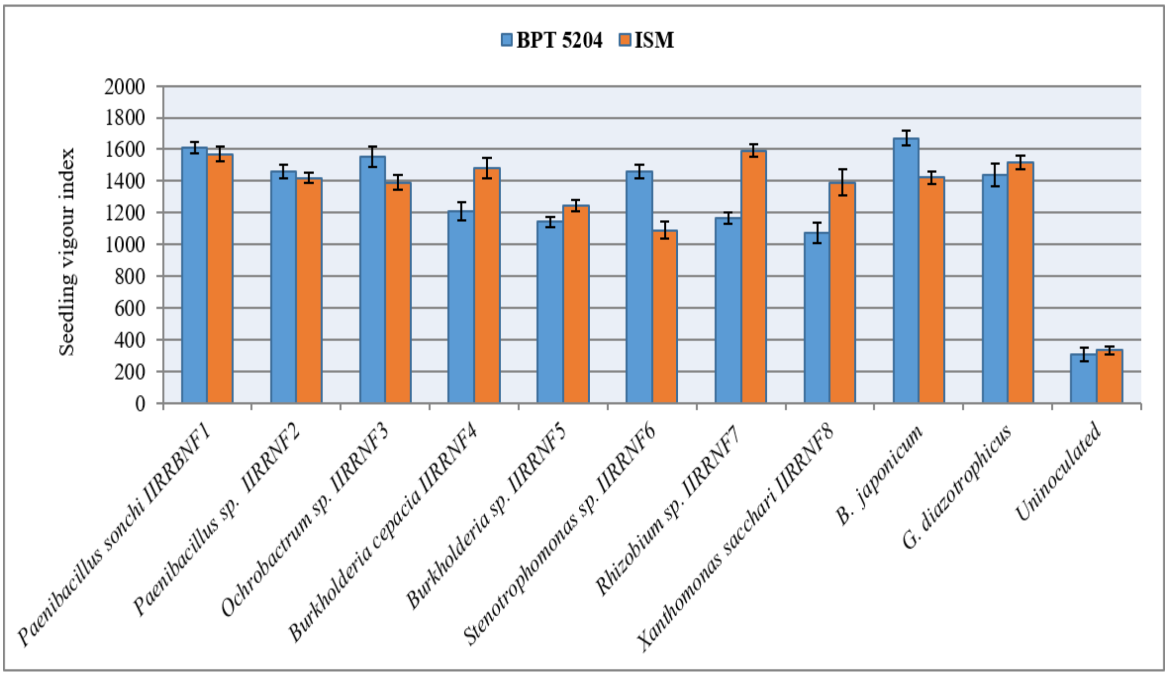

Fig. 1. Effect of PGPBs on the seedling vigour index of rice cultivars (BPT 5204 and ISM)

The error bar indicates the standard deviation

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 ≤ 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 ($P \le 0.05$)	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 small letters indicate significant differences (LSD, $P \le 0.05$)

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 small letters indicate significant differences (LSD, $P \leq 0.05$)

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}	$15.7^{\mathbf{bc}}$	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 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, $P \leq 0.05$)

3.3 *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; Fig. 2).

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, Fig. 3).

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; Fig. 2 and Fig. 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).

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.

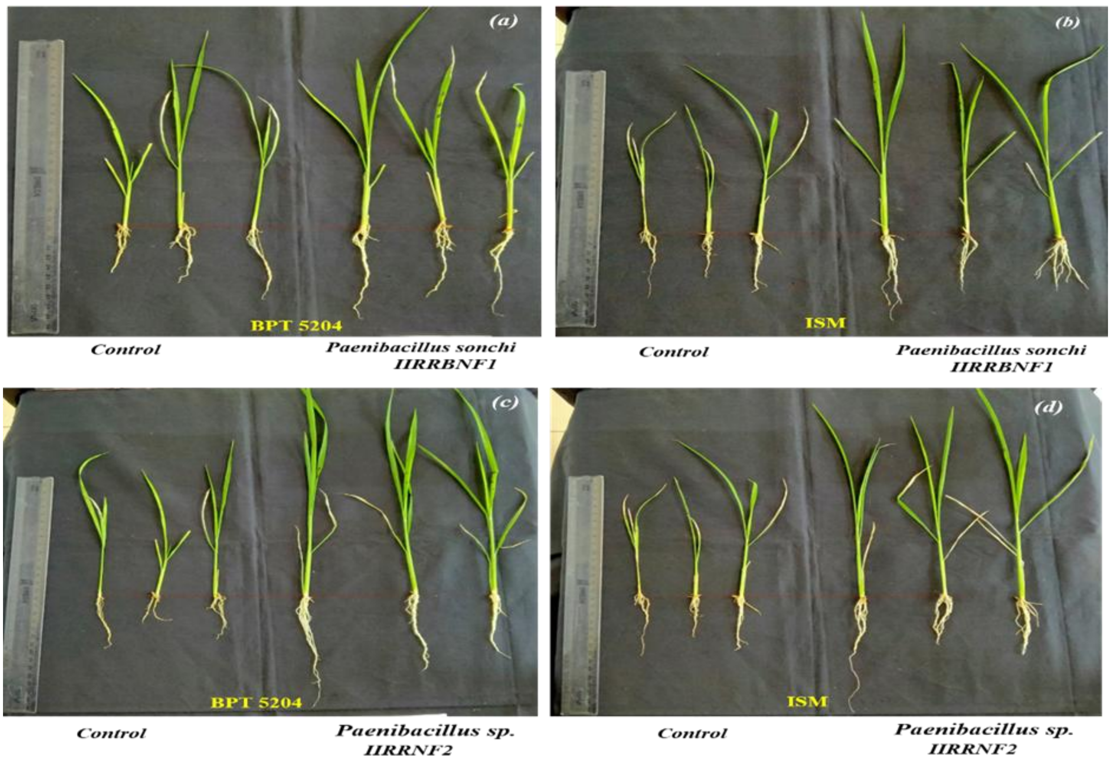

Fig. 2. Growth promotion of rice cultivars in response to *Paenibacillus sonchi IIRRBNF1* and *Paenibacillus sp. IIRRNF2*

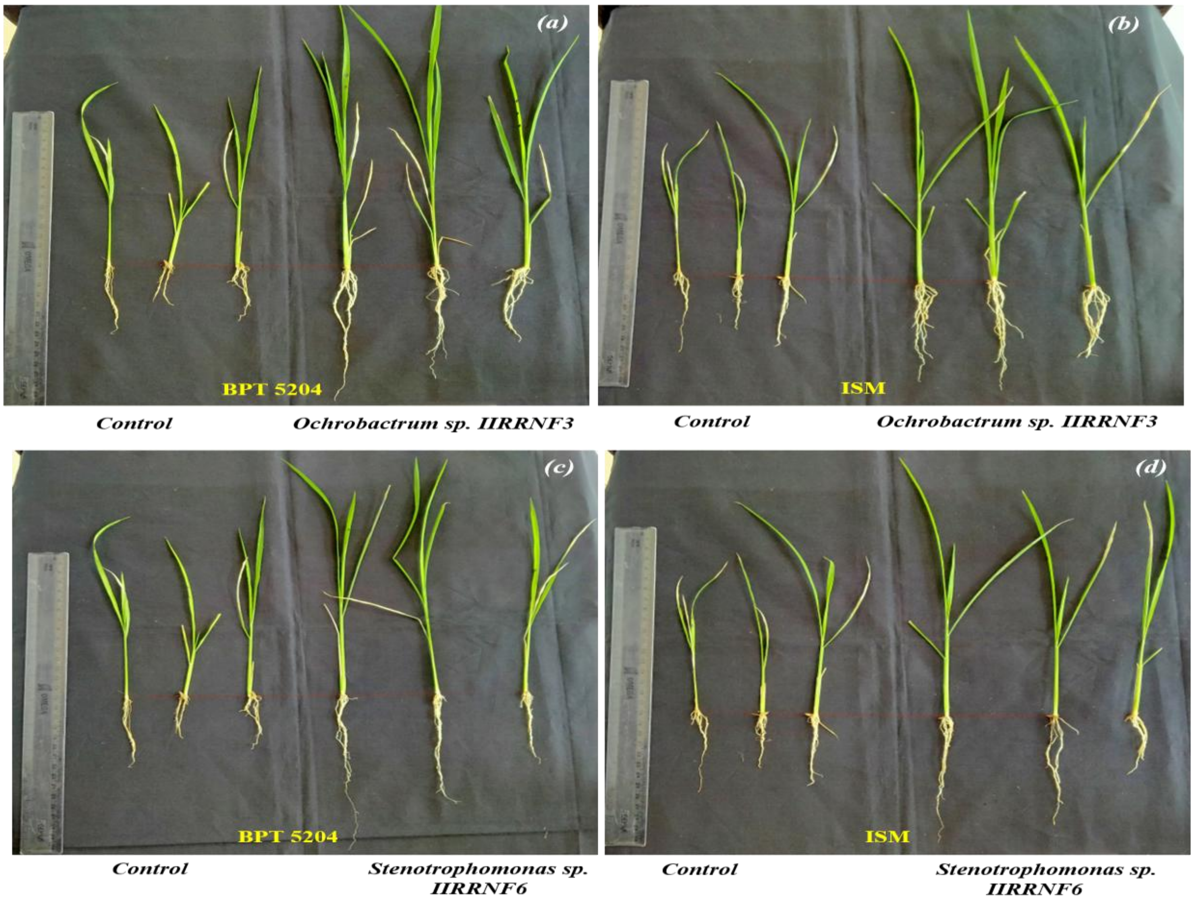

Fig. 3. Growth of rice cultivars in response to *Ochrobactrum sp. IIRRNF3* and *Stenotrophomonas sp. IIRRNF6*

It has been reported that, *Paenibacillus sp.ANR-ACC3* significantly enhanced the growth parameters like root and shoot length over control of rice [18]. Similarly, *Paenibacillus sp.* also enhanced the seedling growth of rice due to their ability to produce IAA and ammonia [17]. Our findings on *Paenibacillus sp.*is in accordance with earlier reports from other crops. Zhao et al. [25] reported that *Paenibacillus sp.* which possessed a positive influence on phosphorous solubilization, siderophore, IAA production and ACC deaminase activity and 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 [19]. Singh et al. [20] 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 [21]. Gholamalizadeh et al. [22] 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 [23]. It has been demonstrated that *Rhizobium sp.* treatment significantly enhanced the root elongation, root dry weight, shoot elongation and shoot dry weight in wheat [26].

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 [27,28]. Silva et al. [29] 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. [24] 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* [30]. 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 [31].

4. CONCLUSION

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 [32]. 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 safety and effective bio-fertilizers for sustainable rice production as an alternative to the application of chemical fertilizers.

ACKNOWLEDGEMENTS

The authors are thankful to ICAR, New Delhi the funding agency for providing financial assistance under the plan scheme network project Entitled-Incentivizing Research Project III 'Genetic modifications to improve biological nitrogen fixation for augmenting nitrogen needs of rice' and to the Director, ICAR-IIRR, Hyderabad for providing necessary facilities for research.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Hegde S, Hegde V. Assessment of global rice production and export opportunity for economic development in Ethiopia. Int. J. Sci. Res. 2013;2:257-260.
2. Ladha JK, Reddy PM. Nitrogen fixation in rice systems: State of knowledge and future prospects Plant Soil. 2003;252:151-167.
3. Araujo AES, Baldani VLD, Galisa PS, Pereira JA, Baldani JI. Response of traditional upland rice varieties to inoculation with selected diazotrophic bacteria isolated from rice cropped at the Northeast region of Brazil. App. Soil Ecol. 2013;64:49-55.
4. Rogers C, Oldroyd GED. Synthetic biology approaches to engineering the nitrogen symbiosis in cereals. J. Exp. Bot. 2014;65: 1939-1946.
5. Singh RK, Singh P, Li HB, Yang LT, Li YR. Soil-plant-microbe interactions" use of nitrogen-fixing bacteria for plant growth and development in sugarcane. In: Singh DP, et al. editors. "Plant-Microbe Interactions in Agro-Ecological Perspectives". Singapore Pte Ltd. Springer Nature; 2017.
6. Bakulin MK, Grudtsyna AS, Pletneva A. Biological fixation of nitrogen and growth of bacteria of the genus Azotobacter in liquid media in the presence of Perfluorocarbons. Appl Biochem Microbiol. 2007;4:399-402.
7. Gupta AK. The complete technology book on biofertilizers and organic farming. Natio Insti of Indus Res Pres. India; 2004.
8. Paungfoo-Lonhienne C, Lonhienne TGA. Yeoh YK, Webb RI, Lakshmanan P, Chan CX, Lim PE, Ragan MA, Schmidt S, Hugenholtz P. A new species of Burkholderia isolated from sugarcane roots promotes plant growth. Microb Biotechnol. 2014;7(2):142-54.
9. Vadakattu G, Paterson J. Free-living bacteria lift soil nitrogen supply. Farming Ahead. 2006;116:40.
10. Sahoo, R.K., M.W. Ansari, T.K. Dangar, S. Mohanty N. Tuteja. Phenotypic and molecular characterisation of efficient nitrogen-fixing Azotobacter strains from rice fields for crop improvement. Protoplasma. 2014;251:511-523.
11. Venieraki A, Dimou M, Pergalis P, Kefalogianni I, Chatzipavlidis I, Katinakis P. The genetic diversity of culturable nitrogen-fixing bacteria in the rhizosphere of wheat. Microb Eco. 2011;61:277-285.
12. Boddey RM, de Oliveira OC, Urquiaga S, Reis VM, Olivares FL, Baldani VLD, DoEbereiner J. Biological nitrogen fixation associated with sugar cane and rice:

contributions and prospects for improvement. Plant Soil. 1995;174:195-209.
13. Bandeppa S, Latha PC, Phule Amol S, Rajani G, Prasad Babu KV, Barbadikar Kalyani M, Chandrakala C, Prasad Babu MBB, Mandal PK, Sundaram RM. Isolation, identification and characterization of efficient free-living nitrogen-fixing bacteria from rice ecosystem. J. Rice Res. 2020;12(2):38-44.
14. Piper A, Das Saatgut VP. Darely Berlin, Hamburg, Germany; 1952.
15. Gupta PC. Seed vigour testing. In: Agarwal KP, editor. Handbook of seed testing. New Delhi; National Seed Corporation. 1993; 245-246.
16. Abdul-Baki AA, Anderson JD. Vigour determination in soybean seed by multiple criteria. Crop Sci. 1973;13:630-33.
17. Bal HB, Das S, Dangar TK, Adhya TK. ACC deaminase and IAA producing growth promoting bacteria from the rhizosphere soil of tropical rice plants. J. Basic Microbiol. 2013;53:972-984.
18. Bal HB, Adhya TK. Alleviation of submergence stress in rice seedlings by plant growth-promoting rhizobacteria with ACC deaminase activity. Front. Sustain. Food Syst. 2021;5:606158.
19. Garcia-Lemos AM, Grobkinsky DK, Saleem Akhtar S, Nicolaisen MH, Roitsch T, Nybroe O, Veierskov B. Identification of root-associated bacteria that influence plant physiology, increase seed germination, or promote growth of the christmas tree species Abies nordmanniana. Front. Microbiol. 2020;11: 566613.
20. Singh YR, Khunjamayum R, Nongthombam A, Chanu TP, Devi KM, Asem RS. Plant growth and grain yield production of black rice as influenced by *Ochrobactrum intermedium* AcRz3, an endophyte associated with medicinal plant. Crop Res. 2018;53:183-191.
21. Vidhyasri MS, Gomathi V, Siva Kumar U. Plant growth promotion of rice as influenced by *Ochrobactrum* sp. (MH685438) an rhizospheric bacteria associated with *Oryzae sativa*. Int. J.Curr. Microbiol. App. Sci. 2019;8(5):901-909.
22. Gholamalizadeh R, Khodakaramian G, Ebadi AA. Assessment of rice associated bacterial ability to enhance rice seed germination and rice growth promotion. Braz. Arch. Biol Technol. 2017;60:1-13.
23. Nevita T, Sharma GD, Pandey P. Composting of rice-residues using lingocellulolytic plant-probiotic *Stenotrophomonas maltophilia*, and its evaluation for growth enhancement of *Oryza sativa* L. Environmental Sustainability. 2018;1:185-196.
24. Cassan F, Perrig D, Sgroy V, Masciarelli O, Penna C, Luna V. *Azospirillum brasilense* Az39 and *Bradyrhizobium japonicum* E109 promote seed germination and early seedling growth, independently or co-inoculated in maize (*Zea mays* L.) and soybean (*Glycine max* L.). Eur. J. Soil. Biol. 2009;45:28-35.
25. Zhao L, Xu Y, Lai XH, Shan C, Deng Z, Ji Y. Screening and characterization of endophytic *Bacillus* and *Paenibacillus* strains from medicinal plant Lonicera japonica for use as potential plant growth promoters. Braz. J. Microbiol. 2015;46: 977-89.
26. Zahir ZA, Arshad A, Frankenberger WT. Plant growth promoting rhizobacteria: application and prospectives in agriculture. Adv. Agron. 2004;81:97-168.
27. Muthukumarasamy R, Cleenwerck I, Revathi G, Vadivelu M, Janssens D, Hoste B, Gum KU, Ki-Do P, Son CY, Sa T, Caballero-Mellado J. Natural association of *Gluconacetobacter diazotrophicus* and diazotrophic Acetobacter peroxydans with wetland rice. Syst. Appl. Microbiol. 2005; 28(3):277-286.
28. Govindarajan M, Balandreau J, Kwon SW, Weon HY, Lakshminarasimhan C. Effects of inoculation of *Burkholderia vietnamensis* and related endophytic diazotrophic bacteria on grain yield of rice. Microbiol. Ecol. 2008;55:2-37.
29. Silva R, Filgueiras L, Santos B, Coelho M, Silva M, Estrada-Bonilla G, Vidal M, Baldani JI, Meneses C. *Gluconacetobacter diazotrophicus* changes the molecular mechanisms of root development in *Oryza sativa* L. growing under water stress. Int. J. Mol. Sci. 2020;21:333.
30. Schultz N, Pereira W, de Albuquerque Silva P, Baldani JI, Boddey RM, Alves BJR, Urquiaga S, Reis VM. Yield of sugarcane varieties and their sugar quality grown in different soil types and inoculated with a diazotrophic bacteria consortium. Plant Production Science. 2017;1-9.
31. Dal Cortivo C, Barion G, Visioli G, Mattarozzi M, Mosca G, Vamerali T.

Increased root growth and nitrogen accumulation in common wheat following PGPR inoculation: assessment of plant-microbe interactions by ESEM. Agric. Ecosyst. Environ. 2017;247:396-408.

32. De Souza R, Ambrosini A, Passaglia LMP. Plant growth-promoting bacteria as inoculants in agricultural soils. Genet. Mol. Biol. 2015;38:401-419.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/84872