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PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR) INCREASES YIELD AND MINERAL CONTENTS OF RICE BY MOBILIZING NUTRIENTS IN THE RHIZOSPHERE

Md. Delwar Hossain[1], Md Shafiul Islam Rion[1, 2], Phalguni Das[1], Atiqur Rahman[1], and Quazi Forhad Quadir[1]*

[1]Laboratory of Plant Nutrition and Environmental Chemistry, Department of Agricultural Chemistry, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh; [2]Division of Plant and Soil Sciences, West Virginia University, Morgantown, WV 26506, USA.

***Corresponding author:** Quazi Forhad Quadir; E-mail: qfq@bau.edu.bd

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ABSTRACT

Plant growth-promoting rhizobacteria (PGPR) are multipurpose entities in case of crop quality and yield improvement. This study evaluated the impact of PGPR inoculation in root zone nutrient release, rice yield, and grain nutrient (P, Fe, Mn, and Zn) content in two popular rice varieties of Bangladesh (viz., BRRI dhan49 and Binadhan7). It was a single factor (bacterial treatment) experiment where B0, B1, B2, and B3 treatments represented the inoculated control, indole acetic acid (IAA) producing bacteria consortium, phosphorus solubilizing bacteria (PSB) consortium, and combination of B1 and B2 treatments, respectively. Nutrients release in pore water was higher at 5 days after transplanting (DAT), compared to 25 DAT. Mostly B2 and B3 treatments performed significantly in the number of tillers/pot, straw yield, grain yield, grain P, Mn, Fe content, and all four nutrients uptake. The highest grain yield observed in B3 treatment is in both Binadhan7 (42.10±1.76 g/pot) and BRRI dhan49 (36.20±1.57 g/pot). PSB containing B2 treatment bio fortified the largest amount of P in both rice varieties. On the other hand, the B3 treatment stored the highest amount of Mn (46.70±1.30 and 44.30±1.37 mg/kg) and Fe (45.30±2.90 and 25.70±2.37 mg/kg) in Binadhan7 and BRRI dhan49, respectively. The B3 treatment resulted in maximum nutrient content (P, Mn, and Fe) and uptake (P, Mn, Fe, and Zn) in both rice varieties. These bacterial isolates seemed promising for rice yield and quality improvement in an eco-friendly and sustainable way.

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INTRODUCTION

Micronutrient deficiency is a sweltering issue in today's world as a large portion of the world population is still malnourished. According to the Food and Agriculture Organization (FAO), almost 792.5 million people worldwide are malnourished, among which 780 million people belong to developing countries (McGuire, 2015). Around two billion people across the world are deprived of adequate micronutrient intake in their daily diet, though overall crop production has increased over the decades (Garg et al., 2018). Previously the global agricultural system was absorbed in food security rather than nutritional security which resulted in increased production of micronutrient deficient grain crops. In recent times, apprehensions are focused about nutritional security with an aim to mitigate micronutrient malnutrition or 'hidden hunger', especially in poor and developing countries where micronutrient deficient crops are the main diet (Khush et al., 2012). One major way to fight the malnutrition issue is to bio fortify essential nutrients in crops. Bio-fortification refers to cost-effective, sustainable, and promising techniques of supplying essential micronutrients and vitamins to a population which has inadequate access to diverse diets (Garg et al., 2018). This decade old concept was suggested to significantly ameliorate micronutrient and vitamin deficiencies (Clemens et al., 2002; Guerinot, 2001; Ye et al., 2000). Researchers are trying to alleviate malnutrition with improved nutrient-rich cultivars of several crops. Over 20 million people worldwide are currently consuming biofortified crops (Bouis and Saltzman, 2017). But, developing a new variety is a lengthy process whereas agronomic bio fortification is not promising enough (Cakmak, 2008). Again, concerns have been intensifying over the effect of synthetic fertilizer application as they are detrimental to the environment. Public health risks are also concentrated because of these chemical residues on food and drinking water (Soares & Porto, 2009; Maroni et al., 2006).

Utilization of plant growth-promoting rhizobacteria (PGPR) for productivity improvement and nutrient biofortification may be a viable alternative to inorganic fertilizers, which will reduce pollution, preserve the environment, and assist in alleviating malnutrition. PGPR affect plant growth through mechanisms such as N_2-fixation, plant growth regulators production (Vessey, 2003), water and nutrient uptake enhancement (Dey et al., 2004), soil-borne plant pathogens inhibition (Sindhu et al., 2002), and so on. Application of a single microbial strain may not be feasible enough, but their effectiveness can be improved through co-inoculation with other strains in consortium or with plant growth regulators i.e., precursor-inoculum interaction (Giri et al., 2023; Gohil et al., 2019). Oluwambe and Kofoworola (2016) found the inoculation of PGPR consortium more efficient over single strain inoculation in tomato growth. The use of PGPR for inoculation of seedlings, seeds or soil helps in the mobilization of nutrients through biological activity and increases the population of micro flora, leading to improved soil health (Das and Singh, 2014). The improvement in soil health indicators such as soil EC, pH, available N, P, K, S, and soil organic matter due to combined use of PGPR and organic manure in rice (*Oryza sativa* L.) fields was also reported in previous studies (Ali et al., 2017). Rice is a staple food for a large part of the world population including Bangladeshi people. Therefore, the objectives of this study were to evaluate the effect of PGPR consortium on nutrient release pattern, grain nutrient content, and growth and yield attributes in two popular rice varieties (viz. BRRI dhan49 and Binadhan7) of Bangladesh.

MATERIALS AND METHODS

Experimental design and treatments

The experiment was conducted on two rice varieties (BRRI dhan49 and Binadhan7) with single factor (bacterial treatment) in the Net House of the Department of Agricultural Chemistry, Bangladesh Agricultural University. Four bacterial treatments (B0 = uninoculated control; B1 = IAA producing bacterial consortium of M-10, M-23, F-37; B2 = phosphorus solubilizing bacteria (PSB) consortium of M-17, PWB-5, FB-4; and B3 = combination of B1 and B2) in triplicate were in the experiment. The bacteria were not tested for antagonism prior to application in consortium. The variety was not considered another factor as they possess distinct growth and yield traits. Therefore, the experimental setup was the same for both rice varieties. Table 1 shows the plant growth-promoting functions of the selected rhizobacteria.

PGPR inoculation, seedling transplanting, and intercultural operations

Clean 30 days old rice seedling roots were surface sterilized with 40% ethyl alcohol for 5 minutes prior to soaking in the treatment broths overnight. Then the bio-primed seedlings were transplanted to plastic pots (3-4 seedlings/pot) containing well pulverized, visibly clean, and fertilized (urea 250 kg/ha, TSP 200 kg/ha, MoP 230 kg/ha, gypsum 75 kg/ha, $ZnSO_4$ 5 kg/ha) soil (FRG, 2012). The full dose of fertilizers (except urea) applied during pot preparation, and the first installment of urea applied 7 days after transplanting (DAT). The second and third urea installment applied at 21 DAT and 45 DAT, respectively. Each pot was properly cared for weeding, irrigation, disease, and pest management as per the requirement.

Sample collection and analysis

Pore-water collected from the rice rhizosphere with rhizon sampler at 5 DAT and 25 DAT for phosphorus (P), manganese (Mn), iron (Fe), and zinc (Zn) release determination. Plants from half of the pots harvested at 50 DAT and rest half at the full agricultural maturity. Plant samples were air dried (48 hours) and oven dried (48 hours at 60°C) prior to grinding. Grounded plant samples were digested using di-acid mixture (HNO_3:$HClO_4$ = 2:1) and plant extracts were analyzed for P, Mn, Fe, and Zn determination in atomic absorption spectrophotometer (Shimadzu AAS-7000, Japan).

Statistical analysis

One-way ANOVA was performed for each parameter and rice varieties with R (R Core Team, 2020). The data are normalized prior to analyses. Significant differences were distinguished by Tukey's Pairwise Comparisons at 95% confidence level using the *agricolae* (Mendiburu and Yaseen, 2020) package, and group-wise statistics was done with the *doBy* (Højsgaard, 2012) package. In addition, all the graphs were prepared in the *ggplot2* (Wickham, 2016) package.

RESULTS

Nutrient release pattern

Nutrient release had a significant difference between 5 DAT and 25 DAT and mostly higher at 5 DAT in both varieties for all inoculated bacterial treatments (Table 2). However, Zn release did not have any substantial change in samples collected in two different times for the two rice varieties under study. In both varieties, B2, and B1 treatment released more P and Zn, respectively, than other treatments. Highest Mn and Fe release observed in B3 treatment in both rice varieties, irrespective of the day of sampling. Overall nutrients release was higher in Binadhan7 than BRRI dhan49.

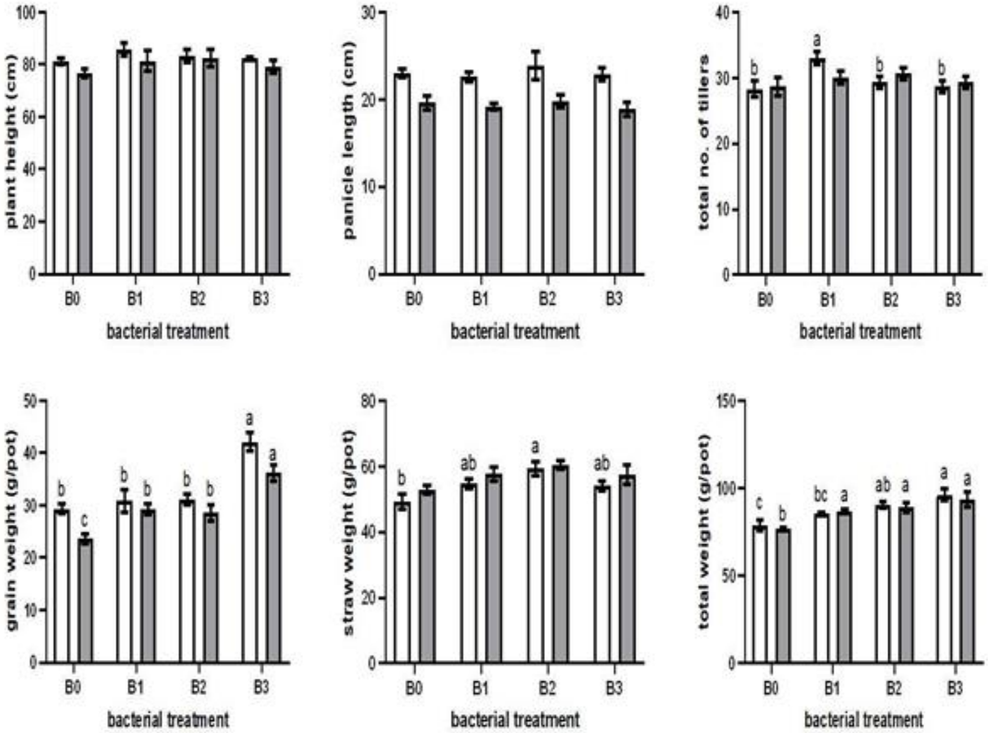

Figure 1. Effect of PGPR on plant growth parameters (e.g., plant height, panicle length, and total number of tillers), grain weight, straw weight, and total weight in two rice varieties, viz. Binadhan7 (white bars) and BRRI dhan49 (gray bars). Here, B0, B1, B2 and B3 is the control, IAA producing rhizobacterial consortium, phosphate solubilizing bacteria (PSB), and consortium of both IAA producing rhizobacteria and PSB, respectively. Bars on each column indicate Mean±SE and columns with unlike letters are significantly different at 5% level of significance by Tukey's HSD test.

Table 1. The functionalities of rhizobacteria used in the study as a consortium.

Bacteria isolate	Plant growth-promoting traits	Source
M-10	IAA	Asha et al., 2015
M-23	IAA	Asha et al., 2015
F-37	IAA	Khatun et al., 2021
PWB-5	PSB	Arifin et al., 2021
M-17	PSB	Asha et al., 2015
FB-04	PSB, metal solubilizing	Taher et al., 2019

Here, "IAA" = indole-3-acetic acid production, "PSB" = phosphorus solubilizing bacteria

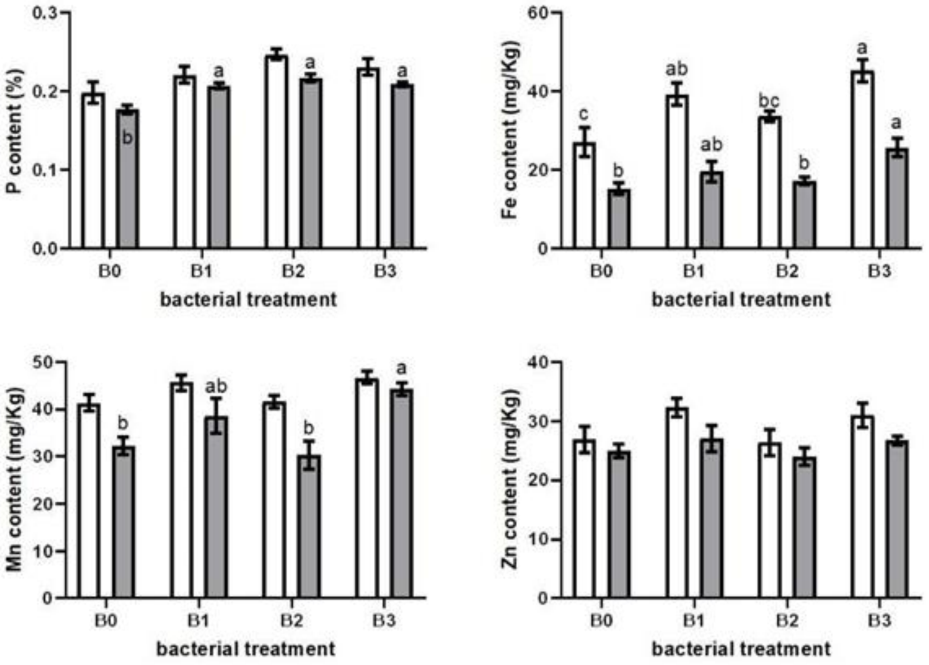

Figure 2. Effect of PGPR on grain nutrient (P, Fe, Mn, Zn) content in two rice varieties, viz. Binadhan7 (white bars) and BRRI dhan49 (gray bars). Here, B0, B1, B2 and B3 is the control, IAA producing rhizobacterial consortium, phosphate solubilizing bacteria (PSB), and consortium of both IAA producing rhizobacteria and PSB, respectively. Bars on each column indicate Mean±SE and columns with unlike letters differ significantly at 5% level of significance by Tukey's HSD test.

Growth and yield attributing characters

An overview of the effect of PGPR treatment over the control is presented in Figure 1, while the detailed values are mentioned in Supplement material 1. Though bacteria inoculation did not have a significant effect on plant height and panicle length, the grain yield and total yield were substantially influenced in both varieties. In Binadhan7 and BRRI dhan49, the highest grain yield (42.1±1.76 and 36.2±1.57 g/pot, respectively) and total yield (96.3± 3.22 and 93.7±4.56 g/pot, respectively) were observed in B3 treatment. For Binadhan7, total number of tillers and straw yield were significantly influenced by the treatments, where the highest no. of tillers (33±1 total tillers/pot) and straw yield (59.4±2.18 g/pot) were found in B1 and B2 treatment, respectively.

Table 2. Effect of PGPR on nutrient release (Mean±SE) in soil pore water, collected using rhizon sampler from two rice varieties (Binadhan7 and BRRI dhan49) at 5 DAT and 25 DAT.

Variety	Treatment	P release (ppm)		Mn release (ppm)		Fe release (ppm)		Zn release (ppm)	
		5 DAT	25 DAT	5 DAT	25 DAT	5 DAT	25 DAT	5 DAT	25 DAT
BRRI dhan49	B0	0.08±0.01	0.04±0.00	4.57±0.14	3.31±0.73	12.72±1.51	4.99±1.72	0.12±0.02	0.16±0.02
	B1	0.07±0.02	0.06±0.00	3.96±0.44	1.29±0.38	10.30±1.65	1.78±0.95	0.43±0.14	0.34±0.13
	B2	0.12±0.04	0.08±0.00	4.68±0.49	1.25±0.05	10.41±1.14	1.53±0.29	0.17±0.12	0.23±0.02
	B3	0.09±0.03	0.06±0.00	4.97±0.31	3.02±0.81	17.25±3.29	7.26±3.24	0.14±0.06	0.26±0.03
p value	Treatment	0.107^{ns}		0.033*		0.009**		0.037*	
	Days	0.030*		<0.001***		<0.001***		0.552^{ns}	
Binadhan7	B0	0.11±0.04	0.06±0.01	5.24±0.54	2.93±0.84	11.91±1.62	3.44±0.96	0.05±0.03	0.19±0.05
	B1	0.13±0.04	0.07±0.01	3.58±0.29	3.42±0.70	8.89±1.19	3.46±0.70	0.47±0.21	0.21±0.06
	B2	0.29±0.07	0.13±0.07	4.73±0.77	4.23±0.72	10.87±1.97	4.23±0.72	0.17±0.07	0.16±0.04
	B3	0.13±0.05	0.09±0.02	6.93±1.43	4.83±0.54	15.59±4.53	4.83±0.54	0.13±0.05	0.16±0.01
p value	Treatment	0.264^{ns}		0.043*		0.235^{ns}		0.119^{ns}	
	Days	0.111^{ns}		0.035*		<0.001***		0.721^{ns}	

Here, B0, B1, B2, and B3 is the control, IAA producing rhizobacterial consortium, phosphate solubilizing bacteria (PSB), and consortium of both IAA producing rhizobacteria and PSB, respectively.

'*', '**', '***', and 'ns' indicates 5% level of significance, 1% level of significance, 0.1% level of significance and non-significant, respectively

Nutrient content and uptake

In case of grain nutrient content, only Fe content in Binadhan7, and P, Fe, Mn content in BRRI dhan49 were significantly affected by PGPR treatments (Figure 2 and Supplementary 2). The B3 treatment provided highest Fe content in both Binadhan7 (45.30±2.90 mg/kg) and BRRI dhan49 (25.70±2.37 mg/kg) (Figure 2 and Supplementary 2). The highest P (0.217±0.004%) and Mn content (44.30±1.37 mg/kg) in BRRI dhan49 reported in B2 and B3 treatment, respectively. PGPR inoculation also significantly influenced nutrient uptake in both rice varieties (Figure 3 and Supplementary 2). The B3 treatment reported highest nutrient uptake (P, Mn, Fe, and Zn) in both Binadhan7 and BRRI dhan49. The P, Mn and Fe uptake were higher in Binadhan7 in most of the treatments.

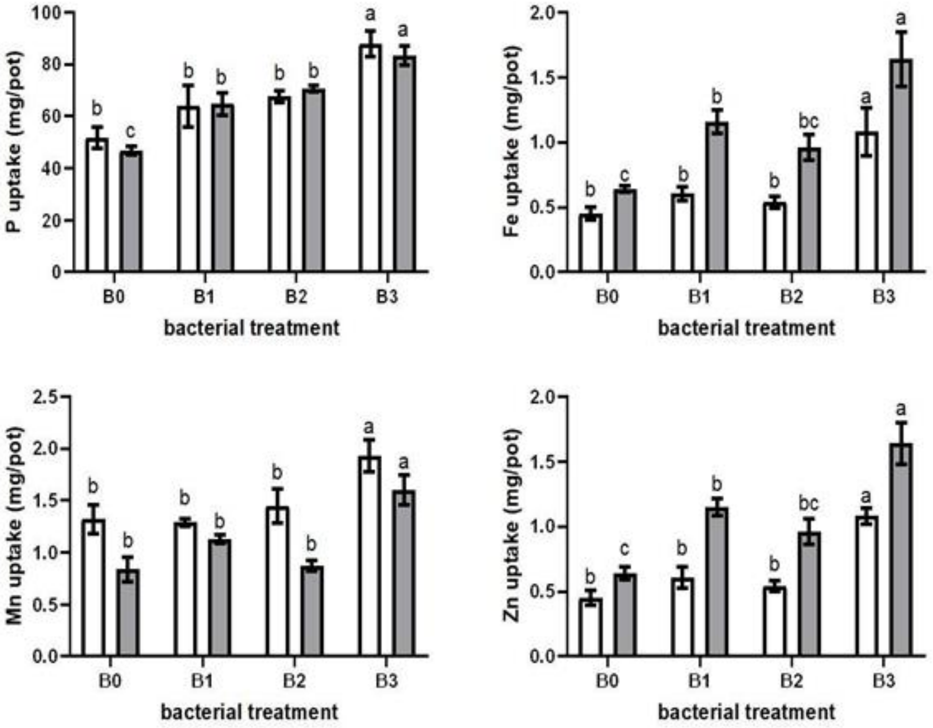

Figure 3. Effect of PGPR on nutrient (P, Fe, Mn, Zn) uptake (mg/pot) in two rice varieties, viz. Binadhan7 (white bars) and BRRI dhan49 (gray bars). Here, B0, B1, B2 and B3 is the control, IAA producing rhizobacterial consortium, Phosphate solubilizing bacteria (PSB), and consortium of both IAA producing rhizobacteria and PSB, respectively. Bars on each column indicate Mean±SE and columns with unlike letters are statistically significantly different at 5% level of significance by Tukey's HSD test.

DISCUSSION

The P release, grain P content, and P uptake in rice plants were higher in PSB containing treatments. PSB can increase P uptake in plants (Gulati et al., 2008; Rodríguez and Fraga, 1999) as they are able to solubilize the insoluble P in soil (Chen et al., 2006). Similar result of increased P content upon PSB inoculation in aerobic rice was reported earlier by (Panhwar et al., 2011). Microbial phosphate solubilization takes places through various mechanisms including, organic acid production and proton extrusion (Banik and Dey, 1982; Dutton and Evans, 2011), chelation of metal ions (Fe, Al, Ca) and reduction of soil pH (Arifin et al., 2021; Stevenson and Cole, 1999), phosphate anion binding (Jones and Darrah, 1994), etc. The organic acid production might be responsible for the P solubilization here as this is regarded as the main mechanism for insoluble P solubilization (Marschner, 1995).

In PSB containing treatments, Zn, Mn and Fe release were also higher, which is consistent with the findings of Liu et al. (2020). In their study on tomato, they found increased Zn, Mn, and Fe availability upon PSB inoculation. All three bacterial treatments enhanced Zn solubilization. These isolates may have Zn solubilizing capability, though they were not tested for Zn solubilization. Kumar et al. (2014) also reported substantial increase in wheat grain micronutrient (Fe, Cu, Zn, and Mn) concentration upon PGPR treatment.

PGPR application significantly influenced the number of total tillers/pot and straw yield in Binadhan7 and grain yield in both varieties. Lavakush et al. (2014) also found an increase in the number of tillers/hill in rice as a result of PGPR application with different phosphorus fertilization levels. PGPR inoculation substantially enhanced grain yield and straw yield in wheat as reported by Kumar et al. (2014). Rion et al. (2022) also demonstrated that Zn solubilizing PGPR can enhance growth of rice seedlings. In most parameters, Binadhan7 came out superior over BRRI dhan49, though they were facilitated with the same conditions and treatments at the same time. Since these two varieties are different from one another in their individual physiology, the difference between them in several parameters studied is not an exception.

CONCLUSION

The study on nutrient release dynamics upon PGPR inoculation. This study showed that these PGPR treatments have potentiality of improving rice yield and bio fortifying essential micronutrients. Further research and field trials are required to reveal more potency of these isolates in rice cultivation prior to suggesting these PGPR treatments as eco-friendly and cost-effective bio fertilization technology instead of synthetic fertilizers.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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Supplementary materials

Supplementary 1. Effect of PGPR on plant growth parameters (e.g., plant height, panicle length and total number of tillers), grain weight, straw weight, total weight in two rice varieties, viz. Binadhan7 and BRRI dhan49. Here, B0, B1, B2 and B3 is the control, IAA producing rhizobacterial consortium, Phosphate solubilizing bacteria (PSB), and consortium of both IAA producing rhizobacteria and PSB, respectively. Each row indicates Mean±SE and columns with unlike letters are statistically significantly different at 5% level of significance by Tukey's HSD test. Here, '*', '**', and 'ns' indicates 5% level of significance, 1% level of significance and non-significant, respectively

Variety	Treatment	Plant Height (cm)	Panicle Length (cm)	Number of Tillers (total tillers/pot)	Grain Yield (g/pot)	Straw Yield (g/pot)	Total Yield (g/pot)	Fresh Weight (g/pot)	Dry Weight (g/pot)
Binadhan7	B0	81.20±1.07	23.00± 0.47	28.30±1.20b	29.30±0.91b	49.30±2.32b	78.70± 3.12c	184.03±10.70	42.02±1.67
	B1	85.70± 2.52	22.60±0.52	33.00±1.00a	30.80±2.15b	54.80±1.41ab	85.70±0.89bc	186.50±6.96	42.55±1.65
	B2	83.40± 2.52	23.90±1.64	29.30±0.88b	31.20±1.00b	59.40±2.18a	90.60± 2.04ab	190.80±30.30	43.95±7.94
	B3	82.40±0.44	22.90± 0.79	28.70±0.88b	42.10±1.76a	54.20±1.49ab	96.30± 3.22a	223.10±14.9	51.29±2.72
	Significance	ns	ns	*	**	*	**	ns	ns
BRRI dhan49	B0	76.70±1.60	19.60±0.77	28.70±1.45	23.70±0.96c	53.00± 1.26	76.70± 1.14b	185.50±9.40	42.48±1.02
	B1	81.30±3.90	19.20± 0.42	30.00±1.00	29.30±0.97b	57.70±2.02	87.00±1.12a	193.10±4.73	43.41±2.96
	B2	82.40±3.23	19.80±0.70	30.70±0.88	28.60±1.59b	60.40±1.38	89.00±2.94a	193.23±7.33	44.57±1.17
	B3	79.10±2.48	18.90±0.81	29.30±0.88	36.20±1.57a	57.50±3.00	93.70±4.56a	195.77±4.84	48.23±5.04
	Significance	ns	ns	ns	**	ns	*	ns	ns

Supplementary 2. Effect of PGPR on grain nutrient (P, Mn, Fe, Zn) content and nutrient uptake in two rice varieties, viz. Binadhan7 and BRRI dhan49. Here, B0, B1, B2 and B3 is the control, IAA producing rhizobacterial consortium, phosphate solubilizing bacteria (PSB), and consortium of both IAA producing rhizobacteria and PSB, respectively. Each row indicates Mean±SE and columns with unlike letters are statistically significantly different at 5% level of significance by Tukey's HSD test. Here, '*', '**', '***', and 'ns' indicates 5% level of significance, 1% level of significance, 0.1% level of significance and non-significant, respectively

Variety	Treatment	P content (%)	Mn content (mg/kg)	Fe content (mg/kg)	Zn content (mg/kg)	P uptake (mg/pot)	Mn uptake (mg/pot)	Fe uptake (mg/pot)	Zn uptake (mg/pot)
Binadhan7	B0	0.198±0.014	41.30±1.75	27.10±3.68c	26.9±2.23	51.8±4.22b	1.320±0.135b	0.450±0.050b	0.450±0.055b
	B1	0.221±0.011	45.70±1.65	39.30±2.93ab	32.3±1.63	63.9±7.93b	1.290±0.041b	0.607±0.052b	0.607±0.082b
	B2	0.247±0.007	41.70±1.34	33.70±1.35bc	26.4±2.17	67.6±2.20b	1.450±0.162b	0.537±0.048b	0.540±0.040b
	B3	0.231±0.0106	46.70±1.30	45.30±2.90a	31±2.06	88.0±5.06a	1.930±0.154a	1.080±0.184a	1.080±0.059a
	Significance	ns	ns	*	ns	**	*	**	***
BRRI dhan49	B0	0.177± 0.006b	32.30±1.86b	15.30± 1.48b	25±1.07	46.7±1.64c	0.837±0.113b	0.640±0.023c	0.643±0.047c
	B1	0.207± 0.004a	38.70±3.71ab	19.70±2.55ab	27±2.25	64.7±4.46b	1.130±0.037b	1.160± 0.087b	1.150±0.066b
	B2	0.217±0.004a	30.30± 3.06b	17.30± 1.04b	24±1.43	70.6±1.35b	0.870±0.051b	0.963±0.098bc	0.963±0.098bc
	B3	0.209±0.004a	44.30±1.37a	25.70±2.37a	26.7±0.771	83.5±3.78a	1.60±0.144a	1.640±0.205a	1.640± 0.161a
	Significance	*	*	*	ns	***	**	**	***