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PLANT NUTRITION | ORIGINAL ARTICLE

Co-inoculation of multi-trait plant growth promoting rhizobacteria promotes growth and nutrient assimilation of transplant *Aman* rice (cv. BRRI dhan49)

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ABSTRACT

Plant growth-promoting rhizobacteria (PGPR) are the rhizosphere bacteria that can enhance plant growth by a wide range of mechanisms. This study was conducted to assess the enhancement of PGPR on growth and nutrient assimilation of rice. Seeds of transplant Aman rice (cv. BRRI dhan49) were collected from Bangladesh Rice Research Institute (BRRI). A pot experiment was conducted with rice plants inoculated with two PGPR isolates MQ1 (zinc solubilizing, IAA producing, phosphate solubilizing and N_2-fixing) and MQ2 (zinc solubilizing, phosphate solubilizing and N_2-fixing) alone and in a consortium ($PGPR_{CONS.}$ = both MQ1 and MQ2) with addition of three different levels of chemical fertilizers ($RF_{0.0}$ = no fertilizer, $RF_{0.5}$ = half of the recommended dose, and $RF_{1.0}$ = full of the recommended dose) following Completely Randomized Design with three replications. Plant height at different days after transplanting, number of tillers $hill^{-1}$ and biomass yield were recorded. Plant samples were analyzed for N, P, Ca, Mg, S, Fe, Zn, Mn, Cu and Cd contents. Rice plants inoculated with the PGPR both individually or in consortium along with different doses of fertilizers showed improved plant growth and increased biomass production. The highest plant height (74.057±3.164 cm) and the maximum number of tillers $hill^{-1}$ (13.000±2.082) were recorded in $PGPR_{MQ1}RF_{1.0}$ treatment and the highest biomass production (10.275±0.541 t ha^{-1}) was recorded in $PGPR_{CONS.}RF_{1.0}$. Nitrogen content and uptake of the individual PGPR inoculated rice plants were also found to be higher in comparison with the uninoculated control plants. Besides these Fe (37.060±0.017 mg%) and Zn (5.472±0.002 mg%) content of the treated rice plants was also found to be higher in comparison with the uninoculated control plants (20.300±0.017 mg%, 4.274±0.002 mg%, respectively). N (0.268±0.046 mg pot^{-1}), P (0.626±0.095 mg pot^{-1}), Fe (6.547±0.537 mg pot^{-1}), Zn (1.237±0.197 mg pot^{-1}) and Mn (11.908±1.879 mg pot^{-1}) uptake were observed higher in $PGPR_{MQ1}RF_{1.0}$ treatment comparing with the uninoculated control plants. PGPR inoculation has immense potential to be used as rice crop inoculants as they promote plant growth as well as nutrient assimilation of rice.

Keywords: Rhizosphere bacteria, rice growth enhancement, biomass yield, essential and trace elements assimilation

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1 Introduction

Rice (*Oryza sativa*) is the most important cereal grain in the world with 503.17 million tons of global production, and it is a staple food for more than half of the world's people (Al Mamun et al., 2021). The world's population is growing, and it is estimated that 14,886 million tons of food will need to be produced by 2050 to satisfy the food demand (Khush, 2005). Additionally, rice is the primary crop grown in Bangladesh, making up about 78 percent of the nation's net cropped land (Al Mamun et al., 2021). In Bangladesh, food security is achieved by meeting up the rice demand for its 169.04 million peoples from 11.55 million hectares of cultivated gross area where rice is grown year-round throughout the *Aus*, *Aman*, and *Boro* seasons. With an output volume of 3.6 crore tonnes, Bangladesh recently ranked third globally in the production of rice, behind China and India (Al Mamun et al., 2021).

Hence, chemical fertilizer is the most important component for rice production. In order to increase agricultural output and meet the demands of the consumer market and the expanding global population, a variety of chemical fertilizers and pesticides are used, which are frequently misused in soil (Meena et al., 2017). However, continuous application of chemical fertilizers to improve soil fertility and agricultural productivity frequently has unintended negative environmental effects, such as nitrate leaching into groundwater, nitrogen (N) and phosphorus (P) surface runoff, and eutrophication of aquatic ecosystems (Adesemoye and Kloepper, 2009). Besides, using these fertilizers could lead to high production costs and potentially soil pollution after they are released into the ground. As a result, interest in environmentally friendly, organic, and sustainable agriculture practices has recently increased. In this context, Plant Growth Promoting Rhizobacteria (PGPR) may have a potential function in the creation of sustainable crop production systems, environmental plant soil soundness, and improved rice production in conjunction with the cost-effective use of chemical fertilizers (Shoebitz et al., 2009; Yanni and Dazzo, 2010).

The crop rhizosphere is home to a variety of rhizobacteria that aid in plant development, including free-living, symbionts, endophytes that colonize plant tissues, and cyanobacteria that either directly or indirectly stimulate plant growth (Farrar et al., 2014; Giri et al., 2023; Persello-Cartieaux et al., 2003).

Rhizobacteria classified as PGPR, are a diverse group of soil bacteria that colonize plant roots or the rhizosphere to promote plant growth, development and yield (Vessey, 2003). Numerous types of research showed the benefits of PGPR on the growth and yield of several crops under various soil, climatic, and temperature conditions. For instance, *Pseudomonas alcaligenes*, *Bacillus polymyxa*, and *Mycobacterium phlei* strains significantly enhanced the dry matter of the maize shoots, roots, and total by up to 38% (Egamberdiyeva, 2007). There are different plant growth promoting soil bacteria such as *Azospirillum*, *Rhizobium*, *Pseudomonas*, *Bacillus*, *Exiguobacterium*, *Chryseobacterium*, *Ralstonia*, *Kocuria*, *Serratia*, *Pantoea*, *Enterobacteria*, *Burkholderia*, and *Cyanobacteria* that are most effective in rice-microbe interactions and also enhance the growth and development of rice plant (Lucas et al., 2014; Pittol et al., 2015; Rêgo et al., 2018). However, the application of PGPR in rice has resulted in an increase in root length, shoot length, aerial biomass, and nutrient uptake (Awlachew and Mengistie, 2022; Sharma et al., 2014). *Acinetobacter*, *Alcaligenes*, *Arthrobacter*, *Azospirillium*, *Azotobacter*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Rhizobium*, and *Serratia* are a few genera where PGPR can be discovered (Karakurt et al., 2011; Sturz and Nowak, 2000; Sudhakar et al., 2000). By fixing nutrients and preventing them from leaching out, PGPR has the capacity to increase the availability of nutrient concentration in the rhizosphere (Vejan et al., 2016). For instance, nitrogen, which is essential for the synthesis of proteins and amino acids, is the most limiting nutrient for plant growth. Prokaryotes are the only organisms that have mechanisms for converting atmospheric nitrogen into plants available form (Lloret and Martínez-Romero, 2005; Raymond et al., 2004). *Azospirillum* is an uncommon type of free-living nitrogen-fixing organism that is frequently found in temperate zones with cereal crops and has also been shown to increase rice crop yields (Tejera et al., 2005). Some PGPR can solubilize phosphate (Wani et al., 2007), increasing the availability of phosphate ions in the soil, which the plants can quickly absorb. *Kocuria turfanensis* strain 2M4 was shown to be an IAA producer, a siderophore producer, and a phosphate solubilizer when it was isolated from rhizospheric soil (Goswami et al., 2014). Lavakush et al. (2014) investigated how PGPR affected rice's ability to absorb nutrients. Nitrogen fixation, phytohormones production, phosphate solubilization and increasing iron availability is the direct mechanism of PGPR that involved to support plant growth, development and nutrient uptake (Kundan et al., 2015). *Pseudomonas fluorescens*, *Pseudomonas putida*, and *Pseudomonas fluorescens* were some of the PGPR inoculants that were used in his study. In addition to promoting plant growth, the zinc and iron status of the rice genotypes was improved by PGPR inoculation, and the overall yield of the rice genotypes also increased (Sharma et al., 2013, 2014).

Biological nitrogen fixation, organic phosphorus (P) and potassium (K) solubilization, nodulation, siderophore, and phytohormone production are the direct action of PGPR, while the production of hydrolytic enzymes, exopolysaccharides, hydrogen cyanide, development of induced systemic resistance, and heavy metal detoxification is the indirect func-

tions (Mahanty et al., 2016; Trivedi et al., 2012) of PGPR. The plant root exudates released in the rhizosphere act as chemical signals for microorganisms (Chaparro et al., 2013) and perform plant-microbe interaction in the environment.

Banerjee et al. (2017) reported that the native microbes to the jhum fields exhibited enhanced seed germination, plant growth promotion, and production of Indole-3-acetic acid (IAA) in upland paddy crop fields of NE India (Banerjee et al., 2017). Further, PGPR exhibits synergistic and antagonistic interactions with the soil microbiota and offers an array of activities of ecological and economic significance (Basu et al., 2021). Genus *Azospirillum*, *Azotobacter*, *Acetobacter*, *Bacillus*, *Paenibacillus*, *Pseudomonas*, *Serratia*, *Kosakoniasacchari* (Giri, 2019; Mahanty et al., 2016), etc. are very common PGPR that helps in enhancing crop yield and overall plant growth substantially. However, the PGPR consortium was found more effective and promising than the single culture inoculation in paddy yield enhancement (Giri et al., 2023). The present study was carried out to evaluate the performance of PGPR individually and in a consortium for productivity enhancement in the transplant *Aman* rice cultivation.

2 Materials and Methods

The study was conducted in the Net House of the Department of Agricultural Chemistry to assess the effects of PGPR on growth and nutrient assimilation of transplant *Aman* rice (cv. BRRI dhan49). PGPR broth culture, pot experiments and chemical analysis of soil and plant samples were done in the postgraduate laboratories of the Department of Agricultural Chemistry, Bangladesh Agricultural University, Mymensingh.

2.1 Isolates collection and inoculums preparation

Two superior PGPR isolates (MQ1 and MQ2) were selected and used in the present study based on their performances (Table 1) in previous studies conducted at the department of Agricultural Chemistry, BAU (Khatun et al., 2021). Glycerol stocks of the two isolates were collected from Professor. Dr. Atiqur Rahman, Department of Agricultural Chemistry, BAU.

These two selected PGPR were either used individually or they were assembled to prepare a consortium for this study. They were cultured in Petri dishes containing nutrient broth agar medium containing 1% nutrient broth, 1.5% sucrose, and 1.5% agar (NBA medium, pH = 6.5) for 3 days at 28±1 °C temperature in an incubator.

2.2 Treatments

The experiments were conducted following completely randomized design (CRD) method with three replications and two factors viz. Factor A: PGPR treatments, and Factor B: Different rate of fertilizer application. Factor A included 4 treatments of PGPR. These were: $PGPR_0$ = Control (without any PGPR), $PGPR_{MQ1}$ = MQ1 inoculation, $PGPR_{MQ2}$ = MQ2 inoculation, and $PGPR_{CONS.}$ = consortium of both MQ1 and MQ2. Factor B included 3 treatments of fertilizer. These were: $RF_{0.0}$ = without any fertilizer, $RF_{0.5}$ = Half of the recommended doses of fertilizers, and $RF_{1.0}$ = Full of the recommended doses of fertilizers. The treatment combinations are as follows:

$PGPR_0RF_{0.0}$	$PGPR_0RF_{0.5}$	$PGPR_0RF_{1.0}$
$PGPR_{MQ1}RF_{0.0}$	$PGPM_{MQ1}RF_{0.5}$	$PGPR_{MQ1}RF_{1.0}$
$PGPR_{MQ2}RF_{0.0}$	$PGPR_{MQ2}RF_{0.5}$	$PGPR_{MQ2}RF_{1.0}$
$PGPR_{CONS.}RF_{0.0}$	$PGPR_{CONS.}RF_{0.5}$	$PGPR_{CONS.}RF_{1.0}$

2.3 Soil collection and pot preparation

For this study, top 15 cm soil (silt loam textured soil with pH 7.02 and electrical conductivity 0.725 dS m^{-1} and 1.063% organic matter content) was collected from Bangladesh Agricultural University farm.The morphological and physicochemical characteristics of the experimental soil have been presented in Table 2. All the morphological, physical and chemical analysis of soil samples were performed following standard methods. The soil was air dried and then mixed thoroughly. The processed soil samples were placed in the pots at the rate of 10 kg pot^{-1}. The Bangladesh Rice Research Institute recommended fertilizer doses of 149.25 kg ha^{-1} urea, 97.17 kg ha^{-1} TSP, 67.16 kg ha^{-1} MoP, 59.70 kg ha^{-1} gypsum, and 11.19 kg ha^{-1} zinc sulphate were used in this study. All the fertilizers except urea at different doses according to the treatments were added and thoroughly mixed with the soil. Urea was applied at three equal splits, 1st installment at 15 days after transplanting (DAT), 2nd installment during tillering (30 DAT) and 3rd installment at booting stage (45 DAT). Irrigation and other intercultural operations were done as required.

2.4 Seedling inoculation and transplanting

For seedling inoculation normal liquid media (1% nutrient broth, 1.5% sucrose) was prepared. All individual rhizobacteria along with the consortium were placed in the conical flask containing liquid media and kept on a mechanical shaker for 24 hours. PGPR culture suspensions with optimum growth having a cell density of $\sim 10^6$ CFU mL^{-1} were used for seedling inoculation before transplanting. Healthy one month old seedlings of BRRI dhan49 were surface sterilized by using 70% ethanol for 10 minutes.

Table 1. Salient features of the PGPR isolates used in the study (Khatun et al., 2021)

	PGPR isolate	
	MQ1	MQ2
Source	Rhizosphere of Sushni shak (*Marsilea quadrifolia*)	Rhizosphere of Sushni shak (*Marsilea quadrifolia*)
Colony morphology	Reddish pink coloured, raised and round shaped	Cream coloured, non-raised and round shaped
Gram staining reaction	Negative	Negative
Functions/ traits	Zinc solubilizing; IAA producing (1.27±0.21 μg mL^{-1}); phosphate solubilizing (Phosphate solubility index 8); N_2-fixing bacteria	Zinc solubilizing; phosphate solubilizing (Phosphate solubility index 11), N_2-fixing bacteria

Table 2. Morphological, Physical and Chemical characteristics of experimental soil

Morphological characteristics	
Location	GPB field laboratory, Bangladesh Agricultural University
AEZ	Old Brahmaputra flood plain (AEZ-9)
AEZ sub region	Non saline non calcareous
Drainage	Well drained
Land type	Medium high land
Cropping pattern	Rice-Dhaincha-Pulse
Physical characteristics	
Sand (%)	33
Silt (%)	54
Clay (%)	9
Chemical characteristics	
pH (soil: water = 1: 2.5)	7.02
Electrical conductivity (EC 1:5)	0.725 (dS m^{-1})
Organic Matter	1.06%

After that, these seedlings were washed with sterile distilled water for 4 times. Then these seedlings were placed in the conical flask containing PGPR broth culture and kept on the mechanical shaker for overnight shaking. The seedlings with roots coated with PGPR culture, were then kept in paper towel for absorbing extra aqueous mass. The rice seedlings without PGPR inoculation were used as control.

2.5 Harvesting and data collection

Observation on plant height, and number of tillers $plant^{-1}$ were recorded at the one-month interval and presented mean values of all the observations and biomass yield were recorded at the time of harvest. The plant height in the individual pot was measured with the help of a meter scale. Final plant height was measured after harvest. The number of tillers $hill^{-1}$ in the individual pot was recorded. The total number of tillers $hill^{-1}$ was recorded after harvest. The biomass yield (t/ha) in the individual pot was recorded. The straw yield was recorded after harvest.

2.6 Analysis of mineral constituents

The plant extract was prepared by wet oxidation method using di-acid mixture (HNO_3: $HCIO_4$ = 2:1) following Singh et al. (1999) to determine the total elemental composition of calcium (Ca), magnesium (Mg), phosphorous (P), potassium (K), copper (Cu), iron (Fe), zinc (Zn) and cadmium (Cd) in the extracts. Calcium and magnesium concentration of plant samples were determined by complexometric method of titration using 0.01M Na_2EDTA ($Na_2H_2C_{10}H_{12}O_8N_2.2H_2O$) as a chelating agent at pH 12 in presence of calcon indicator and at pH 10 in the presence of eriochrome black T (EBT) indicator respectively (Page et al., 1982). The P and S contents in plant samples were determined through colorimetrically using a spectrophotometer (Model: TG-60 U)

by developing phosphomolybdate blue complex at 600 nm wave length as outlined by Jackson (1973) and turbidimetrically at 425 nm wavelength as described by Tandon (1995), respectively. The oven dried grain samples (0.5 g) were digested with 8 mL H_2SO_4-H_2O_2 digestion mixture (6 mL H_2SO_4 and 2 mL H_2O_2) and 1 g catalyst mixture (K_2SO_4:$CuSO_4$.$5H_2O$:Se = 10:1:0.1) for 30 mins. at 120 °C followed by 70-90 mins. at 360-380 °C to analyze N concentration by the modified Kjeldahl method (Page et al., 1982), distillation with 10N NaOH and by titration of the distillate trapped in 2% H_3BO_3 with 0.05 NH_2SO_4 (Bremner and Mulvaney, 1982). Determination of different elements (Fe, Zn, Cu, Mn, Pb, and Cd) in plant samples was done by using an AAS (Model no: SHIMADZU, AA7000, Japan).

2.7 Statistical analysis

The data of the various parameters was analyzed in triplicates and subjected to ANOVA (Analysis of variance) in accordance with the experimental design (Completely randomized design) using 'Graphpad Prism6' statistical package to quantify and evaluate the source of variation. The treatment means were compared at a significance level of 0.05 and the ranking of treatments denoted by alphabets. The treatments denoted by different letters in the each column of tables and figures represent significantly different values among the treatments.

3 Results

The present research work was undertaken to evaluating the effects of plant growth promoting rhizobacteria on growth and nutrient assimilation of transplant *Aman* rice (cv. BRRI dhan49).

3.1 Plant height

Plant height of BRRI dhan49 in response to the inoculation of two selected PGPR isolates both individually and in consortia along with different doses of the recommended fertilizers were increased significantly (ANOVA, P<0.0001) with the progression of growing period. The lowest plant heights at different treatments were observed in 15 DAT and the highest were observed in 120 DAT. Besides, plant height of BRRI dhan49 at different DAT varied significantly with different treatments of PGPR (ANOVA, P<0.0001). However, two way ANOVA followed by Tukey's multiple comparison test revealed no significant variation among $PGPR_0RF_{0.0}$, and different treatments of PGPR. Here, the treatment $PGPR_{MQ1}RF_{1.0}$ produced the highest plant height (74.057±3.164 cm) at 120 DAT. On the other hand, the lowest plant height (26,780±0.588 cm) was resulted by the treatment $PGPR_{MQ2}RF_{1.0}$ at 15 DAT (Fig. 1).

3.2 Number of tillers hill^{-1}

The number of tillers hill^{-1} of BRRI dhan49 in response to the inoculation of two selected PGPR isolates both individually and in consortia along with different doses of the recommended fertilizers were increased significantly (ANOVA, P<0.0001) with the progression of growing period. The lowest number of tillers hill^{-1} at different treatments was observed in 15 DAS and the highest were observed in 120 DAS. However, the treatment $PGPR_{MQ1}RF_{1.0}$ produced the highest number of tillers hill^{-1} (13.000±2.082) at 120 DAT. On the other hand, the lowest number of tillers hill^{-1} (6.000±1.528) was resulted by the treatment $PGPR_{MQ2}$RF0 at 15 DAT (Fig. 2).

3.3 Biomass yield

Biomass yield of BRRI dhan49 in response to the inoculation of two selected PGPR isolates both individually and in consortia along with different doses of the recommended fertilizers were increased significantly (ANOVA, P<0.0001). However, two way ANOVA followed by Tukey's multiple comparison test revealed no significant variation among $PGPR_0RF_{0.0}$, and different treatments of PGPR except $PGPR_0RF_{1.0}$, $PGPR_{MQ1}RF_{1.0}$, $PGPR_{MQ2}RF_{1.0}$, and $PGPR_{CONS.}RF_{1.0}$. However, the treatment $PGPR_{CONS.}RF_{1.0}$ produced the highest biomass yield (10.275±0.541 t ha^{-1}). On the other hand, the lowest biomass yield (3.383±0.352 t ha^{-1}) was resulted by the treatment $PGPR_0RF_{0.0}$ (Fig. 3).

3.4 Mineral contents and their uptake by rice plants

Mineral contents and their uptake by the treated rice plants was estimated to observe whether PGPR application had any effect on the nutrient and other mineral content and uptake by BRRI dhan49. N, P, Ca, S, Fe, Zn, Mn, Cu, and Cd levels in BRRI dhan49 and their uptake by the rice plants were significantly different in response to the inoculation of two selected PGPR isolates both individually and in consortia along with different doses of the recommended fertilizers (ANOVA, P<0.0001). Individual PGPR inoculation had positive effect in increasing N concentration in treated BRRI dhan49 plant with increasing doses of recommended fertilizers when compared with the control plants. Moreover, the performance of individual isolates was better than their consortium in increasing of N concentration in BRRI dhan49. $PGPR_{MQ1}RF_{1.0}$ and $PGPR_{MQ2}RF_{0.5}$ treatments produced the highest N concentration (1.120.017%), whereas $PGPR_{CONS.}RF_{1.0}$ produced the

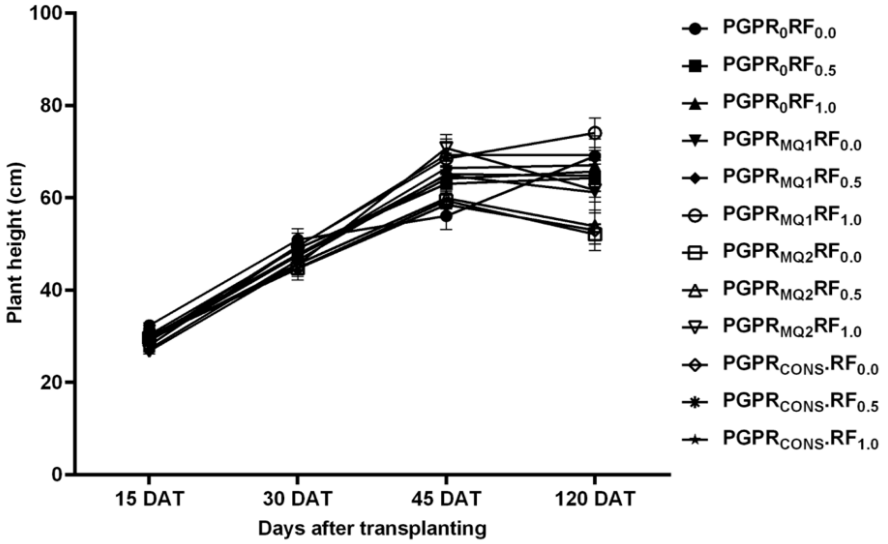

Figure 1. Plant height of BRRI dhan49 at different days after transplanting (DAT) with inoculation of PGPR individual and consortium along with different doses of fertilizer treatments. $PGPR_0$ = PGPR control (without any PGPR), $PGPR_{MQ1}$ = MQ1 isolate, $PGPR_{MQ2}$ = MQ2 isolateand $PGPR_{CONS.}$ = combination of MQ1 and MQ2, $RF_{0.0}$ = Fertilizer control (without any fertilizer), $RF_{0.5}$ = Half of the recommended doses of fertilizers and $RF_{1.0}$ = Full of the recommended doses of fertilizers

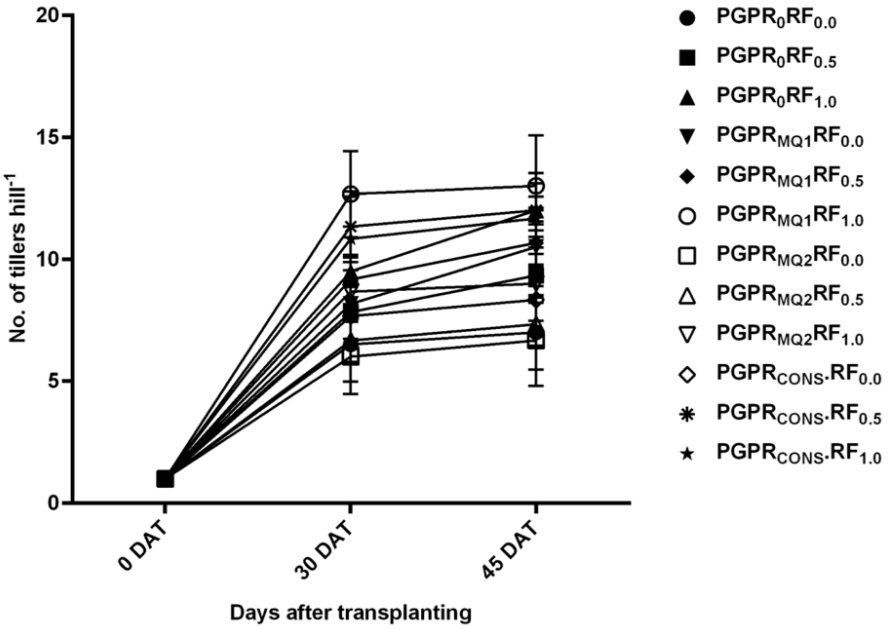

Figure 2. Number of tillers $hill^{-1}$ of BRRI dhan49 at different days after transplanting (DAT) with inoculation of PGPR individual and consortium along with different doses of fertilizer treatments. $PGPR_0$ = PGPR control (without any PGPR), $PGPR_{MQ1}$ = MQ1 isolate, $PGPR_{MQ2}$ = MQ2 isolateand $PGPR_{CONS.}$ = combination of MQ1 and MQ2, $RF_{0.0}$ = Fertilizer control (without any fertilizer), $RF_{0.5}$ = Half of the recommended doses of fertilizers and $RF_{1.0}$ = Full of the recommended doses of fertilizers

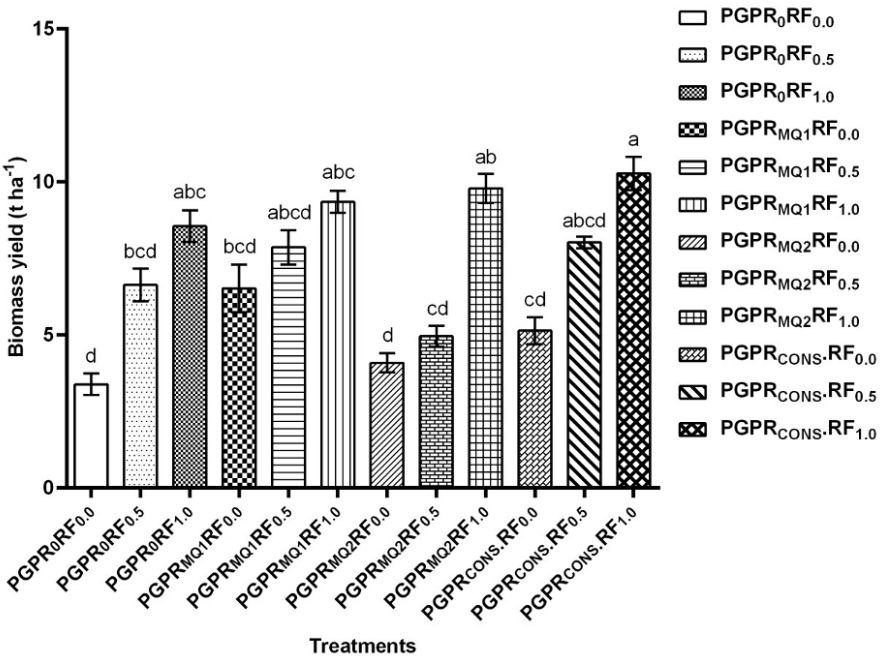

Figure 3. Biomass yield of BRRI dhan49 at 120 DAT after inoculation of PGPR individual and consortium along with different doses of fertilizer treatments. $PGPR_0$ = PGPR control (without any PGPR), $PGPR_{MQ1}$ = MQ1 isolate, $PGPR_{MQ2}$ = MQ2 isolateand $PGPR_{CONS.}$ = combination of MQ1 and MQ2, $RF_{0.0}$ = Fertilizer control (without any fertilizer), $RF_{0.5}$ = Half of the recommended doses of fertilizers and $RF_{1.0}$ = Full of the recommended doses of fertilizers

lowest N content (0.4480.002%) in BRRI dhan49 (Fig. 4).

Individual PGPR inoculation also had positive effect in increasing N uptake by treated BRRI dhan49 plant with increasing doses of recommended fertilizers when compared with the control plants. Moreover, the performance of MQ1 isolate was better than both MQ2 and their consortium in increasing of N uptake by BRRI dhan49. $PGPR_{MQ1}RF_{1.0}$ given the highest (0.268 ± 0.046 mg pot^{-1}) nitrogen uptake and treatment $PGPR_{MQ2}RF_{0.0}$ given the lowest (0.056 ± 0.008 mg pot^{-1}) nitrogen uptake by treated BRRI dhan49 (Fig. 5).

Inoculation of PGPR either individually or consortium had positive effects in increasing P concentration in treated BRRI dhan49 plant and these effects are more prominent with lesser doses of recommended fertilizer applications when compared with the control plants. The maximum P content in BRRI dhan49 plant was found in $PGPR_{CONS.}RF_{0.0}$ (3.3600.017 mg%), and the lowest was found in $PGPR_0RF_{0.0}$ (1.9100.017 mg %) (Fig. 6).

Inoculation of PGPR either individually or consortium had positive effects in increasing P uptake by treated BRRI dhan49 plant and these effects are more prominent with higher doses of recommended fertilizer applications when compared with the control plants. $PGPR_{MQ1}RF_{1.0}$ resulted the highest (0.626 ± 0.095 mg pot^{-1}) and $PGPR_{MQ2}RF_{0.0}$ showed the lowest (0.185 ± 0.045 mg pot^{-1}) P uptake by BRRI dhan49 plant (Fig. 7).

Inoculation of PGPR consortium had positive effects in increasing Ca concentration in treated BRRI dhan49 plant when compared with the control plants. $PGPR_{MQ1}RF_{0.5}$, $PGPR_0RF_{1.0}$, $PGPR_{MQ1}RF_{0.5}$, $PGPR_{MQ2}RF_{1.0}$, $PGPR_{CONS.}RF_{0.0}$ and PGPRCons.$RF_{1.0}$ treatments produced the highest Ca concentration in BRRI dhan49 (0.72 ± 0.012%) and the lowest (0.56 ± 0.029%) was resulted by the treatment $PGPR_{MQ1}RF_{1.0}$ and $PGPR_{MQ2}RF_{0.0}$ (Table 3).

Inoculation of PGPR either individually or in consortium had positive effects in increasing Ca uptake by treated BRRI dhan49 plant when compared with the control plants. The treatment $PGPR_{CONS.}RF_{1.0}$ produced the highest (0.152 ± 0.015 g pot^{-1}) calcium (Ca) uptake by BRRI dhan49 plant and the lowest (0.047 ± 0.006 g pot^{-1}) was resulted by the treatment $PGPR_{MQ2}RF_{0.0}$ (Table 4).

Inoculation of PGPR either individually or in consortium had no significant effect on Mg concentration in treated BRRI dhan49 plant when compared with the control plants. However, the Mg concentrations were decreased with the increase of doses of fertilizer applications. However, the highest Mg concentration (0.29 ± 0.023%) were resulted in $PGPR_0RF0$, $PGPR_{MQ2}RF0$, $PGPR_{MQ2}RF_{0.5}$, and $PGPR_{CONS.}RF_{0.0}$ treatments whereas the lowest (0.19 ± 0.017%) were resulted in $PGPR_0RF_{1.0}$ treatment (Table 3).

Inoculation of PGPR either individually or in

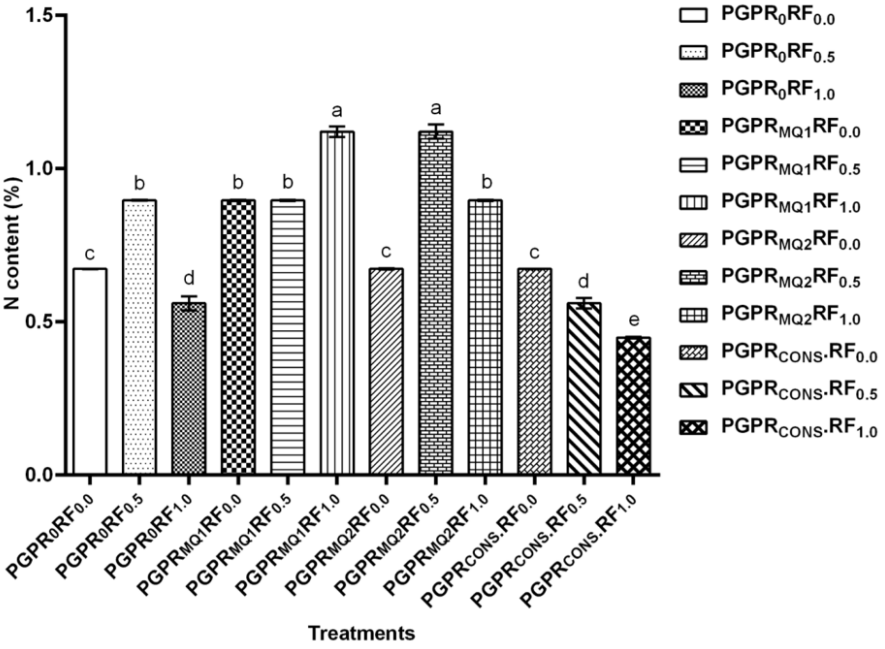

Figure 4. Nitrogen contents of BRRI dhan49 at 120 DAT after inoculation of PGPR individual and consortium along with different doses of fertilizer treatments. $PGPR_0$ = PGPR control (without any PGPR), $PGPR_{MQ1}$ = MQ1 isolate, $PGPR_{MQ2}$ = MQ2 isolateand $PGPR_{CONS.}$ = combination of MQ1 and MQ2, $RF_{0.0}$ = Fertilizer control (without any fertilizer), $RF_{0.5}$ = Half of the recommended doses of fertilizers and $RF_{1.0}$ = Full of the recommended doses of fertilizers

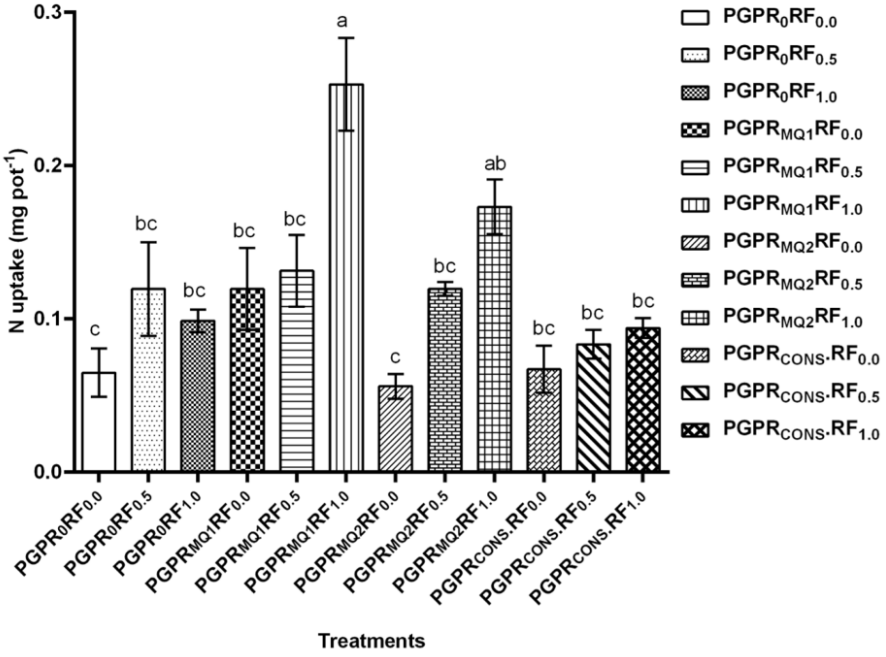

Figure 5. Nitrogen uptake by BRRI dhan49 at 120 DAT after inoculation of PGPR individual and consortium along with different doses of fertilizer treatments. $PGPR_0$ = PGPR control (without any PGPR), $PGPR_{MQ1}$ = MQ1 isolate, $PGPR_{MQ2}$ = MQ2 isolateand $PGPR_{CONS.}$ = combination of MQ1 and MQ2, $RF_{0.0}$ = Fertilizer control (without any fertilizer), $RF_{0.5}$ = Half of the recommended doses of fertilizers and $RF_{1.0}$ = Full of the recommended doses of fertilizers

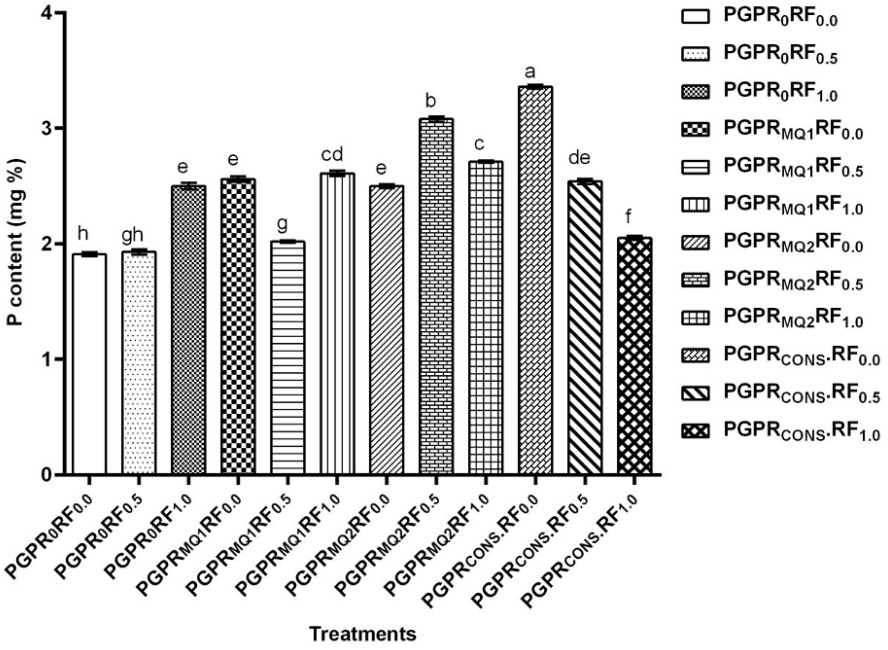

Figure 6. Phosphorus contents of BRRI dhan49 at 120 DAT after inoculation of PGPR individual and consortium along with different doses of fertilizer treatments. $PGPR_0$ = PGPR control (without any PGPR), $PGPR_{MQ1}$ = MQ1 isolate, $PGPR_{MQ2}$ = MQ2 isolateand $PGPR_{CONS.}$ = combination of MQ1 and MQ2, $RF_{0.0}$ = Fertilizer control (without any fertilizer), $RF_{0.5}$ = Half of the recommended doses of fertilizers and $RF_{1.0}$ = Full of the recommended doses of fertilizers

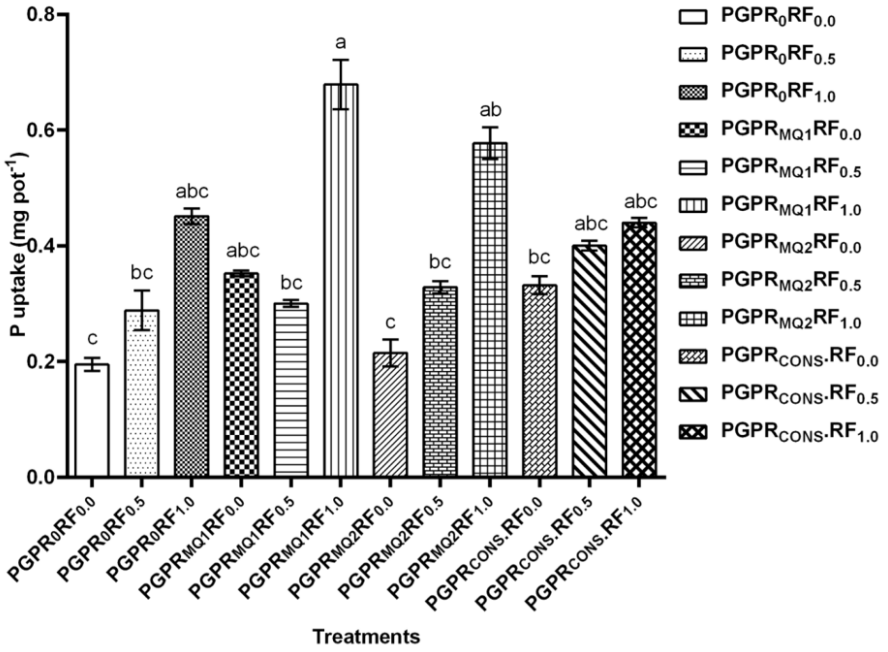

Figure 7. Phosphorus uptake by BRRI dhan49 at 120 DAT after inoculation of PGPR individual and consortium along with different doses of fertilizer treatments. $PGPR_0$ = PGPR control (without any PGPR), $PGPR_{MQ1}$ = MQ1 isolate, $PGPR_{MQ2}$ = MQ2 isolateand $PGPR_{CONS.}$ = combination of MQ1 and MQ2, $RF_{0.0}$ = Fertilizer control (without any fertilizer), $RF_{0.5}$ = Half of the recommended doses of fertilizers and $RF_{1.0}$ = Full of the recommended doses of fertilizers

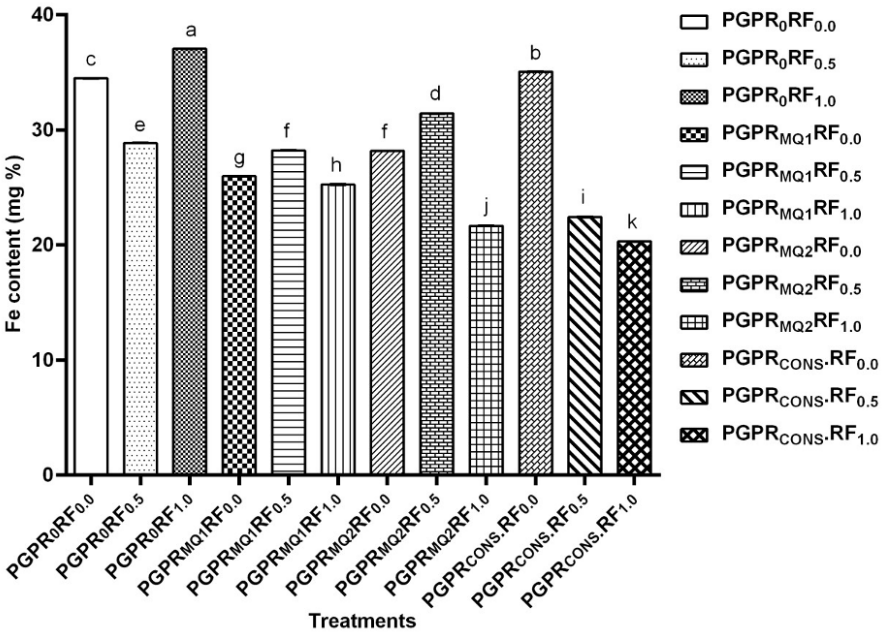

Figure 8. Iron contents of BRRI dhan49 at 120 DAT after inoculation of PGPR individual and consortium along with different doses of fertilizer treatments. $PGPR_0$ = PGPR control (without any PGPR), $PGPR_{MQ1}$ = MQ1 isolate, $PGPR_{MQ2}$ = MQ2 isolateand $PGPR_{CONS.}$ = combination of MQ1 and MQ2, $RF_{0.0}$ = Fertilizer control (without any fertilizer), $RF_{0.5}$ = Half of the recommended doses of fertilizers and $RF_{1.0}$ = Full of the recommended doses of fertilizers

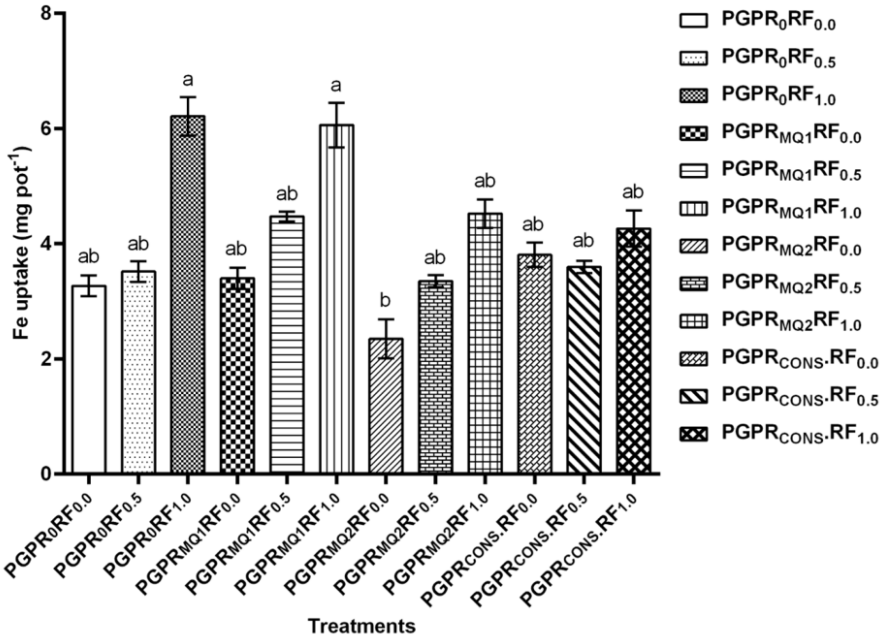

Figure 9. Iron uptake by BRRI dhan49 at 120 DAT after inoculation of PGPR individual and consortium along with different doses of fertilizer treatments. $PGPR_0$ = PGPR control (without any PGPR), $PGPR_{MQ1}$ = MQ1 isolate, $PGPR_{MQ2}$ = MQ2 isolateand $PGPR_{CONS.}$ = combination of MQ1 and MQ2, $RF_{0.0}$ = Fertilizer control (without any fertilizer), $RF_{0.5}$ = Half of the recommended doses of fertilizers and $RF_{1.0}$ = Full of the recommended doses of fertilizers

consortium had significant positive effect on Mg uptake by treated BRRI dhan49 plant when compared with the control plants. Moreover, this effect is more prominent in treatments with higher doses of recommended fertilizers. The highest Mg uptake by BRRI dhan49 plant (0.058±0.015 g pot^{-1}) was resulted in $PGPR_{MQ1}RF_{1.0}$ treatment whereas the lowest (0.0005±0.010 g pot^{-1}) was resulted in $PGPR_{MQ1}RF_{0.0}$ treatment (Table 4).

PGPR inoculation had negative effect in increasing S concentration in treated BRRI dhan49 plant except inoculation of consortium with full recommended dose of fertilizer application when compared with the control plants. The treatment $PGPR_{CONS.}RF_{1.0}$ produced the highest S (7.750±0.017 mg %) and $PGPR_{CONS.}RF_{0.5}$ showed the lowest (1.920±0.012 mg %) S content of BRRI dhan49 (Table 3).

PGPR inoculation had negative effect in increasing S uptake by treated BRRI dhan49 plants except inoculation of consortium with full recommended dose of fertilizer application when compared with the control plants (Table 4). $PGPR_{CONS.}RF_{1.0}$ treatment produced the highest S uptake (1.627±0.115 mg pot^{-1}) and $PGPR_{CONS.}RF_{0.5}$ and $PGPR_{MQ2}RF_{0.0}$ showed the lowest (0.2823±0.040 mg pot^{-1}) sulphur uptake by BRRI dhan49 plants (Table 4).

PGPR inoculation had negative effect in increasing Fe concentration in treated BRRI dhan49 plant except inoculation of consortium without any recommended dose of fertilizers when compared with the control plants. The treatment $PGPR_0RF_{1.0}$ given the highest (37.060±0.017 mg%) and treatment $PGPR_{CONS.}RF_{1.0}$ given the lowest (20.300±0.017 mg %) Fe content of BRRI dhan49 (Fig. 8).

Treatments with or without PGPR inoculation had statistically comparable effect in increasing Fe uptake by treated BRRI dhan49 plants. $PGPR_0RF_{1.0}$ treatment given the highest Fe uptake (6.547±0.537 mg pot^{-1}) and $PGPR_{MQ2}RF_{0.0}$ given the lowest (2.348±0.339 mg pot^{-1}) Fe uptake by BRRI dhan49 plants (Fig. 9).

PGPR inoculation had negative effect in increasing Zn concentration in treated BRRI dhan49 plants except inoculation of PGPR consortium without any recommended dose of fertilizers when compared with the control plants. Moreover, the performance of MQ1 isolate was better than MQ2 in increasing of Zn concentration in BRRI dhan49 plants. $PGPR_{CONS.}RF_{0.0}$ produced the highest Zn contents in plants (5.472±0.002 mg%) and the lowest (4.274±0.002 mg%) was resulted by the treatment $PGPR_{MQ2}RF_{0.0}$ (Fig. 10).

Treatments with or without PGPR inoculation had statistically comparable effect in increasing uptake by treated BRRI dhan49 plants. $PGPR_{MQ1}RF_{1.0}$ treatment resulted the highest Zn uptake (1.237±0.197 mg pot^{-1}) and the $PGPR_{MQ2}RF_{0.0}$ treatment resulted the lowest Zn uptake (0.356±0.051 mg pot^{-1}) by BRRI dhan49 plants (Fig. 11).

Individual PGPR inoculation had positive effect in increasing Mn concentration in treated BRRI dhan49 plants when compared with the control plants. However, the Mn concentration decreased with increasing doses of recommended fertilizers. The treatment $PGPR_{MQ1}RF_{0.0}$ produced the highest Mn content in BRRI dhan49 plants (62.926±0.002 mg%) and $PGPR_{CONS.}RF_{1.0}$ showed the lowest (42.801±0.002 mg%) (Table 3).

Treatments with individual PGPR isolate inoculation and full dose of recommended fertilizers had positive effect in increasing Mn uptake by treated BRRI dhan49 plants when compared with the control plants. However, the Mn uptake increased with increasing doses of recommended fertilizers. $PGPR_{MQ1}RF_{1.0}$ treatment resulted the highest Mn uptake (11.908±1.879 mg pot^{-1}) by BRRI dhan49 plants and the lowest Mn uptake (5.120±0.739 mg pot^{-1}) was resulted from $PGPR_{MQ2}RF_{0.0}$ treatment (Table 4).

Inoculation of PGPR consortium had positive effects in increasing Cu concentration in treated BRRI dhan49 plant when compared with the control plants and the performance of consortium was superior to the individual isolates. The treatment $PGPR_{CONS.}RF_{1.0}$ produced the highest Cu content of BRRI dhan49 (0.472±0.001 mg%) and the lowest copper Cu content was resulted from the treatment $PGPR_{MQ1}RF_{0.0}$ and $PGPR_{MQ1}RF_{0.5}$ which was below detectable level (Table 3).

Inoculation of PGPR consortium had positive effects in increasing Cu uptake by treated BRRI dhan49 plants when compared with the control plants and the performance of consortium was superior to the individual isolates. Moreover, without PGPR inoculation, the Cu uptakes were decreased with increase of fertilizer doses but with inoculation the Cu uptakes were increased with increase of fertilizer doses. The treatment $PGPR_{CONS.}RF_{1.0}$ resulted the highest (0.099±0.007 mg pot^{-1}) Cu uptake by BRRI dhan49 plants and the lowest Cu uptake was resulted by $PGPR_{MQ1}RF_{0.0}$ and $PGPR_{MQ1}RF_{0.5}$ treatments which is below detectable limit.

Inoculation of MQ2 isolate had positive effects in increasing Cd concentration in treated BRRI dhan49 plant when compared with the control plants and the performance of MQ2 isolate was superior to the MQ1 isolate. $PGPR_{MQ2}RF_{0.5}$ treatmentgiven the highest Cd (0.068±0.002 mg %) and $PGPR_{MQ1}RF_{0.0}$ showed the lowest (0.011±0.002 mg%) Cd concentration in BRRI dhan49 (Table 3).

Inoculation of PGPR isolate had positive effects in increasing Cd uptake by treated BRRI dhan49 plants when compared with the control plants and the effect was most prominent in treatments with higher doses of fertilizers. The treatment $PGPR_{MQ2}RF_{0.5}$ produced the highest Cd uptake (0.007±2.961e-004 mg

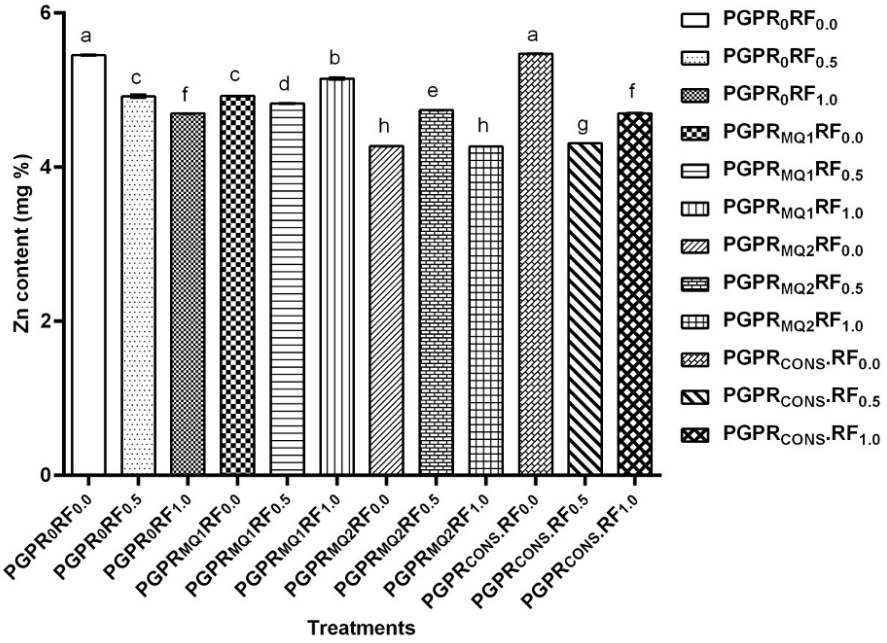

Figure 10. Zinc contents of BRRI dhan49 at 120 DAT after inoculation of PGPR individual and consortium along with different doses of fertilizer treatments. $PGPR_0$ = PGPR control (without any PGPR), $PGPR_{MQ1}$ = MQ1 isolate, $PGPR_{MQ2}$ = MQ2 isolateand $PGPR_{CONS.}$ = combination of MQ1 and MQ2, $RF_{0.0}$ = Fertilizer control (without any fertilizer), $RF_{0.5}$ = Half of the recommended doses of fertilizers and $RF_{1.0}$ = Full of the recommended doses of fertilizers

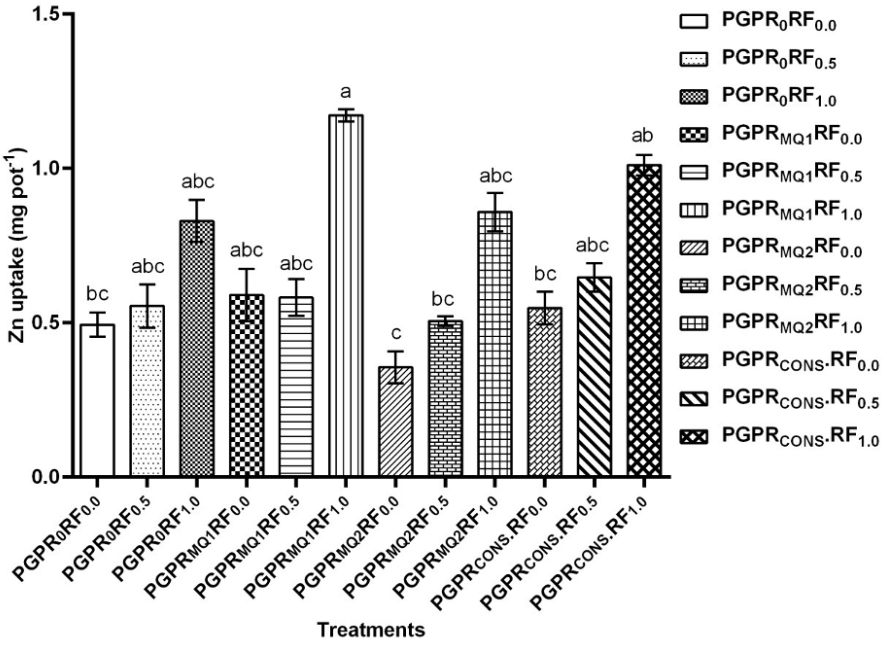

Figure 11. Zinc uptake by BRRI dhan49 at 120 DAT after inoculation of PGPR individual and consortium along with different doses of fertilizer treatments. $PGPR_0$ = PGPR control (without any PGPR), $PGPR_{MQ1}$ = MQ1 isolate, $PGPR_{MQ2}$ = MQ2 isolateand $PGPR_{CONS.}$ = combination of MQ1 and MQ2, $RF_{0.0}$ = Fertilizer control (without any fertilizer), $RF_{0.5}$ = Half of the recommended doses of fertilizers and $RF_{1.0}$ = Full of the recommended doses of fertilizers

Table 3. Other mineral nutrient contents (Mean±SD) in BRRI dhan49 at 120 DAT after inoculation of PGPR individual and consortium along with different doses of fertilizer treatments

Treatments	Ca (%)	Mg (%)	S (mg %)	Mn (mg %)	Cu (mg %)	Cd (mg %)
$PGPR_0RF_{0.0}$	0.64±0.023ab	0.29±0.023a	4.51±0.023c	59.95±0.002d	0.36±0.002b	0.018±0.002bc
$PGPR_0RF_{0.5}$	0.72±0.012a	0.24±0.012a	3.00±0.012g	61.96±0.001b	0.10±0.002d	0.052±0.002a
$PGPR_0RF_{1.0}$	0.72±0.023a	0.19 ±0.017a	4.47±0.017c	55.56±0.001f	0.03±0.001ef	0.014±0.001c
$PGPR_{MQ1}RF_{0.0}$	0.64±0.017ab	0.24 ±0.023a	3.19±0.023f	62.93±0.002a	0.00±0.00f	0.011±0.002c
$PGPR_{MQ1}RF_{0.5}$	0.72±0.017a	0.24±0.012a	2.80±0.029h	43.19±0.002k	0.00±0.00f	0.021b±0.002c
$PGPR_{MQ1}RF_{1.0}$	0.56±0.029b	0.24±0.029a	3.05±0.012g	49.62±0.002i	0.05±0.012e	0.025b±0.001c
$PGPR_{MQ2}RF_{0.0}$	0.56±0.023b	0.29 ±0.023a	3.39±0.029e	61.44±0.002c	0.15±0.002c	0.021±0.002bc
$PGPR_{MQ2}RF_{0.5}$	0.64±0.017ab	0.29±0.023 a	4.71±0.023b	58.33±0.002e	0.16±0.001c	0.068±0.002a
$PGPR_{MQ2}RF_{1.0}$	0.72±0.012a	0.24±0.012a	2.19±0.012i	55.47±0.001g	0.15±0.002c	0.027±0.002bc
$PGPR_{CONS.}RF_{0.0}$	0.72±0.017a	0.29±0.023 a	3.51±0.017d	53.72±0.002h	0.11±0.002d	0.03±0.002b
$PGPR_{CONS.}RF_{0.5}$	0.64±0.023ab	0.24±0.023a	1.92±0.012j	46.73±0.002j	0.37±0.002b	0.01±0.002c
$PGPR_{CONS.}RF_{1.0}$	0.72±0.023a	0.19±0.029a	7.75±0.017a	42.80±0.002l	0.47±0.001a	0.023±0.002bc
Maximum	0.72±0.012	0.29±0.023	7.75±0.017	62.93±0.002	0.47±0.001	0.068±0.002
Minimum	0.56±0.029	0.19±0.029	1.92±0.012	46.73±0.002	0.00±0.00	0.01±0.002
EMS	0.0013	0.0014	0.0012	0.0001	0.0163	0.00003
Sig. level	***	NS	***	***	***	***

PGPR0= PGPR control (without any PGPR), $PGPR_{MQ1}$ = MQ1 isolate, $PGPR_{MQ2}$ = MQ2 isolateand $PGPR_{CONS.}$ = combination of MQ1 and MQ2, $RF_{0.0}$ = Fertilizer control (without any fertilizer), $RF_{0.5}$ = Half of the recommended doses of fertilizers and $RF_{1.0}$ = Full of the recommended doses fertilizers. Means followed by common letter in the column are not significantly different.

Table 4. Mineral nutrients uptake (mean±SD) by BRRI dhan49 at 120 DAT after inoculation of PGPR individual and consortium along with different doses of fertilizer treatments

Treatments	Ca (%)	Mg (%)	S (mg %)	Mn (mg %)	Cu (mg %)	Cd (mg %)
$PGPR_0RF_{0.0}$	0.062±0.015bc	0.028±0.007a	0.435±0.105bcd	5.795±1.399ab	0.035±0.008bc	0.002±4.104e-004cd
$PGPR_0RF_{0.5}$	0.095±0.023abc	0.032±0.010a	0.401±0.103bcd	8.262±2.096ab	0.013±0.004cde	0.007±0.002ab
$PGPR_0RF_{1.0}$	0.12746±0.013abc	0.034±0.005a	0.789±0.064b	9.816±0.807ab	0.006±4.473e-004de	0.002±3.571e-004bcd
$PGPR_{MQ1}RF_{0.0}$	0.086±0.021abc	0.0005±0.010a	0.426±0.097bcd	0.151±1.865ab	0.000±0.00e	2.64e-05±0.001d
$PGPR_{MQ1}RF_{0.5}$	0.105±0.017abc	0.036±0.007a	0.411±0.075bcd	6.33±1.124ab	0.000±0.00e	0.003±4.729e-004a-d
$PGPR_{MQ1}RF_{1.0}$	0.073±0.027ab	0.058±0.015a	0.732±0.118bc	11.908±1.879a	0.012±0.008cde	0.006±0.001abc
$PGPR_{MQ2}RF_{0.0}$	0.047±0.006c	0.024±0.003a	0.282±0.040d	5.121±0.739b	0.012±0.002cde	0.002±3.765e-004cd
$PGPR_{MQ2}RF_{0.5}$	0.068±0.003abc	0.031±0.003a	0.502±0.016bcd	6.222±0.194ab	0.017±0.001cde	0.007±2.961e-004a
$PGPR_{MQ2}RF_{1.0}$	0.139±0.016ab	0.047±0.004a	0.423±0.043bcd	10.725±1.125ab	0.028±0.003cd	0.005±0.001a-d
$PGPR_{CONS.}RF_{0.0}$	0.071±0.015abc	0.029±0.004a	0.352±0.083cd	5.372±1.240ab	0.011±0.002de	0.003±0.002a-d
$PGPR_{CONS.}RF_{0.5}$	0.095±0.010abc	0.037±0.003a	0.288±0.037d	7.009±0.934ab	0.055±0.008b	0.002±0.001cd
$PGPR_{CONS.}RF_{1.0}$	0.152±0.015a	0.041±0.009a	1.628±0.115a	8.988±0.653ab	0.099±0.007a	0.005±1.419e-004a-d
Maximum	0.152±0.015	0.058±0.015	1.628±0.115	11.908±1.879	0.099±0.007	0.007±2.961e-004
Minimum	0.047±0.006	0.0005±0.010	0.282±0.040	5.121±0.739	0.000±0.00	2.64e-05±0.001
EMS	0.0013	0.0006	0.1252	4.99	0.0006	0.000002
Sig. level	***	NS	***	***	***	***

PGPR0= PGPR control (without any PGPR), $PGPR_{MQ1}$ = MQ1 isolate, $PGPR_{MQ2}$ = MQ2 isolateand $PGPR_{CONS.}$ = combination of MQ1 and MQ2, $RF_{0.0}$ = Fertilizer control (without any fertilizer), $RF_{0.5}$ = Half of the recommended doses of fertilizers and $RF_{1.0}$ = Full of the recommended doses fertilizers. Means followed by common letter in the column are not significantly different.

pot^{-1}) and $PGPR_{MQ1}RF_{0.0}$ showed the lowest Cd uptake (0.002±0.001 mg pot^{-1}) by BRRI dhan49 plants (Table 4).

4 Discussion

The vegetative growth phase is the most crucial growth stage for every crop since it determines how much biomass is produced, especially in rice where it is crucial for the growth of tillers. A crop with vigorous vegetative development will have a higher plant height, more leaves and tillers, and consequently more root and shoot dry mass (Sharma et al., 2014). In our investigation, plant height, number of tiller plant-1, and plant dry mass were all found to be higher in response to the PGPR inoculation.

Similar to that, after applying PGPR, rice plants that were 30 days old showed a 20% increase in plant height (Ashrafuzzaman et al., 2009). Along with a rise in plant height, studies by Kumar et al. (2012), de Salamone et al. (2012), Ashrafuzzaman et al. (2009), and Sharma et al. (2014) reported that rice plants treated with the plant growth promoting rhizobacteria also had an increase in the number of tillers and leaves.

Moreover, it was discovered that the treated plants had higher plant dry matter than the untreated control plants. The rise in plant vigor and the improvement in the nutrient uptake of the plants might be correlated with the increase in plant dry mass. In similar studies, Gholami et al. (2009) reported an increase in root dry mass in response to treatment with *P. putida*, *P. fluorescence*, and *A. lipoferum* in maize; besides, de Souza et al. (2012) reported an almost 30% increase in root dry mass in response to bacterial isolates in rice; Rana et al. (2012) also reported an increase in root dry mass in wheat plants in response to PGPR treatment.

Additionally, a rise in the plant's dry matter has a direct impact on productivity (Sharma et al., 2014). In our study, bacterial treatments increased plant vigor and the number of tillers, suggesting that the treated plants may have produced more photosynthates than the untreated control plants, and leading to an increase in the above-ground biomass of the rice plants. In response to bacterial inoculation in maize (Gholami et al., 2009) and rice (de Salamone et al., 2012; Sharma et al., 2014), an increase in shoot dry mass has been documented.

A key characteristic of rhizosphere bacteria that promotes and facilitates plant growth is the generation of indole acetic acid (IAA) (Mohite, 2013). Indole acetic acid (IAA) production of the PGPR isolate also might be responsible for the increased vegetative growth of the inoculated transplant *Aman* rice (cv. BRRI dhan49) plants.

Inoculation with PGPR either alone or in consortium also showed mixed response for assimilation of different mineral elements like N, P, Ca, Mg, S, Fe, Zn, Mn, Cu and Cd in transplant *Aman* rice (cv. BRRI dhan49) plants.

Phosphate solubilizing capacity of the PGPR isolates might be responsible for higher P content and uptake by inoculated transplant rice (cv. BRRI dhan49) plants. Lavakush et al. (2014) reported that PGPR treatment combinations with 30 kg ha^{-1} P were economically more advantageous than PGPR with 60 kg ha^{-1} P.

Due to the cumulative effects of PGPR activities in the rhizosphere especially phytosiderophore production (Sayyed et al., 2012), it is not surprising that the PGPR inoculation also showed significantly higher Fe and Zn uptake by inoculated transplant *Aman* rice (cv. BRRI dhan49) plants over $PGPR_0$. Singh et al. (2016) also reported significantly increased rice yield, nutrients uptake and their soil availability compared to uninoculated conditions.

The increased Cd uptake by transplanted *Aman* rice (cv. BRRI dhan49) plants treated with PGPR inoculation and full doses of chemical fertilizer over $PGPR_0$ has, however, given rise to food safety concerns. More research is needed to determine whether PGPR inoculations play a role in the accumulation of heavy metals in rice.

5 Conclusion

The application of PGPR resulted in improving the vegetative growth of the transplant *Aman* rice (cv. BRRI dhan49) plants and also helped in increasing the dry matter accumulation of the plants. Besides, improving growth of the plants, inoculation with PGPR also resulted in improving the zinc and iron status of transplant *Aman* rice (cv. BRRI dhan49). Hence it can be concluded that the application of PGPRs in rice not only increases the vegetative growth of the plant but also improve the micronutrient status of the crop. Therefore, judicious application of plant growth promoting rhizobacteria to rice holds immense potential that is still to be explored and it can also provide an answer to the global demand for the development of a sustainable strategy for biofortification.

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Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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