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PGPRs inoculations enhances the grain yield and grain nutrient content in four cultivars of rice (*Oryza sativa* L.) under field condition

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Abstract

The effects of the inoculation of plant growth promoting rhizobacterial (PGPR) strains *Bacillus cereus* BSB 38 (14B) *Pseudomonas jessenii* R62, *Pseudomonas synxantha* R81, *Arthrobacter nitroguajacolicus* strainYB3 and strain YB5 on grain yield and grain nutrient content for four genotypes of rice namely, Swarna, Swarna sub1, IR-64, and IR-64 sub1 under field condition. Two consortia, one is *Pseudomonas jessenii* and *Pseudomonas synxantha* (R62 + R81), another consortia of *Arthrobacter nitroguajacolicus* strain YB3 and YB5 (A3 + A5), and one *Bacillus* species (14B) were used as a bioinoculant. Under field condition, PGPRs treated plants showed higher grain yield as compare to their respective control. In all the varieties the grains of inoculated plants had remarkably higher quantity of protein and carbohydrates as well as phosphorus and potassium in respect of their control plants. The grains of treated plants had efficiently higher quantity of four micronutrient, like iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu). Present result indicated that the selected PGPR had greater effect on the nutrients mobilization in rice seeds and thus have shown to be a valid option for sustainable high quality rice production to improve the nutritional status and health of the population.

Keywords: PGPR, rice, micronutrient, protein, carbohydrate

Introduction

Rice (*Oryza sativa*, L) is the leading food grain crop of the world. Worldwide, more than 3.5 billion people depend on rice for more than 20% of their daily calorie intake [1]. In order to ensure food security for growing population agricultural productivity must increase proportionately. Among the different methods of enhancing nutrients quantity and availability for plant utilization is the use of chemical fertilization, which is a fast way of providing plants with necessary macro- and micro-nutrients. To fulfill the nutritional demand of rapid growing world population the use of chemicals has tremendously increased [2]. The increased use of chemical fertilizers is now started to displaying their ill effects such as leaching, polluting water basins, destroying microorganisms and friendly insects, making the crop more susceptible to the attack of diseases, reducing the soil fertility and thus causing irreparable damage to the overall system [3]. One of the alternative methods of providing nutrients for plant growth and yield production is use of soil microbes [4]. Bacteria that colonize the rhizosphere and plant roots, and enhance plant growth by any mechanism are referred to as plant growth-promoting rhizobacteria (PGPR) [5]. These PGPR promote plant growth in two different ways: By direct mechanism PGPR are capable of fixing atmospheric nitrogen, solubilizing phosphorus and iron, and of producing plant hormones, such as auxins, gibberelins, cytokinins, and ethylene [6]. Indirect mechanisms involve the biological control of plant pathogens and thus can improve significantly plant health and promote growth, as evidenced by increases in seedling emergence, vigor, and yield [7]. The plant– microbe interactions in the rhizosphere play a pivotal role in transformation, mobilization, solubilization, etc. of nutrients from a limited nutrient pool. In this regards the use of PGPR as biological approaches is becoming more popular as an additive to chemical fertilizers for improving crop yield in an integrated plant nutrient management system [8].

Present study deals with the PGPRs, *Bacillus cereus* BSB 38 (14B) *Pseudomonas jessenii* R62, *Pseudomonas synxantha* R81, *Arthrobacter nitroguajacolicus* strainYB3 and strain YB5 on grain yield and grain nutrient content for four genotypes of rice namely, Swarna, Swarna sub1, IR-64, and IR-64 sub1 under field condition.

Materials and Methods

Experimental site

The present investigation was carried out during kharif 2012 at the Breeders Seed Production

Centre (BSPC) of Gobind Ballabh Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar (Uttarakhand).

Experimental material
The seeds of four rice genotypes namely, Swarna, Swarna sub1, IR-64, and IR-64 sub1 were collected from the IRRI Office, NASC Complex, Pusa New Delhi, India.

Organic manure used
Before designing the experiment, *Sesbenia aculeata* (Dhaincha) as organic manure was sown in the field at the rate of 20 kg /hectare. The above-ground biomass of 45 days of standing crop of *sesbenia* puddle properly into the pre irrigated field 10 days before rice planting with the help of tractor.

Raising nursery
Sixteen raised nursery beds having a width of 1.5 m and length of 3 m providing 50 cm channels all around were prepared well before sowing of seed. The nursery beds were prepared with a massive mixture of soil. The seeds were spread @ 50 kg /ha in the raised bed. The seeds were treated with PGPR before sowing.

Field preparation for transplanting
In the month of July, after proper mixing of standing crop of *sesbenia* in to soil field was properly leveled. Thereafter, the layout was made and bunds were constructed for separating the plots. Day before transplanting, the field was flooded with water and puddled manually with the help of spade. The bunds were prepared and individual plots were leveled manually.

Bioinoculant treatment
Three bacterial treatments were used for the growth promotion of four rice varieties in organic farming field. Two consortia, one is *Pseudomonas jessenii* and *Pseudomonas synxantha* (R62 + R81), another consortia of *Arthrobacter nitroguajacolicus* strain YB3 and YB5 (A3 + A5), and one *Bacillus* species (14B) were used as a bioinoculant. The bacterial treatment was given twice, at the time of seed sowing (seed treatment) and at the time of transplantation (seedling treatment). The seeds were treated with overnight grown bacterial culture having cfu 10^7- 10^8 and left for 30 min for air drying then sowing in the prepared plot of each treatment. Another treatment was given to rice seedling at the time of transplantation. The seedlings were uprooted and roots of plants were dipped in the overnight grown bacterial culture having cfu 10^7- 10^8 for 20 minute.

Design and Layout
The field experiment was carried out in randomized block design with three replications of each treatment for each variety. The plots were separated from each other with the help of proper bunds in order to prevent leaching of PGPR between the plots having different PGPR treatments.

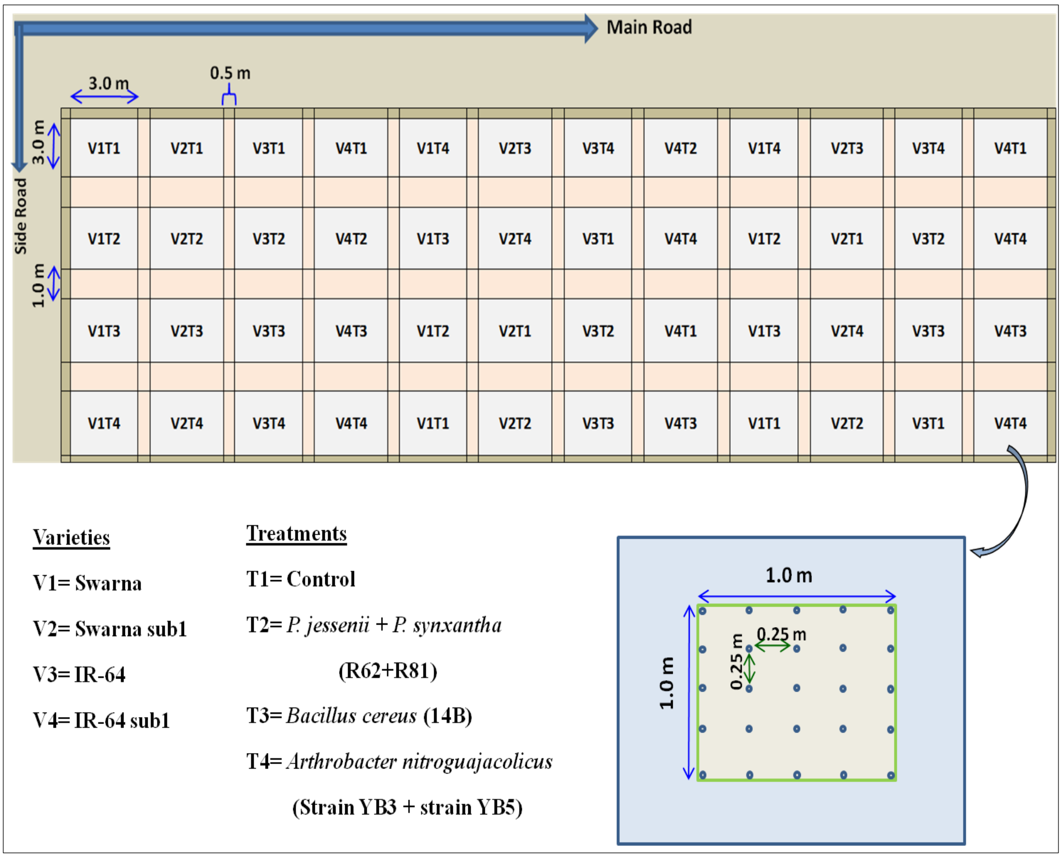

Fig 1: The treatments verities control

Transplanting
After treatments the seedlings were sown in their respective plots. Two seedlings were transplanted per hill and the distance between two hills was 25 x 25 cm. Light irrigation was given after two days of transplanting.

Water management and weed control
Soil was kept moist or slightly flooded during all the growth phase of rice. Water supply was stopped just before harvesting. The weeds were removed from field by hand at regular intervals.

Crop harvesting

Harvesting was done manually when more than 90 per cent of grains in the panicles were fully ripened. After removing the border plants (two rows all around) the sampling area 1m^2 was harvested manually. The produce of individual plot was threshed by Pullman thresher next day of the harvesting. The grain produce was collected separately in cloth bags during threshing and weight of individual plots were recorded after cleaning.

Grain yield

The plant sampling area, 1m × 1 m (1m^2) was harvested from each plot area. The grains were separated by threshing separately from each net plot and were dried under sun for three days. Later winnowed and cleaned and then weight of the grains per net plot was recorded. From the net plot values, the grain yield per hectare was computed and expressed in tones per hectare (t/ha).

Chemical analysis of rice grain samples

For estimation of phosphorus, potassium and micronutrients (Cu, Zn, Mn and Fe), 01 g of ground seed sample was transferred into 150 ml conical flask, 10 ml di-acid mixture (nitric and perchloric acid mixture, 4:1 v/v) was added and left for overnight. Then the contents were digested on a hot plate until digestion was completed. After cooling the flask, 5 ml of 6 N HCL was added to each flask and boiled gently on hot plate. After cooling, the digested material was filtered through a whatman No. 42 filter paper and was transferred in to 100 ml volumetric flask and volume was made up to 75 ml with distilled water. Then the digested samples were analyzed for phosphorus and potassium. For estimation of phosphorus, Five ml aliquot was taken in 50 ml volumetric flask and 10 ml molybdovanadate solution was added. After 25 min when yellow color had fully developed, then absorbance was read on UV-V spectrophotometer at 420 nm. For estimation of potassium, the concentration of potassium in seed sample was determined by flame photometer in digested material after standardizing the flame photometer with known concentrations of potassium. The concentration of four micronutrient (Zn, Cu, Mn and Fe) in seed samples was determined by Atomic Absorption Spectrophotometer (AAS) in digested material after standardizing the AAS with known concentrations of micronutrient.

Statistical analysis

The field experiment was carried out in randomized block design with three replications of each treatment for each variety. The data presented here are mean values ± SD. The data were subjected to factorial analysis of variance (ANOVA), with varieties and treatments used for analysis and the differences between the means were compared using least significant differences at $P<0.05$.

Results and Discussion

Present study deals with three genera of PGPR, *Pseudomonas*, *Bacillus* (14B) and *Arthrobacter*, for their contribution to the growth promotion and nutrient uptake in four cultivar of rice, Swarna, Swarna sub1, IR-64, IR-64 sub1, under field condition. In present study two *Pseudomonas* species *P. jessenii* (R62) and P. *synxantha* (R81) were used as consortia similarly two *Arthrobacter* strain YB3 and YB5 were used as consortia. The experimental findings obtained during the course of investigation are summarized and presented here.

Table 1: Physiochemical properties of field soil

Source	pH	EC (dS/m)	OC (%)	N (kg/ha)	P (kg/ha)	K (Kg/ha)	Micronutrients (µgm/gm soil)			
							Cu	Mn	Fe	Zn
Breeders Seed Production centre (BSPC), GBPUA&T Pantnagar.	7.58	0.87	0.89	186.07	15.24	121.77	0.59 ± 0.03	7.07± 0.07	18.25± 0.03	0.70± 0.06

Grain yield

All the PGPR treated plants showed the higher grain yield as compared to non inoculated plants. When we compared the treatments irrespective of varieties, R62+R81 treated plants showed the maximum grain yield (6.89 ton/h) followed by the consortia of *Arthrobacter* (A3+A5) (Table 2). Among the varieties, irrespective of treatments, Swarna sub1 showed the significantly higher grain yield. When all the treatments compared within varieties, R62+R81 treated plants showed the maximum grain yield in IR-64 sub1 variety of rice.

In present study the PGPR treated plants showed the higher grain yield as compared to untreated plants. Similar results of higher grain yield were obtained by [9] when they evaluated three different biofertilizers (based on *Azospirillum*, *Trichoderma*, or unidentified rhizobacteria) for the growth promotion of rice during four cropping seasons under fully irrigated condition with different rates of inorganic fertilizer. Similarly [10] observed the 25% higher yield in diazotrophic rhizobacteria treated plants of rice as compared to untreated plants. Similar enhanced grain yield were observed [11] when they used *Rhizobium leguminosarum* bv. *Trifolii* as a biofertilizer for rice under field condition. Previous study [12] showed the higher grain yield in rice only in second year cropping system when they used the R62+R81 as a biofertilizer in different location of India under different dozes of nitrogen fertilizers. They also observed the higher grain yield of rice in the area where the *S. aculeata* used as a green manure prior to rice. In present study, results showed that the consortia of two PGPR had the greater effect on grain yield as compared to single PGPR. These results is in agreement with study [13] where they demonstrated that certain mixtures of PGPR strains significantly increased the yield over that by their respective single strains. The PGPR strains are reported to induce plant growth by producing plant growth regulators like gibberellins, cytokinins and indole acetic acid [14] which can either directly or indirectly modulate the plant growth and development. However, enhanced growth promotion depends on the bacterial strains, method of application and amount of inoculums used [13].

Table 2: Effect of PGPR on grain yield (ton/hectare) of rice varieties.

Treatments/Varieties	control	*Arthrobacter* (A3+A5)	R62+R81	14B	Average
Swarna	5.54 ^{abc}	6.71 ^{efgh}	6.75 ^{efgh}	6.67 ^{defg}	6.42 ^{b}
Swarna sub1	6.25 ^{cdef}	7.38 ^{gh}	7.5 ^{gh}	7.29 ^{fgh}	7.10 ^{c}
IR-64	5 ^{a}	5.46 ^{abc}	5.54 ^{abc}	5.13 ^{ab}	5.28 ^{a}

IR-64 sub1	5.63 abcd	5.84 abcde	7.75 h	6.17 bcde	6.35 b
Average	5.60 a	6.35 ab	6.89b	6.31 ab	

Effect of PGPR on nutrient status of rice seeds

Plant growth promoting bacteria (PGPR), when associated with host plants; stimulate the growth of host due to increased mobility, uptake and enrichment of nutrients in the plant [15]. PGPR are known to employ one or more direct and indirect mechanisms of action to improve plant growth and health, although the major mode of action of many PGPR is through increasing the availability of nutrients for the plant in the rhizosphere region [16]. In present study we have also checked the effect of PGPR on the nutrient acquisition in rice grains.

Phosphorus uptake

The *Pseudomonas* consortia of R62+R81 showed the enhenced effect on the phosphorus uptake by rice seeds, when we compared all the treatments with each other irrespective of varieties (Table 3). When we compared the varieties, irrespective of treatments, IR-64 sub1 showed the higher uptake of phosphorus as compared to other varieties of rice. When we compared all the treatments within varieties, R62+R81 treated plants of Swarna variety of rice showed maximum phosphorus uptake. The consortia of *Pseudomonas* R62 and R81 treated plants showed the enhenced phosphorus uptake in the rice seeds as compared to control plants. Similar observation were found [12] when they used R62+R81 as biofertilizer in rice plants under field condition with different doses of nitrogen fertilizers. Similarly, higher phosphorus uptake by *Azospirillum brasilense* and *Pseudomonas fluorescens* treated paddy rice were observed under field condition [17]. Our result showed the varietal differences in phosphorus uptake. It is believed that the nutrients uptake in grains affected by inoculation of strains [18].

Table 3: Effect of PGPR on Phosphorus uptake (%) in grains of four varieties of rice.

Treatments/Varieties	control	*Arthrobacter* (A3+A5)	R62+R81	14B	Average
Swarna	3.06 de	3.34 ef	3.49 f	2.36 a	3.06 bc
Swarna sub1	2.43 ab	2.58 abc	3.02 de	3.36 ef	2.85 a
IR-64	2.76 bcd	2.61 abc	3.28 ef	3.34 ef	3.00 b
IR-64 sub1	3.16 def	2.85 cd	3.39 ef	3.38 ef	3.20 c
Average	2.85 a	2.84 a	3.29 b	3.11 ab	

Potassium uptake

All the PGPR treated plants showed the non significant effect on the potassium uptake in rice grains when compared to control plants, irrespective of varieties (Table 4). Among the varieties, irrespective of treatments, Swarna variety of rice showed the significantly higher uptake of potassium as compared to other varieties. Among all the treatments within varieties R62+R81 and Arthrobacter (A3+A5) showed the maximum potassium uptake in Swarna variety of rice. In previous study of **[12]** the consortia of R62+R81 showed the significant effect on the potassium uptake in rice grains. Some study performed under controlled condition showed the higher potassium uptake in rice grains when the plants were treated with PGPRs [18].

Table 4: Effect of PGPR on Potassium uptake (%) in grains of four varieties of rice

Treatments/Varieties	control	*Arthrobacter* (A3+A5)	R62+R81	14B	Average
Swarna	0.23 d	0.24 d	0.24 d	0.23 d	0.24 c
Swarna sub1	0.21 b	0.22 c	0.21 bc	0.21 c	0.21 b
IR-64	0.21 b	0.21 bc	0.21 b	0.21 b	0.21 b
IR-64 sub1	0.19 a	0.19 a	0.21 b	0.21 b	0.20 a
Average	0.21 a	0.22 a	0.22 a	0.21 a	

Table 5: Effect of PGPR on Total protein content (kg/ton) in grains of four varieties of rice.

Treatments/Varieties	control	*Arthrobacter* (A3+A5)	R62+R81	14B	Average
Swarna	2.79 fgh	2.73 efg	2.95 hi	2.86 ghi	2.83 c
Swarna sub1	1.95 a	2.58 cde	2.95 hi	3.16 j	2.66 b
IR-64	2.51 bcd	2.65 def	2.51 bcd	3.06 ij	2.68 b
IR-64 sub1	1.99 a	2.4 bc	2.32 b	2.32 b	2.26 a
Average	2.31 a	2.59 ab	2.68 b	2.85 b	

Total protein and Carbohydrate

The seeds of treated plants showed the higher proteins as compared to seeds of control plants. When we compared the treatments with control irrespective of varieties, 14B and R62+R81 showed significant effect on total protein content of rice seeds (Table 5). When we compared the varieties with each other, irrespective of treatments, Swarna showed the maximum amount of protein contents in their seeds. When we compared the treatments with control plots, irrespective of varieties, all the treated plot showed the higher amount of carbohydrates in rice seeds (Table 6). Within the varieties, irrespective of treatments, Swarna showed the significantly higher amount of carbohydrates in seeds, whereas Swarna sub1 showed the minimum amount of carbohydrates in seeds. When we compared all the treatments within varieties *Arthrobacter* (A3+A5) showed the maximum amount of total carbohydrates in Swarna variety of rice. In present study two PGPR consortia were used. Consortia of *Pseudomonas* R62+R81 showed the significant effect on the protein content of the rice seeds. The similar consortia of R62+R81 have also been reported for significant effect on crude protein uptake in rice seeds [12].

Table 6: Effect of PGPR on total carbohydrate content (kg/ton) in grains of four varieties of rice.

Treatments/Varieties	control	*Arthrobacter* (A3+A5)	R62+R81	14B	Average
Swarna	146.61 f	148.43 f	147.33 f	146.61 f	147.25 c
Swarna sub1	134.7 a	135.33 ab	137.89 c	137.61 c	136.38 a
IR-64	138.5 cd	138.65 cd	137.63 c	138.65 cd	138.36 b
IR-64 sub1	137.04 bc	139.96 de	141.65 e	137 bc	138.91 b
Average	139.21 a	140.59 a	141.13 a	139.97 a	

Table 7: Effect of PGPR on Fe content (gram/ton rice seeds) of four varieties of rice

Treatments/Varieties	control	*Arthrobacter* (A3+A5)	R62+R81	14B	Average
Swarna	15.33 a	21.33 a	20.2 a	17.58 a	18.61 ab
Swarna sub1	13.04 a	16.04 a	19.3 a	14.8 a	15.79 a
IR-64	15.18 a	16.23 a	25.3 a	17.91 a	18.65 ab
IR-64 sub1	18.89 a	25.71 a	25.83 a	23.91 a	23.58 b
Average	15.61 a	19.83 ab	22.66 b	18.55 ab	

Micronutrients

Micronutrients are essential for plants, humans and animals, and increasing the micronutrient density of staple crops such as rice can play a critical role in improving human nutrition on a global scale. In spite of the yield effect of the selected PGPR on the rice the present study also focus on the effect of PGPR on the micronutrient acquisition in rice grains.

Iron (Fe) uptake

All the treatments showed the higher Fe uptake in rice grains when compared to control plants, irrespective of varieties. R62+R81 treated plots showed the more pronounced enhanced effect on the Fe uptake (Table 7). When we compared all the cultivar of rice with each other irrespective of treatments, IR-64 sub1 showed the maximum effect on Fe uptake, whereas Swarna sub1 showed least effect on iron uptake. When we compared all the treatments within all the cultivar of rice R62+R81 treated plots of IR-64 sub1 showed the maximum Fe uptake in rice grains. Some studies on cereals inoculated with PGPR showed the higher Fe acquisition in grains when the experiments were conducted in the controlled condition [19] and under field condition [20]. In present study the R62+R81 showed the pronounced effect on the Fe acquisition in the rice seeds. The similar results of R62+R81 were also observed [12].

Manganese (Mn) uptake

All the treatments showed the greater effect on Mn uptake by rice plants as compared to control irrespective of varieties. R62+R81 treated plots showed the maximum Mn uptake in rice grains (Table 8). Among the different cultivars of rice, irrespective of treatments, IR-64 sub1 showed the significant effect on the Mn uptake. When we compared the treatments within all the cultivar of rice *Arthrobacter* A (A3+A5) treated plots of IR-64 sub1 showed the maximum effect on the Mn uptake in grains. In present study all the PGPR showed the increased effect on the Mn uptake in rice grains. Similar trends on Mn uptake by cereal grains were also observed [12].

Table 8: Effect of PGPR on Mn content (gram/ton rice seeds) of four variety of rice

Treatments/Varieties	control	*Arthrobacter* (A3+A5)	R62+R81	14B	Average
Swarna	22.41 ab	21.66 ab	28.86 ab	21.17 ab	23.53 a
Swarna sub1	22.98 ab	23.01 ab	23.46 ab	16.26 a	21.43 a
IR-64	21.21 ab	20.61 ab	25.56 ab	27.33 ab	23.68 a
IR-64 sub1	26.73 ab	36.25 b	28.75 ab	35.61 b	31.83 b
Average	23.33 a	25.38 a	26.66 a	25.09 a	

Zinc (Zn) uptake

All the treatments showed the greater effect on the Zn uptake in rice grains when we compared the treatments with control irrespective of varieties. However, only R62+R81 showed the significant effect on Zn uptake as compare to control (Table 9). In the present study all the PGPR inoculums showed the higher Zn mobilization in rice grains. These results were supported [12], who used the consortia of R62+R81 as a biofertilizer in field and [21] in field microplots demonstrated the efficiency of a commercial mixed PGPR consortium (containing *Pseudomonas* sp. and other strains of PGPR) acting as Zn solubilizer and increasing Zn up to 157%.

Table 9: Effect of PGPR on Zn content (gram/ton rice seeds) of four variety of rice

Treatments/Varieties	control	*Arthrobacter* (A3+A5)	R62+R81	14B	Average
Swarna	19.23 abcd	16.45 abc	26.2 d	22.49 bcd	21.09 b
Swarna sub1	13.56 a	18.59 abcd	22.68 bcd	14.76 abc	17.40 a
IR-64	14.69 abc	14.05 ab	19.19 abcd	19.23 abcd	16.79 a
IR-64 sub1	18.93 abcd	26.13 d	22.94 cd	22.75 bcd	22.68 b
Average	16.60 a	18.80 ab	22.75 b	19.81 ab	

Table 10: Effect of PGPR on Cu content (gram/ton rice seeds) of four variety of rice

Treatments/Varieties	control	*Arthrobacter* (A3+A5)	R62+R81	14B	Average
Swarna	9.7 a	11.05 a	9.14 a	11.28 a	10.29 ab
Swarna sub1	9.66 a	10.98 a	9.81 a	10.34 a	10.20 a
IR-64	10.68 a	11.69 a	11.58 a	11.05 a	11.25 b

IR-64 sub1	10.15 [a]	11.2 [a]	10.53 [a]	10.49 [a]	10.59 [ab]
Average	10.05 [a]	11.23 [b]	10.26 [ab]	10.79 [ab]	

Copper (Cu) uptake

Similar to the Zn uptake all the treatments showed the greater effect on the Cu uptake in rice grains as compared to control, irrespective of varieties. The *Arthrobacter* (A3+A5) treated plots showed the significant effect on Cu uptake in grains over control (Table 10). Among the four cultivar of rice IR-64 showed the maximum effect on Cu uptake in grains. When we compared all the treatments within varieties *Arthrobacter* (A3+A5) treated plot of IR-64 showed the maximum Cu uptake. All the PGPRs showed the enhanced Cu mobilization in rice seeds as compared to control plants. Various experiments in the cereal plants inoculated with PGPRs conducted under controlled condition [19] and field condition [12] have also mentioned the similar trends.

Conclusion

The results of this study showed that all the used PGPR had the pronounced effect on rice grain yield under field condition. However the consortia of two PGPR showed the greater effect on the grain yield of rice as compared to single PGPR. The result also indicated that the selected PGPR had greater effect on the nutrients mobilization in rice seeds. In present study, microbial inoculants have shown to be a valid option for sustainable high quality rice production under organic farming field, promising to improve the nutritional status and health of the population.

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