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Short Communications

Influence of plant growth-promoting rhizobacteria inoculation on nutrient availability, soil microbial properties and defence enzymes in rice (*Oryza sativa*) crop

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Rice (*Oryza sativa* L.) is the important staple food in several developing countries including India. Chemical fertilizer is the most important input required for optimal rice yield. The high-yielding rice varieties require large amount of chemical fertilizers, leading to health hazards and environmental pollution. In order to make rice cultivation sustainable and less dependent on chemical fertilizers, it is important to use alternate nutrient sources like plant growth-promoting rhizobacteria (PGPR) and blue green algae (BGA). They can biologically fix nitrogen, solubilize phosphorus and induce plant growth promoting substances like indole acetic acid (IAA) which can contribute in improvement of rice growth (Kloepper 1992). In response to PGPR application, the plants produce defence enzymes including peroxidases (POD), phenylalanine ammonia-lyase (PAL) and polyphenoloxidase (PPO). Peroxidase and PPO are catalysts in the formation of lignin. Phenylalanine ammonia-lyase (PAL) and other enzymes are also involved in the formation of phytoalexins (Pal *et al.* 2011). Induced systemic resistance (ISR) mediated by PGPR against *Pythium aphanidermatum* in cucumber associated with an increase of POD, PPO and PAL was found (Chen *et al.* 2000). So, there is a growing interest in PGPR due to their efficacy as biological control and growth promoting agents in many crops (Thakuria *et al.* 2004). With this background, the present study was undertaken to evaluate the effect of cyanobacterial and bacterial PGPR inoculants on plant defence status and soil chemical and microbial parameters under transplanted rice crop.

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The field experiment was conducted during rainy season (June-October) of 2011 at the research farm of Indian Agricultural Research Institute, New Delhi situated at a latitude of 28°40' N, longitude of 77°12' E and altitude of 228.6 meters above the mean sea level. The initial soil sample of field had 145.3 kg/ha alkaline permanganate oxidizable N, 15.37 kg/ha available P, 254.2 kg 1 N ammonium acetate exchangeable K, 0.52% organic carbon and 7.7 pH. The experiment was laid out in randomized block design with twelve treatments and three replications. The treatments included: N_0 nitrogen(N_0); 2/3 N(80 kg/ha) through urea; N120 (120 kg N/ha through urea, recommended dose of N); 2/3 N (80 kg N)+C@ 5.0 tonnes/ha; 2/3 N+BI; 2/3 N+BI+C@ 5.0 tonnes/ha; 2/3 N+CI; 2/3N+CI+C@ 5.0 tonnes/ha; 2/3N+CI+BI; 2/3N+BI+CI+C@ 5.0 tonnes/ha; 2/3N+*Multani mitti*(Fullers' earth*)* based BGA biofertilizer and 2/3N+C+*Multani mitti* based BGA biofertilizer. In our study one bacterial (*Providencia* sp. PW5), one cyanobacterial strains (*Anabaena* sp CR3) and *Multani mitti* based BGA biofertilizer (composite culture of *Anabaena, Nostoc, Tolypothrix* and *Aulosira* sp) applied @ 2.0 kg/ha were evaluated in a field experiment. PGPR strains and BGA biofertilizer was obtained from Division of Microbiology, IARI, New Delhi. In this experiment equal dose of P (60 kg P_2O_5/ha) and K (60 kg K_2O/ha) were applied through single super phosphate (SSP) and muriate of potash (MOP) fertilizers. Twenty-one days old seedlings of rice variety Pusa Basmati 1401 were transplanted at 20 cm × 10 cm spacing with 2 seedlings/hill. A water depth of 3-5 cm was maintained from transplanting to grain filling stage of crop and later the soil was kept moist only. The gross plot size was 5.0 m × 3.0 m. Soil samples were taken at mid season stage (60 DAT) and analysed for microbial activity in term of dehydrogenase activity (Casida *et al.* 1964), ARA (Prasanna *et al.* 2003) and soil chlorophyll (Nayak *et al.* 2004). ARA was utilized as an index of nitrogen fixation and measured as per the modified method after incubation of soil cores under field conditions for 3 hour (Prasanna *et al.* 2003). POD (436 nm) and PPO (410 nm) activity was done according to Pal *et al.* (2011) as the rate of increase in absorbance at respective wave length with minor modification. Data were performed by one-way analysis of variance (ANOVA) using statistical software (SPSS 10). Duncan's multiple range test (DMRT) was used to detect a significant difference between means at $P \leq 0.05$.

Results showed that soil organic carbon (SOC) in soil at crop maturity stage increased due to the integrated inoculation of PGPR and *Multani mitti* based BGA with application of compost and chemical N fertilizer (80 and 120 kg/ha) over N control (Table 1). The highest SOC (0.56%) were recorded with 2/3N+BI+CI+C@ 5.0 tonnes/ha and it was significantly higher than N_0. SOC contents were increased with the application of compost in addition to PGPR or BGA. Cyanobacteria also add organic matter, synthesize and liberate amino acids, vitamins and auxins, reduce oxidizable matter in soil, provide oxygen to the submerged rhizosphere, buffer the pH, solubilize phosphates and increase fertilizer use efficiency of in crop plants (Kaushik 2004).

Available contents of macro-nutrients like N, P and K and micronutrient Fe, Zn and Cu were increased in soil at crop maturity stage with bacterial, cyanobacterial PGPR and BGA, when inoculated with compost and chemical N fertilizer (80 and 120 kg N/ha) over N_0 (Table 1). The available N at crop maturity was highest with 2/3N+BI+CI+C@ 5.0 tonnes/ha but at par with 2/3N+BI+C, 2/3N+CI+C and 2/3N+C+BGA inoculation of PGPR and BGA and statistically higher over rest treatments. The available P was highest with 2/3N+BI+CI+C @ 5.0 tonnes/ha (21.9 kg/ha) and it was at par with 2/3N+BI+C, 2/3N+CI+ C and 2/3N+C+BGA inoculation of PGPR and BGA. Available K and micronutrient Fe, Zn and Cu also were reported highest with 2/3N+BI+CI+C@ 5.0 tonnes/ha and it was significantly higher over N_0 and 2/3N. Microbial inoculants have capacity to promote plant growth, enhance nutrient availability and uptake, and support the health of plants (Adesemoye *et al.* 2009). Swarnalakshmi *et al.* (2013) also found increased available nitrogen content with the single and combined inoculation of *Anabaena* with bacterial inoculants compared with uninoculated control. Some bacteria may solubilize inorganic P due to excretion of organic acids (Vessey 2003). PGPR help in solubilization of mineral phosphates and other nutrients and increasing organic matter content. PGPR retain more soil organic N, and other nutrients in the plant-soil system, thus reducing the need for fertilizer N and P and enhancing release of the nutrients. (Hayat *et al.* 2010)

Dehydrogenase activity, soil chlorophyll and acetylene reduction activity (ARA) in soil at mid season stage (60 DAT) of rice crop increased due to the integrated inoculation of PGPR and *Multani mitti* based BGA with application of compost and chemical N fertilizer (80 and 120 kg/ha) over N control (Table 2). Dehydrogenase activity was highest with 2/3N+BI+CI+C@ 5.0 tonnes/ha and it was significantly higher than other treatments. The enhancement in dehydrogenase and alkaline phosphatase activities with increasing dose of fertilizer as well as organic amendments is reflections of organic matter build up by incorporation of green manuring and which led to increase in microbial activities (Prasanna *et al.* 2003). Microbial inoculants are reported to significantly increase the activities of urease, alkaline and acid phosphatase and dehydrogenase in the soils of all wheat-rice and wheat-blackgram crops (Mader *et al.* 2011).

Soil chlorophyll was significantly higher with 2/3N+BI+CI+C@ 5.0 tonnes/ha over other treatments (Table 2). Swarnalakshmi *et al.* (2013) also found increased soil chlorophyll from 4 to 14 weeks with inoculation of *Anabaenae-Pseudomonas* bio-film in rice. ARA also was significantly higher with 2/3N+BI+CI+C@ 5.0 tonnes/ha over other treatments while lowest ARA was recorded in N_0. Chebotar *et al.* (2001) also found enhanced nodulation

Table 1 Effect of PGPR on OC, available N, P, K and available Fe, Cu, and Zn in soil of rice at crop maturity stage

Treatment	Soil OC (%)	Availabe N (kg/ha)	Availabe P (kg/ha)	Availabe K (kg/ha)	Available Fe (g/kg)	Available Cu (g/kg)	Available Zn (g/kg)
N_0	0.51	80.7	15.8	253.0	14.2	1.28	2.09
2/3 N(80 kg/ha)	0.52	144.8	16.6	258.5	14.6	1.37	2.14
N_{120}	0.54	159.3	17.9	266.0	15.8	1.57	2.19
2/3N+C	0.54	156.5	16.7	268.3	16.0	1.54	2.17
2/3 N+BI	0.54	149.4	16.8	263.8	15.5	1.52	2.20
2/3 N+BI+C	0.55	177.3	19.1	267.1	16.8	1.61	2.33
2/3 N+CI	0.53	148.0	16.3	266.5	15.5	1.54	2.19
2/3N+CI+C	0.55	179.9	19.2	269.5	16.5	1.69	2.31
2/3N+CI+BI	0.55	156.8	18.2	263.0	15.9	1.66	2.25
2/3N+BI+CI+C	0.56	186.10	21.9	272.1	17.6	1.79	2.39
2/3N+BGA	0.54	163.1	17.2	258.6	15.3	1.62	2.28
2/3N+C+BGA	0.55	181.7	19.7	270.8	16.8	1.65	2.36
SEm±	0.009	5.47	1.02	3.08	0.57	0.06	0.06
LSD (P=0.05)	0.03	16.03	3.0	9.06	1.66	0.18	0.18

BI, Bacterial inoculants; CI, cynobacterial inoculants; C, compost @ 5.0 tonnes/ha; BGA, BGA biofertilizer and ARA of soybean due to the inoculation of *P. fluorescens* in soybean roots.

Peroxidase and PPO enzymes in rice plant leaf stage were significantly enhanced at mid season (60 DAT) due to the integrated inoculation of PGPR and *Multani mitti* based BGA with application of compost and chemical N fertilizer (80 and 120 kg/ha) over N control (Table 2). The treatment having 2/3N+BI+CI+C@ 5.0 tonnes/ha showed the higher enzymatic activity of POD (3.58 unit/g) compared to other treatments. Lowest enzymatic activity was recorded in N_0 (1.782 unit/g). PPO was significantly higher with 2/3N+BI+CI+C@ 5.0 tonnes/ha over 2/3N and N_0. Treatments namely N_{120}, 2/3N+BI, 2/3N+ BI+C, 2/3N+CI, 2/3N+CI+C, 2/3N+BI+CI, 2/3N+BGA and 2/3N+C+BGA showed at par PPO. Plants produce enzymes like POD, phenylalanine ammonia-lyase (PAL) and PPO for the defence. Peroxidase and PPO are catalysts in the formation of lignin. PAL and other enzymes are involved in the formation of phytoalexins (Pal *et al.* 2011). Chen *et al.* (2000) reported that ISR mediated by PGPR against *Pythium aphanidermatum* in cucumber was associated with an increase of POD, PPO and PAL.

Grain and straw yields of aromatic rice significantly increased due to the inoculation of PGPR, and BGA with compost @ 5.0 tonnes/ha, and chemical N fertilizer (2/3N through urea) over N_0 control. Highest grain yield was obtained with the application of 2/3N+BI+ CI+C@ 5.0 tonnes/ha (5.02 tonnes/ha) and it was at par with N_{120} and 2/3N+C+BGA. Khan and Zaidi (2007) also recorded 133% increase in wheat yield due to the combined inoculation of *Bacillus* sp. (PGPR) and *Glomus fasciculatum* (AMF), whilst single application of either *Bacillus* sp or *G. fasciculatum*

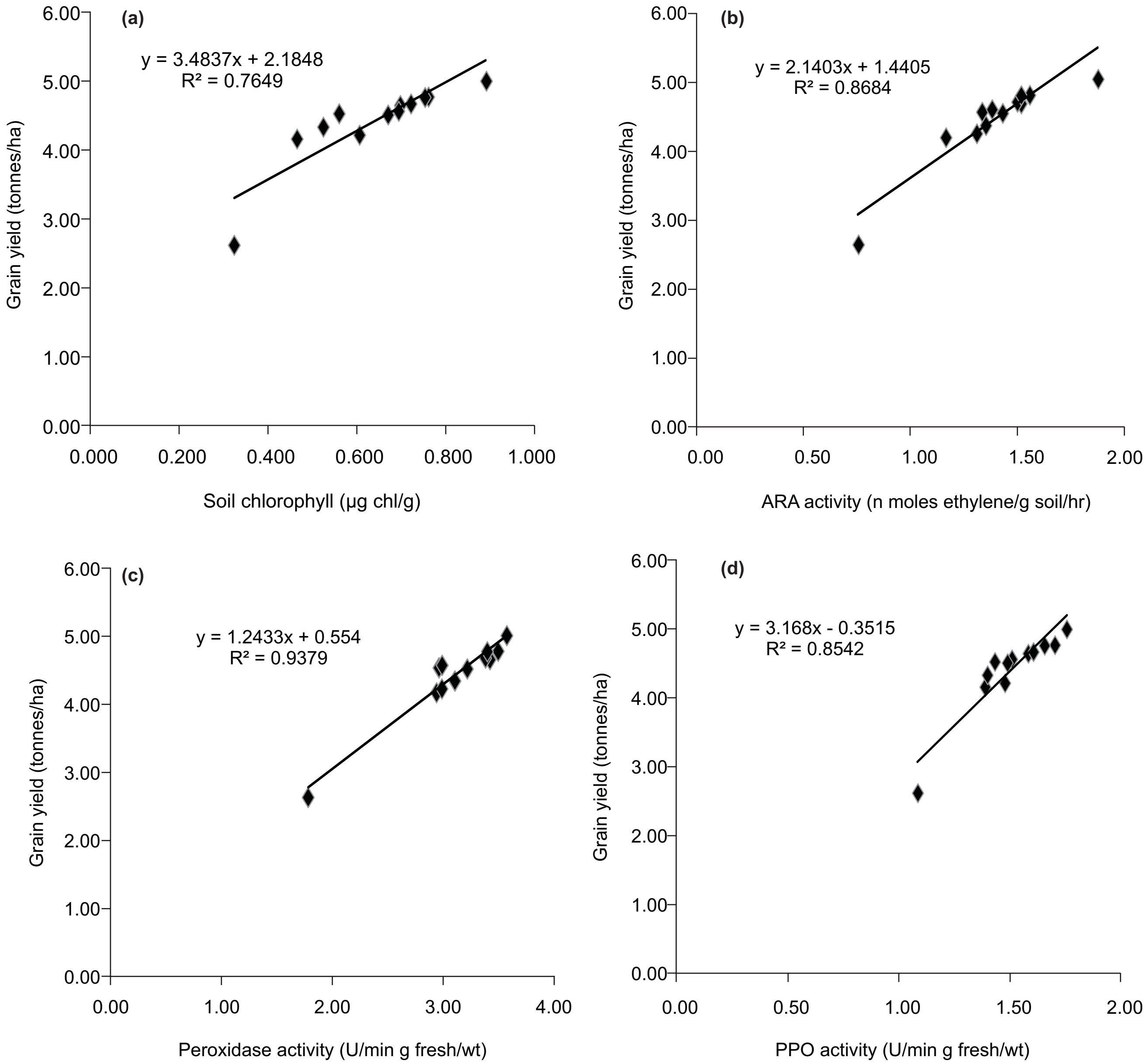

Fig 1 Correlation between soil microbial and plant enzyme with grain yield

Table 2 Effect of PGPR on soil microbial and plant defence enzyme in rice

Treatment	Soil chlorophyll (µg/g)	Dehydrogenase activity (mg TPF/g soil/day)	ARA activity (n moles ethylene/g soil/ha)	POD activity (U/min/g fresh/wt)	PPO activity (U/min g fresh/wt)
N_0	0.324	6.00	0.755	1.782	1.083
2/3 N(80 kg/ha)	0.466	7.65	1.164	2.941	1.387
N_{120}	0.763	11.81	1.555	3.497	1.700
2/3N+C	0.561	8.11	1.333	2.962	1.430
2/3 N+BI	0.607	8.37	1.308	2.989	1.475
2/3 N+BI+C	0.699	11.54	1.517	3.422	1.580
2/3 N+CI	0.525	8.92	1.350	3.106	1.397
2/3N+CI+C	0.723	10.83	1.498	3.383	1.603
2/3N+CI+BI	0.695	10.10	1.379	2.991	1.507
2/3N+BI+CI+C	0.893	14.78	1.875	3.575	1.753
2/3N+BGA	0.671	10.68	1.429	3.218	1.487
2/3N+C+BGA	0.755	12.01	1.515	3.399	1.653
SEm±	0.034	0.719	0.0648	0.147	0.082
LSD (P=0.05)	0.100	2.109	0.190	0.432	0.242

resulted in 22% and 55% higher wheat grain yields, respectively over non-inoculation.

Grain formation in cereals is a complex coordinated process that involves biomass build-up and interaction between soil microbial properties, plant enzymes and grain yield. These processes are strongly affected by crop management conditions. This was shown by the highly positive correlation between the soil microbial properties, plant enzymes and grain yield (Fig 1). The R^2 values for peroxidase and PPO activity were 0.94 and 0.85 respectively which showed their highly positive correlation with grain yield.

SUMMARY

Soil organic carbon, available N, P, K, Zn, Fe and Cu in soil at crop harvest stage significantly increased due to the integrated inoculation of PGPR and *Multani mitti* based BGA with application of compost and chemical N fertilizer (80 and 120 kg/ha) over N control. Status of SOC and available N, P, K, Fe, Zn and Cu in soil at crop maturity stage was highest at 2/3N+BI+CI+C@ 5.0 tonnes/ha. Soil chlorophyll, dehydrogenase and ARA activity were significantly higher at 2/3N+BI+CI+C@ 5.0 tonnes/ha compared to other treatments. Plant enzymatic activity of POD and PPO at 60 DAT also was significantly higher in 2/3N+BI+CI+Compost@ 5.0 tonnes/ha. Application of 2/3N with inoculation of bacterial and cyanobacterial PGPR along with 5.0 tonnes/ha compost not only improved nutrient availability in soil but also enhanced soil microbial, plant enzymatic activity and crop yield.

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