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Original Research Article

Potential of PGPR and its Interactive Effects with TCP on Nutrient Management and Enzyme Activity at Growth Stages of Rice

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A B S T R A C T

Various communities of beneficial plant growth promoting rhizobacteria harbor in rice rhizosphere and plays an important role for improving productivity of rice crop through various direct and indirect mechanisms on interaction with crop by colonizing plant roots. Pot culture experiment was undertaken during kharif season 2019 at Division of Soil Science &Agriculture Chemistry (SS&AC), SKUAST Jammu to study the potential of two cultures of PGPR obtained from soil microbiology laboratory, SS&AC. The treatments were replicated thrice in a factorial CRD design comprising of eight treatments with two factors viz., first with or without TCP ($1g^{-1}$soil) and second with single or combined inoculation of PSRB Isolates along with TCP). Significant increase in available N,P,K content in soil were recorded with TCP $1g^{-1}$soil (P_1) and consortium of PSRB isolates *Pseudomonas fluorescens* and *Bacillus subtilis* strain-2 (B_3) at crop growth stages. Highest increase in available N (16.2% and 21.1%), available P (71.3% and 95.1 %), available K (21.4%, 25.6%) over control were recorded in B_3 (consortium of *Pseudomonas fluorescens* and *Bacillus subtilis strain 2* isolates broth culture. Application of TCP $1g^{-1}$soil (P_1) and consortium of PSRB isolates *Pseudomonas aeruginosa* and *Bacillus subtilis* (B_3) recorded significant increase in dehydrogenase activity at crop growth stages. Highest increase in dehydrogenase activity 26.7%, 34.3 % over control were recorded in B_3. Treatment B_3 along with TCP was found best treatment among all treatments for increase in nutrients and enzyme activity at growth stages of rice.

Keywords

PGPR,
Interactive effects,
TCP,
Enzyme activity

Introduction

Phosphorus is an essential macronutrient for plant nutrition. Its availability to plants is limited due to its low solubility and fixation in soil. Application of phosphatic fertilizers lead to conversion of phosphatic fertilizers to insoluble phosphates which otherwise promotes frequent application of phosphatic fertilizers and are harmful both environmentally and economically. Phosphorus release from insoluble phosphates has been reported by soil microbial inoculants and is considered as an important aspect for crop yield. Rhizosphere is a ecological niche present around the roots of plants and support various microorganisms. Rhizospheric bacteria that are beneficial to plants are referred as plant growth promoting rhizobacteria which exhibit a significant interaction with plant roots and have both direct and indirect positive effects on plant growth and the

reduction of both biotic and abiotic stresses. PGPR includes diverse bacterial taxa that promote plant growth through phosphate solubilization, phytohormone production, stress ethylene suppression, which otherwise inhibits root growth in saline soils and other stress conditions. Phosphate solubilizing rhizobacteria are also reported to solubilize inorganic P by secretion of different kinds of organic acids, siderophores as well as hydroxyl ions (De Souza *et al.,* 2015).Using phosphate solubilizing PGPR with insoluble phosphate sources such as RP, TCP, SSP has also captured immediate attention as these bacteria can solubilize the inorganic phosphate by releasing organic acids, H^+ and chelation compounds (Goldstein, 1995). *Pseudomonas, Rhizobium, Bacillus, Beijerinckia*, *Burkholderia*, *Enterobacter, Flavobacterium, Azotobacter, Erwinia* and *Serratia* are considered as the most prominent PGPR in soil (Ahemad and Kibret, 2014; Rafi *et al*, 2019). Among the predominant genera of PGPR *Pseudomonas* and *Bacillus* in soil. *Pseudomonas* spp. is a rod shaped aerobic bacteria that are known for its association with different plants. Most of the plant associated *Pseudomonas* species belongs to *P. fluorescens*, *P. putida* and *P. aureofaciens*. The activities of these bacteria influence plant growth through, production of organic acids, hydrogen cyanide (HCN), siderophores as well as antimicrobials and lytic enzymes and possess many phytobeneficial traits (Chaiharn and Lumyong, 2009). *Bacillus* species are capable of forming long-lived, stress tolerant spores and secreting metabolites that stimulate plant growth and prevent pathogen infection (Radhakrishan *et al*., 2017). Therefore for improving the population densities of high effective PGPR in the rhizosphere of crop plants introduction of selected strains as seed or soil inoculants should be performed using culture based methods to determine which traits they carry and quantify their potential activity in different laboratory culture media for improved crop plant growth. The present study therefore aimed to study the Potential of PGPR and its interactive effects with TCP on nutrient management and enzyme activity at growth stages of rice under pot culture assays

Materials and Methods

Pot experiment

Pot experiment was conducted at Division of Soil Science &Agriculture Chemistry (SS&AC), SKUAST Jammu to study the potential of PGPR (two cultures of PGPR obtained from soil microbiology laboratory, SS&AC, SKUAST, Jammu) and its interactive effects with TCP after two cultures of PGPR obtained from soil microbiology laboratory, SS&AC.. Pots of dimensions 30*26*17 cm^3 were filled with polythene containing 6kg of soil sterilized with 0.5% formaldehyde. Roots of seven day old rice plantlets were dipped in bacterial broth culture of the two strains obtained from Division of Soil Science and Agriculture Chemistry SKUAST Jammu. The plantlets were planted under two different soil conditions (with or without TCP, added 1 g-kg^{-1} soil) with eight treatments i. Soil (Control 1), ii. Soil +TCP (Control 2), iii. Soil+ PSRB-1 (*Pseudomonas fluorescens*), iv. Soil + PSRB -2 (*Bacillus subtilis strain 2*) v. Soil + Consortium(50:50) vi. Soil +TCP+ PSRB -1 vii. Soil +TCP+PSRB-2 viii. Soil +TCP+ Consortium (50:50). The treatments were replicated thrice. in a factorial CRD design comprising of eight treatments with two factors viz., first with or without TCP ($1g^{-1}$soil) and second with single or combined inoculation of PSRB Isolates along with TCP). The plants were harvested after 120 days and samples were collected at growth stages and further analysed for available nutrients and enzyme activity in soil.

Determination of available nutrients (N, P, K) and enzyme activity in soil

Available Nitrogen: The available nitrogen in soil was estimated by alkaline potassium permanganate method as given by (Subbiah & Asija, 1956).

Available Phosphorus: Stannous Chloride reduced ammonium molybdate method using Olsen's extractant (Olsen *et al*., 1954) was used for the estimation of available phosphorus in soil.

Available Potassium: 1N ammonium acetate was used as extractant and the available potassium was determined by feeding the extract to flame photometer (Jackson, 1973).

Dehydrogenase activity: By monitoring the rate of production of triphenyl formazan (TPF) from tri-phenyl tetrazolium chloride (TTC), using the method of Casida *et al.*, (1964) was used for estimation of dehydrogenase activity in soil and the results were expressed as mg TPF formed per hour per g soil.

Phosphatase activity: Colorimetric estimation of the *p*-nitrophenol released by phosphomonoesterases activity using the method of Tabatabai and Bremner (1969) used for determination of phosphatase activity in soil and the results were expressed as p-nitrophenol released per g of soil per hour.

Statistical analysis

The data recorded from laboratory analysis was subjected to statistical analysis using the technique of analysis of variance for factorial completely randomized design for the interpretation of results as described by Gomez and Gomez (1984). The treatment differences were compared at 5 per cent level of significance (P= 0.05) using OPSTAT program (Sheoran *et al*., 1998).

Results and Discussions

Effect of PSRB isolates alongwith TCP on available nutrients and enzyme activity in soil

Available N ($mgkg^{-1}$)

Data pertaining to Table 1 revealed that TCP and PSRB isolates significantly ($P< 0.05$) increased the available nitrogen in soil at growth stages of rice crop. At both tillering and panicle initiation stages, addition of TCP along with PSRB isolates increased the available nitrogen in soil and highest increase was recorded in B_3 (Consortium) by 16.2%, 21.1 % as compared with control (B_0) at both tillering and panicle initiation stages. due to increase in N fixation by the isolates at growth stages Similar results were found by Vyas *et al*.2009. However, treatments B_3, B_2 and B_1 were statistically at par with each other. Decrease in the available nitrogen in soil at harvest stage was recorded with the addition of both TCP and PSRB isolates and significant interaction was recorded between TCP and PSRB isolates at tillering and panicle initiation stage with highest available N recorded in B_3P_1 (21.4%) at tillering stage and 26.6% at panicle initiation stage. At harvest stage decrease in the availability of available N was due to increase in the absorption of nitrogen by the plants.

Available P ($mgkg^{-1}$)

Data pertaining to Table 2 revealed that TCP and PSRB isolates significantly increased ($P< 0.05$) the available phosphorus in soil at growth stages of rice crop. At both tillering and panicle initiation stages addition of TCP along with PSRB isolates increased the available phosphorus in soil and highest

increase was recorded in B_3 (Consortium) by 71.3 %, 95.1% as compared to control due to increase in phosphate solubilisation activity of both the microorganisms that might have brought P from unavailable form to available form by the secretion of organic acids and phosphates enzymes as reported similarly in the results by Dey *et al.*, 2004 and treatments B_3, B_2, B_1 were statistically at par with each other. Decrease in the available phosphorus in soil was recorded with the addition of both TCP and PSRB isolates at harvest stage due to increase in absorption of phosphorus by plants during their growth and significant interaction was recorded between TCP and PSRB isolates for the available phosphorus in soil at both tillering and panicle initiation stage with highest available P recorded in B_3P_1 (95.4%) at tillering stage and (122.7%) at panicle initiation stage.

Available K (mgkg^{-1})

Data pertaining to Table 3 revealed that TCP and PSRB isolates significantly (P=<0.05) increased the available potassium in soil at growth stages of rice crop. At both tillering and panicle initiation stages addition of TCP along with PSRB isolates increased the available potassium in soil and highest increase was recorded in B_3 (Consortium) by 15.6%, 19.8% as compared with the control (B_0) at both tillering and panicle initiation stages due to less fixation of potassium in soil which was in accordance with Xu *et al*., 2011 However treatments B_3, B_2, B_1 were statistically at par with each other. Decrease in the available potassium in soil was recorded with the addition of both TCP and PSRB isolates at harvest stage due to increased absorption by plants for their growth.

Table.1 Effect of PSRB inoculation and TCP on soil available N status (mgkg^{-1}) at growth stages of rice crop

Treatments	Av. N at Tillering stage (mg kg $^{-1}$)	Av. N at Panicle Initiation stage (mg kg $^{-1}$)	Av. N at Harvest stage (mg kg^{-1})
(TCP)			
P_0(0g^{-1} soil)	85.81	86.35	84.50
P_1(1g^{-1} soil)	88.38	88.94	86.98
SEm(±)	0.18	0.24	0.19
CD	0.55	0.73	0.57
Isolates			
B_0(Control)	79.30	77.87	76.23
B_1(PSRB1)	85.50	85.95	83.8
B_2(PSRB 2)	89.65	90.77	88.74
B_3(PSRB 1+2)	93.93	95.97	94.21
SEm(±)	0.26	0.34	0.27
CD	0.77	1.04	0.81
F- probability test			
P (TCP)	*	*	*
B(PSRB)	*	*	*
P*B	*	*	*

*= significant at P<0.05

Table.2 Effect of PSRB inoculation and TCP on soil available P status ($mgkg^{-1}$) at growth stages of rice crop

Treatments	Av. P at Tillering stage ($mgkg^{-1}$)	Av. P at Panicle Initiation stage ($mgkg^{-1}$)	Av. P at Harvest stage ($mgkg^{-1}$)
TCP			
P_0(0g^{-1} soil)	23.23	23.71	22.07
P_1(1g^{-1} soil)	25.37	26.08	23.85
SEm(±)	0.18	0.23	0.21
CD	0.56	0.68	0.64
Isolates			
B_0(Control)	17.43	15.95	14.55
B_1(PSRB 1)	23.07	23.84	21.45
B_2 (PSRB 2)	26.68	27.62	25.61
B_3 (PSRB 1+ 2)	30.01	32.15	30.22
SEm(±)	0.27	0.32	0.30
CD	0.79	0.97	0.90
F probability test			
P(TCP)	*	*	*
B(PSRB)	*	*	*
P*B	*	*	*

*= significant at P< 0.05

Table.3 Effect of PSRB inoculation and TCP on soil available K status ($mgkg^{-1}$) at growth stages of rice crop

Treatment	Av. K at Tillering stage(mg kg^{-1})	Av. K at Panicle Initiation stage(mg $kg^{-1)}$	Av. K at Harvest stage (mg kg^{-1})
TCP			
P_0(0g^{-1} soil)	84.17	83.77	81.96
P_1(1g^{-1} soil)	86.39	86.18	83.91
SEm(±)	0.18	0.21	0.19
CD	0.56	0.63	0.57
Isolates			
B_0 (Control)	77.84	76.06	74.11
B_1 (PSRB 1)	84.48	83.91	81.82
B_2 (PSRB 2)	87.74	87.71	85.74
B_3 (PSRB 1+ 2)	91.08	92.21	90.06
SEm(±)	0.26	0.29	0.27
CD	0.78	0.89	0.80
F probability test			
P(TCP)	*	*	*
B(PSRB)	*	*	*
P*B	*	*	*

*= significant at P<0.05

Table.4 Effect of PSRB inoculation and TCP on dehydrogenase activity (µg-TPFg^{-1}soil hr^{-1}) at growth stages of rice crop

Treatments	Dehydrogenase activity at Tillering stage(µg-TPFg^{-1}soil hr^{-1})	Dehydrogenase activity at Panicle Initiation stage(µg-TPFg^{-1} soil hr^{-1})	Dehydrogenase activity at Harvest stage(µg-TPFg^{-1}soil hr^{-1})
TCP			
P0 (0g^{-1} soil)	55.91	56.13	54.21
P1 (1g^{-1} soil)	59.19	59.58	57.47
SEm(±)	0.22	0.19	0.18
CD	0.66	0.56	0.55
Isolates			
B_0 (Control)	50.13	48.19	46.08
B_1 (PSRB 1)	55.87	56.88	54.96
B_2 (PSRB 2)	59.60	60.61	58.65
B_3 (PSRB 1+2)	64.62	65.73	63.65
SEm(±)	0.31	0.27	0.26
CD	0.94	0.80	0.77
F probability test			
P (TCP)	*	*	*
B (PSRB)	*	*	*
P*B	*	*	*

*= significant at P<0.05

Table.5 Effect of PSRB inoculation and TCP on phosphatase activity (µg- PNP g^{-1}soil hr $^{-1}$) at growth stages of rice crop

Treatments	Phosphatase activity at Tillering stage (µg-PNP g^{-1}soil hr $^{-1}$)	Phosphatase activity at Panicle Initiation stage (µg-PNP g^{-1}soil hr $^{-1)}$	Phosphatase activity at Harvest stage (µg-PNP g^{-1}soil hr $^{-1)}$
TCP			
P_0 (0g^{-1} soil)	43.59	41.32	39.57
P_1 (1g^{-1} soil)	46.17	44.33	42.27
SEm(±)	0.21	0.19	0.16
CD	0.62	0.58	0.48
Isolates			
B_0 (Control)	34.84	32.01	30.14
B_1 (PSRB 1)	45.34	42.80	40.71
B_2 (PSRB 2)	48.14	46.27	44.28
B_3 (PSRB 1+2)	52.01	50.22	48.53
SEm(±)	0.29	0.27	0.23
CD	0.88	0.82	0.68
F probability test			
P(TCP)	*	*	*
B(Isolates)	*	*	*
P*B	*	*	*

*= significant at P<0.05

There was significant interaction recorded between TCP and PSRB isolates for the available potassium in soil at both tillering and panicle initiation stage with highest increase in B_3P_1 with 21.4% at tillering stage and 25.6% at panicle initiation stage.

Dehydrogenase Activity (µg-TPFg^{-1}soil hr^{-1})

Data pertaining to Table 4 revealed that TCP and PSRB isolates significantly ($P<0.05$) increased the dehydrogenase activity in soil at growth stages of rice crop. At both tillering and panicle initiation stages, addition of TCP along with PSRB isolates increased the dehydrogenase activity in soil and highest increase was recorded in B_3 (Consortium) by 26.7%, 34.3% as compared with control (B_0) due to greater microbial and root activity in the rhizosphere which was in accordance with Angelina *et al*., 2020 However, treatments B_3, B_2, B_1 were statistically at par with each other. Decrease in the dehydrogenase activity in soil was recorded with the addition of both TCP and PSRB isolates at harvest stage due to decrease in microbial activity in the rhizosphere There was significant interaction recorded between TCP and PSRB isolates for the dehydrogenase activity in soil at both tillering and panicle initiation stage. Highest increase was observed in B_3P_1 with 37.4 % at tillering stage and 45.7 % at panicle initiation stage.

Phosphatase activity (µg- PNP g^{-1}soil hr^{-1})

Data pertaining to Table 5 revealed that TCP and PSRB isolates significantly ($P<0.05$) decreased the phosphates activity in soil at growth stages of crop. At both tillering and panicle initiation stages, addition of TCP along with PSRB isolates decreased the phosphatase activity in soil and highest decrease was recorded in B_3 (Consortium) by 51.2%, 55.6% as compared with control (B_0) due to increase in P release as this enzyme has its activity more during P deficiency as reported by Bai *et al*., 2020. Decrease in the phosphatase activity in soil was maximum with the addition of both PSRB isolates and TCP at harvest stage. There was significant interaction recorded between TCP and PSRB isolates for the phosphates activity in soil at both tillering and panicle initiation stage. Highest decrease was recorded in B_3P_1 with 64.3% at tillering stage and 69.8% at panicle initiation stage.

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