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Relative potential of *Rhizobium* sp for improving the rice-wheat crop in the semi-arid regions

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Abstract

Soil Microbiologists have been concentrating on manipulation of rhizosphere microbes in cereals, but many researchers have reported that rhizobia can act as plant growth promoting rhizobacteria (PGPR). *Rhizobium* species impacted the crop ontogeny by root / endophytic colonization, producing phytohormones, efficient nutrient use and nutrient solubilization / mineralization. Field studies were performed at Soil Bacteriology Section and Soil Chemistry Section, Faisalabad to assess the comparative potential of *Rhizobium* species for promoting the growth, yield of wheat and rice. Auxin biosynthesis potential of isolates of *Rhizobium* species (mung (*Vigna radiata*), berseem (*Trifolium alexandrinum*), chickpea (*Cicer arietinum*), lentil (*Lens culinaris*) and peanut (*Arachis hypogaea*)) was determined and isolates of each species having higher values were used for field experiments. Assay for root / shoot elongation, root colonization in plates were carried out under controlled conditions. The rhizosphere soil of wheat and rice were assayed for the Indole Acedic Acid (IAA) content 15 and 30 days after germination / transplanting, respectively. Results revealed that significant increase was observed in the yield parameters of wheat and rice. Highest wheat grains were produced i.e., 4917 kg ha^{-1} with *Rhizobium sp of mungbean* (Mb_3) followed by 4823 with *Rhizobium sp of berseem* (Br_3) than control i.e., 4500 kg ha^{-1}. Similarly, the maximum paddy yield i.e., 4667 kg ha^{-1} with *Rhizobium sp of mungbean* (Mb_3) followed by 4625 *Rhizobium sp of berseem* (Br_3) inoculation was obtained as compared to control i.e., 4208 kg ha^{-1}. Other physical parameters of wheat and rice also showed positive response to inoculation and have elevated levels of IAA in the rhizosphere of inoculated treatments. Results clearly demonstrated that *Rhizobium* species increased the yield of rice and wheat.

Keywords: *Rhizobium* species, IAA equivalents, PGPR, Interaction, wheat, rice.

Introduction

Biofertilizer or microbial inoculants are the substance that either solid carrier based or liquid based contains beneficial microbes that have participated in different functions for promoting plant growth. Biofertilizer are environment friendly, inexpensive (cost effective) and reasonable source that have potential role in the plant growth promotion. The microbial inoculants perform different functions for plant growth viz. by improving

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soil conditions, solubilizing/mineralizing/mobilizing nutrients, restricting pathogens, supplementing/ compensating mineral fertilizers, enhancing nutrient use efficiency, protecting plant micro-environment i.e., rhizosphere from pollutants, abolishing harmful substances, effective in arid, semi-arid or adverse soil conditions and ultimately the higher yield of plants and healthy returns for farmers. Application of mineral fertilizers results in readymade supply of nutrients to plants and on the other hand significant portion of added fertilizer is fixed or lost (Sessitsch et al., 2002; Ibiene et al., 2012; Vargas et al., 2017). The efficient microbes are responsible to restore the soil fertility status by biological means and thus act as restoring agents of soil fertility. The continuous or prolonged and excessive usage of chemical or mineral fertilizers deteriorates/degrades soil, water and air (Vejan et al., 2016; Vargas et al., 2017). Further, injudicious use of mineral fertilizers results in environmental pollution (Lin et al., 2019). Biofertilizer are usually carrier-based formulations of beneficial microbes. The carriers of biofertilizer are carbon source i.e., compost, muds, manures, lignite, leaf molds, peat and water etc. Biofertilizer improve soil fertility, enhance soil water holding capacity, and act as agents of climate change (Vejan et al., 2016; Rachel et al., 2018).

Soil microbes can mobilize soil nutrients, transform nutrients into available forms; store water-soluble nutrients in available forms, tender the plants balanced nutrients, maintain soil aeration required for the roots; improve nutrient uptake/efficiency, provide nutrients during the growing season and especially during growth critical periods of plants (Reddy, 2014; Rachel et al., 2018). Soil microbes arbitrate in various beneficial soil processes like nutrient fixation/mobilization, biodegrade agro-chemicals, improve soil conditions, and suppress soil pathogens (Lupwayi et al., 2011; Przygocka-Cyna and Grzebisz, 2018). Due to competition of crop growers to produce more and more yields to get maximum return enhances the usage of mineral fertilizers and results in ill effect on soil and produce have turn their attention to use biofertilizer in integration with mineral fertilizers (Savci, 2012; Rachel et al., 2018).

Injudicious use of mineral fertilizers to feed the rising population also poses severe threat to environment. Under the prevailing circumstances, the eco-friendly approach to use microbial inoculants is valid option and inspiring the end users (Hardoim et al., 2008; Noreen et al., 2012). The various means i.e., fixing nutrients (nitrogen), solubilizing / mobilizing nutrients, releasing plant hormones, vitamins and antibiotics, inducing stress resistance etc. opted by PGPR for plant growth promotion (Sinha et al., 2014; Bhat et al., 2019). The beneficial rhizosphere microbes are involved in transforming nutrients and supplementing the mineral fertilizers and results in better crop yields (Singh et al., 2011; Vargas et al., 2017). The fixation / losses of nutrients (~60-90%) and about 10-40% is available for plants and microbial inoculants have prime significance in this regard and enhance crop growth/yield and healthy environment (Adesemoye and Kloepper, 2009; Lin et al., 2019).

The best explored PGPR belong to genus i.e., *Rhizobium*. The valuable effect of *Rhizobium* species is recognized splendidly in legumes and non-legumes. The *Rhizobium* sp. due to its root colonizing capability could be used efficiently as potential PGPR (Gouda et al., 2017; Vargas et al., 2017). Species of *Rhizobium* responsible for symbiosis with legumes also reported as asymbiotic means that these species can behave as PGPR in non-legumes (Dobbelaere et al., 2003; Hussain et al., 2009, 2016, 2018). The rhizosphere microbes produce metabolites i.e., primary and secondary for stress relief is well demonstrated and documented (Verbon and Liberman, 2016; Rachel et al., 2018). The exploration of *Rhizobium* species as potential PGPR in non-legumes such as cereals is also established and provides principal rank in agricultural system and can become tool for food security in sustained way (Sessitsch et al., 2002; Gouda et al., 2017; Vargas et al., 2017).

Rhizobium species improve the crop yields by improving root system architecture, increasing lateral roots by producing growth hormones, repressing plant pathogens by releasing antibiotics/siderophores, solubilizing /mobilizing inorganic/organic fixed nutrients, diminishing adverse effects of biotic stresses by producing ACC deaminase, volatile organic compounds and lytic enzymes, and role in systemic resistance (Hardoim et al., 2008; Mehboob et al., 2008; Vargas et al., 2017). Plant nutritionists are in search of such roles by the most promising microbes of agriculture system i.e., *Rhizobium*. Role of *Rhizobium* species in bio-control has also been described (Pacheco-Villalobos et al., 2016; Ullah et al., 2017a; Vargas et al., 2017). *Rhizobium* species can modulate the endogenous plant hormones. It has also been demonstrated that *Rhizobium* sp. influence the various plants processes (Zahir et al., 2010a; Qureshi et al., 2013; Datta and Chakrabartty, 2014; Lin et al., 2019). The use of *Rhizobium* sp. in improving crop growth has been documented by numerous researchers and obtained marvelous results (Hussain et al., 2009; 2016, 2018, 2019; Zahir et al., 2010a,b; Ullah et al., 2017 a,b; Vragas et al., 2017). Studies were aimed to evaluate the relative potential of *Rhizobium* species for the growth and yield promotion of wheat and rice.

Material and Methods

Isolation and Screening of Isolates

Isolations were carried out from the nodules of particular legumes viz. (mungbean, berseem, chickpea, lentil and peanut) on YEM (yeast extract mannitol) (Vincent, 1970). The YEM medium was autoclaved at 15-20 psi pressure and 121°C temperature for 20-30 minutes. The autoclaved YEM medium was plated aseptically in laminar air flow cabinets and exposed to UV light for 30 minutes. The nodules were detached from the roots of legume plants, washed with tap water and then 4-5 washings with autoclaved distilled water (Russell et al., 1982). The nodule sap was collected by puncturing the nodules with sterilized forceps, streaked on YEM and incubated at 28±2°C for 48-72 hours. The bacterial colonies were purified on fresh plates for few times. After repeated purification, the purified growth was preserved at 5±1°C in eppendorf tubes for further studies. The isolates of each specie have been checked for plant infectivity test to assess that isolate was *Rhizobium* sp of that specific legume. The isolates showed promising results have been screened for different biochemical characteristics.

Determination of IAA Equivalents

The IAA content/equivalents with and without L-tryptophan (L-TRP) were analyzed for three isolates of each *Rhizobium* specie. The test tubes containing sterilized general-purpose medium (GPM) inoculated with each bacterial isolate and incubated at 28±2°C for one week. The incubated medium was centrifuged @10000 rpm for 15-20 minutes and filtered. The IAA equivalents were determined using Salkowski's reagent (Sarwar et al., 1992). Then performed the biochemical testing viz. Congo red, organic acid production (BTB test), urease for each isolate. Isolates showed the high IAA content/equivalents (Mb_3, Br_3, Cp_3, Lt_3 and Pt_3) with and without L-TRP were chosen for field studies (Table 1).

Table 1. Different stats/traits of rhizobium species under study.

Rhizobium species	Isolates	L-TRP* [-] (µg mL^{-1})	L-TRP [+] (µg mL^{-1})	Congo red test	BTB* test	Urease test	Root colonization in Rice X 10^4
Mung bean (MB)	Mb_1	2.91	3.52				
	Mb_2	2.28	3.90				
	Mb_3	3.25	4.36	+ve	+ve	+ve	38
Berseem (BR)	Br_1	2.09	2.83				
	Br_2	2.16	2.64				
	Br_3	3.21	3.93	+ve	+ve	+ve	32
Chickpea (CP)	Cp_1	2.50	3.55				
	Cp_2	2.75	4.30				
	Cp_3	3.34	4.19	+ve	+ve	+ve	33
Lentil (LT)	Lt_1	2.53	3.24				
	Lt_2	2.91	3.96				
	Lt_3	3.28	4.23	+ve	+ve	+ve	29
Peanut (PN)	Pt_1	1.97	3.09				
	Pt_2	2.25	3.00				
	Pt_3	2.63	4.07	-ve	+ve	-ve	22

L-TRP* [-]: without L-tryptophan; L-TRP [+]: with L-tryptophan;
Note: Different isolates have been assessed for auxin biosynthesis with and without L-TRP. The biochemical tests have also been carried out and isolate having higher values of IAA equivalents have been mentioned here. The root colonization assay of rice roots of above isolates has been given in the table.

Plate Experiment

The different isolates of *Rhizobium* species were assayed for root colonization of rice in a plate experiment under controlled conditions. The seeds of rice were treated with different tested *Rhizobium* species as seed coating. The rice seeds were moistened with sterilized distilled water at regular intervals. After one week of germination of rice, the seedlings were placed in sterilized distilled water. The serial dilutions of each isolate (treatment) were prepared and inoculated the already prepared plates of yeast extract mannitol agar medium (YEM) and after 48 hours of incubation at 28±2 °C. The bacterial count was carried on colony counter. The root/shoot elongation assay of wheat under axenic conditions for the selected bacterial isolates (Mb_3, Br_3, Cp_3, Lt_3 and Pt_3) was performed. Recorded the root/shoot length/mass after one week of germination. Then analyzed the IAA content/equivalents from root/shoot part after incubating for a week. The root/shoot (1.0 g each) was sterilized separately and placed in the sterilized GPM containing tubes, squashed, agitated repeatedly and incubated for a week. The supernatant was collected after centrifugation of medium at 1000 rpm and utilized for determination of IAA equivalents (Sarwar et al., 1992).

Inoculum Preparation

The yeast extract mannitol (YEM) broth without agar was prepared and autoclaved 15-20 psi pressure and 121°C temperature for 30 minutes. Inoculation of sterilized medium was carried out by specific isolates (Mb_3, Br_3, Cp_3, Lt_3 and Pt_3) separately and incubated at 28±2 for 3 days.

Field Studies

Field studies on wheat and rice were conducted at Soil Bacteriology and Soil Chemistry research area Ayub Agri. Research Institute, Faisalabad with pH 7.88-7.90, EC 1.52-2.0 dS m^{-1}, N 0.032-0.035% and available P 7.14-7.40 mg $kg^{-1}$and organic matter 0.70%, respectively. Plate experiment for wheat and rice for root-shoot elongation and root colonization for bacterium was carried out, respectively. Uniform fertilizer dose i.e., 120-100-60 kg NPK ha^{-1} was applied to wheat while 110-66-62 kg NPK ha^{-1} to rice was applied. Isolates of Rhizobia (mung, berseem, chickpea, lentil and peanut) viz. (Mb_3, Br_3, Cp_3, Lt_3 and Pt_3) were applied as seed coating for 30 minutes to wheat while seedlings of rice were dipped in the suspension of inoculum while control was placed for comparison. There were six treatments in which one control and five inoculation levels with five isolates of *Rhizobium* sp (Mb_3, Br_3, Cp_3, Lt_3 and Pt_3) with three repeats laid out in randomized complete block design (RCBD). Then determined the IAA equivalents from the rhizosphere soil of wheat and rice after 15 and 30 days of germination/transplanting. Wheat and rice were harvested at maturity and recorded yield parameters. Dried the grain and straw samples in oven at 70 °C for 60 minutes and analyzed for N and P. The analyses of soil samples for available P and N at harvest were performed (Bremner and Mulvaney, 1982; Olsen and Sommers, 1982).

Statistical analysis

The statistical analyses were performed using analysis of variance (ANOVA) following Randomized Complete Block Design (RCBD). The statistical analysis was performed using Statistix v8.1 software. The significance was assessed by Least Significance Difference (LSD) test for comparing means at probability level $p \leq 0.05$ (Steel et al., 1997).

Results

Results presented in tables and graphs revealed that *Rhizobium* species enhanced the yield attributes of both crops i.e., wheat and rice. Isolates of *Rhizobium* sp exerted more affirmative effect than the control of both crops. Isolates of *Rhizobium* species either tested in lab or field environment quite amazingly. Results presented in Table 1 clearly demonstrated that three isolates of each host species produced IAA equivalents with and without L-TRP and each isolate showed that the effect was more pronounced with L-TRP (Table 1). The biochemical testing of these isolates was carried out (Table 1). The isolates having higher IAA equivalents with and without L-TRP were observed with Mb_3, Br_3, Cp_3, Lt_3, and Pt_3 as shown in the Table 1. The values of IAA equivalents were observed by Mb_3, Br_3, Cp_3, Lt_3, and Pt_3 i.e., 3.25, 3.21, 3.34, 3.28 and 2.63 µg mL^{-1} and value was enhanced to 4.36, 3.93, 4.19, 4.23 and 4.07 µg mL^{-1} with application of L-TRP, respectively. The root colonization was performed in plate experiment and bacterial count was carried out by standard dilution plate technique. Results presented in Table 1 revealed that different isolates showed variable response for bacterial root colonization. The highest count was observed with Mb_3 i.e., 38 x 10^4 while lowest was observed with Pt_3 i.e., 22 x10^4.

Data regarding root/shoot parameters of wheat and IAA equivalents in root/shoot (Table 2) demonstrated that isolates improved the root/shoot considerably. The root/shoot length / mass significantly higher than control with bacterial isolates. The maximum root/shoot length / mass was observed with isolate (Mb_3) i.e., 14.0, 13.5 cm and 1.26, 2.65 g as compared to control i.e., 10.50, 11.25 cm and 1.07, 1.87 g, respectively. The isolate Mb_3 showed maximum IAA equivalents in root/shoot i.e., 1.81, 2.09 µg g^{-1}, respectively compared to control.

Results relating to biomass/grain or paddy yield of wheat and rice (Figure 1 and 2) explicitly exhibited that inoculation of *Rhizobium* species increased the wheat and rice yield significantly. Each isolate of *Rhizobium* species increased the biomass/paddy of rice and biomass/grain yield of wheat and maximum biomass/paddy and biomass/grains was produced with Br_3 and Mb_3 i.e., 24.20/4.63 and 13.80/4.92 t ha^{-1} in comparison to control i.e., 19.60/4.21 and 12.13/4.50 t ha^{-1}, respectively. The biomass/paddy yield of rice with Mb_3, Br_3 and Cp_3 showed statistically at par with slight variations. The lowest biomass/paddy and biomass/grains yield with *Rhizobium* sp (Pt_3) but it was also higher than control. Percent increase in biomass and paddy yield with different *Rhizobium* species (Mb_3, Br_3, Cp_3, Lt_3, and Pt_3) was observed (21.58, 23.47, 16.99, 14.95, and 8.67%) and (10.93, 9.98, 7.83, 4.99 and 2.85%), respectively. Percent increase in biomass and grain yield of wheat with different *Rhizobium* species (Mb_3, Br_3, Cp_3, Lt_3, and Pt_3) was observed (13.77, 11.13, 9.40, 7.58 and 6.51%) and (9.33, 7.11, 4.89, 1.78, 1.56%), respectively.

Table 2. Root-shoot elongation assay of wheat as affected by different treatments.

Treatments	Shoot length (cm)	Root length (cm)	Shoot Mass (g)	Root mass (g)	IAA in shoot (µg g^{-1})	IAA in root (µg g^{-1})
Control	11.25 d	10.50 d	1.87 d	1.07 c	1.17 d	0.86 d
Rhizobium sp (MB) (Mb_3)	13.50 a	14.00 a	2.65 a	1.26 a	2.09 a	1.81 a
Rhizobium sp (BR) (Br_3)	12.75 b	13.25 b	2.39 ab	1.17 b	1.95 ab	1.64 ab
Rhizobium sp (CP) (Cp_3)	12.55 b	13.00 b	2.32 bc	1.15 b	1.64 bc	1.53 b
Rhizobium sp (LT) (Lt_3)	12.25 bc	11.75 c	2.18 bc	1.12 bc	1.61 bc	1.25 c
Rhizobium sp (PN) (Pt_3)	11.75 cd	10.75 d	2.07 cd	1.10 bc	1.53 cd	1.17 c
LSD	0.7063	0.6841	0.2843	0.0674	0.3601	0.1757

* Mean values with different letter(s) show significant difference (P≤0.05) as Least Significance Difference Test
Note: The bioassay or root/shoot elongation assay of wheat was carried out and IAA equivalents in root/shoot has been determined and mentioned.

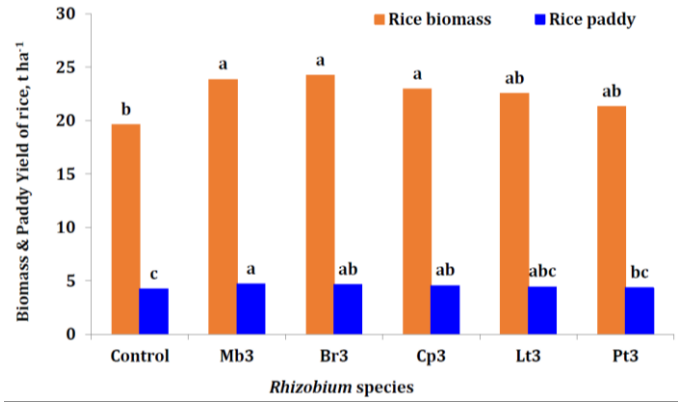

Figure 1. The biomass/paddy yield of rice

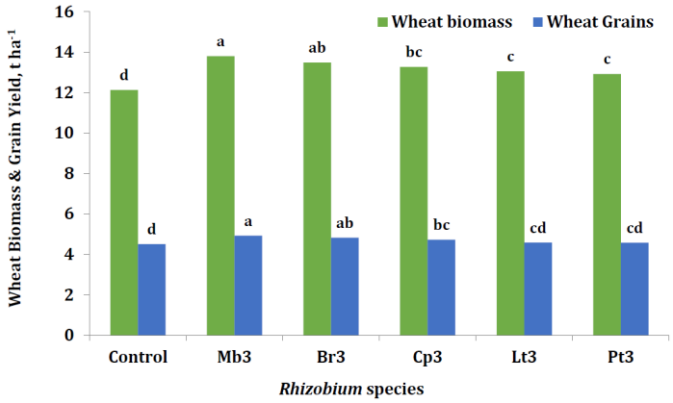

Figure 2. The biomass/grain yield of wheat

Results regarding IAA contents in the rhizosphere soil of rice and wheat after 15 and 30 days transplanting of rice/germination of wheat (Figure 3 and 4) clearly showed that bacterial inoculation of *Rhizobium* species increased the IAA content significantly. Isolates of *Rhizobium* species increased the IAA equivalents in rice and highest IAA was observed with Cp_3 after 15 days of transplanting i.e., 2.50 and 1.62 with Mb_3 after 30 days of transplanting in rice as compared to control i.e., 1.88 and 1.21 µg g^{-1}, respectively. Whereas in wheat, *Rhizobium* species enhanced the IAA content and maximum value was obtained (3.14 µg g^{-1}) after 15 days of germination while after 30 days of germination maximum value was obtained with Cp_3 i.e., 1.83 µg g^{-1} as compared to control (1.78 and 1.36 µg g^{-1}), respectively. The minimum value of IAA content after 15-days and 30-days transplanting/germination was obtained with Pt_3.

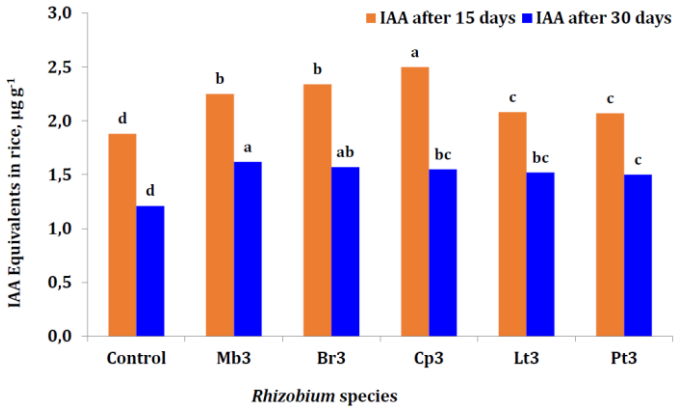

Figure 3. IAA Equivalents in rhizosphere soil of rice after 15 and 30 days of transplanting

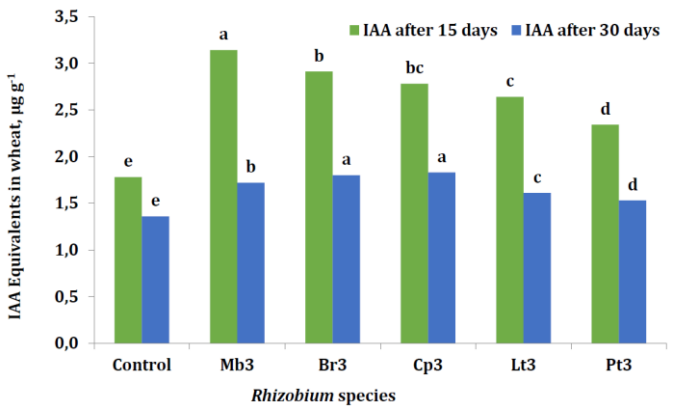

Figure 4. IAA Equivalents in rhizosphere soil of wheat after 15 and 30 days of germination

Results regarding physical attributes i.e., plant height, tillers m^{-2} and 1000 paddy/grain weight in rice and wheat (Table 3). Results clearly demonstrated that *Rhizobium* species enhanced the yield attributes significantly. The maximum plant height, no. of tiller m^{-2} and 1000 paddy weight of rice was obtained with Mb_3 i.e., 118.5, 293, 27.07 while maximum parameters in wheat i.e., 121.1, 466 and 48.70 was obtained and that was significantly higher than control.

Table 3. Rice and wheat yield parameters as affected by different treatments.

Treatments	Rice			Wheat		
	Plant height (cm)	Tillers m^{-2}	1000-Paddy weight (g)	Plant height (cm)	Tillers m^{-2}	1000-Grain weight (g)
Control	111.8 cd	277 c	25.10 c	109.2 c	387 f	42.58 e
Rhizobium sp (MB) (Mb_3)	118.5 a	293 a	27.07 a	121.1 a	466 b	48.70 a
Rhizobium sp (BR) (Br_3)	115.1 abc	289 ab	26.60 a	119.4 a	487 a	47.31 b
Rhizobium sp (CP) (Cp_3)	116.9 ab	286 abc	25.73 b	115.1 b	434 c	45.10 c
Rhizobium sp (LT) (Lt_3)	113.5 bcd	281 bc	25.60 bc	113.5 b	415 d	44.54 cd
Rhizobium sp (PN) (Pt_3)	110.1 d	280 bc	25.50 bc	111.8 bc	402 e	43.62 de
LSD	4.689	10.279	0.5048	3.9238	13.224	1.1317

* Mean values with different letter(s) show significant difference (P≤0.05) as Least Significance Difference Test
Note: The rice and wheat growth/yield parameters recorded at harvesting of both crops was mentioned in this table.

Results regarding plant and grain N, P content of wheat and soil parameters (Table 4) clearly demonstrated the bacterial isolates improved the grain and straw N, P content significantly. *Rhizobium* species isolates (Mb_3) produced the maximum wheat grain N, P content i.e., 1.648, 0.417% and wheat straw N, P content i.e., 0.337, 0.148% that is significantly higher than control (1.596, 0.321 and 0.282, 0.110%), respectively. The soil parameters tested i.e., Available P and soil N showed that soil N had significant higher values in inoculated treatments than control while statistically non-significant higher values was also observed in soil available P. The highest soil N available P was observed with Mb_3 i.e., 0.034% and 10.78 ppm than control i.e., 0.030% and 6.86 ppm, respectively.

Table 4. Influence of treatments on N, P content of wheat and soil analysis at harvest.

Treatments	Grain N (%)	Grain P (%)	Straw N (%)	Straw P (%)	Avail. P (ppm)	Soil N (%)
Control	1.596 d	0.321 e	0.282 c	0.110 d	6.86	0.030 b
Rhizobium sp (MB) (Mb_3)	1.648 a	0.417 a	0.337 a	0.148 a	10.78	0.034 a
Rhizobium sp (BR) (Br_3)	1.639 ab	0.386 b	0.327 a	0.140 ab	9.8	0.032 ab
Rhizobium sp (CP) (Cp_3)	1.629 b	0.369 bc	0.311 b	0.133 abc	8.82	0.032 ab
Rhizobium sp (LT) (Lt_3)	1.615 c	0.359 cd	0.307 b	0.128 bc	8.82	0.031 ab
Rhizobium sp (PN) (Pt_3)	1.609 c	0.335 de	0.301 b	0.122 cd	7.84	0.030 b
LSD	0.013	0.0237	0.0133	0.0159	5.2587	0.003

* Mean values with different letter(s) show significant difference (P≤0.05) as Least Significance Difference Test
Note: The table 4 contains wheat grain N and P content and soil N and available P at harvest.

Results regarding plant and grain N, P content of rice and soil parameters (Table 5) clearly showed that isolates of *Rhizobium* species improved the grain and straw N, P content significantly. *Rhizobium* species isolates (Mb_3) produced the maximum grain N, P content i.e., 1.40, 0.345% and straw N, P content i.e., 0.51, 0.138% that is significantly higher than control (1.33, 0.309 and 0.46, 0.107%), respectively. The tested soil parameters i.e., Available P and soil N showed that soil N was significantly higher in inoculated treatments than control. The highest soil N and available P was observed with Mb_3 i.e., 0.041% and 9.303 ppm than control i.e., 0.036% and 7.367 ppm, respectively.

Table 5. Influence of treatments on N, P content of rice and soil analysis at harvest.

Treatments	Grain N (%)	Grain P (%)	Straw N (%)	Straw P (%)	Avail. P (ppm)	Soil N (%)
Control	1.33 d	0.309 c	0.46 c	0.107 d	7.367 c	0.036 d
Rhizobium sp (MB) (Mb_3)	1.40 a	0.345 a	0.51 a	0.138 ab	9.303 ab	0.041 a
Rhizobium sp (BR) (Br_3)	1.38 b	0.337 a	0.50 a	0.145 a	9.787 a	0.040 ab
Rhizobium sp (CP) (Cp_3)	1.37 b	0.325 b	0.50 ab	0.132 bc	9.303 ab	0.039 bc
Rhizobium sp (LT) (Lt_3)	1.36 c	0.321 bc	0.49 ab	0.129 bc	9.300 ab	0.038 bcd
Rhizobium sp (PN) (Pt_3)	1.35 c	0.313 c	0.48 b	0.122 c	7.85 bc	0.037 cd
LSD	0.014	0.0119	0.0176	0.0102	1.674	0.002

* Mean values with different letter(s) show significant difference (P≤0.05) as Least Significance Difference Test
Note: The table 5 contains rice grain N and P content and soil N and available P at harvest.

Discussion

Isolates of *Rhizobium* species influenced the wheat and rice growth parameters positively while variable response of isolates was observed. The biosynthesis of auxins was observed with and without L-TRP in different isolates of *Rhizobium* species. Five isolates of each *Rhizobium* sp were characterized. Application of L-TRP has more assenting effect on IAA content than without L-TRP. Isolates (Mb_3, Br_3, Cp_3, Lt_3 and Pt_3) were selected on the basis of IAA equivalents. Isolates showed considerable increase in IAA equivalents when L-TRP was applied exogenously. The bacterial isolate Mb_3 produced elevated values of IAA in the presence/absence of L-TRP and biosynthesis of auxin with and without precursor as observed by several researchers (Zahir et al., 2010a, b; Hussain et al., 2013; Qureshi et al., 2013; Verbon and Liberman, 2016).

The bioassay (plate experiment) of bacterial isolates was carried out to assess the growth promoting traits of wheat. The root-shoot elongation assays (bioassay) clearly showed that bacterial isolates boosted the root/shoot length/mass and IAA content in root/shoot. The bacterial isolates exhibited more IAA content in root/shoot (Zahir et al., 2004). The lab bioassay of rice for root colonizing capability of rice showed that bacterial colonized the rice root and showed variable response (Naher et al., 2009; Ullah et al., 2017a, b; Rachel et al., 2018).

Field studies on rice and wheat illustrated higher growth and yield attributes, IAA in the rhizosphere soil, N, P content in grain or straw and soil parameters after harvest might be attributed to the biosynthesis of hormones changing the root architecture, more roots/shoot length / mass results in better crop growth and ultimately yield of crops (Dazzo and Yanni, 2006; Mehboob et al., 2011; Hussain et al., 2014 a,b). The bacterial inoculation due to its root colonizing capability boosted growth and yield of cereals might be ascribed as the hormone/siderophore production, improvement in nutrient uptake and inducing systemic resistance (Akhtar et al., 2013; Vargas et al., 2017; Rachel et al., 2018). The bacterial inoculation increased the plant ontogeny owing to release of auxins and amended the endogenous hormone status and ethylene level repression (Ullah et al., 2017 a,b). The improvement of wheat and rice yield and their yield components by *Rhizobium* inoculation might be accredited to several mechanisms viz. production of biologically active substances, siderophores and organic acids, suppression of plant pathogens and higher nutrient uptake (Mehboob et al., 2011; Parthiban et al., 2016; Vargas et al., 2017).

The bacterial inoculation improved the yield parameters by producing primary and secondary volatiles and enhanced auxin biosynthesis in the rhizosphere (Hossain and Martensson, 2008; Hussain et al., 2014 a,b; Ullah et al., 2017b). The microbial activities in the rhizosphere soil were boosted and impacted the crop growth positively and produced economical yields (Zahir et al., 2010; Gopalakrishnan et al., 2015). Microbial inoculation of cereals improved the yields and bettered nutrient content in plant parts owing to the expanded root system, more lateral roots, better nutrient acquisition to sustain the crop yields (Mehboob et al., 2011; Parthiban et al., 2016).

Inoculation expanded the root surface area by producing the lateral roots for better nutrient acquisition (Naher et al., 2009). The increase in grain and straw N and P might be due to better nutrient mobilization by bacterial inoculation (Ullah et al., 2017b). The rhizosphere microbes having the potential of solubilizing insoluble nutrients improved the nutrient content in plant parts and enhanced the quality of grains and straw (Hemissi et al., 2011; Parthiban et al., 2016; Mukhongo et al., 2017). Isolates released the organic acids that solubilized the phosphates, lowered the rhizosphere soil pH and results in better uptake of nutrients by plants reported by numerous researchers (Berger et al., 2013; Nagy and Pinte, 2015; Parthiban et al., 2016; Mukhongo et al., 2017). The increased levels of N and P content in grain and straw of wheat and rice due to bacteria inoculation owing to enhance root surface area and growth due the presence of plant hormones in the rhizosphere produced by microbes (Hussain et al., 2009; Rachel et al., 2018). The increased levels of soil nitrogen and available P might be ascribed by the solubilization of phosphates, production of organic acids, more root exudations due to microbial activities or microbe meditated processes (Ullah et al., 2017a,b; Vargas et al., 2017; Przygocka-Cyna and Grzebisz, 2018). The interaction of bacterial isolates and roots might enhance the root mass and respiration results in more water and nutrient uptake in shoots and grains. Microbial inoculation improved the crop growth and yield owing to the more nutrient intake by plants, more photosynthetic activity by plants and release of hormones (Parthiban et al., 2016; Przygocka-Cyna and Grzebisz, 2018).

Studies deduced that *Rhizobium* sp can be considered as effective PGPR in non-legumes after thorough screening. *Rhizobium* species responded positively in wheat and rice and may be used as bacterial inoculants for the field crops.

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