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Effect of inoculation of rhizobacterial consortia for enhancement of growth promotion and nutrient uptake in *basmati* rice

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ABSTRACT

A pot experiment was conducted in the phytotron of Indian Agricultural Research Institute, New Delhi (India) to investigate the effects of four rhizobacterial strains viz.,UKA-24 (Rhizobium radiobacter), UKA-27 (Bacillus pumillus), UKA-72 (Stenotrophomonas maltophila)and AKA-1(Pseudomonas putida) on the growth promotion of basmati rice cultivar Pusa Sugandha 4. The treatment with UKA-24 increased the plant height by 11.34%, whereas AKA-1 increased shoot dry weight and root dry weight by 12.6% and 3.48%, respectively in comparison with the control. The maximum uptake of N (75.4%) and P (59.11%) were recorded due to bacterial consortia treatment (N 0.75, $P_2O_5$1.5, K_2O 1.5 +UKA-24 +UKA-27 +UKA-72) than untreated control (N 0.75, $P_2O_5$1.5, K_2O 1.5). Significant correlation was observed among different treatments with all variables. Out of these,the shoot and root dry weights were highly correlated (0.92) to each other. Uptake of nitrogen (N), phosphorus (P) and potassium (K) and were positively correlated with plant height and shoot & root dry weights. Whereas, tiller number was negatively correlated with uptake of N, P and K. Thus, the present study suggested, the rhizobacterial consortia (UKA-24, UKA-27, UKA-72 and AKA^{-1}) were more effective bio-inoculants than single inoculant and could be used effectively for enhancement of growth promotion and nutrient uptake in basmati rice cultivation.

Keywords: *Rhizobacterial consortia;pusa-sugandha 4; indole acetic acid ;phosphate solubilizing bacteria ; siderophore; nutrient uptake*

In the recent years, there has been focus on increasing international concern for food and environmental quality, on the use of plant growth promoting rhizobacteria (PGPR) for reducing chemical inputs and increase of food quality in agriculture (Bowen and Rovira 1999; Glick 1995). PGPRs have been applied to various crops to enhance growth, seed emergence and crop yield (Dey *et al*. 2004; Herman *et al*. 2008; Minorsky 2008). Bacteria that colonize the rhizosphere, plant roots and enhance plant growth are referred to as plant growth-promoting rhizobacteria. PGPRs have shown positive effects in plant growth parameters such as,seed germination rate, tolerance to drought, weight of shoots and roots, nutrient uptake, yield and plant growth (Kloepper *et al*. 2004; Kokalis-Burelle *et al*. 2006). Uses of PGPRs have also been reported in cereal crops including rice (Biswas *et al*. 2000a, 2000b;Yanni *et al*. 1997). In addition to improvement of plant growth, PGPRs are directly involved in enhancing the uptake of nitrogen, synthesis of phytohormones, phosphate solubilization and production of siderophores that chelate iron and make it available to the plant root (Lalande *et al*. 1989; Kumar *et al*. 2016; Kumar *et al*. 2014a; Kumar *et al*. 2014b; Kumar and Dangar 2013a).

Basmati rice is long slender aromatic rice grown mainly in India and Pakistan. The high-yielding basmati rice variety has resulted in an increase in rice production but requires large amounts 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 PGPRs that can biologically fix nitrogen, solubilize phosphorus and induce some substances like indole acetic acid (IAA) that can contribute to the improvement of basmati rice growth. Recently, there is a growing interest in PGPR due to their efficacy as biological control and growth promoting agents in many crops including rice (Holajjer *et al*. 2013; Kumar *et al*. 2012; Kumar *et al*. 2013b; Kumar *et al*. 2013c; Mageshwaran *et al*. 2012; Manjunath *et al*. 2016; Thakuria *et al*. 2004). However, very less information of PGPR was documented as rhizobacteria consortia in basmati rice. Therefore, the present study was undertaken to evaluate the effects of rhizobacterial consortia on growth promotion and nutrient uptake in basmati rice cultivar Pusa Sugandha4.

MATERIALS AND METHODS

PGPR isolates along with reference strains used for pot experiment

All together two hundred rhizobacteria were isolated from rhizosphere soils of ten varieties (Pusa Sugandha-4, Sugandha-5, HBC-19, Super Basmati, Punjab Basmati, Kalajeera, Nua Chandan, Nua Keteki, Nuadesheri and Kala Namak) of basmati rice, grown at research farm of Indian Agricultural Research Institute (IARI), New Delhi, India (28.08° N and 77.12° E, 229 m above MSL) and ICAR-National Rice Research Institute, Cuttack, India (20° N, 86° E, 24 meters above MSL). Based on preliminary screening using standard protocols (Table 1), following four isolates *viz*., UKA-24, UKA-27, UKA-72 and AKA-1 which were efficient in terms of IAA producer, phosphate solubilizer, siderophore producer and nitrogen fixer *in vitro*, respectivelywere selected and identified as *Rhizobium radiobacter*, *Bacillus pumillus*, *Stenotrophomonas maltophilaand Pseudomonas putidare* spectively (Table 1) (Kumar *et al.* 2013). *Pseudomonas striata* (P-solubilizer) and *Azospirillum brazilense* (N-fixer) were obtained from Division of Microbiology, Indian Agricultural Research Institute (IARI), New Delhi and used as reference strains for pot experiment (Table 1). The experiment was conducted at National Phytotron facility, IARI, New Delhi from November, 2009 to January, 2010.

Table 1. PGPR isolates with reference strains used for pot experiment

Isolate no.	PGP@ traits	Methods	Sources	Identified isolates	Reference strains
UKA-24	IAA	Bric *et al*, 1991	Pusa Sugan-dha-5	*Rhizobium radiobacter*	-
UKA-72	PS	Pikovsk-ya,1948	Pusa Sugan-dha-5	*Bacillus pumillus*	-
UKA-27	SP	Schwyn and Neilands, 1987	Super Basmati	*Stenotrophomonas maltophila*	-
AKA-1	NF	Hardy *et al.*, 1968	Bajra Rhizo-sphere	*Pseudomonas putida*	-
PS	PS	-	IARI#	- -	*Pseudo-monas striata*
CD -1	NF	-	IARI#		*Azospirillum brazilense*

@ IAA: Indole acetic acid; PS: Phosphate solubilization; SP: Siderophore production;
NF: Nitrogen fixation # IARI: Indian Agricultural Research Institute, New Delhi, India

Seed treatment

Single colony of UKA24, UKA27, UKA72 and AKA1, each was inoculated in separate 50 ml LB broth and incubated at 29 ±1°C at 180 rpm. The bacterial culture at exponential growth stage (OD: 0.8-1.0) was used as inoculum. Seeds were soaked in respective culture for 30 minutes before sowing in pots. Two ml of each culture was used as inoculums at 15, 30 and 45 days intervals after sowing.

Experimental details

The experiment was conducted in randomized complete block design (RCBD) and replicated three times to determine the impact of PGPR inoculation on basmati rice growth and N, P and K uptake after 90 days of planting in pots. The experimental units were 15 cm diameter plastic pots filled with 2 kg each of sterile soil. Soil was ensured to be moist and wet enough for paddy seed planting. Two seeds treated with bacterial cultures were sown in each pot. The N, P, and K fertilizers were used as urea, triple superphosphate and potassium chloride, respectively. The plants were irrigated with sterilized water at two days interval. Treatments details and parameters studied after 90 days of planting were; T_1-No fertilizer and bacterial inoculation (absolute control); T_2-N 1.5, P_2O_5 1.5, K_2O 1.5 g/pot; T_3-N 0.75, P_2O_5 1.5, K_2O 1.5 g/pot; T_4-N 0.75, P_2O_5 1.5, K_2O 1.5 g/pot + UKA-27; T_5-N 0 .75, P_2O_5 1.5, K_2O 1.5 g/pot + P. striata; T_6-N 0.75, P_2O_5 1.5, K_2O 1.5 g/pot + AKA-1; T_7-N 0.75, P_2O_5 1.5, K_2O 1.5 g/pot + A. brazilense; T_8-N 0.75, P_2O_5 1.5, K_2O 1.5 g/pot + UKA24; T9-N 0.75, P_2O_5 1.5, K_2O 1.5 g/ pot+ UKA72; T_{10}-N 0.75, P_2O_5 1.5, K_2O 1.5 g/pot + UKA-24, 27& 72; T_{11}-No fertilizers+ UKA-24, 27 & 72; T_{12}-No fertilizers+ UKA-24, 27 & AKA1 and T_{13}-No fertilizers+ UKA-24, 27, 72 & AKA1.The parameters studied were plant height (cm), tiller number (shoot/pot), total dry weight (g/pot), root dry weight (g/pot), N uptake (mg/kg), P uptake (mg/kg) and K uptake (mg/kg).

NPK uptake by basmati rice

Straw samples of rice crop were dried in hot air oven at 60^oC for 6 h and ground in a macro-Willey mill to pass through 40 mesh sieves. A representative sample of 0.5 g straw was taken for determination of nitrogen and 0.5 g more for determination of phosphorus and potassium. The nitrogen concentration in the straw sample was determined by modified Kjedahl method (Jackson 1973) and total phosphorus by Vanadomolybdo phosphoric acid yellow colour method and total potassium by flame photometry method, as described by Prasad *et al.* (2006). The NPK concentration in straw was expressed in mg/kg.

Statistical analysis

The data were analyzed using ANOVA, correlation and PCA tools for thirteen treatments including seven variables. The analysis was done using SAS 9.3 software package.

RESULTS AND DISCUSSION

Effect of inoculation offour bacterial isolates along with reference strains on plant growth promoting traits in Pusa Sugandha 4were studied in thirteen treatments. ANOVA was computed for all treatments with seven variables at 5% level of significance which are presented in Table 2.

Table 2.Effectiveness of PGPR on plant growth and NPK uptake of Pusa Sugandha 4 at 90 day

Treat-ments	H	TN	SDW	RDW	N(U)	P(U)	K(U)
T_1	77.06 ^{e}	2.00^e	1.56^e	0.39^h	2.40^h	2.85^i	68.40^d
T_2	106.17^c	3.33^{bcd}	4.50^{bc}	0.76^{cde}	3.46^{cde}	8.77^a	83.30^a
T_3	120.83^b	4.67^a	4.75^{abc}	0.86^{ab}	4.20^{de}	5.65^{de}	81.70^a
T_4	114.67^{bc}	3.33^{bcd}	4.40^{bc}	0.76^{cde}	5.33^{cde}	4.08^h	82.26^a
T_5	133.00^a	3.66^{abcd}	4.84^{abc}	0.78^{bcd}	6.40^{ab}	4.81^{fgh}	73.36^{bcd}
T_6	118.50^b	4.33^{ab}	5.35^a	0.89^a	5.46^{bc}	8.13^{ab}	70.03^{cd}
T_7	120.17^b	2.67^{de}	4.70^{abc}	0.79^{bcd}	5.20^{bcd}	7.45^{bc}	80.66^{ab}
T_8	134.50^a	4.00^{abc}	4.48^{bc}	0.80^{bcd}	6.26^b	4.62^{gh}	76.70^{abc}
T_9	114.00^{bc}	4.00^{abc}	4.70^{abc}	0.76^{def}	6.26^b	7.16^{cd}	83.10^a
T_{10}	121.33^b	3.00^{cde}	4.22^c	0.59^g	7.37^a	8.99^a	78.96^{ab}
T_{11}	106.33^c	3.33^{bcd}	4.97^{ab}	0.85^{abc}	6.50^{ab}	5.65^{ef}	83.87^a
T_{12}	119.50^b	4.00^{abc}	3.45^d	0.69^{ef}	3.46^{ef}	5.09^{fg}	77.36^{abc}
T_{13}	95.00^d	3.00^{cde}	3.61^d	0.68^f	3.46^f	4.34^{gh}	79.30^{ab}
$CD_{5\%}$	9.776	1.081	0.605	0.076	1.037	0.852	7.207

Within columns, means followed by the same letter are not significantly different (P<0.05) using Duncan's multiple range test (DMRT). H: Plant height (cm); TN: Tiller number (per pot); SDW: Shoot dry weight (g/pot); RDW: Root dry weight (g/pot); N(U): Uptake of Nitrogen (mg/kg); P(U):Uptake of Phosphorous (mg/kg); K(U):Uptake of Potassium (mg/kg)

Plant height

Plant height was maximum in UKA-24 treated plants (134.50 cm), followed by *P. striata* (133cm), half dose of nitrogen alone (121.33 cm) and combination of three strains (UKA-24,UKA-27 and UKA-72) (120.83 cm) after 90 days of planting. Significant differences were observed among these treatments. However, no significant difference was noticed between UKA-24 and *P. striata* and between half dose of nitrogen alone and combination of three strains (UKA-24, UKA-27 and UKA-72). They were at par with each other at 90 days of planting with control. Lowest height was recorded in combination of the four strains (AKA-1, UKA-24, UKA-27 and UKA-72) (95 cm) which was at par with untreated plant at 90 days of planting.

Number of tillers

Lowest number of tillers were observed in uninoculated control (2) followed by *A.brazilence* (2.67). No significant difference was observed between other treatments, they were at par with each other. However, the highest value (4.67) was recorded in treatment with half dose of nitrogen followed by AKA-1 (4.33) at 90 days after planting.

Dry weight of shoot

Highest shoot dry weight of 5.35 g was recorded in plants inoculated with AKA-1 followed by 4.97 g in combination of three strains (UKA-24, UKA-27 and UKA-72). Significant differences between all the PGPR treated plants were observed. Lowest shoot dry weight was recorded in untreated control (1.56 g).

Dry weight of root

In Pusa Sugandha-4, highest dry matter accumulation of root was recorded in AKA-1 (0.89 g) followed by treatment with half dose of nitrogen alone (0.86 g) and combination of three isolates (UKA-24, UKA-27 and UKA-72) (0.85g). All the treatments are significant in dry matter accumulation. Lowest dry matter production was registered in untreated control (0.39 g).

Nitrogen uptake

The highest nitrogen uptake at 90 days (7.37 mg/plant) was observed in Pusa Sugandha-4 inoculated with consortia of isolates (UKA-24,UKA-27 and UKA-72) along with half dose of N and full dose of P and K followed by (UKA-24, UKA-27 and UKA-72) without fertilizers (6.5 mg/kg), reference strain *Pseudomonas striata* (6.4 mg/kg) and *Azospirillum brazilense* CD1 (5.2 mg/kg) per plant, all of which were significantly superior over other strains and they also differed significantly among themselves. Both the reference strains and all 4 isolates showed significantly higher nitrogen uptake over absolute control (2.4 mg/kg).

Phosphorus uptake

All isolates including reference strains showed significant increase in the phosphorus uptake by Pusa Sugandha-4 variety of rice plants over uninoculated control (2.85 mg/kg) at 90 days after planting. Among the treatments, consortia of isolates (UKA-24, UKA-27 and UKA-72) along with half dose of N and full dose of P and K recorded the maximum phosphorus uptake (8.99 mg/plant) followed by full dose of NPK (8.77 mg/plant) and the isolates AKA-1 (8.13 mg/kg), both of which were significantly superior over all other strains including two reference strains and absolute control (2.85 mg/kg), however they were not significant among themselves.

Potassium uptake

The highest potassium uptake at 90 days (83.87 mg/kg) was recorded in Pusa Sugandha-4 inoculated with consortia of isolates (UKA-24, UKA-27 and UKA-72) followed by the isolate UKA-72 (83.10 mg/kg). They were at par with control.

Correlation among variables

Significant correlation was observed among different treatments with all variables. Out of these, the shoot and root dry weights were highly correlated (0.92) to each other. Uptake of nitrogen (N), phosphorus (P) and potassium (K) and were positively correlated with plant height and shoot and root dry weight. However, tiller number was negatively correlated with uptake of NP and K (Table 3).

Principle component analysis

Principal Component Analysis (PCA) was done for the seven variables. The first three principal components (PC1, PC2 and PC3) explained nearly 83% of the variability (Data not shown). The pattern plot of principal component 1 (PC1) and PC2 showed that all variables were highly and almost equally correlated with the PC1 (Fig. 1). The uptake of P and K were positively correlated with shoot and root dry weights of plant, however, uptake of N and plant height were positively correlated with each other (Fig. 1). Uptake of N and tiller numbers were negatively correlated (Fig. 1), but other variables were not correlated (Fig. not shown).

The pot experiment conducted in the present investigation on growth promotion in Pusa Sugandha-4 revealed the significant increase in plant growth parameters. This may be attributed to the production of plant growth hormone likeindole acetic acid (IAA) by strain UKA-24. It has often been inferred that rhizobacterially produced indole acetic acid are responsible for growth promotion. A large body of evidence suggested that plant growth promoting

Table 3. Correlation matrix of the variables used in the present study

Varia-bles	H	TN	SDW	RDW	N (U)	P (U)	K (U)
H	1.00	0.65	0.71	0.64	0.47	0.31	0.15
TN		1.00	0.63	0.73	0.05	0.25	0.15
SDW			1.00	0.92	0.50	0.57	0.42
RDW				1.00	0.32	0.36	0.41
N(U)					1.00	0.14	0.11
P(U)						1.00	0.31
K(U)							1.00

H: Plant height; TN: Tiller number; SDW: Straw dry weight; RDW: Root dry weight; N(U): Uptake of nitrogen; P(U):uptake of phosphorous; K(U):Uptake of potassium

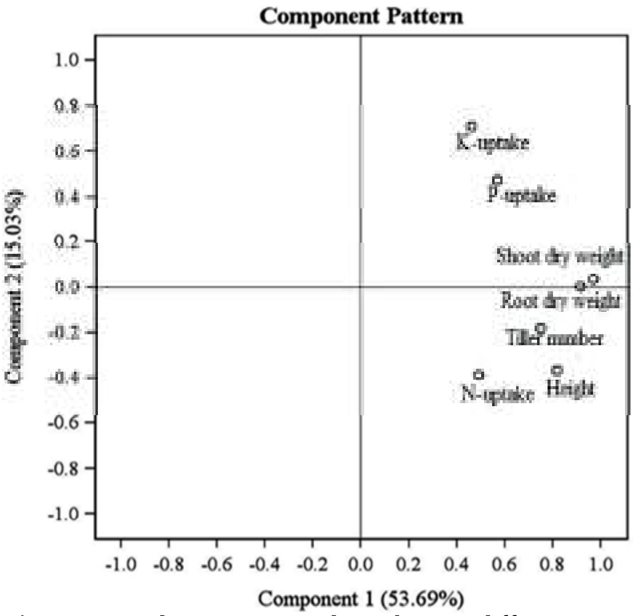

Fig. 1 Principal component analysis showing differentiation of plant variables due to rhizobacterial inoculation in Pusa Sugandha-4

rhizobacteria (PGPR) enhance the growth, seed emergence and crop yield (Dey *et al*. 2004; Glick *et al.* 1999; Kloepper *et al*. 2004; Kokalis-Burelle *et al.* 2006; Herman *et al*. 2008; Minorsky 2008). In addition, the treatment that used either bacterial consortia (UKA-24, UKA-27 & UKA-72) only or along with fertilizers resulted in the highest growth of *basmati* rice. The highest plant nutrient uptake was also observed in these treatments. These results may be due to the fact the bacteria could produce IAA, siderophore, bio-availability of phosphorus and stimulate the assimilation of N, P and K in the plant tissue (Vessey 2003). These findings were similar to Meunchang *et al.* (2006), who reported that rice yield was increased significantly because of inoculation with phosphorous solubilizing bacteria (PSB). On the other hand, control had no significant effect on height, tiller number per hill, total biomass and N, P and K uptake; however, chemical fertilizer had shown their efficacy. Results of this study suggested that the use of these consortia may be used as suitable bacterial inoculants to increase the growth and yield of *basmati* rice. Moreover, the use of PGPR as biofertilizers is an efficient approach to replace chemical fertilizers for sustainable rice cultivation in India. The utilization of biofertilizer can reduce the use of chemical fertilizer and the cost of agricultural production. For long term application, it can lead to healthy soil with less chemical accumulation. Further investigations, including efficiency test under field conditions are needed to clarify the role of these consortia as biofertilizers that exert beneficial effects on plant growth and development in *basmati* rice.

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