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ORIGINAL ARTICLE

Co-inoculation of Urea and DAP Tolerant *Sinorhizobium meliloti* and *Pseudomonas aeruginosa* as Integrated Approach for Growth Enhancement of *Brassica juncea*

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Abstract Two plant growth promoting rhizobacteria—*Sinorhizobium meliloti* RMP1 and *Pseudomonas aeruginosa* GRC_2 were studied for integrated nutrient management to obtain improved yield of *Brassica juncea*. Low concentrations of urea and diammonium phosphate (DAP) stimulated the growth of both *S. meliloti* RMP1 and *P. aeruginosa* GRC_2. 1 M of urea and 0.35 M of DAP was found lethal for RMP1, while 1.3 M and 0.37 M concentrations of urea and DAP proved to be toxic for GRC_2. Lc_{50} was observed as 0.49 M of urea and 0.15 M of DAP for RMP1, and 0.66 M urea and 0.18 M of DAP for GRC_2. Urea and DAP adaptive variants of RMP1 and GRC_2 was isolated. Adaptive bacterial variants had better growth rates at sub-lethal (Lc_{50}) concentrations of urea and DAP as compared to non-adaptive variants. They also retained plant growth promoting attributes similar to non adaptive variants. GRC_2 and RMP1 did not affect the growth of each other and were chemotactically active for DAP, urea as well as root exudates of *B. juncea*. Both the isolates colonized well in the rhizosphere of *B. juncea*, as their populations were recorded ≈ 5 $\log_{10}$ cfu g^{-1} after 120 days. Interestingly, the colonization ability was found even better when both strains were co-inoculated, as their population was recorded in the range of ≈ 6 $\log_{10}$ cfu g^{-1} after 120 days. In field trials, application of RMP1 and GRC_2 resulted in significant increase in biomass and yield of *B. juncea* as compared to control. However, yield was better with application of half dose and full dose of recommended fertilizers. Interestingly, the biomass as well as yield improved further when both isolates were applied together along with half dose of recommended fertilizers.

Keywords *Sinorhizobium meliloti* · *Pseudomonas aeruginosa* · Urea · Diammonium phosphate

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Introduction

The modern cultivation practices exploit millions of ton of chemicals in the form of inorganic fertilizers and pesticides along with various adjuvants, solvents, carriers and diluents, which are applied to the soil or sprayed on the plants. Excessive use of these chemicals exerts deleterious effects on soil microorganism, and also affects the fertility status of soil and pollutes environment [1]. Chemicals used to control pathogens disturb environment, subvert ecology, degrade soil productivity and mismanage water resources [2]. Moreover, because of induction of chemical resistance by fungicides in fungal plant pathogens and non-target side effects on other plant pathogens, alternatives are required to substitute chemicals with bacterial fertilizers and biopesticides [3]. A significant alternative is the use of microorganisms in biological control as non-hazardous strategy [4]. Plant growth-promotion and biological control of soil borne pathogens with plant growth-promoting rhizobacteria (PGPR) has been intensively investigated [5].

The *Brassica* oil seed crops including *Brassica juncea*, *B. napus*, *B. rapa* and *B. campestris* are the world's third most important source of oil seeds and edible oil [6]. Presently, chemical fertilizers such as urea (N-46%) and

diammonium phosphate (DAP; N-18% and P-46%) are applied as source of nitrogen and phosphorus, however, their utilization efficiency remains low in the farmer's field due to loss by volatilization, denitrification, leaching, and conversion into unavailable forms. In the last decade, integrated use of chemical and biofertilizers for improving crop productivity and improvement the soil fertility for sustainable crop production has gained significant importance [7]. To attain this, an approach of blending chemical fertilizers with chemical adaptive bacterial strains derives the synergistic benefits [8].

Pertaining to these facts, in present study, two rhizospheric isolates *Sinorhizobium meliloti* RMP1 and *Pseudomonas aeruginosa* GRC_2 were studied for their potential for integrated nutrient management studies to improve growth and yield of *B. juncea* and reduce use of chemicals. These isolates are well known for their plant growth-promoting (PGP) activities [5, 9]. RMP1 is known to produce siderophore, indole acetic acid (IAA), solubilzed insoluble phosphate and inhibited growth of charcoal rot disease causing *Macrophomina phaseolina*, while GRC_2 is known to have hydrocyanic acid (HCN) and chitinase production ability. GRC_2 is also antagonistic to *Macrophomina phaseolina*, *Fusarium oxysporum* and *Sclerotia sclerotiorum* [5]. Further, to substantiate applicability of *Sinorhizobium meliloti* RMP1 and *Pseudomonas aeruginosa* GRC_2 in rhizosphere, their chemotactic behaviour towards two chemical fertilizers—urea and diammonium phosphate (DAP) was evaluated. Also, they were applied in field along with reduced dose of fertilizers, as individual trials or in co-inoculated conditions. The competence of RMP1 and GRC_2 in *B. juncea* rhizosphere was also experimented.

Materials and Methods

Microorganisms

The rhizobacterial strains viz., *S. meliloti* RMP1 and *P. aeruginosa* GRC_2 were obtained from departmental culture collection, Department of Botany and Microbiology, Gurukul Kangri University, Hardwar (India). *S. meliloti* RMP1 and *P. aeruginosa* GRC_2 were maintained on yeast extract mannitol (YEM) agar medium and Kings' B agar medium. Both strains were stored at −20°C in 20% glycerol until mentioned otherwise.

Isolation of Urea and DAP Adaptive Variants of *S. meliloti* RMP1 and *P. aeruginosa* GRC_2

The lethal concentrations of urea and DAP on bacterial strains were determined by growing them in medium having different concentrations of urea (0.01–1.5 M) and DAP (0.01–1.00 M). For this, YEM broth was used for *S. meliloti* and King's B broth for *P. aeruginosa* GRC_2. The log phase cultures (10^8 cells ml^{-1}) were transferred under aseptic conditions to the respective supplemented medium and incubated at 28°C 150 rpm for 48 h. Optical density was measured at 610 nm after every 6 h interval. Growth stimulatory, sub-lethal (Lc_{50}) and lethal (Lc_{100}) concentrations of urea and DAP were determined by calculating specific growth rate and, growth rate of control/specific growth rate of treatment (Vo/V) as described earlier by Maheshwari and Saraf [10]. The adaptive variants of both RMP1 and GRC-$_2$ were raised against the sub-lethal concentrations (Lc_{50}) of urea and DAP by transferring the surviving colonies on growth medium and medium supplemented with sub-lethal (Lc_{50}) concentrations of urea and DAP, respectively [11]. The plant growth-promoting activities of chemical fertilizer adaptive variants of strains RMP1 and GRC_2 were examined as described earlier [5, 9].

Interaction Study Between Strains RMP1 and GRC_2, and Preparation of Root Exudates

Effect of interaction between the strains RMP1 and GRC_2 on their growth rates was determined according to Sindhu et al. [12]. Supernatants of log phase cultures (10^8cells ml^{-1}) of RMP1 and GRC_2 were prepared by centrifuging the late-log phase cultures at $12{,}000\times g$ for 20 min. The supernatants were passed through membrane filter (0.45 μM). The Whatman filter paper discs (1 cm) soaked in supernatant of GRC_2 were placed on YEM plates pre-inoculated with RMP1 and disc soaked with supernatant of RMP1 was placed on King's B solidified plates pre-inoculated with GRC_2. The inhibition of growth around the filter disc was assessed and recorded after every 6 h of incubation at 28°C, in form of zone of inhibition (if any). For preparation of root exudates, the seeds were surface sterilized with 0.5% sodium hypochlorite (NaOCl) for 5 min and soaked in 0.75% H_2O_2 for 3 min. Germinated seeds with root length of about 5–6 mm were transferred to sterilized glass tubes (170 mm length, 30 mm diameter) containing 20 ml of sterile half strength Knop's solution [13]. Contamination free root exudates solutions were stored at −20°C for further use.

Chemotactic Behavior of Strains for DAP, Urea and Root Exudates

The chemotactic behavior of the strains RMP1 and GRC_2 towards root exudates of *B. juncea*, urea and DAP was determined by capillary assay method [14]. The appropriate concentrations (equal to Lc_{50}) of urea and DAP were added to the buffer and filled into the capillaries, inserted into the cell suspensions of both adaptive variants (10^6–10^7

cells ml^{-1} of buffer) on a glass slide. Root exudates were used in their original concentrations for capillary assay. The cells in the capillaries were plated on to the YEM agar and King's B agar media for RMP1 and GRC_2, respectively after every 30 min of incubation at 28°C, and incubated for 24–48 h at 28°C, after which the total numbers of colonies were scored. Aspartic acid (200 μM) was experimented as positive control while capillary tubes containing buffer alone served as negative control. The chemotaxis index (C.I.) was determined according to Lopez-de-Victoria and Lowell [15], as the ratio of number of bacterial cells accumulated in the test capillaries containing either urea, DAP or root exudate with respect to control.

Seed Bacterization

Method of Weller and Cook [16] was used for seed bacterization. Bacterial strains RMP1 and GRC_2 were grown in YEM broth and King's B broth, respectively for 48 h at 28°C in a bioreactor (*BIOFRRM-L* Scigenics India Pvt. Ltd.). Both the cultures were centrifuged at 7100×*g* for 15 min at 4°C. The culture supernatants were discarded and pellets were washed and resuspended in sterile distilled water (SDW) to get final bacterial population density of approximately 10^8cells ml^{-1}. The cell suspensions of strains RMP1 and GRC_2 (1:1) was mixed with 1% carboxymethylcellulose (CMC) solution to form slurry coated on the surface of seeds, as described earlier [5]. Seeds of *B. juncea* coated with 1% CMC slurry without bacterial strains served as control.

Field Trials

Field trials were carried out in district Haridwar, India (29°66′ 40″N lat., 78°13′E long.) in sandy loam soil (77.3% sand, 13.6% silt, 11.7 clay, total organic C 0.098%, pH 6.4 having 36% water holding capacity). Trials were carried out in 100 m^2 field plots during October 2004 to February 2005. The recommended dose of chemical fertilizers for the crop of *B. juncea* was 100 kg h^{-1} nitrogen, in two split doses in the form of urea and 40 kg h^{-1} phosphate in form of DAP, in two split doses viz. $N_{50+50,}$ P_{20+20} as suggested [17]. Bacterized and non-bacterized seeds were sown on randomized field design in five sets of treatments with three replication of each treatment. (I) seeds bacterized with RMP1, (II) seeds bacterized with GRC_2 (III) non-bacterized seeds + low dose of chemical fertilizers (N_{25+25} P_{20}) (IV) non-bacterized seeds + recommended dose of chemical fertilizers ($N_{50+50,}$ P_{40}) (V) seeds bacterized with adaptive variants of strains RMP1 and GRC_2 (VI) seeds bacterized with adaptive variants of strains RMP1 and GRC_2 + low dose of chemical fertilizers (N_{25+25} P_{20}) and (VII) control (non-bacterized seeds and without chemical fertilizers). The crop was irrigated at different intervals as and when required. Seed germination (%) was recorded on the tenth day after sowing (DAS). Growth and yield parameters were recorded after 120 DAS.

Root Colonization

In our previous studies it was observed that *S. meliloti* RMP1 resistant to 100 μg ml^{-1} ampicillin [9] while *P. aeruginosa* GRC_2 was resistant to 100 μg ml^{-1} streptomycin [5]. *B. juncea* plants, bacterized with strains were sampled after 30, 60, 90 and 120 DAS and bacterial population on the roots was measured. The roots were cut into 1 cm long segments and 1 g of root segments was dipped in 5 ml of SDW and vortex for 5 min. Suitable dilution of the suspension was poured into Petri plates containing YEM agar (ampicillin 100 μg ml^{-1}) and King's B agar (streptomycin 100 μg ml^{-1}) to estimate population of RMP1 and GRC_2, respectively. Cfu per gram of root segment was enumerated after 24 h of incubation at 28°C. Population dynamics of RMP1 and GRC_2 along with other aerobic bacteria were recorded after 30, 60, 90 and 120 DAS.

Statistical Analysis

For each treatment, samples were obtained from three replicate plots per treatment in the completely randomized block design. Bacterial counts (cfu g^{-1}) in broth cultures and soil were analyzed after logarithmic transformation. The data were analyzed and considered to be significantly different at $P \leq 0.05$ and $P \leq 0.01$ [18].

Results

Isolation of Chemical Fertilizers (Urea and DAP) Adaptive Variants of *S. meliloti* RMP1 and *P. aeruginosa* GRC_2

Decrease in growth rate of both RMP1 and GRC_2 strains was observed at higher concentrations of urea and DAP, respectively. The growth of RMP1 was completely inhibited at 1 M of urea and 0.35 M of DAP, while in case of GRC_2, 1.3 and 0.37 M concentrations of urea and DAP proved to be toxic as evidenced by absence of growth on respective medium, hence considered as lethal concentrations. However, the growth rate of RMP1 and GRC_2 were found to increase at low concentrations of urea (0.04 and 0.3 M) and DAP (0.037 M), respectively (Figs. 1, 2). Lc50 was observed as 0.49 M of urea and 0.15 M of DAP for RMP1, and 0.66 M urea and 0.18 M of DAP for GRC_2, as 50% decrease in growth rates of RMP1 and GRC_2 was recorded at these concentrations as compared to control

(data not shown). Interestingly, adaptive bacterial variants showed increase in growth rates at sub-lethal (Lc50) concentrations of urea and DAP. These tolerant variants showed growth pattern identical to non adaptive variants, and also gave PGP activities similar to non adaptive variants (data not shown).

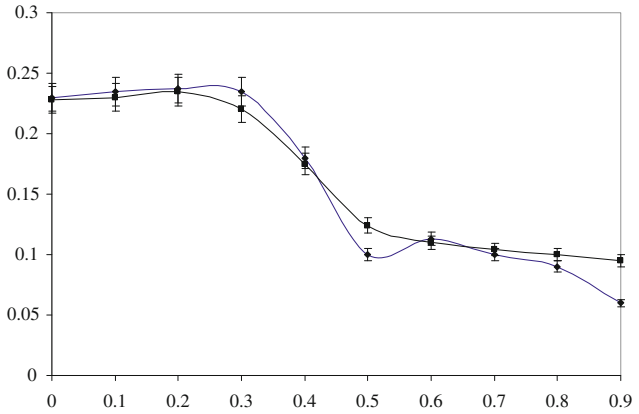

Fig. 1 Effect of urea concentration on specific growth rate of RMP1 (*diamond*) and GRC_2 (*square*). *Error bars* indicate standard error of the mean, where error bars are not visible; they are smaller than the marker

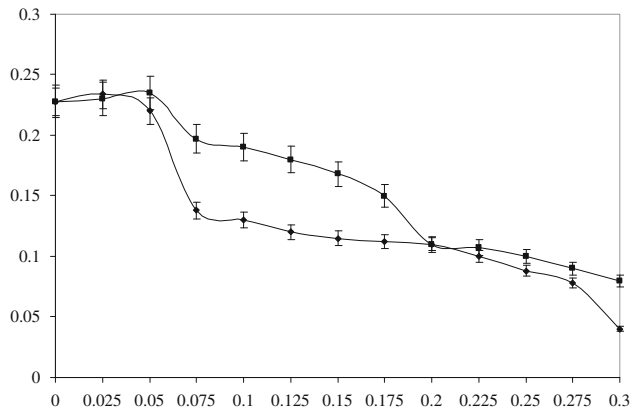

Fig. 2 Effect of DAP concentration on specific growth rate of RMP1 (*diamond*) and GRC_2 (*square*). *Error bars* indicate standard error of the mean, where *error bars* are not visible; they are smaller than the marker

Interaction Between RMP1 and GRC_2, and Chemotaxis Study

P. aeruginosa GRC_2 did not inhibit the growth of *S. meliloti* RMP1 under co-culture conditions in disc test as no inhibition zone was observed around the culture discs of GRC_2 on the lawn of RMP1. Similarly, RMP1 did not suppress the growth of GRC_2.

RMP1 and GRC_2 and their adaptive variants showed good chemotaxis index (C.I.) towards root exudates of crop plant, urea and DAP. The C.I. of adaptive variants for urea and DAP was found invariably higher than respective non adaptive variants, as evidenced by their better chemotactic movement towards crystal of urea and DAP in comparison to wild strains of RMP1 and GRC_2. The C.I. of RMP1 and GRC_2 and their adaptive variants was almost same for root exudates (Table 1).

Root Colonization

Both RMP1 and GRC_2 showed significant increase in rhizospheric population when inoculated individually. The population of RMP1 increased from $\log_{10}$ 4.20 cfu g^{-1} root (after 30 DAS) to $\log_{10}$ 5.83 cfu g^{-1} root (120 DAS), while population of GRC_2 increased from $\log_{10}$ 4.98 cfu g^{-1} root to $\log_{10}$ 5.99 cfu g^{-1} root, in the same duration, in individual trials (Table 2). However, population of both isolates increased considerably when co-inoculated as compared to individual trials. When co-inoculated with GRC_2 the population of RMP1 increased from $\log_{10}$ 4.29 cfu g^{-1} root (30 DAS) to $\log_{10}$ 6.02 cfu g^{-1} root (120 DAS). Similarly, in presence of RMP1, the population of GRC_2 increased from $\log_{10}$ 4.58 cfu g^{-1} root to $\log_{10}$ 6.12 cfu g^{-1} root in same duration (Table 2).

Table 1 Capillary assay of chemotaxis of *Sinorhizobium meliloti* RMP1 and *Pseudomonas aeruginosa* GRC_2 towards root exudates of *Brassica juncea* and chemical fertilizers (urea and diammonium phosphate)

Treatment	RMP1 C.I.	RMP1 Urea-DAP adaptive variant C.I.	GRC_2 C.I.	GRC2 Urea-DAP adaptive variant C.I.
Root exudates	15.67 ± 0.83	17.5 ± 0.89	15.17 ± 0.44	15.88 ± 0.59
Urea	11.27 ± 0.51	13.35 ± 0.88	11.53 ± 0.54	12.96 ± 0.98
DAP	25.16 ± 0.77	28.49 ± 0.67	23.97 ± 0.73	25.39 ± 0.79
Aspartic acid (Positive control)	53.22 ± 4.25	53.42 ± 4.02	52.39 ± 4.77	61.62 ± 3.2
Chemotaxis Buffer (Negative control)	1.0 ± 0.2	1.0 ± 0.31	1.0 ± 0.12	1.0 ± 0.25

C.I. Chemotaxis index (the ratio bacterial number accumulated in the test capillaries containing urea, DAP and root exudates to that of control), *DAP* diammonium phosphate. *data are an average of three replicates

Table 2 Bacterial population in the rhizosphere of *Brassica juncea* var *Pusa Jaikisan*

Bacteria	Log_{10} value cfu g^{-1} root			
	30 DAS	60 DAS	90 DAS	120 DAS
RMP1	4.20 ± 0.14	5.32 ± 0.22	5.72 ± 0.15	5.83 ± 0.14
GRC_2	4.98 ± 0.14	5.74 ± 0.12	5.94 ± 0.22	5.99 ± 0.19
RMP1 (when co-inoculated with GRC_2)	4.29 ± 0.12	5.35 ± 0.21	5.55 ± 0.18	6.02 ± 0.22
GRC_2 (when co-inoculated with RMP1)	4.58 ± 0.11	5.71 ± 0.19	5.82 ± 0.19	6.12 ± 0.24

DAS Days after of sowing. Values are mean of 10 randomly selected plants

Regression coefficient (r) value: RMP1 = 0.969; GRC_2 = 0.963

Table 3 Effect of integrated use of chemical fertilizers and co-inoculants (RMP1 + GRC_2) on growth and yield of *Brassica juncea* after 120 DAS

Treatments	Seed germination (%)	Root			Shoot			Yield	
		Length (cm)	Fresh wt. (g)	Dry wt. (g)	Length (cm)	Fresh wt. (g)	Dry wt. (g)	No of siliquae $plant^{-1}$	Seed yield $Hect^{-1}$ (kg)
RMP1	85	15.2	13.7	8.4	180	86	41	246	631
GRC_2	87	15.9	14.1	8.9	184	89	43	249	639
N_{25+25} P_{20}	89	16.8	15.3	9.2	190	96	52	291	781
N_{50+50} P_{20+20}	88	19.8	20.1	11.3	201	106	62	337	981
RMP1 + GRC_2	90	19.5	20.0	10.1	196	101	60	315	978
RMP1 + GRC_2 + N_{25+25} + P_{20}	95	20.2	21.8	12.4	204	108	69	341	989
Control	79	11.2	10.7	5.9	146	47	28	146	467
SEM	1.46	0.86	0.75	0.37	0.51	0.29	0.43	0.79	0.83
CD @ 1%	6.53	3.85	3.36	1.65	2.31	1.33	1.94	3.56	3.71
CD @ 5%	4.6	2.71	2.36	1.16	1.62	0.93	1.36	2.50	2.61

Values are mean of 10 randomly selected plants from each set

N_{50+50} P_{20+20} full doses of chemical fertilizers, N_{25+25} P_{20} half dose of chemical fertilizers

Field Trial

Dry root and shoot weight, root and shoot length were increased significantly in all the treatments as compared to control 82.97 and 89.36% increase in fresh shoot weight of *B. juncea* plants, and seed yield per hectare was recorded 631 and 639 kg by treatment of RMP1 or GRC_2, respectively. The synergistic effect of RMP1 and GRC_2 was apparent in co-inoculated trial, for growth enhancement of *B. juncea*. 34.24 and 114.89% increase in shoot length and fresh shoot weight was recorded in co-inoculated trial as compared to control. Further, plant growth and yield was significantly improved with application of half dose and full dose of fertilizers. Interestingly, application of urea and DAP along with adaptive bacterial inoculants RMP1 and GRC_2 resulted in maximum increase in number of pods per plant. It was interesting to note that the total grain yield (kg ha^{-1}) was almost similar to that obtained after application of recommended dosages of urea and DAP. Approximately 35% increase in grain yield was obtained with application of either of bacterial strain in individual trials; while co-inoculation of both strains resulted in 109.04% increase in seed yield as compared to control. However, application of both isolates along with reduced dose of fertilizers resulted in 111.77% increase in seed yield with respect to control, which was almost similar to treatment, where full dose of fertilizers were applied (110.06%). Growth and yield parameters obtained from different treatments are summarized in Table 3.

Discussion

In present study, the growth of *P. aeruginosa* GRC_2 and *S. meliloti* RMP1 was subjected to increased concentrations of urea and DAP. The low doses were found to stimulate the bacterial growth, but increasing the concentration of urea and DAP resulted in decreased the growth rate, and also lead to cell lysis and death, which was in accordance to earlier observations [19]. Recently, Bhattacharya and

Roy [20] found that higher concentrations of urea are inhibitory to rhizobial growth, because of alteration in cell membrane permeability and/or effect on cellular DNA synthesis, but extremely low doses urea proved stimulatory for growth rate of rhizobia, which is similar to our findings. Urea and DAP tolerant variants of both strains were obtained and it was observed that their PGP characteristics were restored in vitro. Both RMP1 and GRC_2 were good colonizers of *B. juncea* rhizosphere. The population of both isolates increased ten times after 120 DAS, as compared to population of 30 DAS. Their colonization ability was further substantiated by the finding that both RMP1 and GRC_2 were attracted towards *B. juncea* root exudates. Also, root exudates of *B. juncea* had not any adverse affect on bacterial survival. All the desired characteristics were restored even after acquisition of tolerance to urea and DAP in both isolates. Both strains had urease activity.

DAP when applied to the field release ammonia and provide HPO_4^-, a soluble and available form of inorganic phosphate but soon after it become unavailable due to low solubility and high sorption capacity in soil. Acidification was responsible for inorganic phosphate solubilization by RMP1 which had been reported to form acids [9], resulting drop in pH and cause phosphate soubilization [21]. Whereas, H^+ excreting ATPase seems to be improbable with GRC_2 for phosphate solubilization mechanisms. Ability of phosphate solubilization was restored in RMP1 and GRC_2 after acquisition of resistance to urea and DAP. Variant strains also restored other PGP activities like siderophore production and suppression of the phytopathogenic fungus i.e. *Macrophomina phaseolina* in spite of the presence of urea or DAP, which encourage the utilization of these strains further in rhizosphere. RMP1 and GRC_2 both colonized well in the rhizosphere of *B. juncea* as proved by the regression coefficient (r) values, 0.969 and 0.963 for RMP1 and GRC_2, respectively (Table 2). Earlier it was reported that *S. meliloti* [22] and *P. aeruginosa* [23] secreted acyl homoserine lactones (AHLs) molecules that directly play a role in the root colonization and their behavior in rhizosphere through quorum sensing (intercellular communication) [24]. Since RMP1 and GRC_2 are PGPR strains, hence role played by AHLs is an added advantage that can not be ruled out as these are common among plant-associated species in comparison to that of soil borne species [25].

Distinct microbial populations in rhizosphere frequently interact with each other. Therefore mixed inoculants (combination of microorganisms) that interact synergistically are currently being devised, which yield better and quick results [26]. The secondary metabolites produced by RMP1 and GRC_2 were non-reactive against each other; hence both were able to co-exist. Berggren et al. [27] also observed neutral behavior of *P. putida* towards *R. leguminosarum* under controlled conditions. It has been suggested that bacteria that attain colony-forming units of about $\approx 10^3$ per gram or higher on root mass can be considered as good colonizers [28], and we obtained population of RMP1 and GRC_2 of $\approx 10^5$ per gram in individual trials. The rhizospheric competence of both strains increased in presence of each other as their population was recorded in the range of $\approx 10^6$ per gram. During present study, enhanced plant growth and yield parameters revealed the significance of integrated use of co-inoculant of variants with reduced dose of urea and DAP. The co-inoculation of RMP1 and GRC_2 proved very effective for growth promotion of *B. juncea*, when applied with half dose of fertilizers. 103.73% increase in fresh root weight and 117.8% increase in yield was recorded with the co-inoculation of both strains with half dose of fertilizers. This was in accordance to the findings of root colonization studies. Earlier, Gupta et al. [5] obtained enhanced nodule weight in pea when inoculated with *Pseudomonas* GRC_2. Recently, Pandey and Maheshwari [29] reported commensalism between *Sinorhizobium* and *Burkholderia* sp. resulting in growth enhancement of pigeon pea when applied together. The increase in grain yield with co-inoculation of both strains and half dose of fertilizers was almost similar to that obtained after application of recommended doses of urea and DAP. The results clearly suggest possibility of reduction in chemical use by application of RMP1 and GRC_2. Saraf and Sood [11] suggested possible exploitation of pesticide resistant mutant rhizobial strains for integrated use with chemical fertilizers. Tripathi et al. [30] observed 48% increase in gram yield by using *Rhizobium* with half dose of chemical fertilizers. Similarly, Okon [31] reported 5–30% enhancement in growth and yield of cereals by inoculation with *Azospirillum* sp. with reduced dose of fertilizers. Mohiuddin et al. [32] suggested integrated use of biofertilizers with reduced dose of chemical fertilizers in wheat. However, here we report reduction of chemical fertilizer and growth enhancement of *B. juncea* by integrated use of two PGPR, chemotactically active for these fertilizers, with immense potential for these chemical fertilizer adaptive variants for commercial and environmental benefits.

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