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Effect of Chemical Fertilizer-adaptive Variants, *Pseudomonas aeruginosa* GRC_2 and *Azotobacter chroococcum* AC_1, on *Macrophomina phaseolina* Causing Charcoal Rot of *Brassica juncea*

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ABSTRACT: *Pseudomonas aeruginosa* GRC_2, siderophore-producing strain, inhibited growth of *Macrophomina phaseolina in vitro* and reduced charcoal rot in seeds of *Brassica juncea* in field when coated with adaptive strains. *P. aeruginosa* GRC_2 and *Azotobacter chroococcum* AC_1 produced indole-3-acetic acid and solubilized insoluble phosphate. *A. chroococcum* AC_1 fixed nitrogen asymbiotically. Urea and diammonium phosphate-adaptive variant strains of *P. aeruginosa* and *A. chroococcum* strongly inhibited *M. phaseolina* in comparison to parental strains. Bacterization of seeds induced seed germination, seedling growth, and enhanced yield of *B. juncea* by 10.87% as compared to full doses of urea and diammonium phosphate. Both adaptive strains of chemical fertilizers aggressively colonized roots, showing effectiveness to growth and developments of *B. juncea*.

Key Words: bioinoculants, urea, diammonium phosphate, *Brassica juncea*, *Macrophomina phaseolina*

INTRODUCTION

Species of *Brassica* viz., *B. juncea*, *B. campestris*, *B. napus*, and *B. rapa* are the world's third most important source of edible oil and the second most important oilseed crop after groundnut[1)]. For sustainable crop production and soil fertility restoration, fertilizer management practices involve chemical fertilizers and incorporation of other ecofriendly alternatives in traditional agriculture[2)].

In *Brassica juncea*, *Macrophomina phaseolina* causes charcoal rot, characterized in young plants by black, irregular lesions which form at the base of the cotyledons and extend to the stem causing strangulation and death. In adult plants, the host shows wilting and blockage of the vascular system due to production of microsclerotia which are black or gray in color. Typical symptoms are black or gray lengthwise lesions, premature leaf loss, reduced vigor and finally diminished yields.

Nitrogen and phosphorus are the two essential nutrients required for plant growth. Their major physiological roles are accumulation and release of energy during cellular metabolism[3)]. Chemical fertilizers such as urea (N-46%) and diammonium phosphate (DAP; N-18% and P-46%) are important sources of nitrogen and phosphorus for plant growth; however, their utilization efficiency remains low in the farmer's field due to loss by volatilization, denitrification, leaching, and conversion into unavailable forms. Contamination of ground water by NO_3^- is one of the most serious causes of ground water pollution due to the use of chemical fertilizers.

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Plant growth promotory rhizobacteria (PGPR) induce plant growth and protect crops against various diseases including charcoal rot caused by *Macrophomina phaseolina*. Integration of PGPR with biocontrol potential as well as managed amount of traditional inorganic fertilizers in the field may prove to be effective means to increase the nitrogen and phosphorus availibilities to plants with simultaneous reduction in disease incidences. They take systemic and simultaneous account of environmental aspects, quality of the produce, and profitability of agriculture[4]. To substitute chemicals with bacterial fertilizers and biopesticides, alternatives are needed, especially blending of chemical fertilizers with chemical adaptive strains is an approach to obtain sustainable fertility of the soil[5].

Keeping in view, development of new agro-techniques, particularly a blend of chemical (urea and DAP) and biological nutrient management technologies, the present work was aimed to investigate the management of charcoal rot disease of *B. juncea* by *Pseudomonas aeruginosa* GRC_2 and *Azotobacter chroococcum* AC_1.

MATERIALS AND METHODS

Microorganisms and chemical fertilizers

P. aeruginosa GRC_2 was isolated from potato rhizosphere from a farmer's field using standard microbiological techniques[6] and maintained on tryptic soy agar medium (TSM) at 4℃ for further use. *A. chroococcum* AC_1 was procured from cultures maintained in our laboratory. The morphological, physiological, and biochemical characterizations were determined according to Bergey's Manual of Determinative Bacteriology[7] (data not shown). *M. phaseolina* was isolated from diseased seeds of *B. juncea* var. Pusa Jai Kisan using the blotter technique[8], maintained on Czapek-Dox agar at 4℃, and identified by comparing with the standard culture.

Urea and DAP were procured from a local market, applied as sources of nitrogen and phosphorus, respectively, as per standard quantity (urea at 50 Kg ha^{-1} and DAP at 40 Kg ha^{-1}) and other varying treatments in furrows at the time of sowing, and mixed with the soil. The remaining 50% urea (N 50 Kg ha^{-1}) was applied once 30 days after sowing (DAS).

Physiochemical characteristics of bacterial strains related to plant growth-promoting activities

Phosphate solubilization abilities of both strains were detected by spotting them separately on Pikovskya's agar plates[9]. Plates were incubated and observed for the development of clearing zone around the colonies.

To test indole-3-acetic acid (IAA) production, culture supernatants were collected seperately by centrifugation at 9,300 g for 15 min at 4℃ and passed through 0.2 μm Millipore filter paper. Into two separate test tubes, 2 mℓ each supernatant and 100 μℓ of 10 mM *o*-phosphoric acid were added. The test tubes were incubated at room temperature for 25 min and visually examined for the presence of pink color, which is an indication of IAA production[10].

Siderophore production was estimated qualitatively on Chrom-Azurol-S agar medium (CAS), and type of siderophore was determined following the method of Neilands[11].

For free-living nitrogen fixation ability, *A. chroococcum* AC_1 was grown on nitrogen-free medium[12]. AC_1 strain was streaked on to the medium surface, incubated at 28±1℃ for 3~4 days, and observed for growth. The strains that grew after sequential transferring for 10 times to the same medium, were considered positive for N_2 fixation.

To measure the antagonistic activities of adaptive strains of *P. aeruginosa* GRC_2 and *A. chroococcum* AC_1 against *M. phaseolina*, dual culture technique was performed[13].

Growth inhibition of treatment against control was calculated by percentage, using the following formula.

Inhibition ratio (%) = {1- mycelium growth of treatment (mm) / mycelium growth of control (mm)} × 100

Development of chemical fertilizer (urea and DAP) -adaptive variants of *P. aeruginosa* GRC_2 and *A. chroococcum* AC_1

To determine the lethal, sublethal and growth-inducing levels of fertilizers, graded concentrations of urea (1-200 μg $mℓ^{-1}$) and DAP (1-200 μg $mℓ^{-1}$) were supplemented in sterilized Tryptic Soy Broth for *P. aeruginosa* GRC_2 and nitrogen-free broth for *A. chroococcum* AC_1. The exponentially grown cultures (10^8 cells $mℓ^{-1}$) were transferred under aseptic conditions to the respective supplemented media and incubated at 28 ±1℃ and 150 rpm on gyroratory shaker for 48 h. At 6 h intervals from the time of inoculation, optical density was observed at 610 nm spectrophotometrically. Growth-inducing and sublethal concentrations (LC_{50})

of urea and DAP were determined by calculating the growth rate of control/specific growth rate of treated cultures[14]. The adaptive GRC_2 and AC_1 were raised against LC_{50} of urea and DAP by transferring the surviving colonies singly on to the growth medium supplemented with LC_{50} of urea and DAP, respectively.

Interaction between *P. aeruginosa* GRC_2 and *A. chroococcum* AC_1

Supernatants of exponentially grown cultures of adaptive variants of GRC_2 and AC_1 were prepared by separate centrifugation at 9,600 g for 20 min and passed through Millipore membrane filter (0.45 μm). Whatman filter paper discs (1 cm) soaked in GRC_2 supernatant were placed on the surface of solidified nitrogen -free medium plates pre-seeded with AC_1. Similarly, disc soaked with AC_1 supernatant was placed on TSM plates pre-seeded with GRC_2. Interaction was assessed at 6 h intervals during incubation for 48 h.

Development of antibiotic-resistant marker

Exponentially grown cultures of adaptive variants of GRC_2 and AC_1 strains were spread onto TSM and nitrogen-free agar plates, respectively. Streptomycin and chloramphenicol antibiotic discs (Hi media, Bombay, India) were placed over the surfaces of pre-seeded bacterial cultures, and the plates were incubated at 24 ± 1℃ for 24 h. The width of inhibition zone around the disc was measured. Resistant marker strains were developed by subjecting the cultures successively to different antibiotics from low to high concentrations (100 μg $mℓ^{-1}$ each).

Seed bacterization

Adaptive strains of GRC_2 and AC_1 were grown in TSM and nitrogen-free broths, respectively, for 48 h at 28±1℃ in a fermentor (*BIOFERM-L*, Scigenics India Pvt. Ltd.), and centrifuged at 9,300 g for 15 min at 4℃. After discarding the culture supernatants, pellets were washed and resuspended in sterile distilled water to obtain final bacterial cell density of $1x10^8$ cells $mℓ^{-1}$. Bacterial cell suspensions of chemical fertlizer-adaptive strains of GRC_2 and AC_1 were mixed separately with 1% carboxymethylcellulose solution in the form of slurry to coat the surface of seeds. In the case of mixed inoculum (1:1), final titer was approximately the same ($1x10^8$ cells $mℓ^{-1}$) as required by individual strain for seed bacterization.

Root colonization

B. juncea plants bacterized with chemical fertlizer-adaptive strains were sampled after 30, 60, 90, and 120 DAS, and bacterial population on the roots was measured. Plants were carefully removed with a shovel, and soil particles loosely adhered to the roots were gently removed. The roots were cut into 1 cm segments, and 1 g root segments was dipped in 5 mℓ sterile distilled water and vortexed 4-5 times. One milliliter aliquot of the above suspension was poured into Petri plates containing TSM and nitrogen-free medium supplemented with the antibiotics selectively ineffective for GRC_2 and AC_1, respectively, to evaluate the population of GRC_2 and AC_1. After 24 h incubation, CFU g^{-1} root segment was counted.

Field study

Field trials were carried out in sandy loam soil (77.3% sand, 13.6% silt, 11.7% clay, 0.0976% total organic C, pH 6.4, 36% water-holding capacity) in Haridwar district, Uttaranchal (India) (29066′ 40′′N lat, 78013′E long). *In vivo* disease suppression of charcoal rot was carried out in 16 m^2 (4 x 4 m) plots from October 2002 to February 2003, and integrated nutrient management -based field trial was conducted in 100 m^2 (10 x 10 m) plots from October 2003 to February 2004.

In vivo charcoal rot suppression

Bacterized with adaptive strains and non-bacterized seeds were sown on randomised plot design in seven sets of treatments with three replicates of each treatment as followings: I, soil inoculated with *M. phaseolina* + non-bacterized seeds; II, seeds bacterized with GRC_2; III, seeds bacterized with AC_1; IV, soil inoculated with *M. phaseolina* + bacterized seeds with GRC_2; V, soil inoculated with *M. phaseolina* + bacterized seeds with AC_1; VI, seeds bacterized with GRC_2 + AC_1; and VII (control), seeds unbacterized and without fungal inoculation. The plots were irrigated four times at different intervals. Seed germination (%) was noted on 10 days after sowing (DAS). Seedling growth (shoot and root lengths, and fresh shoot and root weights), total number of pods, and grain yield were recorded at 30, 60, 90, and 120 DAS.

Integrated nutrient management-based field trials

The recommended dose of chemical fertilizers for *B. juncea* crop was 100 Kg ha^{-1} nitrogen, in two split

doses in the form of urea, and 40 Kg ha^{-1} phosphate in single dose viz N_{50+50}, P_{40}[15]. Bacterized with adaptive strains, GRC_2 and AC_1, and non-bacterized seeds were sown on randomised field design in six sets of treatments with three replicates of each treatment as followings: I, seeds coated with GRC_2; II, seeds coated with AC_1; III, seeds coated with GRC_2 + AC_1; IV, non -baterized seeds + recommended dose of chemical fertilizer (N_{50+50}, P_{40}); V, non-bacterized seeds + reduced dose of chemical fertilizers (N_{25+25}, P_{20}); VI, seeds coated with adaptive variants of strains GRC_2 + AC_1 + reduced dose of chemical fertilizers (N_{25+25}, P_{20}); VII, seeds coated with strains GRC_2 + AC_1 + reduced dose of chemical fertilizers (N_{25+25}, P_{20}); and VIII (control), non -bacterized and without chemical fertilizer. The plots were irrigated four times at different intervals. Seed germination rate (%) was noted on 10 DAS. Seedling growth (shoot and root lengths, and fresh shoot and root weights), growth parameters, and grain yield were recorded at 30, 60, 90, and 120 DAS.

RESULTS AND DISCUSSION

Physiochemical characteristics of GRC_2 and AC_1

P. aeruginosa GRC_2 and *A. chroococcum* AC_1 showed phosphate solubilization and IAA production. GRC_2 also produced orange color on CAS agar plates, as well as yellow-orange halo around the colonies, as indication of siderophore production[17]. Supernatant of GRC_2 culture showed a major peak at 400 nm that corresponded with the hydroxamate type of siderophore. AC_1 failed to produce the siderophore, but showed significant nitrogen-fixing ability when repeatedly grown on nitrogen-free medium[16].

Both adaptive strains inhibited the growth of *M. phaseolina* on modified TSM plates and nitrogen-free medium, respectively. For *in vitro* inhibitory effect against *M. phaseolina*, plates were inoculted with adaptive strains of GRC_2 and AC_1 on the modified TSM and nitrogen free medium. A disc of fungal inoculum was removed from a previous culture of *M. phaseolina* and placed upside down in the center of the petri dishes. The plates were incubated for 5-7 days, time by which the growth of control would have reached the edges of the plates. Growth inhibition of *M. phaseolina* was calculated as the percentage of inhibition of radial growth relative to the control along with antifungal effect on mycelia growth. As shown in Fig. 1, GRC_2 caused 80% growth inhibition of *M. phaseolina*. Inhibition in radial growth of the test fungus increased with time. The maximum growth inhibition was recorded 5 days after incubation (Fig. 1). Maximum values of regression coefficients of GRC_2 and AC_1 against *M. phaseolina* were 0.909 and 0.898, respectively, indications of significantly effective correlation between fungal inhibition and incubation time of bacteria.

Development of chemical fertilizer (urea and DAP) -adaptive variants of *P. aeruginosa* GRC_2 and *A. chroococcum* AC_1

The sublethal doses (LC_{50}) of urea and DAP for GRC_2 were 15 and 12 μg $m\ell^{-1}$, respectively. Similarly, LC_{50} values of urea and DAP for AC_1 were 33 and 22 μg $m\ell^{-1}$, respectively. However, lower doses of urea (2.4 and 2 μg $m\ell^{-1}$) and DAP (4.7 and 5.1 μg $m\ell^{-1}$) induced the growth of GRC_2 and AC_1, and were termed as inducer doses (Table 1).

The adaptive strains of GRC_2 and AC_1 grew more

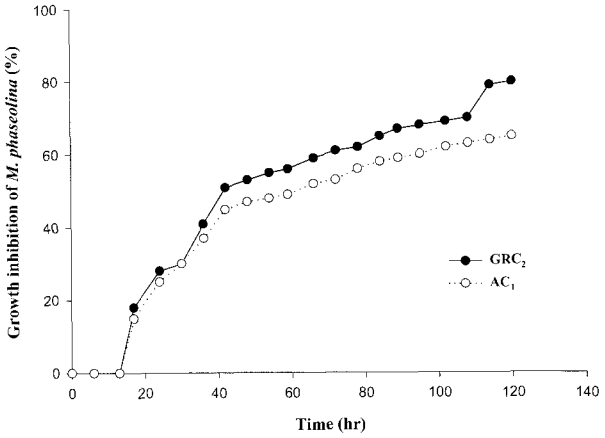

Fig. 1. Growth inhibition of *Macrophomina phaseolina* by *Pseudomonas aeruginosa* GRC_2 and *Azotobacter chroococcum* AC_1.

Table 1. Growth inducer and lethal doses (LC_{50}) of urea and diammonium phosphate for *Pseudomonas aeruginosa* GRC_2 and *Azotobacter chroococcum* AC_1

Chemical fertilizer		Doses/concentrations (μg $m\ell^{-1}$)	
		P. aeruginosa GRC_2	*A. chroococcum* AC_1
Urea	Inducer dose	2.4	2
	LC_{50}	15	12
DAP	Inducer dose	4.7	5.1
	LC_{50}	33	22

successfully than their parental cultures on TSM and nitrogen-free growth media supplemented with LC_{50} of urea and DAP, indicating the metabolically stable adaptive variants of GRC_2 and AC_1 (data not shown).

Interaction between GRC_2 and AC_1 strains

No inhibition zone was observed in lawns of adaptive GRC_2 and AC_1, when supernatant-soaked discs of both strains were placed in oppositely pre-seeded plates, showing that both bacteria did not inhibit the growth of each other. Such mutual interaction led to the conjecture of proto-cooperation.

In vivo charcoal rot suppression

B. juncea seeds bacterized with chemical fertlizer (urea and DAP)-adaptive strains of AC_1 and GRC_2 strains increased seed germination in *M. phaseolina*-infested soil by 15.5% and 17.6%, respectively, as compared to the control. Seed germination in *M. phaseolina* -infested soil was only 32%. Significant reductions in seed germination, plant growth, and yield were recorded (Table 2). The bacterized seeds in pathogen-infested soil lowered disease incidence by 48%.

Root colonization

Adaptive strains of GRC_2 and AC_1 efficiently colonized the root system of *B. juncea*. Adaptive variants of GRC_2 and AC_1 also ggressively colonized the rhizosphere of *B. juncea* as evidenced by 4.65 and 3.98 $\log_{10}^{CFU}$ of GRC_2 and AC_1, respectively, after co-inoculation in the presence of low doses of urea and DAP (Table 3). The antibiotic-resistant marker of adaptive variant strains (GRC_2^{str+} and AC_1^{chl+}) survived well and

Table 2. Effect of adaptive variants of *Pseudomonas aeruginosa* GRC_2 and *Azotobacter chroococcum* AC_1 on suppression of *M. phaseolina* and growth and yield of *Brassica juncea*

Tested organism	Seed germination (%)	Root length (cm)	Root weight (g)	Shoot length (cm)	Shoot weight (g)	Pod $plant^{-1}$	Grain yield $plant^{-1}$ (g)	Disease incidence (%)
M. phaseolina	32.1	5.6	1.5	7.6	5.2	50	3.5	90.2
P. aeruginosa GRC_2	78.3	16.1	11.12	114	66.5	192	13.5	0.9
A. chroococcum AC_1	75.5	15.8	10.85	112	64.8	189	13.1	1.2
GRC_2 + AC_1	79.2	19.3	12.4	118	72.4	221	17.21	0.5
GRC_2 + *M. phaseolina*	75.6	14.8	10.2	109	59.6	180	11.7	2.3
AC_1 + *M. phaseolina*	73.5	13.9	9.80	102	58.9	172	11.29	2.5
Control	58	12.0	7.8	103.2	52.6	112	10.5	5
CD at 1% level[1]	2.50	58.6	12.5	38.4	115.3	7.05	15.3	-
CD at 5% level	0.47	12.0	2.51	7.3	26.8	1.25	2.5	-

CD at 1% level[1] means critical difference at 1% probability.
Values are mean of three replicates of random sampling.
Analysis of variance (ANOVA) with two factors (variety x treatment).

Table 3. Root colonization of *Brassica juncea* cv. Pusa Jaikisan by *Pseudomonas aeruginosa* GRC_2 and *Azotobacter chroococcum* AC_1 and their adaptive variants after 30, 60, 90, and 120 days after sowing (DAS)

Treatments	Bacterial population ($\log_{10}^{CFU}$)			
	30 DAS	60 DAS	90 DAS	120 DAS
P. aeruginosa GRC_2	4.50 ± 0.15	5.33 ± 0.12	5.45 ± 0.14	5.55 ± 0.18
A. chroococcum AC_1	4.20 ± 0.14	4.48 ± 0.14	4.98 ± 0.15	5.10 ± 0.10
GRC_2 with reduced chemical fertilizers	3.21 ± 0.90	3.83 ± 0.99	4.41 ± 0.90	4.65 ± 0.15
AC_1 with reduced chemical fertilizers	3.01 ± 0.14	3.11 ± 0.12	3.81± 0.14	3.98 ± 0.12
GRC_2* with reduced chemical fertilizers	3.80 ± 0.99	5.10 ± 0.90	5.22 ± 0.99	5.60 ± 0.20
AC_1* with reduced chemical fertilizers	4.30 ± 1.1	4.81 ± 0.99	5.40 ± 1.4	5.33 ± 0.15

Values are mean ± SE from three replicates of each treatment.
* means adaptive variant for chemical fertilizers.

effectively colonized the rhizosphere of *B. juncea* when treated with reduced doses of chemical fertilizers. After 120 DAS, 5.60 and 5.33 $\log_{10}^{CFU}$ of adaptive variants of GRC_2 and AC_1 were, respectively, recorded.

Integrated nutrient management-based field trial

After bacterization adaptive GRC_2 and AC_1 enhanced seed germination of *B. juncea*. All treatments showed enhanced seed germination with maximum germination (36.6% increase as compared to the control) recorded in a set co-inoculated with GRC_2 + AC_1 (Table 2). Co-inoculation of adaptive variants (GRC_2 + AC_1) and wild strains, (GRC_2 + AC_1) with half dosage of urea and DAP significantly enhanced the root length (Table 4).

Plant growth and yield were also enhanced with co-inoculation of GRC_2 + AC_1 variants with half dose of chemical fertilizers. An increase in yield was recorded, while comparing with the application of full dose and by inoculating with wild type strains of GRC_2 and AC_1, in combined form with low dose of urea and DAP (Table 4). Fresh and and dry weights of root and shoot were enhanced significantly at 1% level of LSD at 120 DAS in all treatments. Grain yield ha^{-1} was increased by 67, 51, and 45% with co-inoculation of adaptive variant strains of GRC_2 and AC_1 with reduced doses of chemical fertilizers, recommended doses of chemical fertilizers and co-inoculation of wild strains of GRC_2 and AC_1 with reduced dose of chemical fertilizers, respectively, in comparison to untreated plants (Table 4).

Both GRC_2 and AC_1, showing phosphate-solubilizing and PGPR activities, inhibited the growth of *M. phaseolina*. Earlier effects of siderophore[17], antimicrobial substances[16], and growth hormone have been found to affect plant growth and yield directly and indirectly. GRC_2 produced hydroxamate type of siderophore, and also caused a significant reduction in radial growth of *M. phaseolina in vitro*. Inhibition zone between the antagonists GRC_2 and *M. phaseolina* may be attributed to the production of siderophore. GRC_2 and AC_1 solubilized insoluble phosphate to release phosphorus besides IAA production, which is a more common feature in various species of rhizobacteria[18].

A positive correlation was found between *in vitro* antagonism and *in situ* antagonism in field experiments[10,16,17,19]. Seed germination of *B. juncea* increased considerably in *M. phaseolina*-infested soil with introduction of adaptive variants of GRC_2 and AC_1.

Bacterial growth declined with increasing concentrations of urea and DAP. Morphological changes were observed in the GRC_2 cells grown under the influence of higher concentrations of urea. Bhattacharya and

Table 4. Combined effect of *Pseudomonas aeruginosa* GRC_2 and *Azotobacter chroococcum* AC_1 with and without urea and DAP on growth of *Brassica juncea* cv Pusa Jaikisan at 120 days after sowing (DAS)

Treatment	Root			Shoot			Grain yield $Plant^{-1}$	Grain yield (Kg ha^{-1})
	Length (cm)	Fresh weight (g)	Dry weight (g)	Length (cm)	Fresh weight (g)	Dry weight (g)		
P. aeruginosa GRC_2	16.10 **	11.12	8.98	116 .0	69.12 *	47.5	13.86	670
A. chroococcum AC_1	17.28 **	14.85	9.58 **	161.0 **	87.95 *	65.85 *	14.51	788 *
GRC_2 + AC_1	18.79 **	15.51 *	10.35 **	180.5 **	93.68 **	74.11 **	16.60	880 *
Recommended chemical fertilizers (N_{50+50} P_{40})	20.50 **	17.38 *	11.56 **	186.0 **	98.83 **	88.25 **	18.52*	920 **
Reduced chemical fertilizers (N_{25+25} P_{20})	18.35 **	15.01 *	9.98 **	166.8 **	90.98 **	68.50 *	16.12 ns	853 *
GRC_2 + AC_1 (wild strains) with reduced chemical fertilizers	18.89 **	15.58 *	10.85 **	182.6 *	94.23 **	76.32 **	16.95 *	885 *
GRC_2 + AC_1 (adaptive strains) with reduced chemical fertilizers	21.55 **	18.92 **	11.90 **	188.4 **	102.96 **	91.58 **	19.00 **	1020 **
Control (non-bacterized seed and without fertilizer)	12	8.8	4.98	105.5	54.23	39.35	10.22	610

Values are means of 10 randomly selected plants from each set.
* and ** mean significant at 5% and 1% level of least significant difference.

Roy[20] suggested that inhibitory effects were due to the alteration in cell membrane permeability and/or cellular DNA synthesis. Similarly, inhibitory effects of higher concentrations of urea and DAP on GRC_2 and AC_1 are obvious; thus, the possibility of cell membrane alteration can not be ruled out.

Antibiotic markers have been developed, and used to monitor and re-isolate the introduced beneficial rhizobacteria from experimental fields[21]. Adaptive variants of GRC_2 and AC_1 developed resistance towards 100 μg $m\ell^{-1}$ each streptomycin and chloramphenicol, respectively. Results revealed the effective root colonization by both rhizobacteria, suppressed the growth of *M. phaseolina*, while tolerating the influence of chemical fertilizers that actually helped them in specialized doses during their establishment in the rhizosphere. Efficient root colonization resulted in enhanced plant growth and grain yield of *B. juncea.*

In the present study, chemical, biological, and integrated approaches were applied to obtain better growth and yield of *B. juncea* cv Pusa Jaikisan. The enhanced plant growth and yield parameters reveal the significance of integrated use of GRC_2 and AC_1 with lower doses of chemical fertilizers (N_{25+25}, P_{20}). The co-inoculants GRC_2 and AC_1, applied with reduced dose of chemical fertilizers (N_{25+25}, P_{20}), increased the yield to level almost similar to that obtained after application of recommended dose of chemical fertilizers (N_{50+50}, P_{40}) without any aid of allochthonous microorganisms. Chemical fertilizer-adaptive variants of GRC_2 and AC_1 restored the ability to survive in the presence of higher dose of chemical fertilizers without loosing their PGPR traits.

In the presence of recommended dose of chemical fertilizers (N_{50+50}, P_{40}) grain yield of crop ha^{-1} increased by 51% over the control, whereas reduced dose of chemical fertilizers (N_{25+25}, P_{20}) with co-inoculant of chemical fertilizer-adaptive variants of GRC_2 and AC_1 enhanced 67% yield ha^{-1} over the control.

The *Azotobacter* and other free living nitrogen fixers have been reported in relation to their favorable effects on plant dry weght[22]. AC_1 fixed the atmospheric nitrogen as well as solubilized the inorganic phosphate. AC_1 showed a significant inhibition of *M. phaseolina*, as supported by the finding that *Azotobacter* produces an ether-soluble fungistatic substance, which inhibits the growth of fungi like *Alternaria, Helminthosporium* and *Fusarium* spp[23].

These results suggest that adaptive strains of GRC_2 and AC_1 have the potential to be used in seed coating to inhibit charcoal rot caused by *M. phaseolina*. The positive root colonization ability of GRC_2 and AC_1 lies in the strains being successful colonizers of spermosphere, and increasing seedling emergence, and their establishment in the rhizosphere of *B. juncea*, giving protection against targeted pathogen, which resulted in enhanced yield.

About 50% reduction in the levels of chemical fertilizers (urea and DAP) was recorded when GRC_2 and AC_1 were used together, along with managed amount (urea at 50 Kg ha^{-1} and DAP at 40 Kg ha^{-1}) of chemical fertilizers. The strains produced almost similar results when half dose of chemical fertilizers, full dose of fertilizers, and without bacteria were used, all enhancing the growth and yield of *B. juncea.*

In conclusion, chemical fertilizer-adaptive variants of *P. aeruginosa* GRC_2 and *A. chroococcum* AC_1 showed marked reductions in charcoal rot incidences in *B. juncea*, increasing the yield when combined with reduced dosages of chemical fertilizers. Both bacterial strains substantiated the requirements of chemical fertilizers, but with a significant reduction of chemicals in farming system for a better environment and ecology.

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