Research Article

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Rhizobacteria-mediated resistance against the blackeye cowpea mosaic strain of bean common mosaic virus in cowpea (*Vigna unguiculata*)

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Abstract

BACKGROUND: The present study investigated the effect of seven *Bacillus*-species plant-growth-promoting rhizobacteria (PGPR) seed treatments on the induction of disease resistance in cowpea against mosaic disease caused by the blackeye cowpea mosaic strain of bean common mosaic virus (BCMV).

RESULTS: Initially, although all PGPR strains recorded significant enhancement of seed germination and seedling vigour, GBO3 and T4 strains were very promising. In general, all strains gave reduced BCMV incidence compared with the non-bacterised control, both under screen-house and under field conditions. Cowpea seeds treated with *Bacillus pumilus* (T4) and *Bacillus subtilis* (GBO3) strains offered protection of 42 and 41% against BCMV under screen-house conditions. Under field conditions, strain GBO3 offered 34% protection against BCMV. The protection offered by PGPR strains against BCMV was evaluated by indirect enzyme-linked immunosorbent assay (ELISA), with lowest immunoreactive values recorded in cowpea seeds treated with strains GBO3 and T4 in comparison with the non-bacterised control. In addition, it was observed that strain combination worked better in inducing resistance than individual strains. Cowpea seeds treated with a combination of strains GBO3 + T4 registered the highest protection against BCMV.

CONCLUSION: PGPR strains were effective in protecting cowpea plants against BCMV under both screen-house and field conditions by inducing resistance against the virus. Thus, it is proposed that PGPR strains, particularly GBO3, could be potential inducers against BCMV and growth enhancers in cowpea.

Keywords: potyvirus; BCMV-BICM; *Bacillus*; plant-growth-promoting rhizobacteria; virus management

1 INTRODUCTION

Present-day agriculture is increasingly dependent on the use of chemicals for increased yield and disease management. Increased dependence on chemicals is inevitably associated with problems of environmental and health hazards. In this context, plant-growth-promoting rhizobacteria (PGPR) are often novel and potential tools to provide substantial benefits to agriculture, and they present immense potential and promise as effective substitutes for chemicals.

The obligatory nature and intimate relationship of viruses with a host plant are quite complex and nearly impossible to control. However, attempts are made to keep them in check and reduce loss – basically to manage their existence within a crop. Most of the viral management schemes integrate with grower's cultivation practices, e.g. altering planting dates to avoid vector migrations, various mulches to deter vectors and use of trap crops.

Although genetic resistance to virus infection is the preferred approach, effective resistance genes identified are limited in number, and there is a serious need for new sources of virus resistance in many crops. Traditional breeding methods for virus resistance are labour intensive, time consuming, and often undesirable traits must be selected out in order for a new variety to be commercially acceptable. Many virus coat protein genes expressed in transgenic plants deliver only a small percentage of highly resistant lines, with the majority of lines showing moderate levels of resistance or susceptible responses.[1]

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Therefore, there is a continuous search for safe and ecofriendly management practices for plant diseases. PGPR are a wide range of root-colonising bacteria with the capacity to enhance plant growth by increasing seed emergence, plant weight and crop yields,[2] and they have been used to enhance the growth of several crops.[3–8] The induced systemic resistance (ISR) is phenotypically similar to pathogen-induced systemic acquired resistance (SAR) in that it confers an enhanced defensive capacity against diseases caused by fungi, bacteria, viruses and nematodes. SAR is associated with the accumulation of plant-pathogenesis-related proteins, some of which have been demonstrated to possess antimicrobial properties.[9]

PGPR are among the various groups of plant-associated microorganisms that can elicit plant defences.[10] Most reports on using PGPR have been for application of a single bacterial strain. The inconsistent performances by PGPR reported under field conditions may be partially accounted for this, because a single biological agent is not likely to be active in all soil environments. In contrast, when mixed treatments of PGPR strains were applied directly to seeds or seedlings before sowing or transplanting, these mixture treatments improved the effect of plant growth promotion or induced systemic resistance in many cases, compared with single treatment.[3,11,12]

The significant improvement observed as a result of bacterisation with PGPR during various field trials indicates the possibility of evolving ecofriendly input for farmers with limited sources. PGPR strains as promising inducers against the blackeye cowpea mosaic strain of the bean common mosaic virus (BCMV) is the focus of the present study.

2 MATERIALS AND METHODS

2.1 PGPR strains and inoculum preparation

Seven PGPR strains (*Bacillus pumilus* INR7, *B. pumilus* T4, *B. amyloliquefaciens* IN937a, *B. subtilis* IN937b, *B. subtilis* SE34, *B. subtilis* GB03 and *Brevibacillus brevis* IPC11) were originally obtained from the culture collections of the Department of Entomology and Plant Pathology, Auburn University, Alabama (courtesy of Prof. JW Kloepper and Prof. MS Reddy). PGPR strains were stored in tryptic soy broth amended with glycerol (20%) at −80 °C prior to use.

Bacterial cell suspensions were prepared by streaking the isolates onto tryptic soy agar and incubating at 27 °C for 24 h to check for purity, then transferring single colonies to tryptic soy agar plates. After 24 h, the bacterial cells were harvested from plates in sterile distilled water and centrifuged at 6000 rpm (Thermo, USA) for 5 min. The pellet obtained was resuspended in sterile distilled water and again subjected to centrifugation, and the supernatant was discarded. The pellet was reprocessed twice and finally collected with minimum sterile distilled water. The optical density of the bacterial suspension was adjusted using a UV-visible spectrophotometer (Hitachi, Japan) to obtain a final density of 10^8 cfu mL^{-1}.[13]

2.2 Host

The seeds of cowpea, *Vigna unguiculata* Auct. cv. C-152, susceptible to the blackeye cowpea mosaic strain of the bean common mosaic virus (BCMV-BICM), were used throughout the study.

2.3 Pathogen, source and inoculation

The identification of BCMV-BICM was confirmed by immunocapture reverse transcription polymerase chain reaction (IC-RT-PCR) employing polyclonal IgG raised against BCMV-BICM,[14] and degenerate primers for potyvirus detection.[15] The ~700 bp amplicon was cloned and sequenced. Nucleotide blast analysis of the cloned fragment closely matched with BCMV-BICM accession no. AF395678 (Shankar UAC, unpublished). BCMV-BICM was maintained by mechanical passage in cowpea. The primary leaves (eight-day-old seedlings) were dusted with carborundum (600 mesh) and then rub inoculated with BCMV-BICM. Inoculum consisted of BCMV-BICM infected cowpea leaf tissue ground in phosphate buffer (100 mM, pH 7.2; 1 : 10 w/v). Buffer, mortar and pestle were chilled prior to use and maintained on ice during inoculation.

2.4 Mode of PGPR seed treatment

All seven PGPR strains were used as fresh suspension and talc formulations. For fresh suspension, seeds were surface sterilised with 2% sodium hypochlorite for 5 min. The seeds were then soaked in 10^8 cfu mL^{-1} bacterial suspension (100 g 500 mL^{-1}) using sterilised carboxymethyl cellulose (CMC; 0.2%) as an adhesive to facilitate attachment of bacterial cells to the seed coat, incubated at 27 °C in an incubator rotary shaker (Amerex Instruments Inc., Lafayette, CA) at 150 rpm for 6 h and shade dried before use. Seeds treated with sterile distilled water amended with CMC and seeds soaked in distilled water alone served as controls.

PGPR strains in a purified talc powder formulation were prepared by aseptically mixing the bacterial suspension, prepared as described above, with sterilised talc powder. This formulation was mixed with CMC (0.2%) prior to treating seeds. Surface-sterilised seeds of cv. C-152 were mixed with the formulation at a rate of 10 g kg^{-1} seed. Seeds treated with sterile talc powder amended with CMC and seeds treated with talc powder alone served as controls.

2.5 Seed treatment by combination of PGPR strains

Four PGPR preparations, each shown in the above studies to induce resistance, were selected for the present study. Each PGPR treatment consisted of a preparation of two *Bacillus* strains. The seeds were treated with a mixture of these two bacterial strains in equal proportions.

2.6 Effect of PGPR treatment on seed germination and seedling vigour of cowpea under laboratory conditions

The germination test was carried out according to the paper towel method,[16] using seeds treated with both fresh suspension and talc formulations in four replicates of 100 seeds each. Treated and control seeds were seeded onto paper towels soaked in distilled water. One hundred seeds of cowpea were placed equidistantly on the paper and covered with another presoaked paper towel, and rolled up along with polythene wrapping to prevent drying of the towels. The rolled towels were then incubated in an incubation chamber at 24 ± 1 °C. After 8 days, the towels were unrolled and the number of seeds germinated were counted and represented as a percentage of those applied. Seedling vigour was analysed and calculated at the end of 7 days incubation.[17] To assess vigour, the lengths of the roots and shoots of individual seedlings were measured. The vigour index (VI) was calculated using the formula

$$\text{VI} = (\text{mean root length} + \text{mean shoot length}) \times (\text{percentage germination})$$

2.7 Screening of PGPR strains for their potential to elicit systemic protection against BCMV-BlCM under screen-house and field conditions

Treatments were the same as described above. In screen-house experiments, the treated seeds were sown in plastic pots (8 cm diameter) containing a mixture of soil and sand at 2:1 ratio. Each treatment consisted of five replicates, with 20 plants per replicate, with four repeated experiments. The eight-day-old seedlings were challenge inoculated with BCMV-BlCM inoculum as described in Section 2.3. Seedlings inoculated with buffer served as control. Challenge-inoculated plants were maintained in an insect-free screen house and were observed for disease development. BCMV-BlCM disease incidence was recorded 15 and 30 days post-inoculation (dpi). Seeds treated with distilled water amended with CMC and seeds treated with distilled water alone served as negative controls.

Field trials were designed and conducted at the experiment field of the Department of Applied Botany and Biotechnology, Mysore, during 2002–2006. The treatments and controls were the same as given above. One hundred seeds sown in four rows each of 10 m length were considered as one replicate, with four replications per treatment arranged in a randomised block design. Normal agronomic practices were followed to raise the crop. The eight-day-old emerging seedlings were challenge inoculated with BCMV-BlCM inoculum as described in Section 2.3. Seedlings inoculated with buffer served as control. BCMV-BlCM disease incidence was recorded 3 weeks after pathogen inoculation.

2.8 Serological assessment of BCMV-BlCM incidence by ELISA

BCMV-BlCM incidence was evaluated by indirect enzyme-linked immunosorbent assay (ELISA).[18] In screen-house studies, one leaflet from uninoculated trifoliate leaves (45 leaf samples per treatment), and, in field studies, 90 leaf samples per treatment were collected randomly at 15 dpi. The leaf samples were ground in antigen buffer (100 mM phosphate buffered saline + 0.01 M sodium diethyl dithiocarbonate) at 1:10 ratio. Samples were considered positive for the presence of BCMV-BlCM when the absorbance value (410 nm) was twice the negative control. Control plants inoculated with buffer served as negative control.

2.9 Data analysis

Data from repeated laboratory, screen-house and field experiments were combined for analysis. The data from each experiment were subjected to arcsine transformation and analysis of variance (JMP software; SAS Institute Inc., Cary, NC). The significance of the effect of PGPR treatments was determined by the magnitude of the *F*-value ($P = 0.05$). Treatment means were separated by Duncan's multiple range test (DMRT).

3 RESULTS

3.1 Effect of seed treatment with PGPR on seed germination and seedling vigour of cowpea under laboratory conditions

None of the PGPR strains tested as fresh suspension or talc formulation had any phytotoxic effect on cowpea seeds/seedlings. The germination percentage of cowpea seeds treated with fresh suspension of different PGPR strains ranged between 83 and 87%. The seed germination in the talc formulation treatment was between 82 and 86%. The germination of control seeds without PGPR treatment was 81–82%. The vigour index of seedlings was 1402–1863 for seeds treated with fresh suspension and 1405–1846 for seeds treated with talc formulation, compared with 1386–1392 in the control (Table 1). Among the seven PGPR strains tested, the highest germination of 87% was recorded for seeds treated with a fresh suspension of GBO3 and T4, and maximum VI was observed on treatment with strain GBO3 (Table 1).

Table 1. Effect of seed treatment with PGPR strains on seed germination and seedling vigour of cowpea seeds under laboratory conditions[a]

	Germination (%)		Vigour index	
Treatment[b]	Fresh Suspension	Talc formulation	Fresh	Talc
INR7	83 de	83 bc	1472 e	1417 f
SE 34	83 de	82 c	1402 f	1405 g
GBO3	87 a	86 a	1863 b	1846 a
937a	84 cd	83 bc	1517 c	1496 d
IPC 11	86 ab	85 ab	1763 b	1705 c
T4	87 a	86 a	1844 a	1818 b
937b	84 cd	83 bc	1507 d	1480 e
Control 1	**81** f	**82** c	**1392** g	**1388** i
Control 2	**82** ef	**82** c	**1386** h	**1390** h
Control 3	–	**81** c	–	**1360**

[a] Values are the mean from four repeated experiments with four replications of 100 seeds each. Means followed by the same letter(s) in a column do not differ significantly according to Duncan's multiple range test at $P = 0.05$.
[b] Control 1: non-bacterised, non-CMC treated, seeds soaked in water; control 2: CMC-treated, seeds soaked in water; control 3: CMC + sterile talcum powder, seeds soaked in water.

3.2 Effect of seed treatment with PGPR strains on BCMV-BlCM incidence of cowpea under screen-house conditions

Among the PGPR strains evaluated for their efficacy to induce resistance against BCMV-BlCM disease incidence, three formulations of two strains were significantly very effective in this. Varying degrees of protection, ranging from 3 to 42%, against BCMV-BlCM were induced by strains applied as fresh suspension and talc formulation. BCMV-BlCM disease incidence of 51% (42% protection) occurred with seeds treated with pure suspension of strain T4 by comparison with the non-bacterised control (89% BCMV-BlCM incidence) (Table 2). Both fresh suspension and talc formulation of strain GBO3 resulted in 52% BCMV-BlCM disease incidence, offering 41% protection.

3.3 Serological assessment of BCMV-BlCM incidence by ELISA

Immunoreactivity values for all PGPR treatments were lower than the non-bacterised control. The average absorbance values for plants treated with GBO3 and T4 were over twofold lower in comparison with control plants. Among the methods used to deliver PGPR, the seeds treated with pure suspension of PGPR strains resulted in the lowest ELISA values, 0.20 and 0.21 for strains GBO3 and T4 respectively, as opposed to 0.50 for the non-bacterised control (Table 2). Similarly, in the talc powder formulations of GBO3 and T4, absorbance values of 0.32 and 0.33 were recorded, as opposed to 0.51 for the non-bacterised control (Table 2).

Table 2. Effect of seed treatment with PGPR strains on BCMV-BlCM incidence in cowpea under screen-house conditions

Treatment[a]	BCMV-BlCM incidence (%)[bd]		ELISA reactivity at 410 nm[cd]	
	Fresh suspension	Talc formulation	Fresh suspension	Talc formulation
INR-7	86 a (3)	89 a (4)	0.41 g	0.43 d
IPC-11	70 c (21)	72 c (19)	0.35 i	0.41 d
SE-34	75 b (16)	81 c (12)	0.43 f	0.48 c
GBO3	52 d (41)	52 d (41)	0.20 j	0.32 e
937a	68 c (23)	78 c (16)	0.48 e	0.49 c
937b	70 c (20)	73 c (17)	0.39 h	0.42 d
T4	51 d (42)	55 d (38)	0.21 j	0.33 e
Control 1	**89** a	**90** a	**0.50** d	**0.49** b
Control 2	**89** a	**92** a	**0.50** cd	**0.51** ab
Negative control	–	–	**0.09** h	**0.10** f
Positive control	–	–	**0.51** b	**0.50** ab

[a] Control 1: non-bacterised, seeds soaked in water; control 2: CMC + sterile talcum talc, seeds soaked in water.
[b] Percentage of BCMV-BlCM incidence is the mean of four repeated experiments with five replications of 20 seeds each. Figures in parentheses represent percentage protection offered.
[c] ELISA values are the mean from four repeated experiments with two replications of 45 leaf samples per treatment.
[d] Means followed by the same letter in a column do not differ significantly according to Duncan's multiple range test at $P = 0.05$.

Table 3. Effect of seed treatment with PGPR strains on BCMV-BlCM incidence of cowpea under field conditions

Treatment[a]	BCMV-BlCM incidence (%)[bd]		ELISA reactivity at 410 nm[cd]	
	Fresh suspension	Talc formulation	Fresh suspension	Talc formulation
INR-7	81 a (7)	84 a (6)	0.43 d	0.43 de
IPC-11	66 c (24)	72 b (20)	0.41 d	0.48 c
SE-34	77 ab (12)	79 ab (12)	0.40 d	0.40 e
GBO3	57 c (34)	62 c (31)	0.24 e	0.27 f
937a	74 b (14)	77 b (15)	0.39 d	0.46 cd
937b	71 b (18)	73 b (19)	0.41 d	0.41 e
T4	61 c (30)	66 c (26)	0.28 e	0.28 f
Control 1	**87** a	**90** a	**0.50** b	**0.52** ab
Control 2	**87** a	**90** a	**0.51** a	**0.50** ab
Negative control			**0.10** f	**0.10** g
Positive control			**0.58** ab	**0.556** a

[a] Control 1: non-bacterised, seeds soaked in water; control 2: CMC + sterile talcum talc, seeds soaked in water.
[b] Percentage of BCMV-BlCM incidence is the mean of four repeated experiments with four replications of 100 seeds each. Figures in parentheses represent percentage protection offered.
[c] ELISA values are the mean from four repeated experiments with two replications of 45 leaf samples per treatment.
[d] Means followed by the same letter in a column do not differ significantly according to Duncan's multiple range test at $P = 0.05$.

3.4 Effect of seed treatment with PGPR strains on BCMV-BlCM incidence of cowpea under field conditions

The incidence of BCMV-BlCM ranged between 57% (GBO3 strain) and 80% (INR7 strain) when treated with fresh suspension of PGPR, as opposed to 87–88% in the control (Table 3). The absorbance values in ELISA ranged from 0.24 (GBO3 strain) to 0.430 (INR7), whereas in the control the values were 0.50–0.52. Similar results were observed in seed treatments with talc formulation of PGPR strains. Again, the GBO3 strain was the most effective, reducing the disease incidence to 62% with absorbance values of 0.27, as opposed to 0.52 for the control. Under field conditions, the highest protection of 34% (strain GBO3) was obtained with fresh suspension.

3.5 Effect of seed treatment with a combination of PGPR strains on BCMV-BlCM incidence of cowpea under screen-house conditions

Overall assessment of disease protection offered by a combination of PGPR strains was significantly greater than protection offered by strains treated individually. The combination of GBO3 + T4 strains offered the best protection of 69% (27% BCMV-BlCM incidence). Combinations of GBO3 + 937a and GBO3 + IPC11 offered protection of 48 and 40%. The remaining combinations of 937a + IPC11, 937a + T4 and IPC11 + T4 offered protection of 31, 33 and 37% respectively, as against 88% disease incidence in the case of the non-bacterised control (Table 4).

The absorbance values for the GBO3 + T4 combination were reduced approximately 2.5-fold by comparison with the non-bacterised control (Table 4). The combinations of GBO3 + 937a and GBO3 + IPC11 strains as fresh suspension reduced the absorbance to 0.22 and 0.30 respectively, as opposed to 0.54 in the control. Absorbance values of 0.24, 0.33 and 0.32 were recorded for the fresh suspensions of strain combinations 937a + IPC11, 937a + T4 and IPC11 + T4 respectively.

3.6 Effect of seed treatment with combination of PGPR strains on BCMV-BlCM incidence in cowpea under field conditions

The mixture of pure suspensions of strains GBO3 + T4 offered maximum protection of 62%, followed by 37 and 33% offered by GBO3 + IPC11 and GBO3 + 937a pure suspension combinations (Table 4). The remaining pure suspension combinations of 937a + IPC11, 937a + T4 and IPC11 + T4 offered 24, 27 and 30% protection respectively, as against 90% disease incidence in the case of the non-bacterised control (Table 4).

An absorbance value of 0.20 was recorded for the pure suspension mixture GBO3 + T4, as opposed to 0.51 for the other strains and the non-bacterised control. The pure suspension mixtures GBO3 + 937a and GBO3 + IPC11 recorded absorbance values of 0.26 and 0.28 respectively, in comparison with an absorbance value of 0.51 in the non-bacterised control (Table 4).

4 DISCUSSION

The role of beneficial microorganisms in agriculture is gaining worldwide importance and acceptance. Bacterial products that are reliable and that can effectively complement synthetic chemicals are already on the market. In particular, PGPR have the potential to replace the chemical component of agriculture. Treating seeds with PGPR has resulted in increased growth in several crops and induced resistance against pathogens.

The role of PGPR in the induction of resistance against plant viruses has been reported in genus *Cucumovirus*, family *Bromoviriridae*,[4,8,12,19,20] genus *Necrovirus*, family *Tombusviridae*,[21]

Table 4. Effect of seed treatment with combination of PGPR strains on BCMV-BlCM incidence under screen-house and field conditions

Treatment[a]	BCMV-BlCM incidence (%)[be]		ELISA reactivity at 410 nm	
	Screen-house conditions	Field conditions	Screen-house conditions[ce]	Field conditions[de]
GBO3 + 937a	46 e (48)	60 de (33 c)	0.22 f	0.26 f
GBO3 + IPC11	53 d (40)	57 e (37 b)	0.35 c	0.28 e
GBO3 + T4	27 f (69)	34 f (62 a)	0.18 g	0.20 g
937a + IPC11	61 b (31)	68 b (24 f)	0.24 e	0.28 e
937a + T4	59 bc (33)	65 bc (27 e)	0.33 d	0.33 d
IPC11 + T4	56 cd (37)	63 cd (30 d)	0.32 d	0.35 c
Control 1	**89** a	**90** a	**0.50** b	**0.51** a
Negative control	–	–	**0.10** h	**0.11** h
Positive control	–	–	**0.50** a	**0.50** b

[a] Control 1: non-bacterised, seeds soaked in water.
[b] Percentage of BCMV-BlCM incidence is the mean of four repeated experiments with four replications of 100 seeds each. Figures in parentheses represent percentage protection offered.
[c] ELISA values are the mean from four repeated experiments with two replications of 45 leaf samples per treatment.
[d] ELISA values are the mean from four repeated experiments with two replications of 90 leaf samples per treatment.
[e] Means followed by the same letter in a column do not differ significantly according to Duncan's multiple range test at $P = 0.05$.

and genus *Begmovirus*, family *Geminiviridae*.[22] Here, the effect of plant-growth-promoting rhizobacteria on the induction of resistance against seed-borne potyvirus is reported.

In general, all seven strains of PGPR, as pure suspension or talc formulation, promoted the vegetative and reproductive growth of cowpea plants, as assessed in terms of seed germination and seedling vigour. The strains GBO3 and T4 were found to be the best growth promoters. *Pseudomonas* spp. have been shown to be effective in plant growth promotion in several crops.[4,23–25]

The strain GBO3 offered 56% protection against BCMV-BlCM when seeds were treated with pure suspension. In the treatment of tomato plants that were mechanically inoculated with CMV, the four PGPR strains SE34, 973a, 937b and IN114 resulted in significant reductions in the percentage of plants infected, the amount of CMV in young tissue and the areas under the disease progression curve by comparison with non-treated, CMV-inoculated controls.[8] Seed treatment with *P. fluorescens* and *Serratia marcescens* reduced the cucumber mosaic virus infection in *Cucumis sativus* L. and *Lycopersicon esculentum* Mill. under screen-house conditions.[20] PGPR strains of *Bacillus amyloliquefaciens, B. subtilis* and *B. pumilus* applied as seed treatment/powder amendment to planting medium under field conditions successfully reduced ToMoV disease severity in tomato.[22]

The protection offered by PGPR strains against BCMV-BlCM in the present studies correlated very well with serological data. The ELISA absorbance values for PGPR-treated cowpea seedlings were found to be lower in most cases compared with the non-bacterised control. The decrease in concentration of BCMV-BlCM that was evident from absorbance values in GBO3- and T4-treated plants might have resulted from PGPR-induced resistance against BCMV-BlCM.

The present results are in line with the observation that no viral antigen could be detected by ELISA in any asymptomatic tomato and cucumber plants treated with PGPR strains, whereas CMV was evident in every leaf of non-bacterised plants.[20] Similarly, in the field experiment, the areas under the disease progression curve for CMV, indicating disease symptom progression over time, were significantly lower in all PGPR-treated tomato seedlings compared with the disease control. ELISA values in all PGPR treatments and the percentage of infected plants (based on ELISA) in three PGPR treatments were significantly lower than in the disease control.[8] Southern dot blot analysis for the detection of ToMoV DNA corresponded to the symptom severity ratings, e.g. the percentage of tomato plants infected by ToMoV was lower in all PGPR-powder-based treatments compared with control treatment in tomato.[22]

Following the trend observed in screen-house experiments, the best strains in reducing BCMV-BlCM disease incidence under field conditions were GBO3 and T4, which recorded 52 and 54% BCMV-BlCM incidence when cowpea seeds were treated with pure suspension of PGPR strains, as opposed to 87% BCMV-BlCM recorded in the non-bacterised control. The protection offered by PGPR strains was less under field conditions than under screen-house conditions. GBO3 and T4 strains applied as fresh suspension and talc formulation recorded a reduction in BCMV-BlCM incidence to 57–66%, as against 87–92% BCMV-BlCM incidence in the control.

The PGPR strains offered better protection in combination than they did individually. A highest protection of 69% was recorded for strains GBO3 + T4 when treating cowpea seeds under screen-house conditions. A total of 21 combinations of PGPR and seven individual PGPR were tested in the greenhouse for induced resistance activity against bacterial wilt of tomato caused by *Ralstonia solanacearum*, anthracnose of long cayenne pepper caused by *Colletotrichum gloeosporioides*, damping off of green kuang futsoi (*Brassica chinensis* var. *parachinensis* Tsen & Lee) caused by *Rhizoctonia solani* and cucumber mosaic virus (CMV) on cucumber. Four mixtures of PGPR and one individual strain treatment significantly reduced the severity of all four diseases compared with the non-bacterised control: 11 mixtures reduced CMV of cucumber, 16 mixtures reduced bacterial wilt of tomato, 18 mixtures reduced anthracnose of long cayenne pepper and seven mixtures reduced damping off of green kuang futsoi. Most mixtures of PGPR provided greater disease suppression than individual PGPR strains.[11] *Bacillus amyloliquefaciens* strain IN937a and *Bacillus pumilus* strains IN937b, SE34, SE49, T4 and INR7 were evaluated for induction of resistance capabilities against southern blight of tomato caused by *Sclerotium rolfsii*, anthracnose of long cayenne pepper caused by *Colletotrichum gloeosporioides* and mosaic disease of cucumber caused by CMV. A PGPR mixture, *Bacillus amyloliquefaciens* strain IN937a + *B. pumilus* strain IN937b, significantly protected plants against all tested diseases in both seasons.[12]

A variety of substances produced by PGPR have been implicated in the mechanisms to limit the damage to plants by phytopathogens. These include siderophores, antibiotics, other small molecules and a number of enzymes.[26,27] It can be concluded from the results of the present study that the application of PGPR as a seed treatment would prove beneficial and could be a potential component of integrated pest management. Apart from offering protection against phytopathogens, these bacteria are also good growth promoters, which is an added advantage for any practical agricultural system. It is evident that rhizobacteria could possibly

serve as ecofriendly and sustainable alternatives to the hazardous chemicals used for growth promotion and management of crop diseases.

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