Journal of Microbiology (2014) Vol. 52, No. 9, pp. 778–784
DOI 10.1007/s12275-014-3496-3

Two Rhizobacterial Strains, Individually and in Interactions with *Rhizobium* sp., Enhance Fusarial Wilt Control, Growth, and Yield in Pigeon Pea[§]

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(Received Sep 27, 2013 / Revised Feb 4, 2014 /Accepted Mar 24, 2014)

A *Pseudomonas aeruginosa* strain, RRLJ 04, and a *Bacillus cereus* strain, BS 03, were tested both individually and in combination with a *Rhizobium* strain, RH 2, for their ability to enhance plant growth and nodulation in pigeon pea (*Cajanus cajan* L.) under gnotobiotic, greenhouse and field conditions. Both of the rhizobacterial strains exhibited a positive effect on growth in terms of shoot height, root length, fresh and dry weight, nodulation and yield over the non-treated control. Co-inoculation of seeds with these strains and *Rhizobium* RH 2 also reduced the number of wilted plants, when grown in soil infested with *Fusarium udum*. Gnotobiotic studies confirmed that the suppression of wilt disease was due to the presence of the respective PGPR strains. Seed bacterization with drug-marked mutants of RRLJ 04 and BS 03 confirmed their ability to colonize and multiply along the roots. The results suggest that co-inoculation of these strains with *Rhizobium* strain RH 2 can be further exploited for enhanced growth, nodulation and yield in addition to control of fusarial wilt in pigeon pea.

Keywords: fusarial wilt control, nodulation, pigeon pea, PGPR, *Rhizobium*, volatile compounds

Introduction

Pulses are the second most important crop group after cereals, with a global production of 61.5 million tons from 70.6 million hectares, and an average yield of 871 kg/ha. Pigeon pea (*Cajanus cajan* (L.) Millsp.) is one of the important members of this group, comprising 4% of the world pulse production. India is a leading producer of pigeon pea, contributing 75% of the global production. Estimates for 2010-2011 indicated that the production of pulses in India was 14.66 million tons from an area of 22.47 million hectares, with an average production of 637 kg/ha. In spite of having the largest area devoted to chickpeas, pigeon peas, lentils, and dry beans, India still has to import 3.0 to 4.0 million tones of pulses every year to meet its domestic requirements (Anonymous, 2011). To meet the demand for protein from pulses, at least 32 million tones of pulses are required by 2030. This necessitates an annual growth rate of 4.2% in pulse production. Major factors affecting the yield and efficiency in pulse production are pests and diseases. Wilt of pigeon pea caused by *Fusarium udum* is one of the most dreaded diseases, leading to a crop loss of about $US 36 M in India (Vidhyasekaran *et al*., 1997). The fungus is soilborne and chemical control is impractical after the fungus is established (Reddy *et al*., 1990). Therefore, agriculturists are now looking for naturally occurring free-living rhizobacteria, which can promote plant growth as well as suppress diseases, as a viable alternative. Such beneficial rhizobacteria, commonly known as Plant Growth Promoting Rhizobacteria (PGPR), enhance plant growth and yield and/or suppress disease in many crop plants (Ogoshi *et al*., 1997; Dutta *et al*., 2008; Perez *et al*., 2012). *Rhizobium* or *Bradyrhizobium*, on the other hand, are widely used for crop improvement because of their ability to fix atmospheric nitrogen. Co-inoculation of PGPR and rhizobia for improving growth and yield in legume crops has been attempted by only a few workers (Dileep Kumar *et al*., 2001; Estevez De Jensen *et al*., 2002; Rani and Arundhathi, 2012).

The present study deals with the effect of co-inoculation of two rhizobacterial strains and *Rhizobium* RH 2 on growth, yield and suppression of fusarial wilt of pigeon pea.

Materials and Methods

Organisms

Bacterial strains: A *Pseudomonas aeruginosa* strain designated as RRLJ 04, and a *Bacillus cereus* strain BS 03, which showed plant growth and disease controlling properties in our earlier investigation (Dutta *et al*., 2005) were used for the present investigation. Both of the strains were identified from the Microbial Type Culture Collection and Gene Bank (MTCC) Division of the Institute of Microbial Technology (IMTECH), a constituent establishment of the Council of Scientific and Industrial Research (CSIR), Chandigarh, India. The accession

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[§]Supplemental material for this article may be found at http://www.springerlink.com/content/120956.

numbers of these strains are MTCC 7278 and 7277 for BS 03 and RRLJ 04, respectively. The *Rhizobium* strain RH 2, capable of producing nodules in pigeon pea, was isolated from the root nodule of a pigeon pea plant from Jorhat, Assam, India, and was selected for the interaction study of the present work.

Fungal pathogen: *Fusarium udum*, causing wilt of pigeon pea, was procured from the International Crop Research Institute for Semi-Arid Tropics (ICRISAT), Hyderabad, India.

In vitro antibiosis test

The *in vitro* antagonism of the two rhizobacterial strains against *F. udum* was tested in King's medium B (KB), Nutrient Agar (NA), and Potato Dextrose Agar (PDA) as described by Dileep Kumar *et al.* (2001). Antagonism of the *Rhizobium* strain RH 2 was examined in Yeast Mannitol Agar (YMA). For this, an actively growing mycelial disc (≈ 6 mm^2) of *F. udum* was placed at one side of the Petri plate containing YMA medium (2.0 cm inside of the periphery) and a loopful of the bacterial strain was streaked on the opposite side of the mycelial disc. The plates were then incubated at 28±2°C and the inhibition zone was measured as the distance (in cm) between the bacterial antagonist and the test fungal pathogen after seven days of growth. Plates without introduction of bacterial strains served as control.

Seed bacterization

Bacterization studies were done according to Dileep Kumar *et al.* (2001). Pigeon pea seeds were surface sterilized by placing them in 1% mercuric chloride solution for 1 min followed by rinsing in 1:29 mixture of hydrogen peroxide and distilled water for 30 min and drying under a sterile air stream. The strains RRLJ 04, BS 03, and RH 2 grown in KB, PDA and YMA for 48 h, respectively, were harvested with a sterile glass rod and suspended in 20 ml of sterile 1% carboxymethylcellulose (CMC) solution. Five grams of surface sterilized seeds were steeped in this bacterial suspension for 1 h and then surface dried overnight under a sterile air stream. The treated seeds were examined for colony forming units (CFU) on KB, PDA, and YMA and the bacterial suspension was adjusted to give 1.2×10^7 CFU/seed for individual treatments and 1.0×10^4 CFU/seed for each organism in combination (co-inoculation). Seeds treated with only 1% CMC served as the control.

Effect on plant growth promotion and yield

Seeds bacterized with RRLJ 04, BS 03, and RH 2, individually and combinations of rhizobacterial strains with RH 2, were sown into earthen pots (Size 28 × 30 cm; 5 seeds/pot) in a completely randomized design. The percentage of seed germination was noted at 7 days after sowing (DAS) and the shoot height, root length, fresh, dry weight and number of root nodules were recorded at 90 DAS. Chlorophyll content of the leaves was estimated after 75 days of growth. Yield in terms of number, fresh and dry weight of pods and protein content in seeds were recorded at harvesting (270 DAS).

For field studies, seeds bacterized (as above) were sown into plots (3.16 × 2.36 m) in a randomized block design (8 treatments, 3 replications, 40 plants/ replication) and the data were recorded for the pot culture studies.

Disease suppression studies

For this, bacterized seeds were sown in pots infested with fungal conidia (soil mixed with 25 ml of fungal conidial suspension containing 1.0×10^7 conidia/ml). Plants were monitored for occurrence of disease symptoms up to 270 DAS.

Gnotobiotic studies

The system consisted of a glass tube, 26 cm long and 1.5 cm in diameter, connected with a 100 ml conical flask at the bottom (Fig. 3). The tube was filled with sand up to 12 cm by securing it with a piece of muslin cloth at the bottom of the tube. The tube was then fixed tightly to the conical flask containing Hoffland's Plant Nutrient Solution (PNS), consisting of 5 mM $Ca(NO_3)_2$, 5 mM KNO_3, 2 mM $MgSO_4$, 1 mM KH_2PO_4, and micronutrients (g/L of $MnSO_4$ - 0.61, $ZnSO_4{\cdot}7H_2O$ - 0.1, H_3BO_3 - 1.27, $Na_2MoO_4{\cdot}2H_2O$ - 0.40 and $CuSO_4$ - 0.04). The bottom portion of the tube was immersed in the nutrient solution to keep the sand column moist. The system was autoclaved and cooled then pre-germinated pigeon pea seedlings, (roots dipped in a 5% solution of a 24 h culture of the bacterial suspension for 15 min), were aseptically placed 5 mm below the surface of the sand column. The treatments were given in two sets - the first consisted of individual treatments with the rhizobacterial and *Rhizobium* strains as well as combinations of the rhizobacterial strains together with Rhizobium RH 2. The second set consisted of the same treatments in pathogen-infested sand columns. For this, 2 ml of the conidial suspension were added to the sand column. Seed treated with only 1% CMC served as control. Shoot height, root length, fresh and dry weight of shoot and root were recorded after 15 days of growth.

Effect of volatile compounds

The effect of volatile compounds on growth of pigeon pea seeds was monitored according to Ryu *et al.* (2004). Surface-sterilized seeds (three per treatment) were placed on one side of the Petri dish containing MS medium (separated into two halves by placing a glass plate in the centre) and the other side was inoculated with 20 µl of the test PGPR, Rhizobium, or combination (as described earlier), respectively. Plates inoculated with sterile distilled water served as the control. All the plates were sealed with parafilm and allowed to grow at 25°C and 2500 lux light. Shoot height, root length, and fresh and dry weight of the seedlings were recorded after 15 days of growth.

Root colonization studies

To monitor the colonization of roots by the introduced bacteria, antibiotic-resistant strains were used (Dileep Kumar, 1998). For this, plants bacterized with drug-resistant strains of RRLJ 04 (resistant to streptomycin sulphate and chloramphenicol @ 50:50 mg/L) and BS 03 (resistant to 50 mg/L chloramphenicol) were sampled for root colonization by the introduced bacteria after 7, 14, 21, 28, and 35 days. For this, one gram of fresh root (cut into one cm segments) from

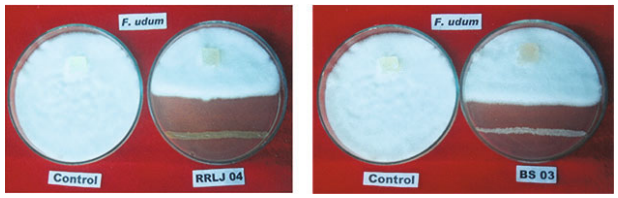

Fig. 1. *In vitro* antagonism exhibited by PGPR strains to *F. udum* in PDA medium. Bacterial strains, RRLJ 04 and BS 03 and fungal mycelium were inoculated at the same time (0 days of preincubation). Mycelial growth of fungi was determined after 7 days of incubation.

bacterized plants was dipped in 5.0 ml sterile distilled water and shaken for 3–4 min to release the rhizoplane bacteria into the water. Dilutions of the bacterial suspensions were pour plated on (i) KB medium amended with streptomycin sulphate and chloramphenicol (@ 50 mg/L each) or PDA media containing chloramphenicol (@ 50 mg/L) for enumerating the introduced bacteria (RRLJ 04^{+} and BS 03^{+}), and (ii) NA for the total aerobic bacteria in the rhizoplane sample. The CFU for 1 g fresh root segments were counted after 48 h of incubation.

Statistical analysis

All the data obtained were subjected to Duncan's Multiple Range Test (DMRT) using a computer software program.

Results

In vitro antibiosis studies

Both of the test rhizobacterial strains showed statistically significant inhibition zones against *F. udum* in all the media tested (Fig. 1 and Supplementary data Table S1). Among the two rhizobacterial isolates, RRLJ 04 showed the strongest inhibition zone in all the test media. The results confirmed that *Rhizobium* strain RH 2 had no inhibitory property to the test pathogen in YMA medium.

Seed bacterization under nursery and field conditions

Effect on growth: The effect of different treatments was found to be statistically significant in enhancing shoot height, root length, fresh weight, dry weight and number of nodules at 90 DAS (Table 1). The highest increase in shoot height was recorded in the combination treatment of RRLJ 04 + RH 2

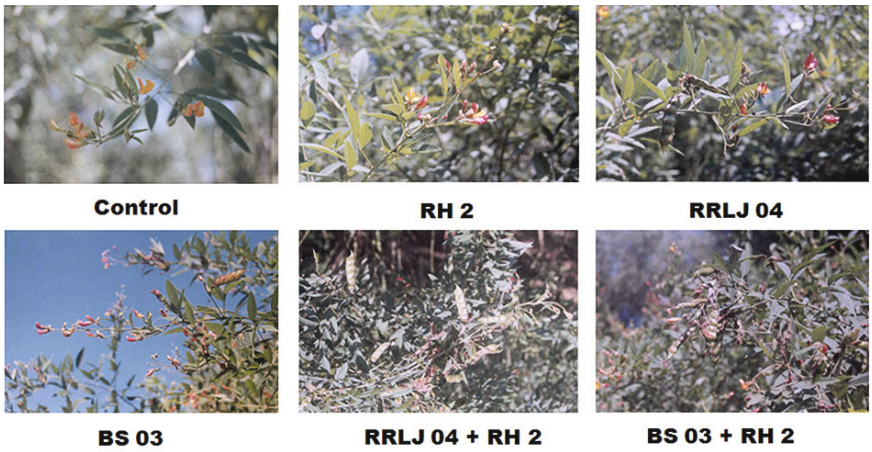

Fig. 2. Effect of different treatments on growth under gnotobiotic conditions. (1) Control (2) RRLJ 04 (3) RH 2 (4) RRLJ 04 + RH 2 (5) *F. udum* (6) RRLJ 04 + *F. udum* (7) RH 2 + *F. udum* (8) RRLJ 04 + RH 2 + *F. udum*. The results were taken after 15 days incubation.

Table 1. Effect of seed bacterization on growth under nursery condition (90 DAS)

Treatments	Shoot height (cm)	Root length (cm)	Fresh weight (g)	Dry weight (g)	No. of nodules
RRLJ 04	92.00^{c} (46.03)	22.65^{d} (35.55)	12.983^{c} (81.30)	3.289^{c} (99.82)	42^{b} (147.06)
BS 03	90.00^{d} (42.86)	24.18^{c} (44.70)	12.368^{d} (72.71)	3.117^{d} (89.37)	42^{b} (147.06)
RH 2	75.50^{e} (19.84)	17.33^{e} (3.71)	9.320^{e} (30.15)	2.626^{e} (59.54)	19^{c} (11.76)
RRLJ 04 + RH 2	125.25^{a} (98.81)	29.99^{b} (79.47)	17.925^{a} (150.31)	5.165^{a} (213.79)	65^{a} (282.35)
BS 03 + RH 2	121.30^{b} (92.54)	30.33^{a} (81.51)	17.328^{b} (141.98)	4.206^{b} (155.53)	68^{a} (300.00)
Control	63.00^{f}	16.71^{f}	7.161^{f}	1.646^{f}	17^{c}
S.Ed.(±)	0.75	0.16	0.063	0.008	2.13
CD-5%	1.60	0.34	0.134	0.017	4.54

Data within parentheses represent percent increase over control
The figures followed by same letter(s) in a column do not differ significantly.

Table 2. Effect of seed bacterization on growth under field condition (90 DAS)

Treatments	Shoot height (cm)	Root length (cm)	Fresh weight (g)	Dry weight (g)	No. of nodules
RRLJ 04	126.17^{c} (32.35)	41.00^{bc} (39.79)	108.500^{d} (58.00)	36.414^{d} (17.04)	152.33^{d} (52.83)
BS 03	119.42^{d} (25.27)	40.83^{c} (39.21)	110.340^{d} (60.68)	37.086^{cd} (19.20)	199.33^{c} (99.99)
RH 2	100.13^{e} (5.04)	38.17^{d} (30.14)	116.670^{c} (69.90)	38.055^{c} (22.32)	119.00^{e} (19.39)
RRLJ 04 + RH 2	166.50^{a} (74.66)	50.67^{a} (72.73)	135.660^{a} (97.55)	41.136^{a} (32.22)	210.00^{b} (110.70)
BS 03 + RH 2	162.00^{b} (69.94)	42.33^{b} (44.32)	125.000^{b} (82.03)	39.085^{b} (25.63)	251.67^{a} (152.50)
Control	95.33^{f}	29.33^{e}	68.670^{e}	31.112^{e}	99.67^{f}
S.Ed.(±)	1.37	0.66	1.932	0.464	1.22
CD-5%	2.93	1.41	4.12	0.989	2.59

Data within parentheses represent percent increase over control
The figures followed by same letter(s) in a column do not differ significantly.

(98.81%) followed by BS 03 + RH 2 (92.54%). Treatment of BS 03 + RH 2 gave the largest increase in root length (81.51%), whereas maximum increment in fresh and dry weight was recorded for RRLJ 04 + RH 2 (150.31 and 213.79%). The number of nodules was noted to be highest in BS 03 + RH 2 (300%) but it was found to be statistically *at par* with RRLJ 04 + RH 2 (282.35%).

Results similar to those obtained for the nursery condition, were observed in field experiments. It was observed that the treatment RRLJ 04 + RH 2 gave the highest increase in shoot height. The largest root length and fresh and dry weight of plants were recorded with the RRLJ 04 + RH 2 application (72.73, 97.55, and 32.22%, respectively). The number of nodules, however, was highest in BS 03+RH 2 (152.50%) followed by RRLJ 04 + RH 2 (110.70%) and BS 03 alone (99.99%) respectively (Table 2). The highest chlorophyll content was recorded in the RRLJ 04 + RH 2 treatment followed by BS 03 + RH 2 (data not shown).

Table 3. Effect of seed bacterization on yield and plant growth under nursery condition (270 DAS)

Treatment	No. of pods	Fresh weight (g)	Dry weight (g)	Protein content in seeds (%)	No. of nodules
RRLJ 04	74.79^{c} (26.31)	50.37^{c} (43.87)	48.31^{c} (44.94)	29.19^{c} (41.97)	42^{b} (147.06)
BS 03	71.60^{d} (20.93)	44.47^{d} (27.02)	41.88^{d} (25.65)	24.50^{d} (19.16)	42^{b} (147.06)
RH 2	68.00^{e} (14.85)	40.21^{e} (14.85)	38.38^{e} (15.15)	24.50^{d} (19.16)	19^{c} (11.76)
RRLJ 04+RH 2	100.41^{a} (69.58)	88.08^{a} (151.59)	85.62^{a} (156.89)	31.50^{a} (53.21)	65^{a} (282.35)
BS 03+RH 2	92.01^{b} (55.40)	79.19^{b} (126.19)	77.84^{b} (133.54)	30.31^{b} (47.42)	68^{a} (300.00)
Control	59.21^{f}	35.01^{f}	33.33^{f}	20.56^{e}	17^{c}
S.Ed.(±)	0.235	0.439	0.127	0.235	2.13
CD-5%	0.50	0.94	0.27	0.50	4.54

Data within parentheses represent percent increase over control
The figures followed by same letter(s) in a column do not differ significantly.

Table 4. Effect of seed bacterization on yield/plant growth under field condition (270 DAS)

Treatments	No. of pods	Fresh weight (g)	Dry weight (g)	Protein content in seeds (%)
RRLJ 04	104.41^{c} (42.34)	54.91^{c} (21.13)	52.42^{c} (29.85)	25.13^{d} (9.55)
BS 03	99.76^{d} (36.01)	51.88^{d} (14.45)	48.10^{d} (19.15)	25.00^{d} (8.98)
RH 2	88.63^{e} (20.83)	47.33^{f} (4.41)	44.59^{f} (10.45)	34.38^{c} (49.87)
RRLJ 04 + RH 2	136.53^{a} (86.13)	68.68^{a} (51.51)	66.38^{a} (64.43)	38.88^{a} (69.49)
BS 03 + RH 2	113.27^{b} (54.42)	65.58^{b} (44.67)	63.10^{b} (56.30)	37.31^{b} (62.64)
Control	73.35^{f}	45.33^{e}	40.37^{e}	22.94^{e}
S.Ed.(±)	0.10	0.50	0.22	0.68
CD-5%	0.22	1.07	0.47	1.44

Data within parentheses represent percent increase over control
The figures followed by same letter(s) in a column do not differ significantly.

Fig. 3. Effect of different treatments on flowering and pod formation under field conditions after 205 DAS, which indicate that the combined application of PGPR and *Rhizobium* is better than the other treatments.

Effect on chlorophyll content of leaves: A statistically significant increase in chlorophyll content of leaves was observed in all the treatments over the non-treated control. The highest total chlorophyll content was observed in RRLJ 04 + RH 2 followed by BS 03+ RH 2 (Supplementary data Table S2). It is evident from this table that the increase in chlorophyll content was due to an increase in chlorophyll b.
Effect on yield: A statistically significant increase in yield, in terms of number of pods, fresh and dry weight of pods and protein content in seeds, was recorded for all the treatments over their non-treated controls (Table 3). RRLJ 04 + RH 2 exhibited the maximum increase followed by BS 03 + RH 2.

Bacterized plants showed early flowering and pod formation as compared to the control. Control plants and those in RH 2 treatments flowered at 205 DAS (Fig. 2). Plants treated with RRLJ 04 and BS 03, respectively showed flowering at this time with the initiation of pod formation. However, in the combination treatments of RRLJ 04 + RH 2 and BS 03 + RH 2 the flowering stage was over and all plants were in the pod stage. It is evident from Table 4 that yield after 9 months, in terms of number of pods, fresh and dry weight of pods and protein content in seeds, was highest in RRLJ 04 + RH 2.

Disease suppression studies under nursery condition

Bacterization of seeds with the rhizobacteria delayed the occurrence of disease as compared to the other plants when grown in pathogen-infested soil. Disease symptoms occurred in *F. udum* treatments of non-bacterized and RH 2-treated plants from 12 DAS onwards. Individual treatments with RRLJ 04 and BS 03 showed disease symptoms after 60 DAS. In RRLJ 04 + RH 2 and BS 03 + RH 2 treatments, disease symptoms did not occur up to the last day of observation (90 DAS). At 90 DAS, no plants survived in pathogen-treated, non-bacterized control plants, whereas, 100% of plants survived in RRLJ 04 + RH 2 and BS 03 + RH 2 treated plants (Supplementary data Table S3).

Gnotobiotic studies

Plants grown under the gnotobiotic conditions, showed the best effect on shoot height, root length, and fresh and dry weight by the treatment with BS 03 + RH 2, followed by RRLJ 04 + RH 2 except root length which was second best in BS 03 alone (Supplementary data Table S4, Fig. 3). In pathogen infested soil, greatest shoot height and root length were recorded in BS 03 + RH 2 (66.82 and 105.54%) followed by BS 03 alone (50.67 and 71.63%). Fresh and dry weights of plant were also recorded as highest in the BS 03 + RH 2 combination (Table 5).

Effect of volatile compounds on growth

The data confirmed that the presence of RH 2 alone and the co-inoculation with RRLJ 04 and BS 03 had no significant effect on growth enhancement (Supplementary data Table S5). It is evident from the Supplementary data Table S5 that seeds treated with RRLJ 04 and BS 03 showed a statistically significant enhanced effect on growth. Application of BS 03 gave the maximum increment in shoot height, root length, fresh, and dry weight of the seedlings.

Table 5. Effect of different treatments on growth in pathogen infested soil under gnotobiotic condition

Treatments	Shoot height (cm)	Root length (cm)	Fresh weight (g)	Dry weight (g)
RRLJ 04	15.41^{cd} (14.66)	6.04^{cd} (4.50)	0.416^{b} (67.74)	0.080^{b} (66.67)
BS 03	20.25^{ab} (50.67)	9.92^{ab} (71.63)	0.375^{c} (51.21)	0.071^{c} (47.92)
RH 2	17.68^{bc} (31.55)	8.06^{bc} (39.45)	0.317^{d} (27.82)	0.056^{d} (16.67)
RRLJ04+RH 2	15.74^{cd} (17.11)	7.33^{cd} (26.82)	0.412^{b} (66.13)	0.078^{b} (62.50)
BS 03+RH 2	22.42^{a} (66.82)	11.88^{a} (105.54)	0.474^{a} (91.13)	0.087^{a} (81.25)
F. udum	9.53^{e} (-29.09)	3.77^{e} (-34.78)	0.086^{f} (-65.32)	0.021^{f} (-56.25)
Control	13.44^{d}	5.78^{de}	0.248^{e}	0.048^{e}
S.Ed.(±)	1.26	1.00	0.014	0.003
CD-5%	2.73	2.17	0.031	0.007

Data within parentheses represent per cent increase over control
The figures followed by same letter(s) in a column do not differ significantly.

Root colonization studies

Data collected for seeds bacterized with drug-resistant strains of RRLJ 04^+ and BS 03^+, respectively showed that the introduced bacteria were able to colonize the growing roots of the plants (Supplementary data Fig. S1). Their population increased from 7 days of growth onwards up to 28 days, after which they attained stability.

Discussion

Pseudomonas strain RRLJ 04 and *Bacillus* strain BS 03 in combination with a Rhizobium strain RH 2 improved plant growth and yield over the individual treatments and control. The combination treatments were also more effective for disease suppression. Co-inoculation of PGPR strains with *Rhizobium* and *Bradyrhizobium* has been reported to enhance nitrogen fixation, nodulation, biomass, yield and disease suppression in many legume plants (Geetha *et al*., 2012; Saini and Khanna, 2012; Singh, 2012). The control of root infecting fungi and nematodes of *Vigna mungo* by co-inoculation of *P. aeruginosa* and *Bradyrhizobium* has been reported earlier (Siddiqui *et al*., 2000). Field tests with some pseudomonad strains have recorded yield increases (Raj *et al*., 2003), delayed leaf senescence at the later stages of growth (Sarig *et al*., 1990) and promotion of legume nodulation by nitrogen-fixing rhizobia (Leij *et al*., 2002). Pre-incubation of *B. japonicum* with PGPR can increase soybean nodulation and nitrogen fixation in the early part of the growing season when the soil temperatures is low (Dashti *et al*., 1998). Inoculation with root colonizing bacteria and *Rhizobium* has been demonstrated to affect symbiotic nitrogen fixation by enhancing root nodule number or mass (Kutcher *et al*., 2002; Roopa *et al*., 2012). It was reported that co-inoculation of *Pseudomonas* and *Bacillus* species with *Rhizobium* strains enhanced the nodule weight, root length, shoot biomass and total plant nitrogen in chickpea, when grown in sterilized jars or under pot culture conditions (Parmar and Dadarwal, 1999). An enhanced level of flavonoid-like compounds produced by these nodule-enhancing rhizobacteria was observed, which may trigger the plant to be more susceptible towards rhizobial infections. Podile (1995) reported enhancement in seedling emergence, growth and nodulation in pigeon pea with co-inoculation of *Rhizobium* and a *Bacillus* strain AF 1 under greenhouse conditions. The phytohormone ethylene is known to inhibit nodulation in various legumes (Nukui *et al*., 2000). Plant growth-promoting bacteria have been reported to decrease ethylene levels in plants (Penrose and Glick, 2001; Ghosh *et al*., 2003). Thus, plants inoculated with ACC deaminase-producing bacteria have longer roots under gnotobiotic conditions (Glick *et al*., 1999) and have better ability to resist the inhibitory effects of ethylene stress on plant growth imposed by heavy metals (Burd *et al*., 2000), pathogens (Wang *et al*., 2000), and flooding (Grichko and Glick, 2001).

Co-inoculation of BS 03 and RRLJ 04 with RH 2 has confirmed their ability to control wilt disease, thus qualifying them as bio-control agents for control of fusarial wilt in pigeon pea. That the positive effect on plant growth promotion and disease control is due to the presence of these treatments has been confirmed through the gnotobiotic studies, where combination treatment of bacterial strains, RRLJ 04 and BS 03 with the *Rhizobium* strain RH 2 showed enhancement in growth both with and without the pathogen. It was observed that under gnotobiotic conditions, the combination treatment of BS 03 + RH 2 gave the best results; whereas, in pot culture and in the field RRLJ 04 + RH 2 came out as the best treatment.

In conclusion, the results confirmed the potential of the combined use of PGPR together with rhizobial strains for enhancement of growth, nodulation and yield in addition to suppression of fusarial wilt in pigeon pea.

Acknowledgements

The authors are thankful to Director, North East Institute of Science and Technology (CSIR), Jorhat, for his keen interest and help in this work. Dutta and Morang thank the Department of Science and Technology, Government of India, and the University Grant Commission, New Delhi, respectively for the financial assistance to carry out a part of this work. Dileep Kumar and Nishanth Kumar S. thanks Dr. Suresh Das, Director, National Institute for Interdisciplinary Science and Technology (CSIR), Thiruvanathapuram, for his help to publish this work.

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