ORIGINAL PAPER

Evaluation of fluorescent *Pseudomonas* spp. with single and multiple PGPR traits for plant growth promotion of sorghum in combination with AM fungi

**G. Praveen Kumar · N. Kishore · E. Leo Daniel Amalraj ·
S. K. Mir Hassan Ahmed · Abdul Rasul ·
Suseelendra Desai**

Abstract Traits involved in plant growth promotion by bacteria are ambiguously decided as phytohormones, siderophores, HCN, proteases, chitinases, cellulases, ammonia, exopolysaccharide production and phosphate solubilization or antagonistic activity. A total of 40 fluorescent *Pseudomonas* strains were isolated from diverse soils of various agro-ecosystems of India. Among these 7 strains viz, P1, P10, P13, P18, P21, P28 and P38 were selected depending on their character of possessing single or multiple PGPR traits. These isolates individually and in combination with AM fungi (*Glomus fasciculatum* and *Glomus aggregatum*) were used for treating sorghum seeds. 25 days after sowing, plants were analysed for different plant growth promoting parameters. It was observed that strains in presence of mycorrhizae performed well compared to the strains devoid of mycorrhizae. *Pseudomonas* sp. P1 possessing GA3, EPS production and 'P' solubilization performed well. *Pseudomonas* sp. P38 which was a volatile (HCN) producer but a good phosphate solubilizer did not perform well. Strain P28 with multiple PGPR traits did not show the expected out come. Results varied when mycorrhizae was used in combination. P10 and P13 which were good in IAA, GA3, EPS, siderophore and 'P' solubilization performed well with mycorrhizae, with an overall increase in plant biomass, leaf area, total chlorophyll and mycorrhizal infection compared to other combinations. Here, strain P1 in combination with mycorrhizae did not show significant increase in plant growth compared to P10 and P13. Hence, mere possession of multiple PGPR traits does not confer fluorescent *Pseudomonas* strains as PGPR. Further studies have to be carried out in order to evaluate the other traits which may be involved in plant growth promotion.

Keywords AM Fungi · *Pseudomonas* spp. · PGPR traits · Sorghum · Plant growth promotion

Introduction

A large number of different interactions between fungi and bacteria occur in association with plants, and depending on the nature of the species involved, the plant can be positively or negatively affected. Microbial activity in rhizosphere soil affects plant health and growth. Arbuscular mycorrhizal fungi (AMF) impart differential effects on the bacterial community structure in the mycorrhizosphere (Lynch 1990; Marschner and Baumann 2003). Arbuscular mycorrhizal fungi improve phosphorus nutrition by scavenging available phosphorus through the large surface area of their hyphae. Plant growth promoting rhizobacteria (PGPR) also improve plant phosphorus acquisition by solubilizing organic and inorganic phosphorus sources through phosphatase synthesis or by lowering soil pH (Rodriguez and Fraga 1999). Mycorrhizal helper bacteria (MHB) are defined as bacteria associated with mycorrhizal roots and mycorrhizal fungi, which collectively promote the establishment of mycorrhizal symbiosis (Garbaye 1994). Rhizobacteria include mycorrhization helper bacteria (MHB) and PGPR, which assist AMF in colonizing

G. Praveen Kumar · E. Leo Daniel Amalraj ·
S. K. Mir Hassan Ahmed · A. Rasul · S. Desai
Division of Crop Sciences, Central Research Institute
for Dryland Agriculture (ICAR), Santoshnagar, Hyderabad,
Andhra Pradesh 500 059, India

N. Kishore (✉)
Department of Microbiology, Palamuru University,
Mahbubnagar 509 001, Andhra Pradesh, India
e-mail: kishore_micro2003@yahoo.co.in

the plant root (Andrade et al. 1997), phosphorus solubilizers, free-living and symbiotic nitrogen fixers, antibiotic-producing rhizobacteria, plant pathogens, predators and parasites (Sun et al. 1999).

Although the mechanisms by which PGPR promote plant growth are not yet fully understood, many different traits of these bacteria are responsible for growth promotion activities (Cattelan et al. 1999). Plant growth promoting rhizobacteria directly stimulate growth by nitrogen fixation (Han et al. 2005), production of growth hormones and ACC deaminase (Correa et al. 2004) and indirectly by inhibiting the pathogenic fungi by production of chitinases, β-1,3-glucanases, antibiotics, fluorescent pigments and cyanide (Pal et al. 2001).

The most common bacteria in the mycorrhizosphere are *Pseudomonas* spp. (Vosatka and Gryndler 1999). Many fluorescent *Pseudomonas* strains have been reported as plant growth enhancing beneficial rhizobacteria. They are studied extensively in agriculture for their role in crop improvement. The ability of *Pseudomonas* spp. to enhance plant growth has been attributed to various mechanisms like phytohormones production, solubilization of nutrients, HCN, siderophore and biocontrol activity. But very few studies were conducted which explains the importance of a particular PGPR trait in plant growth promotion. With this objective, to identify the importance of PGPR traits in plant growth promotion, an experiment was conducted using fluorescent *Pseudomonas* isolates possessing single to multiple PGPR traits in combination with two different arbuscular mycorrhizal fungi.

Materials and methods

Plant growth experiments were conducted under standard controlled conditions (temperature 28 ± 2°C; 65% relative humidity) with sterile soil as a substrate. Plants were watered with sterile de-ionized water regularly whenever required. Experiment was conducted in small pots of 25×15 cm holding a total of 500 g of soil.

Bacterial cultures

Twenty-five fluorescent *Pseudomonas* isolates, were isolated from rhizosphere and non-rhizosphere soils of different crops of diverse agro-ecosystems of India using King's B medium (King et al. 1954).

Arbuscular mycorrhizal fungi

Two wild strains of arbuscular mycorrhizal fungi (AM) viz., *Glomus fasciculatum* (G1) and *Glomus aggregatum* (G2) were isolated from 2 different rainfed agro-ecosystems.

Pure cultures of these isolates were maintained as soil based cultures with Styloxanthys (*Hordeum vulgare*) as host plant. Resting spores from these cultures were collected and identified by manual provided by Hall (1983).

Evaluation of PGPR traits

All the 40 *Pseudomonas* isolates were qualitatively and quantitatively characterized by standard protocols for the presence of PGPR traits which are known to play an essential role in growth promotion of plants.

Detection and estimation of indole acetic acid (IAA)

IAA production in the culture medium of *Pseudomonas* isolates was detected following the method described by Brick et al. (1991). The quantitative analysis of IAA was carried out as per the modified method of Loper and Schroth (1986). Bacterial cultures were grown for 48 h at 28 ± 2°C in mineral salts medium amended with 1% L-tryptophan. After their growth, cultures were centrifuged at 5,000 rpm for 15 min. The clear supernatant (2 ml) was mixed with few drops of orthophosphoric acid and 2 ml of Salkowski reagent. Generation of pink colour indicates presence of IAA. The optical density of the colour produced was recorded at 530 nm using a UV–Visible spectrophotometer (Elico, India) and concentration of the IAA produced in culture broth was estimated.

Estimation of gibberellic acid

Gibberellic acids were quantified using the method of Holbrook et al. (1961) with minor modifications. Bacterial cultures in King's B broth medium were centrifuged as described above to separate the cells. The pellet was used for protein estimation. The supernatants pH was adjusted to 2.5 using 0.1 M HCl and using equal volume of ethyl acetate the extraction of gibberellic acid was done into organic phase twice. To 1.5 ml of extract, 0.2 ml of potassium ferrocyanide was added and centrifuged at 1,000 rpm for 10 min. To the supernatant an equal volume of 30% HCl was added and mixture was incubated at 20°C for 75 min. 5% HCl was used as blank and absorbance was measured in a UV–Visible spectrophotometer (Elico, India) at 254 nm. The concentration of gibberellic acid was deduced using a standard graph and the quantity was expressed as mg gibberellic acid mg^{-1} protein.

Siderophores detection and quantification

Siderophore production by *Pseudomonas* isolates was detected by observing orange halos production around the

bacterial colony on CAS agar plates (Schwyn and Neilands 1987) after 72 h of growth. For quantification of siderophores, to 0.5 ml of cell free culture supernatant grown in liquid CAS medium, 0.5 ml of CAS reagent was added and absorbance was measured at 630 nm against a blank. Siderophores content was expressed as percentage siderophore units using the formula:

$$\% \ \text{Siderophore units} = Ar - As/Ar \times 100$$

where Ar = absorbance of reference at 630 nm (CAS reagent) and As = absorbance of sample at 630 nm.

Hydrogen cyanide (HCN) detection

King's B medium amended with 0.44% of L-Glycine was used for detection and quantification of hydrogen cyanide following the method of Bakker and Schippers (1987). Whatman no. 1 filter paper strips soaked in a 0.5% picric acid + 2% Na_2CO_3 solution were inserted in to test tubes with the liquid media inoculated with 100 μl of bacterial inoculum. The tubes were sealed with cotton plugs and parafilm and incubated at $28 \pm 2°C$ for 48 h. HCN production was indicated by change in colour of the filter paper from yellow to light brown or reddish brown. The total cyanides (in ppm) content in the filter paper was estimated using the following equation.

$$\text{Total cyanides content (ppm)} = 396 \times A_{510} \text{ nm}$$

Detection of ammonia production

Isolates under test were checked for ammonia production, after their growth in test tubes containing peptone water medium (10.0 g peptone; 5.0 g NaCl; 1,000 ml distilled water; 7.0 pH (Dye 1962). The tubes were inoculated with 100 μl of 24 h grown cultures and incubated at $28 \pm 2°C$ for 72 h. The accumulation of ammonia was detected by addition of 0.5 ml of Nessler's reagent to each tube. A faint yellow colour indicated a small amount of ammonia (+), and deep yellow to brownish colour indicated high production of ammonia (++).

Quantification of phosphate solubilization

For quantification of P-solubilization, all the test isolates were grown in 150 ml conical flasks containing 50 ml of Pikovskaya's broth (Pikovskaya 1948) having 50 mg of insoluble tri-calcium phosphate. Flasks were inoculated with 100 μl of 24 h grown cultures and incubated at $28 \pm 2°C$, in a shaking incubator (Orbitek, India) at 140 rpm for 10 days. Available 'P' in the culture supernatant was assayed following the method explained by Olsen and Sommers (1982) and 'P' solubilized by test isolates was expressed in ppm.

Estimation of exo-polysaccharides (EPS)

Pseudomonas isolates were grown in 50 ml of tryptone soy broth in 150 ml conical flask, incubated at $28 \pm 2°C$, 120 rpm in an incubator shaker (Orbitek, India) for 48 h. Cells were separated by centrifugation at 10,000 rpm for 10 min. Pellet was dissolved in 0.4% KCl and re-centrifuged at 5,000 rpm for 10 min. To 1 ml of supernatant 3 ml absolute ethanol was added and incubated overnight at 4°C in a refrigerator. The solution was centrifuged at 15,000 rpm for 20 min. Pellet obtained was washed with sterile distilled water followed by centrifugation at 10,000 rpm for 15 min. Pellet was dissolved in 0.5 ml sterile distilled water. 100 ml of this sample was used for the total EPS estimation by anthrone method using glucose as a standard (Robert and William 1960).

Detection of chitinase and protease

Test for the detection of chitinase was carried out as per the method of Hirano and Nagao (1988). Screening was carried out in petri plates containing sterile nutrient agar amended with 0.1% colloidal chitin (for protease nutrient agar medium was amended with 1% skimmed milk powder). Bacterial cultures grown for 24 h were spot inoculated on to media in plates and incubated for 5 days (for chitinase) and 48 h (for protease). Isolates that produced a clearing zone around the colony were considered positive for chitinase or protease.

In vitro antagonism against phytopathogenic fungi

Pseudomonas isolates were tested for their ability to inhibit the growth of soil-borne phyto pathogenic fungi (Fusarium ricini, Rhizoctonia solani, Botrytis ricini, Sclerotium rolfsii and Macrophomina phaseolina) by dual culture technique (Skidmore and Dickinson 1976). Petri plates containing sterile malt-dextrose agar (peptone: 2.0 g; malt extract: 20.0 g; yeast extract: 2.0 g; dextrose: 5.0 g; agar–agar: 15.0 g; distilled water: 1000 ml; pH: 7.0) were inoculated in the centre with a 5 mm disc of fungal culture grown for 7 days and in the periphery the bacterial strains were inoculated perpendicularly by single streak. Plates were incubated at $28 \pm 2°C$ for 5 days. A bacterial isolate was considered positive for inhibition of fungi when the growth of pathogen under test was absent.

Pot trials

The experimental setup consisted of 23 treatments comprising of single and dual agent inoculation with AM fungi. Seeds of sorghum cv. CSV-15 procured from The Directorate of Sorghum Research, Hyderabad, India were

surface sterilized with 1% sodium hypochlorite for 5 min and washed five times with sterile distilled water. Sterile seeds were bacterized individually with P1, P10, P13, P18, P21, P28 and P38 *Pseudomonas* strains at the population of 2×10^6 CFU/seed employing talc-cmc as carrier material. Mycorrhizae were applied to these pots at the rate of 1,000 propagules/pot at a depth of 15 cm below the seed. An un-inoculated control devoid of both bacteria and mycorrhizae was maintained. Each treatment was replicated at least 6 times with 3 seeds per pot, thinned down to 2 seeds/pot after germination. Experiment was continued for 25 days, later plants were excavated carefully from the pot and analysed for root volume, plant biomass, leaf area (measured by LI 3100 Lincoln Nebraska USA leaf area meter), chlorophyll (measured by Minolta spad chlorophyll meter-502 and expressed as spad units) and percentage of mycorrhizal infection.

Statistical analysis

The pooled data was subjected to statistical analysis using two way ANOVA at a probability of 0.001. The means of the values among the treatments were compared with one another by applying Fisher's least significant difference test ($P < 0.05$).

Results

All the 7 isolates (Table 1) were initially evaluated for different PGPR traits besides testing for their ability to inhibit soil borne fungal pathogens like *F.ricini, B. ricini, S.rolfsii, R.solani* and *M. phaseolina*. Among the 7 isolates P18 and P21 inhibited all the 5 pathogens. All these isolates were further screened for qualitative production of IAA, GA, HCN, NH$_3$, EPS, siderohpore and P-solubilisation. The critical review of the Table 2 reveals that each of this isolate possesses one of the above PGPR trait at maximum i.e., P1 for GA (124.6 mg/l), P10 for IAA (11.4 mg/l), P13 for EPS (86 µg/l), P18 for siderophores and P-solubilisation (60 SU and 65% respectively) and P28 for HCN (29.04 ppm). P21 which was a HCN and

siderophore producer inhibited all the 5 pathogens whereas P10 and P28 were able to inhibit *M. phaseolina*. Ammonia production was observed in all the isolates. Whereas chitinase was observed in P1 and P18. All the isolates were found producing protease enzyme except P1 (Table 2).

All the isolates were observed to increase plant growth in sorghum compared to uninoculated control. Strain P18 which was possessing multiple PGPR traits did not show plant growth promotion (PGP) as expected compared to P1, which was a high GA producer besides solubilising P and producing EPS. Increase in root volume, plant biomass and chlorophyll content were significantly higher ($P < 0.001$) compared to other isolates and un-inoculated control. However, leaf area was more pronounced in the case of P38 (43.3 sq cm) (Table 3).

In combination with AM fungi these isolates showed varied results for PGP in sorghum. P10 and P13 which were efficient IAA and EPS producers respectively performed unexpectedly well with mycorrhizae (Fig. 1). P1 which was found to be best isolate in individual inoculation, did not prove its ability in combination with mycorrhizae. P10 and P13 in combination with two mycorrhizae showed significantly higher root volume and plant biomass compared to other isolates and mycorrhizae alone. Leaf area was more pronounced when G2 was used in combination with P10. However, this increase was not significantly different when compared to G1 in combination with P28. Photosynthetic activity was significantly high in treatment with P10 + G2 (total chlorophyll 22) ($P < 0.005$). Even though P13 + G1 treated plants showed higher photosynthetic activity (total chlorophyll 17) this was not significantly different when P18 + G2 was used. Mycorrhizal infection was more facilitated in presence of P10 and P13 over other isolates (62.4 and 72.8, respectively) (Table 3; Fig. 2).

Discussion

Combined interactions between AM fungi and bacteria can enhance plant growth and that some of these interactions may be very specific (Artursson et al. 2006). In the present

Table 1 Details of soil samples crop, location and state used for the isolation of *Pseudomonas* spp.

Isolate	Crop	Location	District	State
P1	Greengram	Hayathnagar	Ranga Reddy	Andhra Pradesh
P10	Fingermillet	Hayathnagar	Ranga Reddy	Andhra Pradesh
P13	Bulk soil	Udaipur	Udaipur	Rajasthan
P18	Groundnut	Kadiri	Anantapur	Andhra Pradesh
P21	Sorghum CSH-9	Hayathnagar	Ranga Reddy	Andhra Pradesh
P28	Cotton	Warangal	Warangal	Andhra Pradesh
P38	Oats	Hisar	Hisar	Haryana

Table 2 List of *Pseudomonas* isolates and their PGPR traits selected for experiment

Isolate	IAA (mg/mL)	GA (mg/mg protein)	Siderophore (SU)	HCN (ppm)	Protease	Chitinase	NH₃	EPS (µg/mL)	P-Sol (ppm)	F	R	B	S	M
P1	−	124.6	−	−	−	+	+	49	32	−	−	−	−	−
P10	11.4	−	20	−	+	−	+	7	26	−	−	−	−	+
P13	11.2	96.4	42	−	+	−	++	86	17	−	−	−	−	−
P18	8.1	46.5	60	−	+	+	+	26	65	+	+	+	+	+
P21	−	−	58	11.88	+	−	+	24	50	+	+	+	+	+
P28	6.2	31.4	12	29.04	+	−	+	23	−	−	−	−	−	+
P38	−	−	−	6.6	+	−	+	−	60	−	−	−	−	−

F = *Fusarium oxysporum*; R = *Rhizoctonia solani*; B = *Botrytis ricini*; S = *Sclerotium rolfsii*; M = *Macrophomina phaseolina*

Table 3 Increase in various plant parameters recorded after single and co-inoculation of *Pseudomonas* and AM fungi in sorghum

Treatment	Root volume (cc)	RDW (gm)	SDW (gm)	TDW (gm)	Leaf area (cm²)	Total chlorophyll (spad reading)	Percentage of mycorrhizal infection
P1	1.83 (±0.042)ᵃ	0.480 (±0.011)	0.322 (±0.0074)ᶠᵍ	0.416	25.0 (±0.576)ᵈ	17.3 (±0.399)	−
P10	1.16 (±0.027)ᵈᵉ	0.262 (±0.006)	0.194 (±0.0045)ⁱ	0.816	19.6 (±0.452)ᵉᶠ	12.0 (±0.277)ᵉ	−
P13	1.50 (±0.035)ᵇ	0.310 (±0.0071)ᵉᶠ	0.623 (±0.0144)	0.926	18.5 (±0.426)ᶠᵍ	10.0 (±0.230)ᵍ	−
P18	1.50 (±0.036)ᵇ	0.318 (±0.0073)ᵉ	0.228 (±0.0053)ʰ	0.835	17.3 (±0.399)ᵍʰ	11.7 (±0.270)ᵉᶠ	−
P21	1.50 (±0.038)ᵇ	0.234 (±0.0054)ᵍʰ	0.756 (±0.0174)	1.326	23.1 (±0.532)	13.0 (±0.300)ᵈ	−
P28	1.02 (±0.024)	0.243 (±0.0056)ᵍ	0.503 (±0.0116)ᵃ	1.34	30.9 (±0.712)	10.0 (±0.230)ᵍ	−
P38	1.11 (±0.026)	0.228 (±0.0053)ʰⁱ	0.188 (±0.0043)ⁱ	0.593	43.3 (±0.998)	15.6 (±0.359)ᵇ	−
P1+G1	1.11 (±0.025)ᵈᵉ	0.422 (±0.0097)ᵇ	0.394 (±0.0091)ᵇᶜ	0.887	20.2 (±0.465)ᵉ	14.0 (±0.323)ᶜ	55.9
P1+G2	1.55 (±0.036)ᵇ	0.512 (±0.011)	0.414 (±0.0095)ᵇ	0.837	17.3 (±0.399)ᵍʰ	13.0 (±0.301)ᵈ	58.6
P10+G1	1.11 (±0.026)ᵈᵉ	0.463 (±0.010)ᵃ	0.372 (±0.0086)ᶜᵈᵉ	0.926	17.1 (±0.394)ʰ	11.0 (±0.253)ᶠ	56.4
P10+G2	1.55 (±0.037)ᵇ	0.770 (±0.017)	0.556 (±0.0128)	0.993	28.8 (±0.664)ᵃ	22.0 (±0.507)	62.4
P13+G1	1.87 (±0.043)ᵃ	0.838 (±0.019)	0.502 (±0.0116)ᵃ	1.057	25.6 (±0.590)ᶜᵈ	17.0 (±0.392)ᵃ	72.8
P13+G2	1.11 (±0.027)ᵈᵉ	0.301 (±0.0069)ᶠ	0.292 (±0.0067)ᵍ	0.695	14.7 (±0.339)ⁱʲ	10.0 (±0.230)ᵍ	50.9
P18+G1	1.57 (±0.036)ᵇ	0.464 (±0.010)ᵃ	0.423 (±0.0097)ᵇ	0.803	26.0 (±0.599)ᶜ	18.0 (±0.415)	57.4
P18+G2	1.50 (±0.035)ᵇ	0.492 (±0.0113)	0.345 (±0.008)ᵉᶠ	0.764	17.2 (±0.396)ᵍʰ	17.0 (±0.392)ᵃ	58.4
P21+G1	1.22 (±0.028)ᵈ	0.556 (±0.0128)	0.370 (±0.0085)ᶜᵈᵉ	0.695	17.1 (±0.394)ʰ	12.0 (±0.277)ᵉ	59.2
P21+G2	1.77 (±0.041)ᵃ	0.611 (±0.0141)	0.382 (±0.0088)ᶜᵈ	0.63	26.6 (±0.613)ᵇᶜ	16.0 (±0.369)ᵇ	59.6
P28+G1	1.77 (±0.039)ᵃ	0.629 (±0.0145)	0.428 (±0.0099)ᵇ	0.413	27.5 (±0.634)ᵃᵇ	11.1 (±0.256)ᵉᶠ	60.8
P28+G2	0.77 (±0.018)ᶠ	0.337 (±0.0078)	0.358 (±0.0082)ᵈᵉ	0.416	15.8 (±0.364)ⁱ	12.0 (±0.277)ᵉ	53.1
P38+G1	1.88 (±0.043)ᵃ	0.405 (±0.0093)ᶜᵈ	0.398 (±0.0092)ᵇᶜ	0.816	18.5 (±0.426)ᶠᵍ	14.0 (±0.323)ᶜ	56.4
P38+G2	1.42 (±0.033)ᵇᶜ	0.412 (±0.0095)ᵇᶜ	0.352 (±0.0081)ᵈᵉᶠ	0.926	26.5 (±0.611)ᵇᶜ	14.0 (±0.327)ᶜ	55.2
G1	1.33 (±0.031)ᶜᵈ	0.396 (±0.0091)ᵈ	0.299 (±0.0069)ᵍ	0.835	20.0 (±0.461)ᵉ	14.0 (±0.328)ᶜ	52.6
G2	1.33 (±0.032)ᶜᵈ	0.402 (±0.0093)ᶜᵈ	0.228 (±0.0053)ʰ	1.326	14.3 (±0.330)ʲ	15.5 (±0.357)ᵇ	49.8
Control	1.00 (±0.023)ᵉᶠ	0.221 (±0.0051)ⁱ	0.192 (±0.0044)ⁱ	1.34	12.8 (±0.295)	9.0 (±0.207)	−
C.D at 1%	0.18	0.01	0.03		1.32	0.8	−

Values in the columns super scribed by same alphabet are not significantly different

Values in parentheses are standard errors

Values are means of 6 replicates significant at (ANOVA) $P < 0.001$. Means were compared with each other following Fischer's least significant difference test ($P<0.05$)

rdw/sdw/tdw = root, shoot and total dry weights

experiment some of the plant growth parameters of sorghum were improved with AMF and *Pseudomonas* strains (Table 3). The extent of AM fungal colonization of sorghum roots was dependent on the combinations of mycorrhiza and PGPR strains used. Clear preferences were observed for the microbial associations and beneficial
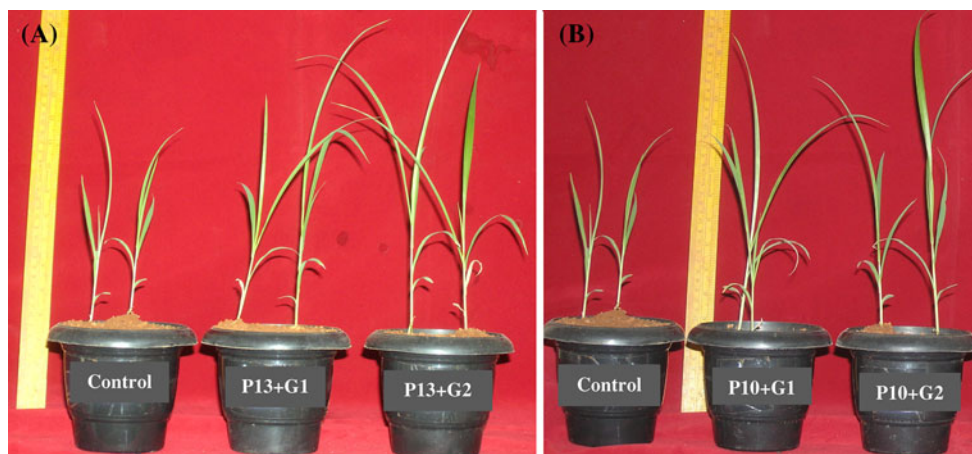
Fig. 1 Plant growth promotion of sorghum by **a** *Pseudomonas* sp. P13 + *Glomus fasciculatum*-G1 and *Glomus aggregatum*-G2 **b** *Pseudomonas* sp. P10 + *Glomus fasciculatum*-G1 and *Glomus aggregatum*-G2
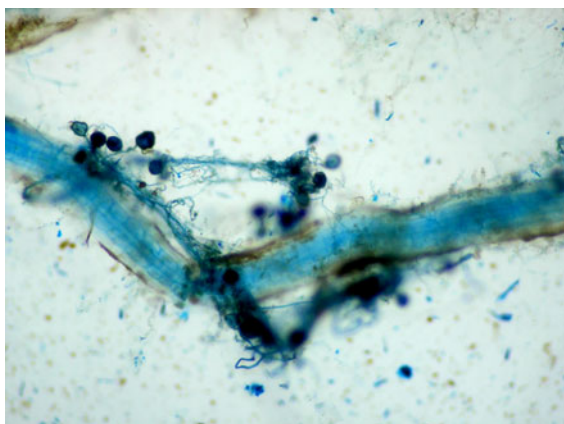

Fig. 2 Extensive mycorrhizal infection (extramatricular vesicles) in roots of *Pseudomonas* sp. P13 + *Glomus fasciculatum*-G1 inoculated plants

effects of the bacterial strains on two different AM fungi. For example, higher mycorrhizal colonization was observed when G1 and G2 were co-inoculated along with P13 than individually. These results suggest that the different AM fungi react differently when inoculated together with the same bacterium. Similar results were obtained with P10 bacterial strain. Similar observations were made by Azcon (1989) on tomato using three AM fungi (*Glomus mosseae*, *Glomus fasciculatum* and another *Glomus* sp.; E3 type) and two bacterial strains (*Azotobacter* and *Enterobacter*). They also found effects that were related to specific interactions between each AM fungus together with either of the bacteria. Meyer and Linderman (1986) also reported enhanced mycorrhization of clover in the presence of PGPR rhizobacterium *Pseudomonas putida*.

There is a growing evidence that diverse microbial populations in the rhizosphere play a significant role in sustainability issues (Barea et al. 2000) and that the manipulation of AMF and certain rhizobacteria such as

PGPR and MHB is important. Vivas et al. (2003) used a dual AM fungus–bacterium inoculum to study the effect of the drought stress induced in lettuce grown in controlled-environment chambers. Their results showed that there was a specific microbe–microbe interaction that modulates the effectiveness of AMF on plant physiology.

The significant positive effects on plant fresh and dry weights were determined upon AM fungal inoculation. When only bacterial strains were inoculated fresh and dry masses were relatively less whereas, 221 and 224% increase in dry mass was observed up on P10 with G2 and P13 with G1, respectively.

Mosse (1962) showed that cell wall degrading enzyme producing *Pseudomonas* sp. enhanced the germination of AM fungal spores of *Glomus mosseae* and promoted the establishment of AM on clover roots under aseptic conditions. AM and PGPR symbioses not only induce physiological changes in the host plant but also modify morphological architecture of the roots such as total root length and root tip numbers (Atkinson et al. 1994). In the present study, P10 which is a high IAA producing bacterium in association with AMF (G1/G2) which could have had influenced the root system of the host plant, thereby increasing the absorption of crucial nutrients from soil resulting in higher plant productivity. Because many of the PGPR inoculants tested, altered rooting patterns, it seems likely that nutrient and water uptake by the plant roots could be affected. Moreover, altered rooting patterns may affect the development of AMF colonization, indirectly influencing mineral nutrition and water uptake attributable to the fungal endophyte.

Behl et al. (2003) studied the effects of wheat genotype and *Azotobacter* survival on AMF and found that the genotype tolerant to abiotic stresses had higher AMF infection and noticed a cumulative effect of plant–AMF–PGPR interaction. Similar observations were reported by

Chaudhry and Khan (2002; 2003) who studied the role of symbiotic AMF and PGPR nitrogen-fixing bacterial symbionts in sustainable plant growth on nutrient-poor heavy metal contaminated industrial sites and found that the plants surviving on such sites were associated with nitrogen-fixing rhizobacteria and had a higher arbuscular mycorrhizal infection, that is, a cumulative and synergistic effect.

Cattelan et al. (1999) identified 22 isolates from soybean rhizosphere positive for PGPR traits. Not all bacteria that had PGPR traits stimulated soybean growth, but six isolates positive for ACC deaminase production, four isolates positive for siderophore production, three isolates positive for β-1,3-glucanase production, and two isolates positive for P solubilization increased at least one aspect of early soybean growth. Similarly in the present study all the PGPR traits possessing Pseudomonas strains did not showed higher plant biomass. Antoun et al. (1998) surveyed 266 strains of rhizobia and found 83% produced siderophores, 58% produced IAA, and 54% could solubilize phosphorus. Inoculation of radish with these strains revealed 25% of the stains to be PGPR, but 64% to have no effects, and 11% to actually have detrimental effects on plant growth. Belimov et al. (2001) investigated 15 strains of bacteria isolated from the rhizoplane of pea and Indian mustard representing a variety of bacterial species. They found that of five Pseudomonas isolates which stimulated some aspect of growth of rape grown in uncontaminated or Cd-contaminated soils, three were positive for ACC-deaminase and phosphate solubilization activity, and two were positive for ACC deaminase activity, phosphate solubilization activity, and IAA production. These studies indicate that PGPR will often have multiple modes of actions. We can also infer from these studies that PGPR co-existing in the rhizosphere that have single modes of action may act synergistically to stimulate the growth of the host plant, such as that indicated by Rojas et al. (2001). Likewise, in the present study, IAA producing P10 and EPS producing P13 improved the seedling biomass when co-inoculated with G2 and G1, respectively. The reason that P13 has enhanced the plant growth could be due to its higher EPS producing nature which lead to better soil aggregation and increased soil aggregate stability thereby helping the plants to take higher volume of water and nutrients from rhizosphere soil resulting in better growth of plants. Similar observations were reported by Miller and Wood (1996).

Conclusion

In summary, the microbial interactions in our experiments show that soil microbial components can play an essential role in helping the plant to establish and thrive. Particular microbial combinations might determine which interactions can be exploited to enable inoculated plants to behave in a more competitive way and to survive when established in the field. P10 and P13 strains which were higher IAA and EPS producers respectively performed well with mycorrhizae for plant growth promotion of sorghum. IAA and EPS producing PGPR strains are known to be efficient plant growth promoters. Further, structural identification and typing of actual compounds involved in improved growth of sorghum is yet to be deciphered. Mass spectral analysis of gibberellins, auxins, EPS etc., and their impact on plant growth promotion are to be carried out.

References

Andrade G, Mihara KL, Linderman RG, Bethlenfalvay GJ (1997) Bacteria from rhizosphere and hyphosphere soils of different arbuscular-mycorrhizal fungi. Plant Soil 192:71–79

Antoun H, Beauchamp CJ, Goussard N, Chabot R, Lalande R (1998) Potential of Rhizobium and Bradyrhizobium species as plant growth promoting rhizobacteria on non-legumes: effect on radishes (Raphanus sativus L.). Plant Soil 204:57–67

Artursson V, Finlal RD, Janet KJ (2006) Interactions between arbuscular mycorrhizal fungi and bacteria and their potential for stimulating plant growth. Environ Microbiol 8(1):1–10

Atkinson D, Berta G, Hooker JE (1994) Impact of mycorrhizal colonization on root architecture, root longevity, and the formation of growth regulators. In: Gininazzi S, Scheupp H (eds) Impact of arbuscular mycorrhizas on sustainable agriculture and natural ecosystems. Birkhauser Verlag, Basal, pp 89–99

Azcon R (1989) Selective interaction between free-living rhizosphere bacteria and vesicular-arbuscular mycorrhizal fungi. Soil Biol Biochem 21:639–644

Bakker AW, Schippers B (1987) Microbial cyanide production in the rhizosphere in relation to potato yield reduction and Pseudomonas spp. mediated pant growth stimulation. Soil Biol Biochem 19:451–457

Barea JM, Toutant P, Balazs E, Galante E, Lynch JM, Shepers JS, Werner D, Werry PA (2000) Rhizosphere and mycorrhiza of field crops. INRA and Springer, Berlin

Behl RK, Sharma H, Kumar V, Narula N (2003) Interactions amongst mycorrhiza, Azotobacter chroococcum and root characteristics of wheat varieties. J Agron Crop Sci 198:151–155

Belimov AA, Safronova VI, Sergeyeva TA, Egorova TN, Matveyeva VA, Tsyganov VE, Borisov AY, Tikhonovich IA, Kluge C, Preisfeld A, Dietz KJ, Stepanok VV (2001) Characterization of plant growth promoting rhizobacteria isolated from polluted soils and containing 1-aminocyclopropane-1-carboxylate deaminase. Can J Microbiol 47:642–652

Brick JM, Bostock RM, Silverstone SE (1991) Rapid in situ assay for indole acetic acid production by bacteria immobilized on nitrocellulose membrane. Appl Environ Microbiol 57:535–538

Cattelan AJ, Hartel PG, Fuhrmann JJ (1999) Screening for plant growth promoting rhizobacteria to ptomote early soyabean growth. Soil Sci Soc Am J 63:1670–1680

Chaudhry TM, Khan AG (2002) Role of symbiotic organisms in sustainable plant growth on heavy metal contaminated industrial sites. In: Rajak RC (ed) Biotechnology of microbes and sustainable utilization. Scientific Publishers, Jodhpur, pp 270–279

Chaudhry TM, Khan AG (2003) Proceedings of the 7th international conference on the biogeochemistry of trace elements. In: Gorban GR, Lepp N (eds) Swedish University of Agricultural Sciences, Uppsala, pp 134–135 (June 15–19)

Correa JD, Barrios ML, Galdona RP (2004) Screening for plant growth-promoting rhizobacteria in Chamaecytisus proliferus (tagasaste), a forage tree-shrub legume endemic to the Canary Islands. Plant Soil 266:75–84

Dye DW (1962) The inadequacy of the usual determinative tests for identification of Xanthomonas spp. NZT Sci 5:393–416

Garbaye J (1994) Helper bacteria: a new dimension to the mycorrhizal symbiosis. New Phytol 128:197–210

Hall IR (1983) A summary of the features of endogonaceous taxa. Technical report no. 8, 3rd edn. Intermay Agricultural Research Centre, New Zealand, p 33

Han J, Sun L, Dong X, Cai Z, Yang H, Wang Y, Song W (2005) Characterization of a novel plant growth promoting bacteria strain Delftia tsuruhatensis HR4 both as a diazotroph and a potential biocontrol agent against various pathogens. Sys Appl Microbiol 28:66–76

Hirano S, Nagao N (1988) An improved method for the preparation of colloidal chitin by using methanesulfonic acid. Agric Biol Chem 52:2111–2112

Holbrook AA, Edge WJW, Baily F (1961) Spectrophotometric method for determination of gibberellic acid. Adv Chem Ser 28:159–167

King EO, Ward MK, Raney DE (1954) Two simple media for the demonstration of pyocyanin and fluorescin. J Lab Clin Med 44:301–307

Loper JE, Scroth MN (1986) Influence of bacterial sources on indole-3 acetic acid on root elongation of sugarbeet. Phytopathol 76:386–389

Lynch JM (1990) The rhizosphere. Wiley, West Sussex

Marschner P, Baumann K (2003) Changes in bacterial community structure induced by mycorrhizal colonisation in split-root maize. Plant Soil 251:279–289

Meyer RJ, Linderman RG (1986) Response of subterranean clover to dual inoculation with vesicular-arbuscular mycorrhizal fungi and a plant growth-promoting bacterium, Pseudomonas putida. Soil Biol Biochem 18:185–190

Miller KJ, Wood JM (1996) Osmoadoptation by rhizosphere bacteria. Annu Rev Microbiol 50:101–136

Mosse B (1962) The establishment of vesicular-arbuscular mycorrhiza under aseptic conditions. J Gen Microbio 27:509–520

Olsen SR, Sommers LE (1982) Phosphorous. In: Page AL, Miller RH (eds) Methods of soil analysis. Part 2, Chemical and Microbiological Properties, 2nd edn. Agronomy Monograph 9. American Society of Agronomy and Soil Science Society of America, Madison, WI, pp 403–430

Pal KK, Tilak KVBR, Saxena AK, Dey R, Singh CS (2001) Suppression of maize root diseases caused by, Macrophomina phaseolina, Fusarium moniliformae and Fusarium graminearum by plant growth promoting rhizobacteria. Microbiol Res 156: 209–223

Pikovskaya RE (1948) Mobilization of phosphorous in soil in connection with vital activity of some microbial species. Mikrobiol 17:362–370

Robert S, William B (1960) Determination of protein-bound carbohydrate in serum by modified anthrone method. Anal Chem 32(7):885–886

Rodriguez H, Fraga R (1999) Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol Adv 17:319–339

Rojas A, Holguin G, Glick BR, Bashan Y (2001) Synergism between Phyllobacterium sp. (N2-fixer) and Bacillus licheniformis (P-solubilizer), both from a semiarid mangrove rhizosphere. FEMS Microbiol Ecol 35:181–187

Schwyn B, Neilands JB (1987) Universal chemical assay for the detection and determination of siderophores. Anal Biochem 160:47–56

Skidmore AM, Dickinson CH (1976) Colony interactions and hyphal interference between Septoria nodorum and phylloplane fungi. Trans Br Mycol Soc 66:57–64

Sun YP, Unestam T, Lucase SD, Johanson KJ, Kenne L, Finlay R (1999) Exudation-reabsorption in a mycorrhizal fungus, the dynamic interface for interaction with soil and soil microorganisms. Mycorr 9:137–144

Vivas A, Marulanda A, Ruiz-Lozana JM, Barea JM, Azcon R (2003) Influence of a Bacillus sp. on physiological activities of two arbuscular mycorrhizal fungi and on plant responses to PEG-induced drought stress. Mycorrhiza 13(5):249–256

Vosatka M, Gryndler M (1999) Treatment with culture fractions from Pseudomonas putida modifies the development of Glomus fistulosum mycorrhiza and the response of potato and maize plants to inoculation. App Soil Ecol 11:245–251