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Phylloplane bacteria increase seedling emergence, growth and yield of field-grown groundnut (*Arachis hypogaea* L.)

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ABSTRACT

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Aim: To isolate and characterize groundnut-associated bacterial isolates for growth promotion of groundnut in field.

Methods and Results: Three hundred and ninety-three groundnut-associated bacteria, representing the geocarposphere, phylloplane and rhizosphere, and endophytes were applied as seed treatment in greenhouse. Maximum increase in plant biomass (up to 26%) was observed following treatment with a rhizosphere isolate identified as *Bacillus firmis* GRS 123, and two phylloplane isolates *Bacillus megaterium* GPS 55 and *Pseudomonas aeruginosa* GPS 21. There was no correlation between the production of L-tryptophan-derived auxins and growth promotion by the test isolates. Actively growing cells and peat formulations of GRS 123 and GPS 55, and actively growing cells of GPS 21, significantly increased the plant growth and pod yield (up to 19%) in field. Rifampicin-resistant mutants of GRS 123 and GPS 21 colonized the ecto- and endorhizospheres of groundnut, respectively, up to 100 days after sowing (DAS), whereas GPS 55 was recovered from both the habitats at 100 DAS.

Conclusion: Seed bacterization with phylloplane isolates promoted groundnut growth indicating the possibility of isolating rhizosphere beneficial bacteria from different habitats.

Significance and Impact of the Study: Identification of phylloplane bacteria as effective plant growth-promoting rhizobacteria (PGPR) broadens the spectrum of PGPR available for field application.

Keywords: bacterial formulation, peanut, rhizosphere colonization, seed bacterization.

INTRODUCTION

Some rhizosphere bacteria, termed plant growth-promoting rhizobacteria (PGPR) (Kloepper and Schroth 1978), colonize the roots and promote growth and yield of crop plants in addition to disease control. Direct beneficial effects of PGPR are as a result of the production of plant growth hormones (mainly auxins), enhanced availability of nutrients to the host plant by production of siderophores and phosphate solubilization (Kloepper 1993), and production of volatiles such as 2,3-butanediol and acetoin (Ryu *et al.* 2003). Most often, it is difficult to distinguish growth promoting and biocontrol PGPR, as bacterial isolates selected for *in vitro* antibiosis frequently demonstrate growth promotion even in absence of the target pathogen. Similarly, PGPR selected for growth promotion had an ability to suppress the pathogens when challenge inoculated (reviewed by Kloepper 1993).

Seed treatment with rhizobacteria or their formulations increased the growth of maize (Jacoud *et al.* 1999), wheat (Khalid *et al.* 2004), rice (Preeti *et al.* 2002), and several other crops (Podile and Dube 1988; Kloepper *et al.* 1991). The observed growth promotion is based on several parameters including increase in plant nutrient uptake, root length, shoot length, branching, nodulation (in legume crops), dry biomass, yield and seed weight. The use of these

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beneficial bacteria as biofertilizers has increased interest worldwide, to attain sustainability in agriculture.

Until now, a large majority of the beneficial bacteria identified for use as PGPR were isolated from the rhizosphere and a few of these have been classed as endophytes (Manjula *et al.* 2002; Sessitsch *et al.* 2004). There is a possibility for selection of PGPR from other plant habitats to broaden the spectrum of PGPR and identify more potent PGPR. Bacteria that colonize the aerial plant parts such as the phylloplane are exposed to higher temperature and moisture fluctuations with limited nutrient availability and such bacteria have a better chance to survive and multiply in a nutritionally rich, buffered rhizosphere soil. The phylloplane provides a diversity of beneficial bacteria because of the frequent drift in the microbial communities. Phylloplane bacteria have been identified as biocontrol agents in the phylloplane (Andrews 1992) but have not been tested in other habitats such as the rhizosphere.

Groundnut or peanut (*Arachis hypogaea* L.) is an important oilseed crop in rain-fed areas of Asia and Africa, and soil fertility is an important constraint for high pod yields. The majority of the small-scale farmers in these regions are reluctant to invest in chemical fertilizers because of the unassured crop returns owing to the high incidence of fungal diseases and unpredictable terminal drought. In this context, there is a greater scope for development and popularization of bioinoculants in these groundnut production systems. Earlier attempts for selection of PGPR for groundnut growth promotion in Indian soils identified an increase in pod yield following seed treatment with *Pseudomonas* sp. (Pal *et al.* 2000). In the present study, we have evaluated the effects of groundnut-associated bacteria on the growth of groundnut both in greenhouse and field. Bacteria including endophytic and phylloplane isolates were compared with rhizospheric isolates for their possible use as PGPR.

MATERIALS AND METHODS

Isolation of bacteria

Groundnut plants collected from 17 different fields of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT, Patancheru, India), and 55 farmers' fields in nine districts of Andhra Pradesh (India) (Table 1) were used for isolation of groundnut-associated bacteria. The groundnut cultivars in the sampled fields were the traditional cultivars adopted by the farmers and locally multiplied, and resembled TMV 2/JL 24. In each field three apparently healthy plants of 70–100 days old were collected from two different sites, which were considered as two replications. The collected plants were used for isolation of bacterial strains associated with different habitats of groundnut, that is, geocarposphere (soil region around the pod), phylloplane and rhizosphere, and endophytes of leaf, root and seed. Ten grams of rhizosphere/geocarposphere soil and leaves were suspended in 90 ml of 20 mM phosphate buffer, pH 7·0 and incubated for 1 h at 200 rev min^{-1} and 30°C. For isolation of endophytic bacteria, 5 g of leaf/root/seed, surface sterilized for 5 min with 70% ethanol, was homogenized in 20 ml of the sterilized phosphate buffer using a mortar and pestle. Appropriate dilutions of these suspensions were plated on one-fourth strength of Luria–Bertani (LB) agar (composition of full strength LB agar: tryptone 10 g, yeast extract 5 g, NaCl 10 g and agar 15 g per 1000 ml distilled water and pH adjusted to 7·0) and incubated for 72 h at 30°C. In each sample, single colonies of predominant strains with distinct morphologies and well separated from the others, were subcultured and preserved as glycerol stocks at −70°C. Bacterial isolates were designated based on their habitat of isolation: GRS – rhizosphere, GPS – phylloplane, GGS – geocarposphere, GSE – seed endophytes, GRE – root endophytes and GLE – leaf endophytes (G stands for groundnut).

Table 1 Village/locations distributed in nine districts of Andhra Pradesh, India, from which field-grown groundnut plants were collected for isolation of groundnut-associated bacterial strains

District	Village/location
Anantapur	Danduvaripalli, Kadiri, Kottapalli*
Chittoor	Ballaiahgaripalli, Doulatkhanpalli, Gollapalli
Cuddapah	Akkampeta, Dennemeedipalli, Dennepadu, Erakatollapalli, Kolomollapally, Maddimadugu, Nagavurpalli, Pakherpalli, Veeraponianipalli, Venkatareddigaripalli
Guntur	Kandlakunta*, Yallamanda
Kurnool	Bastipadu, Brahmanakotkuru, Errakota*, Gonegundla, Goramanakonda tanda, K. Nagalapuram, Meedi vemula, Mugithi, Nannuru, Narnur, Pedakottala, Penchikilapadu, Puttapasham, Pyalakurthi and Vishwanathapuram
MahaboobNagar	Elikicherla*, Kottalagadda*, Vuyyalavada*
Medak	Inavolu*, Kolluru* and Sankarapalli
Nalgonda	Dupadu*, Gaddipally* and Macharam*
RangaReddy	Rajendra Nagar, Gacchibowli

In each village, groundnut plants were collected from one field. *The collection of samples from two fields in the same village. All the fields in Guntur and Medak districts had black soil, Nalgonda and Mahaboobnagar are red soil fields and others are red sandy soils. The collected plants were of locally adopted groundnut cultivars and sampling was carried out at 70–100 days after sowing.

Greenhouse evaluation of plant growth promoting activity of groundnut-associated bacteria

Three hundred and ninety-three groundnut-associated bacterial isolates were evaluated for their plant growth-

promoting activity on groundnut in the greenhouse. Seeds of groundnut cv. TMV 2 were surface sterilized with 0·02% (w/v) $HgCl_2$ and washed three times with sterile distilled water (SDW) to remove traces of $HgCl_2$. Bacterial isolates were grown as a lawn on LB agar in 90 mm diameter Petri plates for 48 h at 30°C. The cells were scraped into 20 ml of 0·5% carboxy methyl cellulose (CMC) and the surface-sterilized seeds were suspended in this cell suspension for 30 min. Bacterized seeds were dried under a flow of sterile air in a laminar flow for 4–5 h before sowing. The viable cell count as determined by dilution plating was 10^6–10^7 CFU seed^{-1}.

Five bacterized seeds with 0·5% CMC-treated seeds as control were planted in 15 cm diameter plastic pots filled with red alfisol and sand (3 : 1). The pH of the alfisol was 6·8 and its mineral content was as follows: organic C, 0·68%, P, 6·1 ppm, N, 813 ppm, B, 0·26 ppm, Zn, 1·42 ppm, Cu, 23·4 ppm, Mn, 2·82 ppm, Fe, 21·08 ppm, K, 186 ppm and Mg, 336 ppm. The temperature in the greenhouse was maintained at 28 ± 2°C and the pots were adequately watered daily. The emergence of seedlings was recorded 7 days after sowing (DAS). The plants were uprooted 20 DAS to measure the root and shoot lengths. The plants were washed, dried in an oven at 80°C for 24 h and the dry weight was recorded. In each treatment, 10 seeds were planted per replication. The experiment was conducted in a completely randomized block design with three replications in each treatment and repeated twice.

Auxin production and mineral phosphate solubilization by groundnut-associated bacteria

The 393 groundnut-associated bacterial isolates were tested for production of auxins in L-tryptophan-amended medium by immobilization on to a nitrocellulose membrane (Bric *et al.* 1991). Mineral phosphate solubilization (MPS) was noted as a clearance zone of $Ca_3(PO_4)_2$ in Pikovsky's agar medium (Pikovsky 1948). The diameter of the clearance zone was measured in mm 7 days after incubation. The experiments were conducted twice with three replications.

Identification of selected bacterial isolates

Bacterial isolates with potential plant growth promoting activity were identified at Microbial Type Culture Collection and Gene Bank of Institute of Microbial Technology (Chandigarh, India) based on morphological, growth and biochemical characteristics.

Evaluation of peat-based formulations of selected isolates in field

Peat-based formulations of three bacterial isolates, that is, *Bacillus firmis* GRS 123, *Bacillus megaterium* GPS 55 and *Pseudomonas aeruginosa* GPS 21, selected for plant growth promotion in greenhouse, were further tested in field. Neutralized peat (Biocare Technology Pvt. Ltd, Chatswood, NSW, Australia) was packed in high molecular and high density polyethylene bags, and sterilized at 121°C for 20 min. Bacterial cultures grown in LB broth for 16 h at 30°C and 180 rev min^{-1} were centrifuged for 5 min at 3600 *g* and 4°C, and the harvested cells were resuspended in 10 mM phosphate buffer, pH 7·0 at a 100-fold dilution. One hundred grams of neutralized peat was asceptically inoculated with 50 ml of the diluted cell suspension with uniform adsorption of the bacterial cells into peat and incubated at 30°C. Moisture loss of the formulation determined from the loss in initial weight was frequently compensated by the addition of SDW. Viable cell count of the formulations was determined by dilution plating on nutrient/LB agar and expressed as log CFU g^{-1}. The experiment was repeated twice with four replications.

The experiment was conducted in red alfisols of ICRISAT, Patancheru during khariff (rainy season) of 2001 and repeated in 2002. The experimental field, with a soil pH of 6·7, has been used for cultivation of groundnut/sorghum/pearl millet for nearly 30 years. Single super phosphate and gypsum (375 kg ha^{-1}) during ploughing and peg initiation, respectively, were applied. Chlorothalonil (2 g l^{-1}, Kavach®, Syngenta India Ltd, Mumbai, India) was applied twice at 55 and 80 DAS to control foliar diseases. In both years, planting was carried out in the fourth week of June and the plants were harvested in the second week of October. Weekly averages of the weather parameters (rainfall (mm), maximum and minimum temperatures (°C)) during the crop season in both the years is provided in Fig. 1.

Each treatment consisted of four rows of 9 m length with an intra- and inter-row spacing of 15 and 60 cm, respectively, and replicated three times in a completely randomized block design. Seed bacterization with actively growing bacteria was similar to that described for greenhouse evaluations. For seed bacterization with bacterial formulations, 100 g of surface sterilized seeds were mixed thoroughly with 2 g of the 90-day-old peat formulation using 0·5% CMC as an adhesive. Seeds treated with CMC alone served as control. The experimental plots were irrigated 1 day before planting and the seeds were planted at a 10-cm depth in a furrow made at the time of planting and immediately covered to retain the moisture. All the treatments were also planted in two additional rows of 4 m length to uproot the plants for periodical observations. Starting from 20 DAS, four randomly selected plants were uprooted from each treatment for measurement of root length, shoot length and dry biomass. At physiological maturity (110 DAS), the experimental plots were irrigated and the plants were uprooted manually. The pods were hand picked, sun-dried and the recorded yield in individual treatment was calculated as t ha^{-1}.

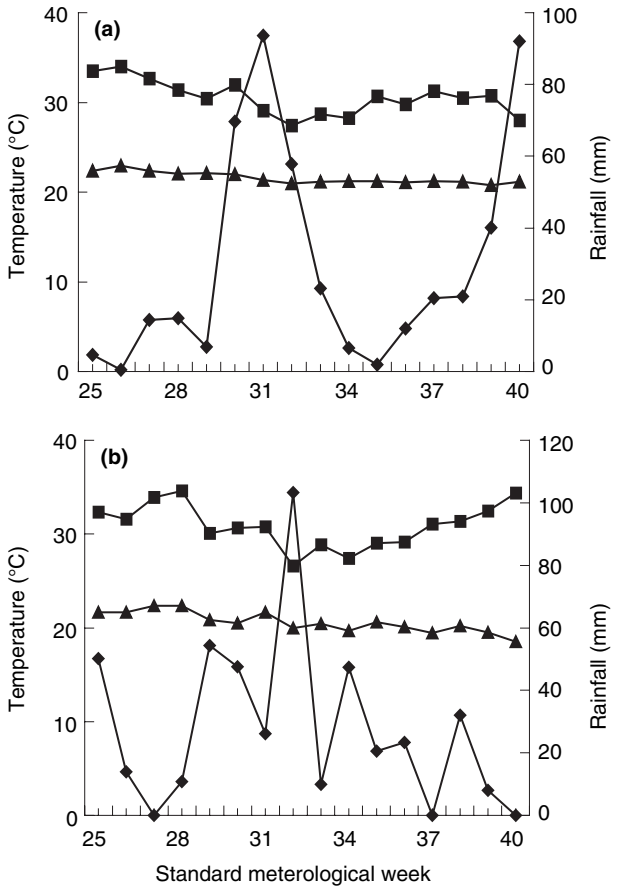

Fig. 1 Weather parameters (♦) rainfall in mm, (■) maximum temperature and (▲) minimum temperature near the experimental field at ICRISAT, Patancheru, India. The average of the weather parameters for each standard meteorological week during the experiment in the years (a) 2001 and (b) 2002 is presented

Rhizosphere colonization of growth promoting bacteria in field

Rhizosphere colonization of the rhizosphere isolate *B. firmis* GRS 123 and phylloplane isolates *B. megaterium* GPS 55 and *P. aeruginosa* GPS 21 in field was determined by using rifampicin resistance as a marker. Growth of GRS 123 and GPS 55, and GPS 21 was observed sensitive to the presence of 1 and 5 μg ml^{-1} rifampicin, respectively, in nutrient/LB agar. Spontaneous mutants of these bacteria with tolerance to higher concentrations of rifampicin were obtained by plating on nutrient/LB agar with 50 and 100 μg ml^{-1} rifampicin. The mutants, observed after 96 h of incubation, were evaluated for stable antibiotic resistance by repeated subculturing (at least 20 times) on rifampicin (100 μg ml^{-1}) added agar medium. Stable mutants of GRS 123, GPS 55 and GPS 21 designated as GRS 123-R_1, GPS 55-R_1 and GPS 21-R_1, similar to the wild isolates in their morphology, *in vitro* growth and plant growth promotion in greenhouse, were applied to the seed and their survival in the ecto- and endorhizosphere of groundnut in field was quantified. Seed bacterization with the rifampicin-resistant mutants was performed as described for greenhouse evaluation and the bacterized seeds were used for field planting. The location, design and method of field experiment was similar to that previously described except that only a single row of 4 m length was planted in each treatment.

For each treatment two plants per replication were uprooted at a 20-day-interval and a 2–3-cm segment from the middle portion of the root along with the tightly adhering soil was suspended in 50 ml of 2 mM phosphate buffer, pH 7·0. The suspension was incubated for 1 h at 180 rev min^{-1} and 30°C. To quantify the colonization of endorhizosphere by the introduced bacteria, the root segments were surface sterilized with 70% ethanol and homogenized in sterile 20 mM phosphate buffer, pH 7·0. Serial dilutions of both the suspensions were plated on nutrient/LB agar with 100 μg ml^{-1} rifampicin, with three plates per dilution and incubated for 48 h at 30°C. Bacterial population in individual treatment was expressed as log CFU g^{-1}. The experiment was conducted in the 2001 and 2002 rainy seasons, with three replications in each treatment.

Data analysis

All the greenhouse and field experiments for plant growth promotion were arranged in a completely randomized block design. The data were subjected to analysis of variance (ANOVA) using the Genstat 5 statistical package (Lawes Agricultural Trust, Rothamsted, UK). The survival of growth promoting isolates in formulations and in rhizosphere was log transformed before subjecting to ANOVA. The mean values in each treatment were compared using least significant differences at 5% ($P = 0{\cdot}05$) level of significance.

RESULTS

Isolation of bacteria

Three hundred and ninety-three groundnut-associated bacteria were isolated from different habitats of field-grown groundnut plants representing all major groundnut growing areas of Andhra Pradesh (India). Isolates from individual fields differed in their colony morphology. The number of bacterial strains isolated from individual habitats is as follows: rhizosphere 250 (63·6%), phylloplane 67 (17%), geocarposphere 13 (3·3%), leaf endophyte 1 (0·3%), root endophytes 5 (1·3%) and seed endophytes 57 (14·5%).

Greenhouse evaluation of plant growth promoting activity of groundnut-associated bacteria

Of the 393 bacterial isolates tested, 27, representing the geocarposphere, rhizosphere, phylloplane and seed of groundnut, significantly ($P = 0{\cdot}05$) improved the dry biomass and root length in repeated greenhouse experiments (Table 2). All of the 27 isolates except GRS 49, GRS 60 and GRS 86, increased shoot length of treated seedlings. Maximum root length (60% over control) was observed following seed treatment with GRS 180, while the highest increase in dry biomass occurred with a phylloplane isolate *B. megaterium* GPS 55 (26%) followed by another phylloplane isolate *P. aeruginosa* GPS 21 (24%) and a rhizobacterium *B. firmis* GRS 123 (24%). These three bacteria also effectively increased the root and shoot length by >43 and >32% respectively. Four of the 393 bacterial isolates were deleterious to the groundnut growth as observed by a significant reduction of plant biomass (data not shown).

Table 2 Effect of seed treatment with groundnut-associated bacteria on growth of groundnut in greenhouse

Isolate*	Habitat of isolation	Root length (cm)	Shoot length (cm)	Dry weight (g)	Auxin†	MPS‡
GGS 6	geocarposphere	18·6 (37)	12·1 (22)	2·73 (17)	–	1·0
GPS 21	phylloplane	19·5 (43)	13·1 (32)	2·91 (24)	+	–
GPS 28	phylloplane	17·8 (31)	12·2 (23)	2·75 (18)	–	–
GPS 32	phylloplane	18·6 (37)	12·4 (25)	2·74 (17)	–	0·2
GPS 38	phylloplane	16·2 (19)	12·3 (24)	2·70 (15)	+	–
GPS 55	phylloplane	20·1 (48)	14·2 (43)	2·95 (26)	+	–
GRS 2	rhizosphere	17·6 (29)	11·9 (20)	2·80 (20)	+	–
GRS 7	rhizosphere	15·8 (16)	11·0 (11)	2·71 (16)	+	–
GRS 11	rhizosphere	16·2 (19)	11·7 (18)	2·80 (20)	–	–
GRS 18	rhizosphere	17·1 (26)	11·0 (11)	2·71 (16)	–	–
GRS 49	rhizosphere	17·4 (28)	10·9 (10)	2·68 (15)	+	–
GRS 60	rhizosphere	16·4 (21)	10·5 (6)	2·69 (15)	–	–
GRS 69	rhizosphere	18·6 (37)	12·2 (23)	2·71 (16)	–	–
GRS 73	rhizosphere	16·4 (21)	12·3 (24)	2·68 (15)	–	–
GRS 86	rhizosphere	15·9 (17)	10·8 (9)	2·74 (17)	–	–
GRS 96	rhizosphere	17·7 (30)	12·9 (30)	2·68 (15)	+	–
GRS 115	rhizosphere	18·9 (39)	12·9 (30)	2·77 (18)	+	–
GRS 123	rhizosphere	19·5 (43)	13·4 (35)	2·89 (24)	–	–
GRS 128	rhizosphere	17·5 (29)	12·3 (24)	2·77 (18)	–	–
GRS 143	rhizosphere	18·8 (38)	12·5 (26)	2·74 (17)	+	0·1
GRS 180	rhizosphere	21·8 (60)	12·2 (23)	2·74 (17)	+	0·1
GRS 183	rhizosphere	16·8 (24)	12·0 (21)	2·79 (19)	–	–
GRS 192	rhizosphere	18·2 (34)	12·5 (26)	2·75 (18)	–	–
GRS 203	rhizosphere	19·1 (40)	11·6 (17)	2·78 (19)	–	0·3
GRS 241	rhizosphere	18·7 (38)	11·7 (18)	2·70 (15)	–	–
GSE 18	seed endophyte	16·3 (20)	11·5 (16)	2·69 (15)	–	0·7
GSE 28	seed endophyte	20·0 (47)	12·0 (21)	2·70 (15)	–	–
Control		13·6 (0)	9·9 (0)	2·34 (0)		
LSD ($P = 0{\cdot}05$)		1·41	1·05	0·33		

Values in parenthesis indicate the percentage increase over control.

*Twenty-seven bacterial isolates that significantly increased the biomass of groundnut, among the evaluated 393 groundnut-associated bacterial isolates, are listed. Bacterized seeds of groundnut cv. TMV 2 planted in a potting mixture (red alfisol and sand, 3 : 1) in greenhouse, and root length, shoot length and dry biomass were measured 20 DAS. Data is the mean of 90 plants in nine replications.

†Auxin production was determined by immobilization on to nitrocellulose membrane and colour development with Salkowski's reagent.

‡Mineral phosphate solubilization (MPS) of the bacterial isolates was measured as diameter (in millimetres) of the clearance zone on Pikovsky's medium containing $Ca_3(PO_4)_2$ after 7 days of incubation.

Auxin production and MPS by groundnut-associated bacteria

Of the 393 groundnut-associated bacterial isolates, 39 (10%) produced auxin on L-tryptophan-amended medium and 50 (13%) solubilized $Ca_3(PO_4)_2$. The percentage of auxin producers and phosphate solubilizers among the 27 plant growth-promoting isolates was high compared with their percentage among all the test isolates (Fig. 2). Few of the bacterial isolates that did not produce auxin *in vitro* were as effective as auxin producers in promotion of root growth. Compared with phosphate-solubilizing bacteria, the majority of the auxin producers enhanced the plant growth.

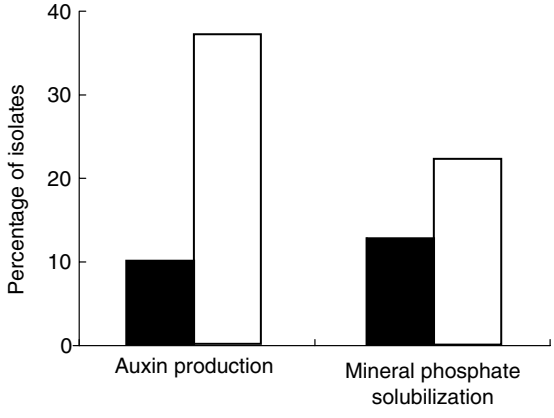

Fig. 2 *In vitro* auxin production and mineral phosphate solubilization by groundnut-associated bacteria. (■) Percentage of isolates positive for individual characteristic among the total 393 bacterial isolates was compared with (□) percentage of isolates positive among the 27 growth promoting isolates

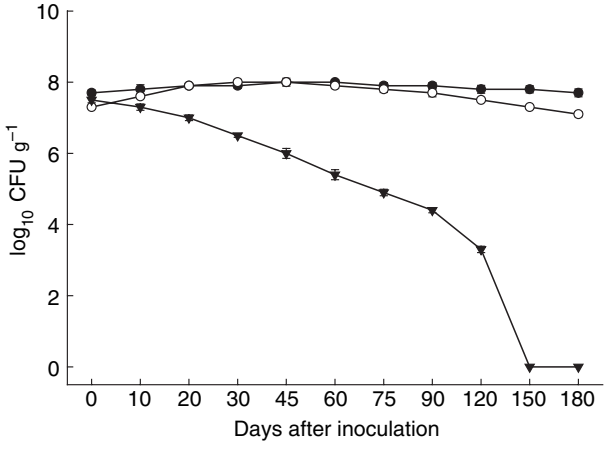

Fig. 3 Survival of selected groundnut-associated bacteria (●) *Bacillus firmis* GRS 123, (○) *Bacillus megaterium* GPS 55 and (▼) *Pseudomonas aeruginosa* GPS 21 in peat-based formulations. Neutralized sterile peat was inoculated with 50% (v/w) of a suspension of mid-log phase cells and incubated at 30°C. Viable cell count was determined at regular time intervals by dilution plating. Data points are the mean and standard error of 12 replications in each treatment

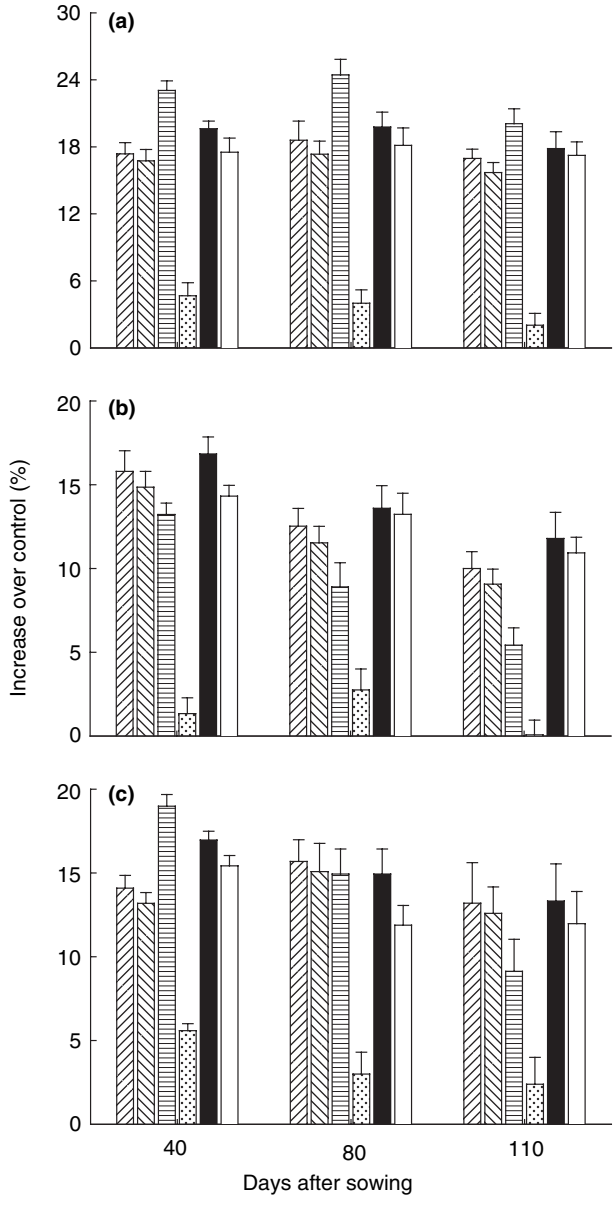

Fig. 4 Effect of selected groundnut-associated bacteria and their peat formulations applied as seed treatment on the growth of groundnut in field, in terms of (a) root length, (b) shoot length, and (c) dry biomass. The treatments evaluated were as follows: (▨) actively growing cells of GRS 123, (▧) peat formulation of GRS 123, (▤) actively growing cells of GPS 21, (▦) peat formulation of GPS 21, (■) actively growing cells of GPS 55, and (□) peat formulation of GPS 55. Data presented is the mean and standard error of six replications in a repeated field experiment

Evaluation of peat-based formulations of selected isolates in field

Bacillus firmis GRS 123 and *B. megaterium* GPS 55 had a good shelf-life of log 7·7 and 7·1 CFU g^{-1}, in peat up to 180 DAI (Fig. 3). Initial population of *P. aeruginosa* GPS 21, log 7·5 CFU g^{-1}, decreased to log 3·3 CFU g^{-1} by 120 DAI, beyond which the bacterium was not recovered from the formulation.

Seed treatment with actively growing cells of *B. firmis* GRS 123, *B. megaterium* GPS 55 and *P. aeruginosa* GPS 21 increased the seedling emergence, shoot and root length, dry biomass and yield of groundnut in the field (Fig. 4; Table 3). Peat formulations of GRS 123 and GPS 55 were as effective as the actively growing cells, whereas the peat formulation of GPS 21 was similar to the CMC control in plant growth promotion. Maximum increase in root length (25%) and shoot length (17%) was recorded in GPS 21- and GPS 55-treated seedlings at 60 and 40 DAS respectively (Fig. 4a,b). Maximum dry weight was observed in GPS 21-treated plants up to 60 DAS. At harvest, GPS 55-treated plants had a maximum dry biomass, which is 13% higher than the CMC-treated control (Fig. 4c).

Seed treatment with GRS 123, GPS 55 and GPS 21 increased the pod yield of field-grown groundnut by 16, 19 and 15% respectively (Table 3). Peat formulations of zGRS 123 and GPS 55 were comparable ($P = 0{\cdot}05$) to the actively growing cells, while the peat formulation of GPS 21 had no effect on the pod yield compared with control.

Rhizosphere colonization of growth promoting bacteria in field

In the groundnut ectorhizosphere, GRS 123-R_1 and GPS 55-R_1 were detectable (log 3·6 and 3·1 CFU g^{-1} respectively) up to 100 DAS (Fig. 5). In contrast, the population of GPS 21-R_1 decreased to log 2·9 CFU g^{-1} by 60 DAS and was not detectable further. In the endorhizosphere, GPS 21-R_1 and GPS 55-R_1 were recovered from 40 and 80 DAS respectively. Maximum populations of these two isolates in the endorhizosphere were recorded as log 3·8 and 3·0 CFU g^{-1}.

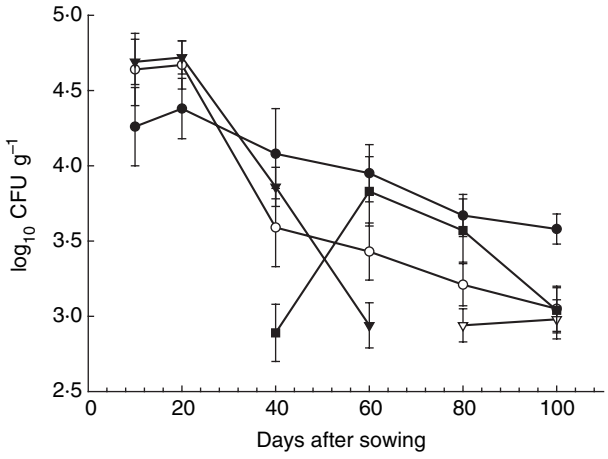

Fig. 5 Survival of rifampicin resistant mutants (●) *Bacillus firmis* GRS 123-R_1 in the ectorhizosphere, (○) *Bacillus megaterium* GPS 55-R_1 in the ectorhizosphere, (▼) *Pseudomonas aeruginosa* GPS 21-R_1 in the ectorhizosphere, (▽) GPS 55-R_1 in the endorhizosphere, and (■) GPS 21-R_1 in the endorhizosphere of groundnut in field. GRS 123-R_1 was not observed in the endorhizosphere throughout the crop season. Mean values and standard errors are calculated based on six replications of each treatment in a repeated field experiment

DISCUSSION

Groundnut-associated bacteria isolated from various habitats, that is, rhizosphere, phylloplane, geocarposphere and seed endophytes promoted the early plant growth of groundnut in the greenhouse. Maximum increase in plant

Table 3 Emergence promotion and yield increase by selected groundnut-associated rhizosphere and phylloplane bacteria, and their peat formulations

Isolate	Treatment*	Emergence (%)	Yield (t ha^{-1})
Bacillus firmis GRS 123	Actively growing ells	95·3 ± 3·1	1·32 ± 0·10
Bacillus firmis GRS 123	Peat formulation	94·4 ± 2·2	1·29 ± 0·14
Bacillus megaterium GPS 55	Actively growing cells	97·9 ± 1·0	1·35 ± 0·11
Bacillus megaterium GPS 55	Peat formulation	92·4 ± 4·5	1·34 ± 0·15
Pseudomonas aeruginosa GPS 21	Actively growing cells	96·3 ± 1·7	1·30 ± 0·13
Pseudomonas aeruginosa GPS 21	Peat formulation	85·7 ± 1·9	1·14 ± 0·06
Control		82·4 ± 4·4	1·13 ± 0·10
LSD ($P = 0{\cdot}05$)		2·97	0·13

*Actively growing cells and peat formulation were applied as seed treatment in groundnut cv. TMV 2 using 0·5% CMC as an adhesive. Seedling emergence was observed 15 days after sowing (DAS) and pod yield was recorded at harvest (110 DAS). Data points are the mean of six replications of a repeated field experiment.

biomass was observed by seed treatment with *B. firmis* GRS 123 from rhizosphere, and *B. megaterium* GPS 55 and *P. aeruginosa* GPS 21 from phylloplane of groundnut. PGPR-mediated growth promotion has been observed in other legumes such as chickpea (Trapero-Casas *et al.* 1990) and soyabean (Zhang *et al.* 1997). Bacterial isolates that promote early plant growth are rapid colonizers of the root system. The use of unsterilized soil for initial screening further facilitates the identification of growth-promoting isolates with rhizosphere competence.

In the present study, the percentage of auxin producers and mineral phosphate solubilizers was high among the growth-promoting isolates compared with the 393 test isolates. However, there was no relation between the *in vitro* auxin production and increase in root length/plant growth by the bacterial isolates. A positive correlation between L-tryptophan-derived auxin production and growth promoting activities of PGPR has been reported (Asghar *et al.* 2002; Khalid *et al.* 2004). The differences in the performance of auxin-producing PGPR could be due to the greater dependency on the availability of L-tryptophan in the root exudates for production of bacterial auxins. Moreover, the optimal concentration of auxin required for plant growth promotion is extremely narrow (Xie *et al.* 1996) and doses of auxin above the threshold levels are deleterious for root growth.

Selected bacterial isolates *B. firmis* GRS 123, *B. megaterium* GPS 55 and *P. aeruginosa* GPS 21 promoted seedling emergence, root length, shoot length, dry weight and pod yield of groundnut in the field. Earlier studies on groundnut PGPR were focused on rhizobacterial isolates of *Pseudomonas* sp., which effectively colonized the rhizosphere and enhanced biomass, nitrogen and phosphorous uptake, and yield (Pal *et al.* 2000). *Pseudomonas* sp. GRC_2 increased the germination, plant growth, nodule weight and grain yield of groundnut in addition to control of charcoal rot caused by *Rhizoctonia bataticola* (Gupta *et al.* 2002). In the present study a similar increase in the growth and yield of groundnut was observed with a phylloplane isolate of *P. aeruginosa*. GRS 123, GPS 55 and GPS 21 were potent siderophore producers on chrome azurol S medium, while GPS 21 was also a broad-spectrum antifungal strain (Kishore *et al.* 2005).

Peat formulations of *B. firmis* GRS 123 and *B. megaterium* GPS 55 maintained high populations up to 180 DAI, while *P. aeruginosa* GPS 21 was not recovered beyond 120 DAI. *Pseudomonas* spp. have a short shelf-life compared with spore-forming bacilli (Georgakopoulos *et al.* 2002). Storage of *Pseudomonas* spp. for >6 months in peat without decrease in populations and effectiveness (Vidhyasekaran and Muthamilan 1995; Georgakopoulos *et al.* 2002) further indicates the possibility of developing formulations of pseudomonads with long shelf-life. In the present study, the field performance of peat formulations of rhizobacteria and phylloplane bacteria as groundnut PGPR was related to the viable cell numbers in these formulations.

Root colonization by PGPR is critical in biological control and plant growth promotion (Kloepper and Beauchamp 1992). Root colonization is highly variable as it is affected by complex interactions of chemical, physical and biological factors (Weller 1988). Determining the dynamics of root colonization by the introduced PGPR is essential for their effective use. We compared the rhizosphere colonization of selected growth-promoting phylloplane bacteria with a rhizobacterium for further understanding of the growth promotion by phylloplane bacteria. The selected phylloplane bacteria, when introduced into the rhizosphere, were able to colonize the ecto- and endorhizospheres of groundnut. The results showed that *B. megaterium* GPS 55 colonized the ecto- and endorhizosphere till 100 DAS, whereas *P. aeruginosa* GPS 21 colonized the ectorhizosphere up to 60 DAS and was present in the endorhizosphere till 100 DAS. It has been previously observed that *Bacillus* and *Pseudomonas* sp. applied as seed treatment colonize the endorhizosphere and increase the plant biomass (Chanway *et al.* 2000).

The present study indicated that the bacterial isolates from habitats like phylloplane and endophytes were also effective, like rhizobacteria, in plant growth promotion when applied as seed treatment. Phylloplane isolates normally survive in low moisture and other adverse conditions, thus may effectively colonize the nutrient-rich rhizosphere. Majority of the selected PGPR were rhizobacteria and it may be possible to select better plant growth-promoting bacteria from parts of the plants other than roots.

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