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Growth promotion and yield enhancement of peanut (*Arachis hypogaea* L.) by application of plant growth-promoting rhizobacteria

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Yield enhancement

Summary

Although plant growth-promoting rhizobacteria (PGPR) have been reported to influence plant growth, yield and nutrient uptake by an array of mechanisms, the specific traits by which PGPR promote plant growth, yield and nutrient uptake were limited to the expression of one or more of the traits expressed at a given environment of plant–microbe interaction. We selected nine different isolates of PGPR from a pool of 233 rhizobacterial isolates obtained from the peanut rhizosphere on the basis of ACC-deaminase activity. The nine isolates were selected, initially, on the basis of germinating seed bioassay in which the root length of the seedling was enhanced significantly over the untreated control. All the nine isolates were identified as *Pseudomonas* spp. Four of these isolates, viz. PGPR1, PGPR2, PGPR4 and PGPR7 (all fluorescent pseudomonads), were the best in producing siderophore and indole acetic acid (IAA). In addition to IAA and siderophore-producing attributes, *Pseudomonas fluorescens* PGPR1 also possessed the characters like tri-calcium phosphate solubilization, ammonification and inhibited *Aspergillus niger* and *A. flavus* in vitro. *P. fluorescens* PGPR2 differed from PGPR1 in the sense that it did not show ammonification. In addition to the traits exhibited by PGPR1, PGPR4 showed strong in vitro inhibition to *Sclerotium rolfsii*. The performances of these selected plant growth-promoting rhizobacterial isolates were repeatedly evaluated for 3 years in pot and field trials. Seed inoculation of these three isolates, viz. PGPR1, PGPR2 and PGPR4, resulted in a significantly higher pod yield than the control, in pots, during rainy and post-rainy seasons. The contents of nitrogen and phosphorus in soil, shoot and kernel were also enhanced significantly in treatments inoculated with these rhizobacterial isolates in pots during both the seasons. In the field trials, however, there was wide variation in the performance of the PGPR isolates in enhancing the growth and yield of peanut in different years. Plant growth-promoting fluorescent

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pseudomonad isolates, viz. PGPR1, PGPR2 and PGPR4, significantly enhanced pod yield (23–26%, 24–28% and 18–24%, respectively), haulm yield and nodule dry weight over the control in 3 years. Other attributes like root length, pod number, 100-kernel mass, shelling out-turn and nodule number were also enhanced. Seed bacterization with plant growth-promoting *P. fluorescens* isolates, viz. PGPR1, PGPR2 and PGPR4, suppressed the soil-borne fungal diseases like collar rot of peanut caused by *A. niger* and PGPR4 also suppressed stem rot caused by *S. rolfsii*. Studies on the growth patterns of PGPR isolates utilizing the seed leachate as the sole source of C and N indicated that PGPR4 isolate was the best in utilizing the seed leachate of peanut, cultivar JL24. Studies on the rhizosphere competence of the PGPR isolates, evaluated on the basis of spontaneous rifampicin resistance, indicated that PGPR7 was the best rhizoplane colonizer and PGPR1 was the best rhizosphere colonizer. Although the presence of growth-promoting traits in vitro does not guarantee that an isolate will be plant growth promoting in nature, results suggested that besides ACC-deaminase activity of the PGPR isolates, expression of one or more of the traits like suppression of phytopathogens, solubilization of tri-calcium phosphate, production of siderophore and/or nodulation promotion might have contributed to the enhancement of growth, yield and nutrient uptake of peanut.
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Introduction

Beneficial plant–microbe interactions in the rhizosphere are the determinants of plant health and soil fertility (Jeffries et al., 2003). In the era of sustainable agricultural production, the interactions in the rhizosphere play a pivotal role in transformation, mobilization, solubilization, etc. from a limited nutrient pool in the soil and subsequent uptake of essential plant nutrients by the crop plants to realize full genetic potential of the crop. Soil microorganisms are very important in the biogeochemical cycles of both inorganic and organic nutrients in the soil and in the maintenance of soil health and quality (Jeffries et al., 2003). Thus, the need of the hour is to enhance the efficiency of the meager amount of external inputs by employing the best combinations of beneficial microbes for sustainable agricultural production. Soil–plant–microbe interactions are complex and there are many ways in which the outcome can influence the plant health and productivity (Kennedy, 1998). The interaction may be harmful, beneficial and neutral to the plants. However, our focus should be to exploit the beneficial interaction of plants and microbes.

While considerable attention has been given to the immense potential of using fluorescent pseudomonads as biocontrol agents against soil-borne fungal pathogens, efforts are now being made to use the fluorescent pseudomonads for enhancing crop growth and yield in a sustainable manner. Beneficial free-living soil bacteria isolated from the rhizosphere, which have been shown to improve plant health or increase yield, are usually referred to as plant growth-promoting rhizobacteria (PGPR) (Kloepper and Schroth, 1978; Suslow and Schroth, 1982) or by one group of workers in China as yield increasing bacteria (YIB) (Tang, 1994) and include a number of different bacteria such as *Azotobacter*, *Azospirillum*, pseudomonads, *Acetobacter*, *Burkholderia* and bacilli (Glick, 1995).

The mechanisms of PGPR-mediated enhancement of plant growth and yields of many crops are not yet fully understood. However, the possible explanations include: (i) the ability to produce ACC deaminase to reduce the level of ethylene in the roots of the developing plants thereby increasing the root length and growth (Jacobson et al., 1994; Glick et al., 1995; Li et al., 2000; Penrose and Glick, 2001); (ii) ability to produce hormones like indole acetic acid (IAA) (Mordukhova et al., 1991; Patten and Glick, 2002), gibberellic acid (Mahmoud et al., 1984) and cytokinins (Tien et al., 1979); (iii) asymbiotic nitrogen fixation (Kennedy et al., 1997); (iv) antagonism against phytopathogenic microorganisms by producing siderophores, β-1,3-glucanase, chitinases, antibiotics, fluorescent pigment and cyanide (Scher and Baker, 1982; Voisard et al., 1989; Renwick et al., 1991; Catellan et al., 1999; Pal et al., 2001); and (v) solubilization of mineral phosphates and mineralization of other nutrients (De Freitas et al., 1997).

Subsequently, a number of PGPR, e.g. *Pseudomonas putida* GR12-2 (Jacobson et al., 1994), *Enterobacter cloacae* UW4 and CAL2 (Shah et al., 1998; Li et al., 2000; Penrose and Glick, 2001), *Bacillus subtilis* A13 (Turner and Backman, 1991), *B. licheniformis* CECT5106 (Probanza et al., 2002), *B. pumilus* CECT5105 (Probanza et al., 2002) and

others like *P. fluorescens* Pf-5, *P. fluorescens* 2-79, *P. fluorescens* CHA0 (Wang et al., 2000), etc., have been identified. Bacteria, especially pseudomonads and bacilli found in the rhizosphere of various leguminous crops, have been found to assist in root colonization by rhizobia and in suppressing soil-borne plant pathogens (Parmar and Dadarwal, 2000). The interactions between these PGPR and rhizobia may be synergistic or antagonistic and beneficial effects of such interaction may be exploited for enhancing the biological nitrogen fixation and crop yield (Dubey, 1996). There is also report of the presence of plant growth-promoting *Bacillus* strains in the root nodules of soybean plants (Yu Ming et al., 2002).

Beneficial effects of PGPR have been reported in many crops, including horticultural, oilseed crops, etc. Reports on oilseed crop like canola (Kloepper et al., 1988) are available but in peanut reports are scanty. Peanut is nodulated by an array of *Rhizobium* species because of the promiscuity of the crop. Peanut seedlings suffer high mortality because of serious seed and soil-borne fungal pathogens like *Aspergillus niger* and *Sclerotium rolfsii*. Therefore, in the present study, attempts were made to identify potent PGPR for peanut which may prove better inoculants for the crop. Thus, 233 rhizobacterial isolates were obtained from peanut rhizosphere on the basis of ACC-deaminase activity. A total of nine isolates were selected on the basis of germinating seed bioassay in which root length was enhanced significantly (Pal et al., 1999). The selected isolates were further tested for other plant growth-promoting attributes, which were also quantified. All the nine isolates were further evaluated to examine the influence of these rhizobacteria on the growth, yield (biomass and pod) and nutrient uptake of peanut. We also tried to understand the possible mechanism(s) involved in the promotion of peanut growth upon PGPR inoculation.

Materials and methods

Cultivars used in the experiments

GG2 (*Arachis hypogaea* spp. *fastigiata* var. *vulgaris*): This Spanish peanut cultivar was released in the year 1983 by the Gujarat Agricultural University, Junagadh, Gujarat, India and developed by a cross between J11 and EC 16659.

JL24 (*A. hypogaea* spp. *fastigiata* var. *vulgaris*): This Spanish peanut cultivar was released in the year 1978 by the Agricultural Research Station, Maharshi Phule Krishi Vidyapith, Jalgaon, India and is a selection from EC 94943. Both are susceptible to iron-chlorosis and soil-borne fungal diseases caused by *S. rolfsii* and *A. niger* but less susceptible to foliar diseases like early and late leaf spots.

Isolation of plant growth-promoting rhizobacteria

Rhizosphere soil samples were collected from peanut growing fields when the crop was 45 days old and brought to the laboratory in polythene bags. Rhizobacteria were isolated from peanut (cultivar JL24) rhizosphere on the basis of ACC-deaminase activity, using the method of Glick et al. (1995). Colonies of different morphologies were picked up and purified. A total of 233 rhizobacterial isolates having ACC-deaminase activity were obtained. All the isolates were sub-cultured at monthly intervals and maintained as stabs at 4 °C in a refrigerator.

Germinating seed bioassay

For seedling bioassay, the bacterial isolates, a total of 233, were grown in King's B medium agar plates at 28 °C for 24 h. The inoculants for treating seeds were prepared by suspending cells from agar plates in a standard nutrient broth (SNB) as described earlier (Gerhardson et al., 1985; Pal et al., 1999). Nine pre-germinated seeds per Petri dish with three replications for each treatment were used and incubated at 28 °C. The length of the root of each seedling was measured after 7 days and expressed in cm and compared with roots treated on day first with sterile SNB. A total of nine isolates were found to enhance the root length significantly. A separate bioassay was conducted taking all the nine isolates to examine the consistency. The isolates were identified by morphological, physiological and biochemical characteristics following Bergeys' Manual of Systematic Bacteriology (8th Edition) and the taxonomy of pseudomonads (Bossis et al., 2000).

Quantification of plant growth-promoting attributes

All the nine isolates, which resulted in the enhancement of root growth, were further tested for their ability to produce siderophore in CAS agar plates and typed and quantified in broth, IAA-like substances, solubilization of tri-calcium phosphate and ammonification.

Siderophore production was detected by observing orange halos around bacterial growth on CAS agar plates (Schwyn and Neilands, 1987) after 72 h of growth. Catechol type of siderophore was quantified by the method of Arnow (1937), modified by Carson et al. (1992) in iron-free liquid medium. Absorbance was measured at 550 ηm keeping pyrocatechol as standard. The quantity of catechol type of siderophore produced was expressed as $mg\,mg^{-1}$ protein. The amount of bacterial protein was quantified using Bradford reagent (Bradford, 1976) using bovine serum albumin as standard. The absorbance was measured at 595 ηm.

The presence of IAA-like substances was detected and quantified following the method of Sarwar and Kremer (1995) in L-tryptophan agar. A 1 ml each of 24 h growth of the isolates in Kings' B (King et al., 1954) broth was pour plated into L-tryptophan agar in triplicate and incubated at $28\pm2\,^{\circ}C$ for 24 h in the dark. After incubation, the agar growth beads (three beads, approximately $0.24\,cm^3$) were placed in freshly prepared Salkowsky reagent (Sarwar and Kremer, 1995) in triplicate, from each Petri dish and incubated in the dark for 30 min for development of pink colour and measured spectrophotometrically at 595 ηm using IAA as standard. The amount of IAA produced was expressed as $mg\,l^{-1}$.

Solubilization of tri-calcium phosphate was quantified in Pikovskaya's (1948) broth. Each flask containing 100 ml of Pikovskaya broth having 500 mg of tri-calcium phosphate was inoculated with 0.5 ml of 24 h broth culture of each isolate in triplicate and incubated in a rotary shaker (240 rpm) at $28\pm2\,^{\circ}C$ for 4 days. The culture was centrifuged at 15,000 rpm for 10 min and the supernatant was collected in 100 ml volumetric flasks. Volume of the supernatant was made to 100 ml with distilled water. Water-soluble phosphorus in the supernatant was determined by the chloromolybdic acid method of King (1932) modified by Jackson (1967). Spectrophotometric measurement was taken at 660 ηm.

For the detection of ammonia production, all the isolates were grown in test tubes containing peptone water: 10.0 g peptone; 5.0 g NaCl; 1000 ml distilled water; 7.0 pH (Dye, 1962). The tubes were inoculated with 100 μl of 24 h grown cultures in broth and incubated at $30\,^{\circ}C$ for 4 days. The accumulation of ammonia was detected by adding Nessler's reagent ($0.5\,ml\,tube^{-1}$). A faint yellow colour indicated a small amount of ammonia, and deep yellow to brownish colour indicated maximum production of ammonia.

In vitro antagonism against *Aspergillus flavus*, *S. rolfsii* and *A. niger*.

Bacterial suspension (100 μl of 24 h growth) was spotted at the centre of Kings' B agar plates and incubated at $28\,^{\circ}C$ for 3 days. After 3 days, the bacterial growth was removed by scrapping and the plates were exposed to chloroform vapour for half an hour to kill the remaining cells. Then the plates were overlayered with 10 ml of soft Kings' B agar amended with the spores of *A. flavus* or *A. niger* and incubated at $28\pm2\,^{\circ}C$ for 24 h. The radii of the inhibition zones were measured in mm. For studying the antifungal activity against *S. rolfsii*, 100 μl of the overnight grown suspension of each rhizobacterial isolate was spread at one periphery of Petri dishes and at the opposite periphery, one sclerotium was placed and the plates were incubated at $28\pm2\,^{\circ}C$ for 3 days. The inhibition zone was measured in mm by inhibition zone measuring scale.

Fungal strains used

The fungal isolates, *S. rolfsii*, *A. niger* and *A. flavus*, used for the experiment were obtained from the fungal pathogen collection of Mycology and Plant Pathology Section of the National Research Centre for Groundnut, Junagadh, Gujarat, India and the pathogens were originally isolated from sick plots at Junagadh, Gujarat, India. The isolates were pathogenic to peanut plant and produced stem rot (*S. rolfsii*), collar rot (*A. niger*) and aflaroot (*A. flavus*) diseases, respectively, on inoculation.

Intrinsic antibiotic resistance patterns

Agar plates of King's B medium were prepared in duplicates after adding the appropriate amount of antibiotics, such as ampicillin (Ap), kanamycin (Km), streptomycin (Str), chloramphenicol (Cm), and nalidixic acid (Nal) from their respective stock solutions ($10\,mg\,ml^{-1}$). All the isolates were grown overnight in Kings' B broth and spots were made by putting 5 μl of each cultural broth of isolates in duplicates in the respective antibiotics plates keeping two plates as control (without any antibiotics). Growth of the isolates was observed after 48 h of incubation at $28\pm2\,^{\circ}C$.

Growth curve of PGPR isolates capable of utilizing seed leachates as the sole source of C and N

For determining the ability of the selected PGPR in utilizing the seed leachate as the sole source of C

and N, seed leachate was extracted from 1 kg of peanut seed (cultivar GG2) soaked overnight in 3 l of deionized water. The entire amount was sterilized by passage through membrane filter assembly (0.22 μm). The seed leachate (50 ml, pH 7.0) was taken in 250 ml Erlenmeyer flask. The plant growth-promoting rhizobacterial isolates were grown overnight in Kings' B broth and 100 μl of each isolate (OD 1.2 at 600 ηm) was used for inoculating the seed leachate, in triplicate. The flasks were incubated at $28 \pm 2\,^{\circ}C$ in a rotary shaker (240 rpm). The OD of each isolate, in triplicate, was measured in a spectrophotometer at 0, 20, 26, 44 and 68 h of growth at 600 ηm. The growth curve was prepared by plotting OD against time.

Development of spontaneous rifampicin resistant mutants

Each plant growth-promoting rhizobacterial isolate was grown overnight in Kings' B broth at $28 \pm 2\,^{\circ}C$ in a rotary shaker at 240 rpm. After centrifugation at 5,000 rpm for 5 min, the pellet of each isolate was dissolved in 10 ml of sterile 0.1 M citrate buffer (pH 5.0). After washing two times with citrate buffer, the pellet was dissolved in 100 ml of citrate buffer containing 200 $\mu g\,ml^{-1}$ rifampicin and incubated for 2 h in a rotary shaker at $28 \pm 2\,^{\circ}C$. After the incubation, the cells were pelleted down and washed twice with 0.1 M phosphate buffer (pH 6.7) and the pellet was redissolved in 100 ml of phosphate buffer and serially diluted up to 10^{-4} in phosphate buffer and 100 μl each of the dilutions of 10^{-3} and 10^{-4} were plated onto Kings' B agar plates containing 200 $\mu g\,ml^{-1}$ rifampicin. Individual colonies were purified and used for studying the population densities of each PGPR isolate in pots.

Pot trials

Based on the above-mentioned tests, a total of nine cultures were selected to evaluate their effects on the growth, yield and nutrient uptake of peanut in earthen pots. The pots had a size of 14″ in diameter and capacity to hold 20 kg of soil (medium black and calcareous, pH 7.9, organic carbon 0.52%, total nitrogen content 0.2125 $g\,kg^{-1}$, available phosphorus 0.0075 $g\,kg^{-1}$ and K_2O 0.120 $g\,kg^{-1}$). Unsterile soil was used for the experiments. There were a total of 10 treatments, each having six replications. Three replications were used for determining population densities of the inoculant strains (during the rainy season of 1999 using intrinsic antibiotic resistance) and counting the nodule number and the nodule dry weight and root length. The remaining three replications were used for observation after harvest. Peanut cultivar, JL24, a Virginia Bunch variety, was raised during the rainy and post-rainy seasons of 1998, 1999 and 2000. Pots were kept in the open and watered at regular intervals (alternate days during post-rainy season at 2 l pot^{-1} and in rainy season as and when required). Nitrogen at 0.01 $g\,kg^{-1}$ as ammonium sulphate and P_2O_5 at 0.02 $g\,kg^{-1}$ as single super phosphate were applied just before sowing. Each isolate of PGPR was grown overnight in Kings' B broth. Each broth was centrifuged at 12,000 rpm, washed with phosphate buffer three times and then pellets were dissolved in 0.1 M phosphate buffer (pH 7.0) and OD was adjusted to 1.2 before being used for pot experiments. An OD of 1.2 was equivalent to 2.0×10^8 cfu ml^{-1}. The seeds for each treatment were soaked for an hour in phosphate saline buffer (PSB) containing the suspension of the PGPR isolates to maintain a population of approximately 10^8 cfu $seed^{-1}$. In each pot, eight seeds (95% germination) were sown at a depth of 5 cm. After germination, five seedlings were maintained in each pot. Effective nodule (pink coloured) number, nodule dry weight and root length were recorded at 45 days after sowing (DAS) whereas plant height and biomass and pod yield were observed after harvest. After the crop harvest, the soil in each pot was discarded; pots were cleaned and filled with fresh soil at the time of the next sowing.

Field trials

Field trials were conducted during the rainy seasons (average annual rainfall of 650 mm; day and night temperatures of 30–35 and 25–30 °C, respectively) of 1998, 1999 and 2000 in $5 \times 4.5\,m^2$ plots in a randomized complete block design (RBD) keeping 10 treatments and four replications for each treatment. The field soil was black calcareous having a pH of 7.9, an organic carbon content of 0.52%, a total nitrogen content of 425 $kg\,ha^{-1}$, an available phosphorus content of 15 $kg\,ha^{-1}$, an available K_2O of 240 $kg\,ha^{-1}$ and iron (5–7 ppm). In the field, recommended doses of fertilizers (20 kg N ha^{-1} as ammonium sulphate and 40 kg $P_2O_5\,ha^{-1}$ as single super phosphate) were used. Peanut cultivar, GG2, was used for field trials. Sowing was completed in the first week of July in each year. Row-to-row and plant-to-plant spacing were maintained at 45 and 10 cm, respectively. The seed was coated with charcoal-based PGPR cultures (10^9–10^{10} cfu g^{-1} carrier). Seeds (120 $kg\,ha^{-1}$) were sown at a depth of 5 cm in rows. A plant population of 440–450 $plot^{-1}$ was maintained by thinning, if

required. Recommended doses of insecticides were sprayed to maintain the pest population below the economic threshold level. Nodule number, nodule dry weight, plant biomass, plant height and root length were recorded at 45 DAS and other parameters like haulm yield, pod yield, pod number, shelling percentage, 100 seed mass, etc. were recorded after harvest. The crop was harvested after maturity at 110 DAS. The crop was raised in the same piece of land for three consecutive years, keeping the land fallow during the post-rainy season.

Disease incidence

Under field condition, the incidence of soil-borne fungal diseases like collar rot caused by *A. niger* and stem rot caused by *S. rolfsii* was monitored. No artificial inoculation was done in the experimental plots. As collar rot usually appears between 20–30 days of crop growth, seedling mortality was monitored at 30 DAS. Seedlings infected with *A. niger* were identified by observing the black spores of *A. niger* and softening of the tissues of the collar region. The infected seedlings dried up after a couple of days. The number of seedlings dying was counted and mortality per cent was calculated in each plot. Similarly, incidence of stem rot usually starts from 45 DAS and it is typically identified by the presence of white mycelial growth of the organism at the stem region and it also attacks the developing pods. The number of plants infected with *S. rolfsii* was counted in each plot at 60 DAS and the mortality of the plants was determined.

Estimation of the contents of N and P in soil, shoot and kernel

The total nitrogen of soil, plant and kernel samples was estimated by Kjeldahl's method. The per cent nitrogen content in the samples was read from the N-autoanalyzer. The total phosphorus in plant and kernel samples was estimated by the method of Jackson (1967). The available phosphorus in soil was estimated by the standard method (Olson, 1954).

Population densities

Population densities in the rhizosphere, rhizoplane and geo-carposphere were measured, in pots, on the basis of intrinsic antibiotic resistance patterns during the rainy season of 1999 in cultivar, JL24 at 45 DAS and expressed as a log number of cells g^{-1}. Ten grams each of the rhizosphere soil (the volume of soil adjacent to and influenced by root), rhizoplane (root surface) and geo-carposphere (pod surface and its attached soil) were taken for determining the population of the inoculant strains. During sampling, plants in each pot were uprooted along with soil adhering to it. Then, roots were shaken repeatedly to remove the loose soil adhering to the roots. Thus, rhizosphere soil was collected. Thereafter, roots were gently dipped in sterile tap water to remove the still adhering soil particles and these roots were used for getting the rhizoplane population. For PGPR isolates, appropriate dilutions were plated onto King's B Petri dishes containing appropriate antibiotics (as in Table 1) and cycloheximide (100 $\mu g\,ml^{-1}$) to inhibit the fungal growth. The colonies were counted and expressed as a log number of cells g^{-1}. Colony morphology and characteristics and fluorescens were also taken into consideration while counting to avoid the counting of the spontaneously growing population.

Rhizosphere competence on the basis of spontaneous rifampicin resistance

A separate experiment was conducted, in pots with six treatments, during the post-rainy season of 2000 to evaluate the rhizosphere competence of the promising PGPR isolates with the cultivar, JL24. A total of six spontaneous rifampicin resistant mutants (one each of the six PGPR isolates, viz. PGPR1, PGPR2, PGPR4, PGPR5, PGPR7 and PGPR8) were monitored in the rhizosphere and rhizoplane of cultivar, JL24 at 30, 45, 60, 75, 90 DAS and at harvest (105 DAS). Appropriate dilutions were plated onto King's B agar medium containing rifampicin at 200 $\mu g\,ml^{-1}$ along with the other appropriate antibiotics and colonies were counted after 48 h of incubation at $28 \pm 2\,^{\circ}C$ and expressed as the log number of cells g^{-1}.

Statistical analyses

Statistical analyses of the experimental data were done following the *SPSS* package. All results were subjected to the least significant difference (LSD) test between means. The correlation co-efficient between a pair of means of related traits of the pooled data over 3 years was determined. Standard deviation was determined following the standard procedures wherever required. The population densities of the isolates were estimated after log transformation of individual estimation.

Table 1. Germinating seed bioassay and quantification of plant growth promoting attributes of selected plant growth promoting rhizobacterial isolates

Isolate	Siderophore		IAA-like substance (mg l^{-1})	Root length of seedling $plant^{-1}$ (cm)[a]	TCP solubilization (mg 100 ml^{-1} broth)	Intrinsic antibiotic resistance patterns	Inhibition zones against (diameter in mm)[b]			NH_4^{+c}	Identified as
	Orange halo (mm)	Catechol (mg mg^{-1} protein)					*A. flavus*	*A. niger*	*S. rolfsii*		
Control	–	–	–	6.03	–	–	–	–	–	–	
PGPR1	5.0	0.106	3.6	8.40*	48.52	$Ap^{100}Str^{100}Cm^{100}$	7.0 (±0.86)	7.0 (±0.56)	–	++	*P. fluorescens*
PGPR2	7.6	0.121	7.8	9.10*	16.6	$Ap^{100}Str^{100}Cm^{100}$	6.7 (±1.06)	3.0 (±0.42)	–	–	*P. fluorescens*
PGPR3	–	–	–	7.90*	–	$Ap^{50}Km^{25}Cm^{50}$	–	–	–	–	*Pseudomonas* sp.
PGPR4	12	0.137	9.3	8.87*	60.0	$Ap^{100}Nal^{50}Cm^{100}$	7.0 (±0.75)	14.0 (±1.41)	10.0 (±2.16)	++++	*P. fluorescens*
PGPR5	4.4	0.102	–	8.03*	38.6	$Ap^{100}Nal^{25}Cm^{100}$	5.6 (±0.64)	–	7.0 (±1.06)	++	*P. fluorescens*
PGPR6	4.6	0.075	3.9	7.97*	–	$Ap^{50}Km^{50}$	6.3 (±0.48)	5.0 (±0.52)	–	–	*P. fluorescens*
PGPR7	9.5	0.109	11.8	9.00*	–	$Ap^{100}Km^{50}Cm^{50}$	–	–	–	+	*P. fluorescens*
PGPR8	4.3	0.054	–	8.07*	–	$Km^{25}Nal^{25}$	–	–	–	–	*Pseudomonas* sp.
PGPR9	4.5	0.072	–	7.6*	23.8	$Ap^{100}Km^{25}Cm^{50}$	–	–	–	+	*Pseudomonas* sp.
LSD (0.05)	–	–	–	0.58	–	–	–	–	–	–	–

[a]Mean of three replications repeated thrice, cultivar JL24.

[b]Data in the parentheses indicate standard deviation from mean.

[c]± Indicates degree of reaction for ammonia production in peptone water broth (colouration of broth from faint yellow to brown with Nessler reagent); – indicates no ammonification activity; + faint yellow colour and small amount of ammonia production; ++ dark yellow colour and medium amount of ammonia production; ++++ dark brown colour and maximum amount of ammonia production; – means absent.

Results

Germinating seed bioassay

All the ACC-deaminase positive isolates were evaluated, in a germinating seed bioassay (Fig. 1A), for enhancement of root growth of the peanut seedlings in vitro. Out of the 233 isolates, only nine isolates enhanced root length of peanut significantly in vitro (Table 1). Results indicated that PGPR1, PGPR2, PGPR4 and PGPR7 were the best among the cultures in increasing the root length of peanut, cultivar JL24. All these nine cultures were identified as pseudomonads in which PGPR1, PGPR2, PGPR4, PGPR5, PGPR6 and PGPR7 were fluorescent types and the rest were non-fluorescent *Pseudomonas*. All fluorescent pseudomonads produced fluorescent pigment on King's B agar (Fig. 1C).

Determination and quantification of other PGPR attributes

All the nine isolates were screened for the production of siderophore (Fig. 1E), IAA, ammonification and solubilization of tri-calcium phosphate (Fig. 1F) in vitro, besides studying their inhibitory effect against soil-borne fungal pathogens like *A. niger* (Fig. 1B), *S. rolfsii* (Fig. 1D) and *A. flavus* (Table 1). Results indicated that four of the isolates, PGPR1, PGPR2, PGPR4 and PGPR7 (all fluorescent pseudomonads), were the best in exhibiting attributes like siderophore production (5.0, 7.6, 12.0 and 9.5 mm of orange halos in CAS agar plates and 0.106, 0.121, 0.137 and 0.109 mg of catechol siderophore mg^{-1} protein in broth, respectively) and production of IAA-like substances (3.6, 7.8, 9.3 and 11.8 $mg\ l^{-1}$, respectively). Five of the PGPR isolates (Table 1), viz. PGPR1, PGPR2, PGPR4, PGPR5 and PGPR9, solubilized tri-calcium phosphate (48.52, 16.6, 60.0, 38.6 and 23.8 $mg\ 100\ ml^{-1}$ broth, respectively). Other isolates did not show all the above characteristics. Five of these isolates, PGPR1, PGPR2, PGPR4, PGPR5 and PGPR6, also showed strong inhibition (Table 1), in vitro, against *A. flavus* (produced 5.6–7.0 mm inhibition zones in King's B after 72 h of incubation). While PGPR1, PGPR2, PGPR4 and PGPR6 strongly inhibited *A. niger* (3.0–14.0 mm inhibition zones), PGPR4 and PGPR5 inhibited *S. rolfsii* (7.0–10.0 mm inhibition zones) strongly (Table 1). Besides, few isolates like PGPR1, PGPR4, PGPR5, PGPR7 and PGPR9 exhibited the ammonification property.

Intrinsic antibiotic resistance patterns

Antibiotic resistance patterns (Table 1) of all these nine isolates were determined for using these markers to evaluate the population densities and rhizosphere competence of the isolates in the rhizotic zones of peanut along with spontaneous rifampicin resistance. Except for PGPR8, all the isolates showed Ap resistance (50 or 100 $\mu g\ ml^{-1}$). Cm resistance was showed by all isolates except PGPR6 and PGPR8 (Table 1). Few of the isolates also exhibited Km, Nal and Str resistance.

Utilization of seed leachate as the sole source of nutrients for growth of isolates

For the initial root colonization, capability of utilizing the seed leachates provides added advantage to PGPR cultures. Thus, growth patterns of the PGPR isolates were evaluated for utilization of peanut kernel leachates of cultivar JL24 as the sole source of C, N and other nutrients. It was observed that all the PGPR isolates could utilize the seed leachates for their growth. Plant growth-promoting rhizobacterial isolates, viz. PGPR1, PGPR2, PGPR3, PGPR4 and PGPR5, showed the optimum growth at 26 h and thereafter, the growth steadily declined (Fig. 2). However, PGPR7 showed a prolonged lag phase of about 20 h and thereafter it reached the maximum growth at 44 h followed by a steady decline. But PGPR4 was the best in utilizing seed leachates as evident from the maximum OD it could achieve throughout its growth cycle (Fig. 2).

Pot trials

Rainy season

During the rainy seasons of 1998, 1999 and 2000, in general, mixed response of the inoculation of plant growth-promoting rhizobacterial isolates on the growth and yield of peanut was obtained. In the first-year trial, *P. fluorescens* PGPR2 was the best in enhancing the number of nodules and dry weight, root length, plant biomass, pod yield and plant height over control, significantly (Table 2). There was also improvement in the growth, yield and nutrient uptake of peanut, cultivar JL24 in pots with the inoculation of other isolates. However, inoculation effects of fluorescent pseudomonads were better than the non-fluorescent types. In terms of pod yield, three fluorescent pseudomonad isolates, viz. PGPR1, PGPR2 and PGPR4, enhanced the pod yield by 24.0%, 26.0% and 23.0%; 25.0%, 26.0% and 23.0%; and 27.0%, 28.0% and 21.0%, respectively, over uninoculated control in the

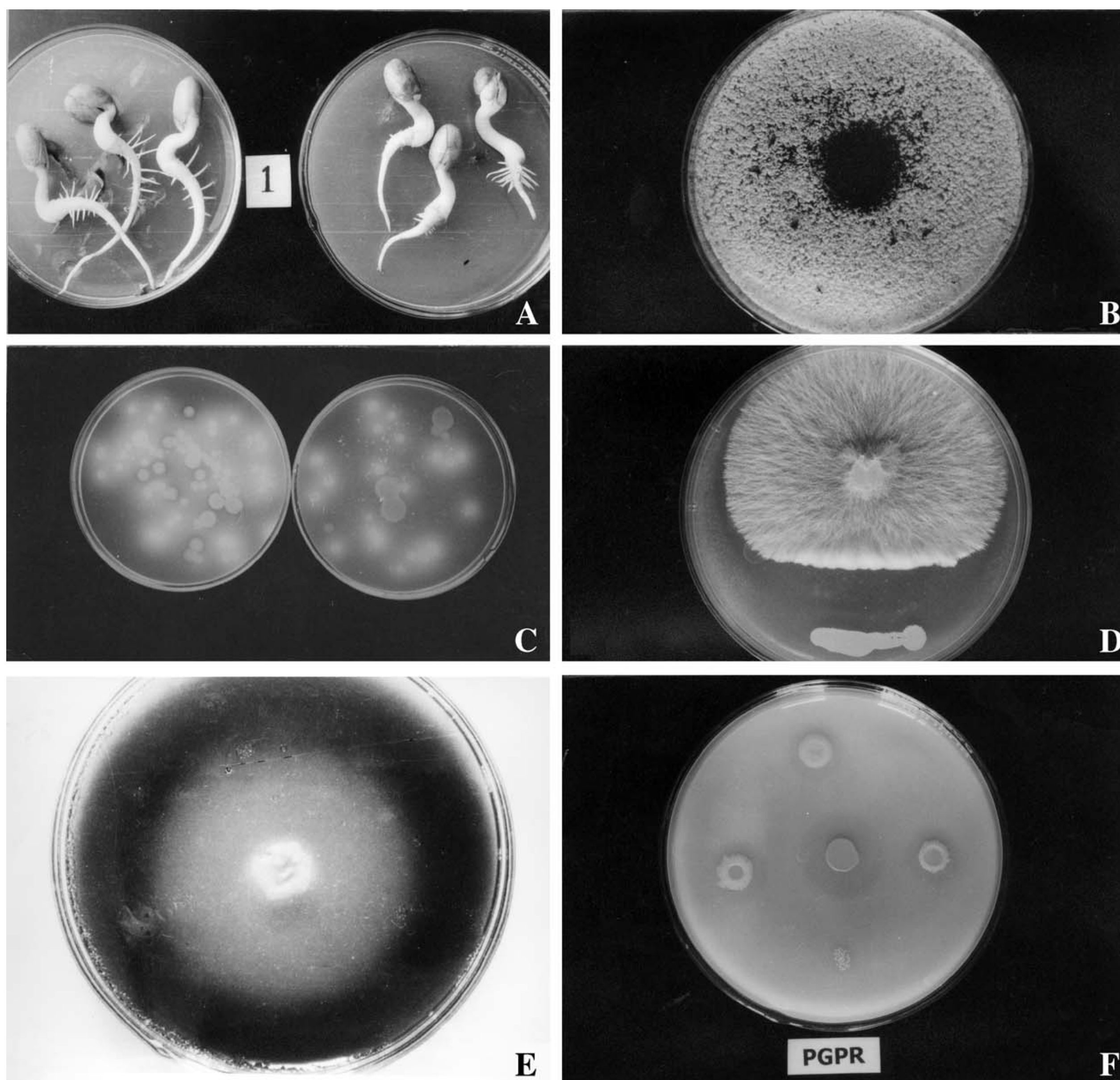


Figure 1. (A) Germinating seed bioassay with cultivar JL24: (left) treated with *P. fluorescens* PGPR4; (right) untreated control; (B) antifungal activity of *P. fluorescens* PGPR4 against *A. niger*; (C) production of fluorescent pigments by *P. fluorescens* PGPR4 (polaroid photograph); (D) antifungal activity of *P. fluorescens* PGPR2 against *S. rolfsii*; (E) production of siderophore by *P. fluorescens* PGPR1, halos indicate the production of siderophore in CAS agar; (F) solubilization of tri-calcium phosphate by PGPR isolates.

first-, second- and third-year trials, respectively (Table 2). A mixed response was obtained when other parameters were analyzed. Interestingly, all the inoculated treatments enhanced the plant biomass significantly over the control in the year 2000. However, only one (PGPR2) and three (PGPR1, PGPR2 and PGPR4) isolates enhanced the plant biomass in the first- and second-year trials. Inoculation of *P. fluorescens* PGPR7 significantly reduced the nodulation, root length and pod yield in the first-year experiment. However, the inoculation effect of this isolate was progressively improved in the second- and third-year trials (Table 2). Sporadically, inoculation of few other isolates also enhanced the growth parameters.

While inoculation of PGPR1, PGPR2, PGPR4 and PGPR9 significantly increased the total nitrogen in soil and kernels in all the 3 years over control, the total nitrogen content in the shoot varied considerably among the trials. In the shoot, inoculation of

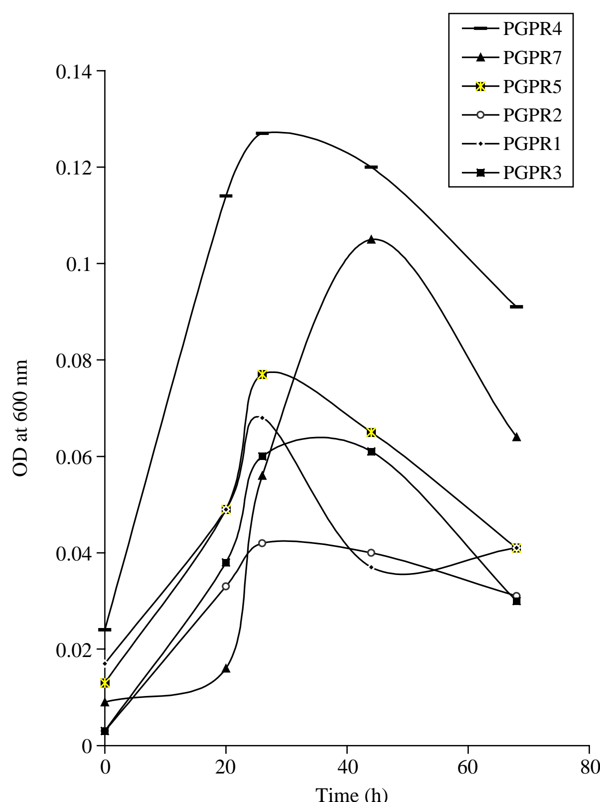


Figure 2. Growth patterns of different PGPR isolates utilizing the seed leachate of peanut cultivar JL24 as the sole source of carbon and nitrogen at 28 °C, OD values are mean of three replications.

PGPR1 and PGPR2 in the first year, PGPR2 and PGPR9 in the second year and PGPR1 and PGPR9 in the third year significantly enhanced the total nitrogen content (Table 3). However, seed bacterization of PGPR7 in the second and third year also improved the total nitrogen content in the soil significantly. Consistently significant inoculation effects of the rhizobacterial isolates, on the available phosphorus content in soil and total phosphorus content in shoot and kernel, were obtained with the inoculation of four *P. fluorescens* isolates, viz. PGPR1, PGPR2, PGPR4 and PGPR5, and *Pseudomonas* sp. PGPR9 in all the 3 years (Tables 3 and 6).

Post-rainy season

In general, seed bacterization of plant growth-promoting rhizobacterial isolates stimulated the growth, yield and nutrient uptake of peanut, cultivar JL24, in pots in each of the 3 years. There was significant enhancement in the number and dry weight of nodules and root length at 45 DAS; plant biomass, plant height and pod yield at harvest; and N and P content in soil and plant as compared to control by the inoculation of fluorescent pseudomonad isolates like PGPR1, PGPR2 and PGPR4 consistently in all the 3 years during the post-rainy seasons (data not shown).

Population densities/rhizosphere competence on the basis of intrinsic antibiotic resistance markers

Population densities of the PGPR isolates were evaluated at 45 DAS, on the basis of intrinsic antibiotic resistance patterns, during the rainy season of 1999. It was observed that PGPR1, PGPR4, PGPR7 and PGPR9 gave population densities of log 6.4, log 6.7, log 6.1 and log 6.4 cfu g^{-1} of soil in the rhizosphere (Fig. 3), respectively. In the rhizoplane, however, higher population densities (as compared to rhizosphere population of those isolates) were obtained after seed bacterization with PGPR5, PGPR6 and PGPR7 (log 6.2, log 5.7 and log 6.4 cfu g^{-1} of root, respectively). However, in the geo-carposphere, PGPR1 and PGPR7 gave the higher population densities compared to other PGPR isolates (Fig. 3).

Rhizosphere competence using spontaneous rifampicin resistance marker

For studying the rhizosphere competence of the PGPR isolates, spontaneous rifampicin resistant mutants of six PGPR (PGPR1, PGPR2, PGPR4, PGPR5, PGPR7 and PGPR8) were developed and tested in pots during the post-rainy season of 2000. However, spontaneous rifampicin resistant mutants for the remaining isolates could not be obtained. The population densities showed high variability based on the different colonization capabilities of the isolates. However, all the isolates were able to maintain population densities over 10^5 cfu g^{-1} rhizosphere soil and mostly 10^6 cfu g^{-1} root up to 105 days (Fig. 4A and B). While *P. fluorescens* PGPR1 was the best rhizosphere colonizer, another *P. fluorescens* PGPR7 was the best rhizoplane colonizer.

Field trials

Root length

Inoculation of two isolates, PGPR1 and PGPR2, significantly enhanced the root length of peanut in all the 3 years. However, inoculation of *P. fluorescens* PGPR4 enhanced the root length in the second- and third-year trials only (Tables 4 and 6). In the third-year trial, in addition to the significant effect of PGPR1, PGPR2 and PGPR4, two non-fluorescent isolates, PGPR8 and PGPR9, also

Table 2. Effect of plant growth promoting rhizobacteria on the nodulation, growth and yield of peanut cultivar JL24 in pots (rainy seasons of 1998, 1999 and 2000) (data mean of three replications)

Growth parameters observed at 45 DAS

Isolate	Nodule number plant^{-1}			Nodule dry weight plant^{-1} (mg)			Root length plant^{-1} (cm)		
	1998	1999	2000	1998	1999	2000	1998	1999	2000
Control	95	83	89	96.4	79.4	81.8	38.9	36.7	32.5
PGPR1	145*	121*	147*	121.3*	106.8*	116.9*	45.4*	45.2*	33.4
PGPR2	161*	134*	114*	125.9*	108.6*	98.5*	48.6*	46.9*	39.7*
PGPR3	102	92	130*	94.9	82.1	112.3*	42.8	35.1	39.0*
PGPR4	145*	129*	117*	118.7*	106.8*	101.4*	43.0	42.6	39.4*
PGPR5	97	102	99	89.0	89.0	86.7	45.1*	41.8	44.0*
PGPR6	90	78	89	87.6	65.8	80.0	41.6	38.4	38.2*
PGPR7	63	74	111*	54.8	71.6	93.4*	31.8	35.9	36.1
PGPR8	112	93	108*	101.3	84.3	91.7*	41.7	40.3	35.2
PGPR9	139*	122*	102	116.8*	104.9*	89.7	46.1*	44.2*	38.9
LSD (0.05)	21.4	22.6	19.0	16.4	11.2	9.56	6.2	6.9	5.39

Growth parameters observed at harvest

	Biomass plant^{-1} (g)			Plant height plant^{-1} (cm)			Pod yield plant^{-1} (g)		
	1998	1999	2000	1998	1999	2000	1998	1999	2000
Control	10.12	11.91	13.10	25.6	27.0	26.7	3.66	3.25	3.57
PGPR1	12.92	16.08*	17.88*	32.8*	32.5*	30.6*	4.53*	4.06*	4.54*
PGPR2	14.62*	17.32*	17.65*	34.2*	31.9*	33.1*	4.62*	4.11*	4.58*
PGPR3	10.91	13.25	16.85*	27.9	26.0	28.4	3.47	3.82	3.65
PGPR4	11.49	15.83*	18.16*	31.8*	31.6*	34.1*	4.49*	3.99*	4.32*
PGPR5	10.99	12.68	17.83*	24.3	27.2	25.9	3.05	3.89	3.87
PGPR6	08.49	14.75	18.22*	22.9	25.9	27.8	3.55	3.25	3.68
PGPR7	08.05	12.95	17.91*	25.0	26.4	28.0	2.62	3.40	3.70
PGPR8	10.14	14.85	16.93*	26.0	26.8	30.1*	4.05	3.60	4.08
PGPR9	11.15	15.25	17.98*	27.8	29.7	28.9	3.72	3.42	3.73
LSD (0.05)	4.07	3.68	1.75	2.98	3.18	3.04	0.82	0.73	0.68

*Indicates significant difference over control.

enhanced the root length significantly. All other treatments performed at par to that of control.

Plant biomass

Dry masses of the plants were recorded on per plant basis (mean of five plants from each plot at 45 DAS). While inoculation of PGPR1 and PGPR2 enhanced the plant biomass (by 28.0% and 28.0%, respectively) in 1998, seed bacterization of PGPR1, PGPR2 and PGPR4, and PGPR1, PGPR2, PGPR3, PGPR8 and PGPR9 enhanced the plant biomass over the control in 1999 (by 37.0%, 52.0% and 37.0%, respectively) and 2000 (by 14.0%, 15.0%, 33.0%, 33.0% and 26.0%, respectively), respectively (Table 4). Seed bacterization of all other isolates resulted in plant biomass at par to that of control. It was also found that treatments inoculated with siderophore-producing PGPR isolates resulted in green foliage as compared to treatments inoculated with siderophore non-producing isolate like PGPR3 and in an uninoculated control.

Nodule number

There was year-to-year variation in the number of nodules. It was observed that inoculation of four (PGPR1, PGPR2, PGPR4 and PGPR5) out of the nine PGPR isolates resulted in a significant increase in nodule number in the first-year trial. In the second-year trial, six out of the nine PGPR isolates enhanced the nodule number significantly. In the third year (2000), however, only three (out of nine) PGPR isolates could enhance the number of nodules. These three PGPR isolates, viz. PGPR1, PGPR2 and PGPR4, consistently enhanced the nodule number in all the 3 years (Tables 4 and 6). In the first-year trial, inoculation of *P. fluorescens*

Table 3. Nutrient status under potted conditions during rainy seasons of 1998, 1999 and 2000 at harvest (data mean of three replications)

Isolate	N content in soil (%)			N content in shoot (%)			N content in kernel (%)		
	1998	1999	2000	1998	1999	2000	1998	1999	2000
Control	0.051	0.057	0.055	1.730	1.752	1.700	4.044	4.037	3.967
PGPR1	0.071*	0.069*	0.068*	1.968*	1.894	1.930*	4.482*	4.375*	4.325*
PGPR2	0.067*	0.069*	0.068*	1.897*	1.957*	1.857	4.458*	4.477*	4.517*
PGPR3	0.049	0.054	0.053	1.689	1.759	1.700	4.005	3.967	4.067
PGPR4	0.062*	0.065*	0.068*	1.852	1.748	1.833	4.550*	4.455*	4.448*
PGPR5	0.051	0.061	0.058	1.723	1.765	1.800	3.958	4.058	4.133*
PGPR6	0.054	0.063	0.059	1.688	1.763	1.733	3.992	4.028	4.067
PGPR7	0.054	0.069*	0.063*	1.693	1.761	1.783	4.082	3.990	3.967
PGPR8	0.056	0.055	0.055	1.713	1.762	1.683	4.120	3.975	3.983
PGPR9	0.064*	0.069*	0.067*	1.816	2.005*	1.950*	4.367*	4.382*	4.325*
LSD (0.05)	0.009	0.007	0.005	0.140	0.145	0.158	0.150	0.270	0.153
	P content in soil (ppm)			P content in shoot (%)			P content in kernel (%)		
	1998	1999	2000	1998	1999	2000	1998	1999	2000
Control	12.79	12.59	11.40	0.211	0.211	0.212	0.403	0.398	0.409
PGPR1	18.56*	21.02*	23.42*	0.255*	0.246*	0.245*	0.442*	0.452*	0.444*
PGPR2	22.70*	19.39*	21.28*	0.243*	0.251*	0.233*	0.440*	0.468*	0.455*
PGPR3	13.19	12.75	11.16	0.200	0.212	0.212	0.403	0.390	0.398
PGPR4	24.70*	21.72*	23.35*	0.254*	0.250*	0.245*	0.461*	0.451*	0.452*
PGPR5	18.40*	19.86*	22.32*	0.252*	0.253*	0.225*	0.449*	0.457*	0.452*
PGPR6	14.97	11.01	10.87	0.205	0.211	0.215	0.398	0.403	0.415
PGPR7	11.26	13.66	13.09	0.210	0.202	0.208	0.412	0.409	0.420
PGPR8	13.05	13.10	12.09	0.211	0.204	0.202	0.400	0.405	0.390
PGPR9	25.17*	21.12*	26.71*	0.253*	0.251*	0.243*	0.454*	0.458*	0.433*
LSD (0.05)	2.80	2.53	2.24	0.018	0.014	0.011	0.022	0.021	0.021

*Indicates significant difference over control.

PGPR7 resulted in significantly reduced number of nodules per plant.

Nodule dry weight

In general, majority of the inoculated treatments resulted in higher nodule dry weight, except in treatments receiving PGPR7 and PGPR9 in 1998 (Tables 4 and 6). Out of the nine isolates tested, inoculation of five isolates, viz. PGPR1, PGPR2, PGPR4, PGPR5 and PGPR6, resulted in significantly higher (by 42.0%, 62.0%, 33.0%, 32.0% and 16.0%, respectively) nodule dry weight than control. However, in 1999, all the inoculated treatments had better nodulation and significantly higher nodule dry mass than the control. In the third year, however, only four out of the nine isolates exhibited significantly higher nodule dry mass as compared to the control (Tables 4 and 6). Again, in majority of the cases, *P. fluorescens* isolates performed better than that of the non-fluorescent ones.

Plant height

It was observed that inoculation of plant growth-promoting rhizobacterial isolates *P. fluorescens* PGPR1 and *P. fluorescens* PGPR2 significantly enhanced the plant height in all the 3 years. Seed bacterization of another isolate, *P. fluorescens* PGPR4, resulted in significantly higher plant height in the second- and third-year trial (Table 4) only. In the first-year trial, however, inoculation with *P. fluorescens* PGPR7 resulted in a reduced plant height. Application of all the other rhizobacterial isolates resulted in plant height at par to that of the control (Table 4).

Pod yield

The crop was harvested after 110 days of sowing, when it was fully mature, each year. There was seasonal variation in terms of total rainfall and temperature which might have affected the growth and yield parameters. Inoculation of a number of rhizobacteria enhanced the growth (Fig. 5) and pod

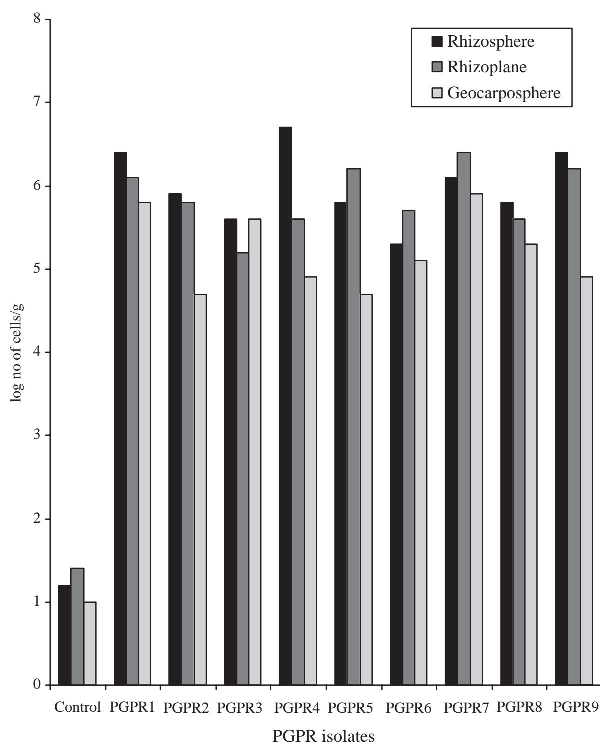


Figure 3. Population densities of PGPR isolates in the rhizotic zones of peanut, cultivar JL24, determined on the basis of intrinsic antibiotic resistance patterns of the organisms, in the rainy season of 1999 at 45 DAS under potted conditions.

yield of peanut. The maximum pod yield was recorded in the third year of experimentation, i.e. in the year 2000. During 1998, out of the nine isolates tested, only two isolates, viz. PGPR1 and PGPR2, significantly enhanced (by 23.0% and 28.0%, respectively) the pod yield of peanut than the control (Table 4). All other treatments yielded at par to that of the control except isolate PGPR7 which gave significantly reduced pod yield (Tables 4 and 6). Compared to the 1998 rainy season, marked variation in pod yield was observed in the rainy season of 1999. Bacterization of peanut with PGPR isolates like PGPR1, PGPR2, PGPR3, PGPR4, PGPR5 and PGPR6 (by 26.0%, 24.0%, 16.0%, 24.0%, 15.0% and 16.0%, respectively) resulted in significantly higher pod yields while the treatments inoculated with the three remaining isolates recorded yields at par to that of the control (Table 4). In the third year, however, six (PGPR3–PGPR8) out of the nine PGPR isolates exhibited a significantly higher pod yield (by 13.0%, 18.0%, 22.0%, 32.0%, 19.0% and 31.0%, respectively) than the control while treatments inoculated with PGPR1, PGPR2 and PGPR9 yielded at par to that of control (Tables 4 and 6).

Haulm yield

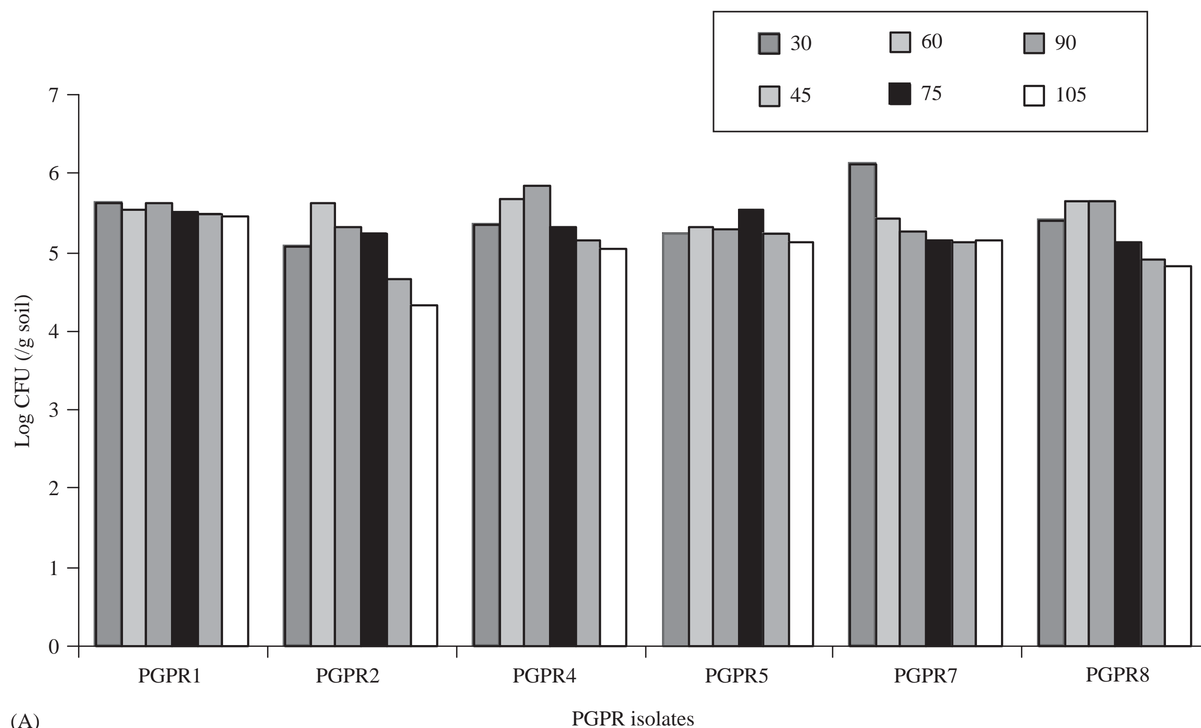
As evident from the results (Table 4), there was marked seasonal variation in the haulm yield of peanut by the inoculation of rhizobacterial isolates. Both fluorescent and non-fluorescent pseudomonads could enhance the haulm yield of peanut, significantly over the control, under field conditions. In the first year, five out of the nine PGPR isolates enhanced the haulm yield significantly. However, maximum haulm yield was obtained with the inoculation of *P. fluorescens* PGPR2. Inoculation of two PGPR isolates, viz. PGPR3 and PGPR7, however, reduced the haulm yield significantly over control. In the second-year trial, only three, viz. PGPR1, PGPR2 and PGPR4 (all *P. fluorescens* isolates), out of the nine isolates tested enhanced the haulm yield significantly (by 48.0%, 52.0% and 37.0%, respectively) over control. But results obtained in the third-year trial were quite different from the results obtained in the previous 2 years. Except PGPR6 (at par), inoculation of the eight remaining PGPR isolates enhanced the haulm yield significantly over the control (Table 4). It was also observed that inoculation of siderophore-producing strains of fluorescent and non-fluorescent pseudomonads gave non-chlorotic (green) foliage as compared to pale green foliage (chlorotic lesions in between the veins) in untreated and in treatment inoculated with siderophore non-producing isolate, *Pseudomonas* sp. PGPR3.

100 seed mass

The mass of 100 kernels was recorded after harvest. Results indicated that in the first year (1998), seed bacterization with the plant growth-promoting rhizobacterial isolates significantly enhanced the kernel mass in case of only four (PGPR2, PGPR4, PGPR5 and PGPR6) out of the nine isolates tested. The best result was obtained with the inoculation of PGPR6 (*P. fluorescens*). However, in the next years' trial (1999), inoculation of all the nine PGPR isolates enhanced the mass of 100 kernels significantly over control (Table 6). But in the year 2000, the effect was not significant.

Pod number

Inoculation of *P. fluorescens* isolates, viz. PGPR1, PGPR2 and PGPR4, resulted in a significantly higher pod number plant^{-1} at harvest in the first 2 years trials. In the third-year trial, inoculation of PGPR3, PGPR4, PGPR5, PGPR6, PGPR7 and PGPR8 significantly enhanced the pod number. Therefore, only one PGPR isolate, *P. fluorescens* PGPR4, enhanced the pod number plant^{-1} in all the 3 years.



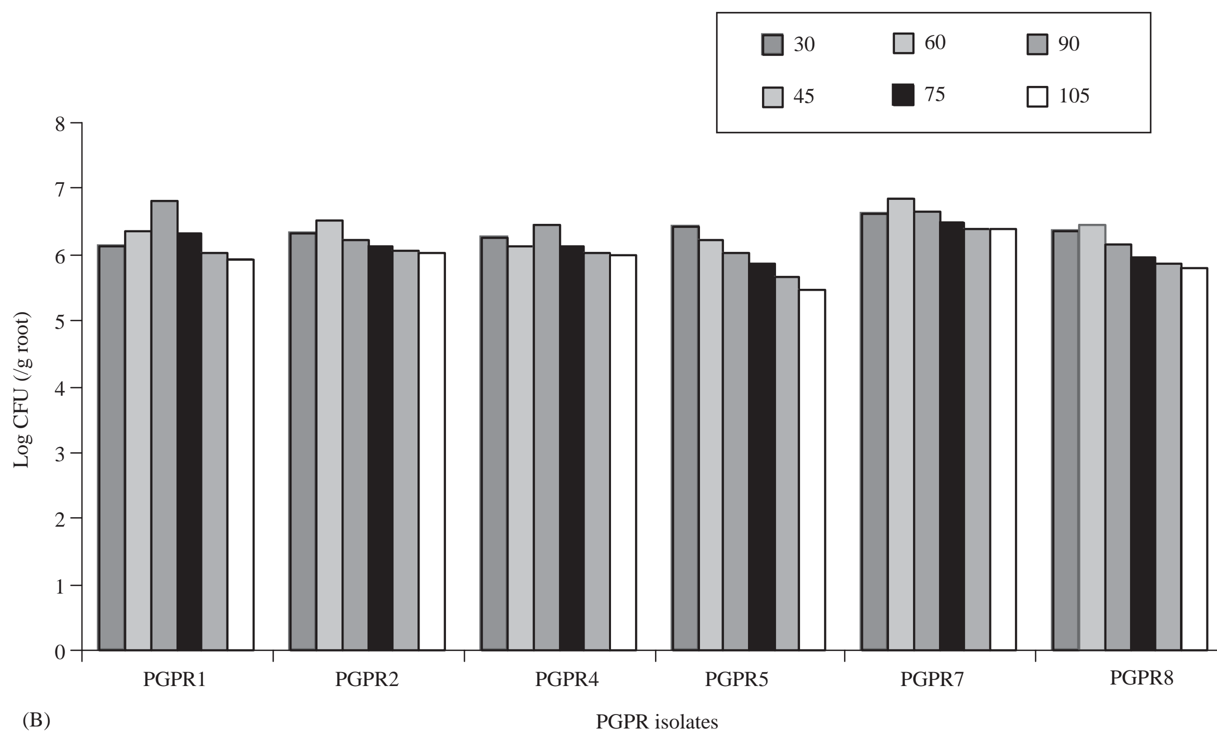


Figure 4. (A) Population densities of different spontaneous rifampicin resistant mutants of plant growth-promoting rhizobacterial isolates in the rhizosphere of peanut, cultivar JL24 at different crop growth stages (30, 45, 60, 75, 90 and 105 DAS). (B) Population densities of spontaneous rifampicin resistant mutants of plant growth-promoting rhizobacterial isolates in the rhizoplane of peanut, cultivar JL24 at different crop growth stages (30, 45, 60, 75, 90 and 105 DAS).

Shelling (%)

Inoculation of only one rhizobacterial isolate, *P. fluorescens* PGPR4, significantly enhanced the shelling out-turn over the control in the 1998 trial. All other treatments performed at par to that of the control. However, in the second-year trial

Table 4. Effect of PGPR on the nodulation, growth and yield of peanut, cultivar GG2, in the rainy seasons of 1998, 1999 and 2000 under field conditions (data mean of four replications)

Growth parameters observed at 45 DAS

Isolate	Root length plant^{-1} (cm)			Plant biomass plant^{-1} (g)			Nodule number plant^{-1}			Nodule dry weight plant^{-1} (mg)			Plant height plant^{-1} (cm)		
	1998	1999	2000	1998	1999	2000	1998	1999	2000	1998	1999	2000	1998	1999	2000
Control	19.67	20.00	16.33	18.65	17.91	14.74	83	78	97	71.15	86.4	102.0	35.42	36.35	31.24
PGPR1	25.33*	26.33*	20.67*	23.83*	24.48*	16.76*	117*	112*	121*	101.0*	116.4*	119.5*	43.44*	44.95*	37.25*
PGPR2	24.00*	27.00*	21.00*	23.95*	27.28*	16.98*	123*	109*	117*	115.2*	103.0*	116.8	42.80*	46.25*	38.25*
PGPR3	18.00	21.33	15.00	14.57	21.48	19.56*	79	84	86	72.90	91.4	137.3*	31.93	38.25	30.25
PGPR4	19.00	25.00*	22.67*	18.20	24.48*	15.73	106*	98*	115*	94.80*	103.4*	114.3*	34.61	45.41*	40.12*
PGPR5	21.33	18.67	17.33	21.60	19.06	16.18	103*	88	98	93.75*	108.0*	127.0*	35.04	36.25	33.42
PGPR6	20.67	19.33	16.33	20.20	20.94	16.03	82	96*	102	82.85*	104.2*	121.0*	34.61	32.54	32.65
PGPR7	17.33	20.67	14.67	16.70	20.55	15.46	49	101*	87	39.80	108.1*	115.5	30.72	33.65	31.65
PGPR8	17.00	22.00	20.67*	17.05	18.48	19.60*	78	110*	101	68.00	105.2*	113.3	38.25	35.65	38.21*
PGPR9	18.00	18.33	19.33*	16.19	19.52	18.57*	72	89	89	59.25	95.6*	103.8	37.61	37.25	37.55*
LSD (0.05)	3.29	4.21	2.59	5.10	4.72	1.69	12.6	16.8	14.2	6.69	5.15	15.7	4.28	4.21	5.42

Growth parameters observed at harvest

	Pod yield (kg ha^{-1})			Haulm yield (kg ha^{-1})			100 seed mass (g)			Number of pod plant^{-1}			Shelling %		
	1998	1999	2000	1998	1999	2000	1998	1999	2000	1998	1999	2000	1998	1999	2000
Control	1678	1872	1938	2349	2687	2332	31.91	26.25	37.61	17.6	20.3	20.0	70.89	71.12	74.45
PGPR1	2071*	2350*	1900	2899*	3985*	2932*	34.28	34.25*	36.41	22.3*	25.6*	19.6	72.15	73.25	74.77
PGPR2	2154*	2320*	1917	3016*	4092*	2830*	36.21*	33.75*	36.40	22.6*	25.4*	18.3	71.96	74.00*	74.17
PGPR3	1422	2170*	2192*	1991	3222	3252*	31.25	37.25*	38.38	16.2	22.2	25.3*	70.12	74.50*	73.77
PGPR4	1936	2315*	2288*	2710*	3672*	2950*	37.24*	35.50*	36.77	20.6*	24.6*	24.6*	73.25*	74.50*	73.10
PGPR5	1685	2157*	2366*	2359	2860	2722*	36.12*	35.50*	37.70	18.4	22.6	24.0*	70.36	75.25*	74.50
PGPR6	1488	2175*	2566*	2083	3142	2550	37.45*	35.75*	36.71	15.6	21.6	27.6*	69.23	75.00*	73.83
PGPR7	1326	2045	2315*	1856	3082	2640*	33.72	34.25*	36.86	15.0	21.2	24.3*	71.23	73.75*	74.67
PGPR8	1791	1955	2538*	2686*	2772	2775*	34.32	35.50*	37.20	18.6	20.4	26.3*	72.56	75.25*	73.80
PGPR9	1576	1945	1785	2679*	2927	3037*	34.85	32.50*	37.54	16.3	21.0	18.6	69.21	73.25	74.32
LSD (0.05)	287	258	193	301	956	268	3.21	2.59	NS	2.45	3.21	3.78	1.87	2.57	NS

*Indicates significant difference over control.

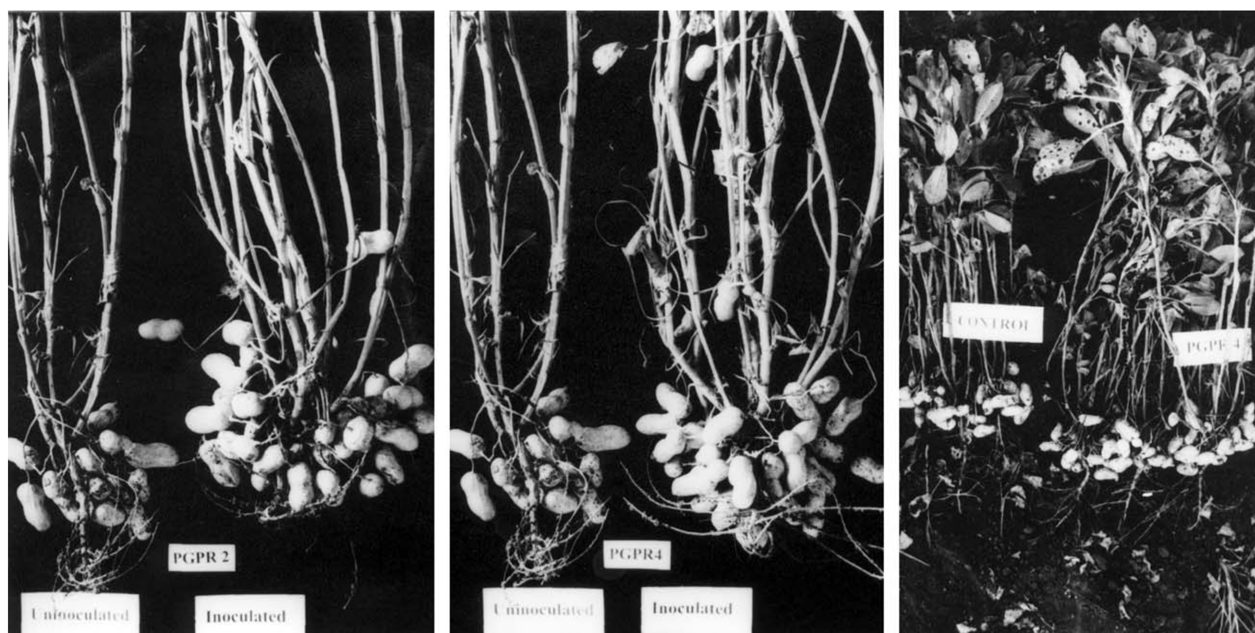


Figure 5. Effect of the inoculation of PGPR on the growth and pod yield of peanut, cultivar GG2, in field. (Left) Effect of *P. fluorescens* PGPR2; (middle) effect of *P. fluorescens* PGPR4; (right) effect of *P. fluorescens* PGPR4.

(1999), seven (PGPR2-PGPR8) out of the nine isolates, significantly enhanced the shelling per cent as compared to control. However, the best result was obtained with the inoculation of PGPR5 and PGPR8 (Table 4). In the third year, none of the PGPR isolates could enhance the shelling out-turn significantly.

Incidence of stem and collar rot diseases

The incidence of both collar and stem rot in peanut, cultivar GG2, was monitored at 30 and 60 DAS, respectively, under field conditions. In general, incidence of both the diseases was low and seedling and plant mortality due to collar rot caused by *A. niger* and stem rot caused by *S. rolfsii* were below 10% in the uninoculated control (Table 5). Though the cultivar was susceptible to collar and stem rot, under natural conditions, at the site of experimentation the disease incidence was low. However, inoculation of PGPR isolates, viz. PGPR1, PGPR2 and PGPR4, reduced the seedling mortality from 8.6% in the uninoculated control to 3.6%, 3.5% and 3.0%, respectively (Table 5), for collar rot caused by *A. niger*. Although PGPR6 showed in vitro inhibition to *A. niger*, it could not reduce the incidence of collar rot and the same intensity of disease severity was found in the treatments to that of uninoculated control and other remaining strains (Table 5). Inoculation of PGPR4 and PGPR5, which showed strong inhibition to *S. rolfsii*, reduced the incidence of stem rot severity and subsequently, the plant mortality from 8.3% in uninoculated control to 4.0% and 3.9%, respectively (Table 5).

Nitrogen content in soil, shoot and kernel

Inoculation with PGPR isolates resulted in enhanced nitrogen content in the soil, shoot and kernels in several treatments. In the first-year trial, inoculation of PGPR1 and PGPR2 significantly enhanced the total nitrogen content in soil, shoot and kernels. All other treatments except PGPR8 (in case of soil) performed at par to that of the control. In the second-year trial, five, four and three out of the nine rhizobacterial isolates tested, significantly enhanced the total nitrogen in soil, shoot and kernels, respectively (Tables 5 and 6). However, in the third-year trial, three (PGPR1, PGPR2 and PGPR7), four (PGPR1, PGPR2, PGPR4 and PGPR9) and three (PGPR1, PGPR2 and PGPR4) rhizobacterial inoculations resulted in significantly enhanced total nitrogen content in soil, shoot and kernels, respectively, over the control. An overall assessment revealed that inoculation of PGPR1 and PGPR2 isolates always resulted in the significant enhancement of N in soil, shoot and kernels (Tables 5 and 6). Inoculation of all other rhizobacterial isolates performed at par to that of the control.

Phosphorus content in soil, shoot and kernel

Since many of the rhizobacterial isolates had phosphate solubilizing ability, the available phosphorus content in the soil increased significantly

Table 5. Comparison of nutrient status and disease incidence under field conditions during rainy seasons of 1998, 1999 and 2000 at harvest (mean of four replications)

Isolate	N content in soil (%)			N content in shoot (%)			N content in kernel (%)			Seedling mortality (%) due to collar rot[a]
	1998	1999	2000	1998	1999	2000	1998	1999	2000	Mean of 3 years
Control	0.052	0.044	0.048	2.148	2.155	2.171	4.203	4.183	4.125	8.6 (±0.56)
PGPR1	0.065*	0.069*	0.078*	2.363*	2.370*	2.355*	4.571*	4.660*	4.675*	3.6 (±0.63)
PGPR2	0.071*	0.060*	0.067*	2.359*	2.368*	2.315*	4.625*	4.595*	4.525*	3.5 (±0.50)
PGPR3	0.046	0.053	0.044	2.178	2.290	2.125	3.975	4.072	4.025	7.7 (±1.25)
PGPR4	0.060	0.046	0.059	2.255	2.371*	2.375*	4.455	4.623*	4.525*	3.0 (±1.00)
PGPR5	0.057	0.059*	0.055	2.205	2.305	2.175	3.972	3.978	4.050	7.2 (±0.76)
PGPR6	0.056	0.062*	0.057	2.170	2.225	2.241	4.012	4.130	4.100	6.7 (±0.58)
PGPR7	0.069*	0.072*	0.069*	2.160	2.217	2.193	3.982	4.155	4.150	7.0 (±0.90)
PGPR8	0.039	0.048	0.043	2.056	2.283	2.175	4.230	4.195	4.200	6.9 (±0.63)
PGPR9	0.050	0.060*	0.053	2.242	2.590*	2.372*	4.120	4.070	4.050	6.7 (±0.25)
LSD (0.05)	0.012	0.015	0.015	0.206	0.212	0.135	0.323	0.347	0.238	
	P content in soil (ppm)			P content in shoot (%)			P content in kernel (%)			Plant mortality (%) due to stem rot[a]
	1998	1999	2000	1998	1999	2000	1998	1999	2000	Mean of 3 years
Control	14.26	13.97	13.73	0.181	0.192	0.187	0.267	0.282	0.359	8.3 (±0.76)
PGPR1	18.25*	17.22*	21.67*	0.214*	0.227*	0.229*	0.352*	0.367*	0.375*	8.2 (±0.38)
PGPR2	21.25*	22.17*	16.37	0.202*	0.221*	0.229*	0.321*	0.344*	0.371*	8.9 (±0.38)
PGPR3	12.39	14.30	15.76	0.193	0.201	0.191	0.311	0.309	0.363	8.5 (±0.50)
PGPR4	18.75*	19.72*	20.22*	0.216*	0.225*	0.231*	0.351*	0.368*	0.389*	4.0 (±0.50)
PGPR5	17.32	19.62*	18.77*	0.211*	0.223*	0.214	0.337*	0.352*	0.371*	3.9 (±0.38)
PGPR6	16.12	14.77	17.49*	0.177	0.242*	0.222*	0.248	0.296	0.356	8.7 (±0.43)
PGPR7	15.10	14.10	14.99	0.189	0.238*	0.217	0.276	0.305	0.356	7.7 (±0.25)
PGPR8	16.18	13.75	14.60	0.187	0.245*	0.214	0.281	0.293	0.361	8.6 (±0.38)
PGPR9	13.16	14.40	14.33	0.216*	0.218	0.234*	0.324*	0.332*	0.398*	8.6 (±0.95)
LSD (0.05)	3.29	3.19	2.77	0.020	0.028	0.031	0.052	0.047	0.012	–

*Indicates significant difference over control.
[a]Data in the parentheses indicate standard deviation.

due to the inoculation of PGPR. Seed bacterization of PGPR1 and PGPR4 significantly enhanced the available phosphorus content in the soil in all the three trials. However, application of other PGPR isolates like PGPR2 in the first 2 years' trials, PGPR5 in second- and third-year trial and PGPR6 in the third-year trial also enhanced the available phosphorus content in soil significantly after harvest. When the total phosphorus content in shoot and kernels was evaluated, it was found that inoculation of PGPR1, PGPR2 and PGPR4 significantly enhanced the total phosphorus content in shoot and kernels in all the three trials (Table 5). However, inoculation of PGPR9 in the first- and third-year trials enhanced the total phosphorus content in shoot and kernels. But in the second-year field evaluation, inoculation of PGPR5, PGPR6, PGPR7 and PGPR8 resulted in significantly higher phosphorus content in shoot (Table 5). The maximum increase in available phosphorus in soil was obtained with the inoculations of PGPR4 and PGPR2. It was also evident from the results that inoculation of PGPR4, which was a potent phosphate solubilizer, gradually increased the available phosphorus content in the soil and the total phosphorus in shoot and kernels over the years (Table 5).

Correlation studies

To understand the possible trait(s) of PGPR isolates involved in enhancing the growth, yield and nutrient uptake of peanut, correlation was studied between the trait(s) of the PGPR isolates and its related traits in plants which could have been influenced by the traits of the PGPR isolates. Since, under field conditions the real situation exists, the correlation between the related trait(s) was studied taking the pooled data of field trials over 3

Table 6. Summary table of the results obtained on the effect of PGPR on the growth, yield and nutrient uptake of peanut[a]

Isolates	PY		Plant biomass		Nodule number		Nodule dry weight		Root length		Plant height	
	Pot	Field	Pot	Field	Pot	Field	Pot	Field	Pot	Field	Pot	Field
PGPR1	+	(+)	(+)	+	+	+	+	+	(+)	+	+	+
PGPR2	+	(+)	+	+	+	+	+	(+)	+	+	+	+
PGPR3	—	(+)	—	—	—	—	—	—	—	—	—	—
PGPR4	+	(+)	(+)	—	+	+	+	+	—	(+)	+	(+)
PGPR5	—	(+)	—	—	—	—		+	(+)	—	—	—
PGPR6	—	(+)	—	—	—	—	—	+	—	—	—	—
PGPR7	—	—	—	—	—	—	—	—	—	—	—	—
PGPR8	—	—	—	—	—		—	—	—	—	—	—
PGPR9	—	—	—	—	(+)	—	+	—	+	—	—	—
	N in soil		**N in plant**		**N in kernels**		**P in soil**		**P in plant**		**P in kernel**	
	Pot	Field	Pot	Field	Pot	Field	Pot	Field	Pot	Field	Pot	Field
PGPR1	+	+	(+)	+	+	+	+	+	+	+	+	+
PGPR2	+	+	(+)	+	+	+	+	(+)	+	+	+	+
PGPR3	—	—	—	—	—	—	—	—	—	—	—	—
PGPR4	+	—	—	(+)	+	(+)	+	+	+	+	+	+
PGPR5	—	—	—	—	—	—	+	(+)	+	(+)	+	+
PGPR6	—	—	—	—	—	—	—	—		(+)	—	—
PGPR7	(+)	+	—	—	—	—	—	—	—	—	—	—
PGPR8	—	—	—	—	—	—	—	—	—	—	—	—
PGPR9	+	—	(+)	(+)	+	—	+	—	+	(+)	+	+

[a]+ Indicates significant increase over control in all the three seasons; (+) indicates significant increase over control in two out of three seasons;— indicates no significant increase or increase in 1 year only.

years. It is an established fact that ACC-deaminase activity would enhance the root length and thereby increase the active rhizosphere zone. However, only five isolates, viz. PGPR1, PGPR2, PGPR4, PGPR8 and PGPR9, could enhance the root length of peanut significantly in 1, 2 or 3 years (Table 4). The correlation co-efficient between root length enhancement by these strains with different plant growth, yield and nutrient uptake parameters indicated that enhancement in root length had high positive correlation co-efficients with plant biomass (0.93), nodule number (0.91), nodule dry weight (0.99), plant height (0.97), pod yield (0.73), haulm yield (0.92), pod number (0.75), soil nitrogen content (0.80), nitrogen content in kernel (0.98) and available phosphorus in soil (0.94). However, enhancement in root length could not contribute enough to 100 seed mass (0.09), shelling per cent (0.46), nitrogen in shoot (0.21) and phosphorus content in shoot (0.10) and in kernel (0.45) as correlation co-efficients were less than 0.5.

Very low correlation (co-efficient between −0.60 and 0.31) was found between the soil nitrogen content (assuming that ammonification could enhance the soil nitrogen content) and all other yield and yield-related traits studied.

Five PGPR isolates, viz. PGPR1, PGPR2, PGPR4, PGPR6 and PGPR7, possessed IAA-producing trait. However, among these strains, only three strains, viz. PGPR1, PGPR2 and PGPR4, significantly enhanced the growth, yield and nutrient uptake of peanut, cultivar GG2, under field conditions and the other two strains failed to do so. Thus, correlation study between plant height (assuming that IAA influences plant height due to hormonal effect) data of treatments inoculated with PGPR1, PGPR2 and PGPR4 with other plant growth, yield and nutrient uptake traits indicated that enhancement in plant height was associated with growth and yield-related traits of peanut as indicated by high correlation co-efficients between plant height and plant biomass (1.00), haulm yield (0.99), nodule number (0.97), nodule dry weight (0.96) along with nitrogen content in shoot (0.71) and kernel (0.71). However, plant height showed negative correlation with pod yield (−0.90), 100 seed mass (−0.86), shelling per cent (−0.98), shoot phosphorus (−0.74) and kernel phosphorus contents (−0.80).

It was observed that although PGPR9 showed in vitro phosphate solubilization, it failed to enhance the available soil phosphorus content significantly under field conditions (Table 5). Assuming that

phosphate solubilization would enhance the available phosphorus content in soil, the correlation between significantly enhanced available soil phosphorus (due to inoculation with PGPR1, PGPR2, PGPR4 and PGPR5) and other growth and yield-related parameters was studied. The correlation revealed that a significantly higher available soil phosphorus content influenced plant biomass (0.62), nodule number (0.65), plant height (0.78), pod yield (0.76), root length (0.70), haulm yield (0.78), nitrogen content in shoot (0.71) and kernel (0.73). However, available soil phosphorus showed negative influence on kernel phosphorus content (−0.17) and nodule dry weight (−0.12). It also showed little influence on shelling per cent (0.33) and phosphorus content in shoot (0.20).

It was also observed (Tables 4 and 6) that inoculation of PGPR isolates enhanced the nodule dry weight. Thus, in order to evaluate the role of nodulation promotion by inoculation of PGPR isolates, correlation between nodule dry weight and other plant growth and yield parameters was studied. Nodulation positively influenced plant biomass (0.80), nodule number (0.83), available soil phosphorus (0.85), root length (0.72), pod yield (0.79), number of pods per plant (0.80), haulm yield (0.56) and nitrogen content in kernel (0.56). It also gave positive correlation with plant height (0.53), 100 seed mass (0.39), shelling per cent (0.41) and kernel phosphorus (0.43). Very low influence of nodulation was found over the shoot phosphorus content (0.08), nitrogen content in shoot (0.19) and soil (0.26).

Discussion

The plant rhizosphere is a versatile and dynamic ecological environment of intense microbes–plant interactions for harnessing essential micro- and macro-nutrients from a limited nutrient pool (Jeffries et al., 2003).

In the present investigation, nine PGPR isolates were evaluated for their effect on plant growth, yield and nutrient uptake of peanut in pots as well as under field conditions for 3 years and to ascertain the involvement of possible PGPR trait(s) in enhancing peanut growth. Among these nine strains, eight produced siderophore and five produced IAA, ammonia, solubilized inorganic phosphate and inhibited fungal pathogens like *A. niger* and *S. rolfsii*, causing collar and stem rot, respectively. *P. fluorescens* isolates, viz. PGPR1, PGPR2 and PGPR4, exhibited multiple PGPR traits like IAA production, ammonification, phosphate solubilization and siderophore production besides ACC-deaminase activity.

In the present study, it was also ascertained that the majority of the PGPR isolates could utilize the seed exudates, the best being *P. fluorescens* PGPR4. In the initial stages of the multiplication and establishment of rhizobacteria in the rhizosphere, it has been found essential for the rhizosphere bacteria to have the capability of utilizing the seed exudates as the sole source of C and N (Weller, 1988). This capability gives an added advantage (rhizosphere competence) to the inoculant strains compared to the ones which cannot utilize the seed exudates as the sole source of C and N and are hence less competent. However, the present studies revealed some interesting information. From the growth curve (Fig. 2), it is evident that PGPR2 was least able to utilize seed leachate and thus it was supposed to be the least competent. But PGPR2 was one of the best in enhancing the growth, yield and nutrient uptake of peanut under potted and field conditions. The situation under in vitro may vary from the field conditions. The seed leachate may provide the sources of carbon and nitrogen in the initial few days but thereafter, the translocation of the quantum and nature (qualitative and quantitative) of photosynthates in the form of root exudates would determine the proliferation of the inoculant bacteria and thus, the desired effect. It was evident from the rhizosphere competence studies that PGPR2 was able to maintain a population of more than 10^5 and 10^6 cfu g^{-1} of rhizosphere soil or rhizoplane, respectively, until harvest. Moreover, the utilizing capability of complex sets of carbon sources of the root exudates by the organisms might have ultimately determined the outcome of PGPR–plant interactions. Thus, the least ability of utilizing seed leachate under in vitro conditions might not always be a handicap for performance under in situ conditions.

Seed bacterization of these three isolates (PGPR1, PGPR2 and PGPR4) increased the root length, plant biomass, nodulation, plant height and pod yield in pots significantly over control and consistently over the years under potted conditions. These isolates also helped in better nitrogen fixation as revealed from significantly higher N content in shoot and in kernels in peanut, cultivar JL24. As only pink-coloured nodules (active nodules) were selected during observation, there was definite enhancement in nodulation promotion and biological nitrogen fixation. The content of phosphorus in soil, shoot and kernels was also enhanced significantly in all the 3 years, both in rainy and post-rainy seasons.

Under field conditions also, inoculation with these three isolates produced significant improvement in pod yield, plant biomass, nodule dry weight, N and P contents in soil, shoot and kernels, etc. Although significant enhancement in various growth and yield parameters was obtained with the inoculation of the PGPR isolates like PGPR1, PGPR2, PGPR4, etc., both under potted and field conditions in all the 3 years consistently, there were seasonal variations among the treatments over the years. This might be attributed to variations in bacterial population due to rapid wetting and drying of soil under rain-fed farming systems, rainfall distribution, temperature variations, pest and disease incidence, etc., which affected the performance of PGPR.

To investigate the possible role of different plant growth-promoting trait(s) of PGPR strains, correlation co-efficients between a trait of PGPR and the related traits in plants supposed to be influenced by PGPR trait were studied. It provided very interesting information regarding the possible involvement of PGPR traits of fluorescent pseudomonad strains in enhancing the growth, yield and nutrient uptake of peanut under field conditions with cultivar GG2.

As the soil was deficient in available phosphorus and soil pH was very conducive for phosphate solubilization, microbial phosphate solubilization would have played a role in better plant growth, yield and nutrient uptake. Even biological nitrogen fixation requires a bulk of the absorbed phosphate from soil to produce ATP which is required by the plant for atmospheric nitrogen fixation through peanut–rhizobia symbiosis. Thus, continuous supply of available phosphorus was required for sustaining the enhanced biological nitrogen fixation process. It would have been possible through phosphate solubilization. The possible role of phosphate solubilization by PGPR isolates in enhancing peanut growth, yield and nutrient uptake is further substantiated by the fact that significantly higher available soil phosphorus (indicator of phosphate solubilization) had very high positive correlation with pod yield (0.76), haulm yield (0.78) and other parameters mentioned in the results. However, available soil phosphorus showed negative influence on kernel phosphorus content (−0.17) and also showed little influence on shelling per cent (0.33) and phosphorus content in shoot (0.20), which could be explained on the basis of the possible sink of phosphorus in the plant to meet the excess demand of phosphorus flow to sustain the requirement of ATP generation to meet the demand of enhanced biological nitrogen fixation. It has been reported earlier that inoculation of phosphate solubilizing microorganisms enhanced the growth and yield of canola but not the phosphorus uptake (De Freitas et al., 1997). The present study also indicated a similar effect as low correlation was found between soil phosphorus content and P uptake in shoot and kernels, though uptake was found to be enhanced significantly in quantitative terms. Further, some significant effect between PGPR and *Rhizobium* interaction might have resulted in better nodulation and hence a high degree of biological nitrogen fixation, the energy for which would have come from phosphate solubilization and higher available soil P content. The process was facilitated further by the enhancement in the active root zone by increase in the root length and growth due to ACC-deaminase activity of the inoculant strains and thus enhanced nutrient mobilization, capture, harvest and uptake by the plant from the limited pool of nutrients in the rhizosphere. Chelation of iron by microbial siderophores and phosphate solubilization has been reported earlier to increase crop yield (Kloepper et al., 1988; Glick, 1995).

Ammonification, an important step in the transformation of organic nitrogen to ammoniacal form, would enhance soil nitrogen content by the ammonifying character of the PGPR isolates. However, very low correlation (co-efficient between −0.60 and 0.31) was found between soil nitrogen content and all other yield and yield-related traits studied. Hence, involvement of ammonification trait of *P. fluorescens* strains might not be significant.

Glick et al. (1995) observed that seven bacterial strains positive for ACC-deaminase activity promoted canola seedling elongation under gnotobiotic conditions. ACC-deaminase activity of PGPR strains is known to enhance the root length and growth by sequestering and hydrolyzing ACC from germinating seeds and thereby increasing the active rhizosphere zone. An ACC-deaminase minus mutant of *E. cloacae* UW4 has been found not to promote root growth of canola (Li et al., 2000). In the present study, only five isolates, viz. PGPR1, PGPR2, PGPR4, PGPR8 and PGPR9, out of the nine tested could enhance the root length of peanut, cultivar GG2, significantly in 1, 2 or 3 years, though ACC-deaminase activity was found in all the inoculant strains, in vitro. The expression of a particular trait under soil condition is governed by the interaction of the inoculant strain with the host plant, other microorganisms in the rhizosphere, environmental factors and its own genetic makeup. However, enhancement of root length due to ACC-deaminase activity of the inoculant strains like PGPR1, PGPR2 and PGPR4 had a pronounced effect on the growth, yield and nutrient uptake of peanut,

cultivar GG2 under field conditions, as evident from the high positive correlation co-efficient between root length enhancement and different plant growth, yield and nutrient uptake parameters like plant biomass (0.93), nodule number (0.91), dry weight (0.99), plant height (0.97), pod yield (0.73), etc. as mentioned earlier. However, enhancement in root length could not contribute enough to 100 seed mass (0.09), nitrogen in shoot (0.21) and phosphorus content in shoot (0.10). As enhancement of root length by ACC-deaminase activity of the inoculant PGPR strains had a significant effect on the majority of the growth, yield and nutrient uptake parameters, expression of ACC-deaminase activity must have been involved in yield enhancement in peanut by inoculation of *P. fluorescens* strains PGPR1, PGPR2 and PGPR4. This was in consistence with the observations in canola and other crops (Kloepper et al., 1988; Jacobson et al., 1994; Glick et al., 1995; Li et al., 2000; Penrose and Glick, 2001). Despite all the strains being ACC-deaminase positive, only few strains enhanced the root length and growth significantly both under potted and field conditions. Thus, ACC-deaminase activity alone was not responsible for enhancement in growth, yield and nutrient uptake of peanut.

Only three (PGPR1, PGPR2 and PGPR4) out of the five PGPR isolates possessing IAA-producing trait, enhanced the growth, yield and nutrient uptake of peanut, cultivar GG2, under field condition. If it is hypothesized that IAA influences plant height due to hormonal effect, and if enhanced plant height had high positive correlation with pod yield, it could be presumed that IAA was involved in enhancing growth and yield. In spite of a very high positive correlation of plant height with plant biomass (1.0), haulm yield (0.99) and nodule dry weight (0.96), it failed to take advantage of the enhancement in plant growth parameters to translate it into yield (−0.90). Production of IAA also failed to have any influence on 100 seed mass (−0.86), shelling per cent (−0.98), plant phosphorus (−0.74) and kernel phosphorus (−0.80). Moreover, tryptophan and indole-3-acetamides are the key intermediates in the IAA biosynthesis pathways (Barbieri and Galli, 1993; Patten and Glick, 2002) and how much of the precursor and intermediates were available in the rhizosphere of peanut, at a given point of time, was also a question. Tien et al. (1979) did establish that *Azospirillum brasilense* produced IAA, gibberellins, and cytokinins and that this bacterium could increase the number of lateral roots and root hairs in pearl millet, but under gnotobiotic conditions only. There are some reports that rhizobacteria that overproduce IAA inhibit root elongation, which is attributed to the stimulation of ethylene synthesis by IAA (Xie et al., 1996; Glick et al., 1998). Thus, involvement of IAA production by PGPR strains in enhancing growth, yield and nutrient uptake in peanut could be ruled out.

There was significant enhancement in the number as well as in the nodule dry weight of peanut due to inoculation of PGPR strains, PGPR1, PGPR2 and PGPR4, both in pots and in the field. The enhancement in the number of nodules might be attributed either to increase in root length and growth and thus, providing more number of active sites and access to nodulation by soil rhizobial strains. Fluorescent pseudomonads have been reported to promote nodulation in chickpea and thereby enhancing biological nitrogen fixation (Parmar and Dadarwal, 2000). Combined inoculation of *Bradyrhizobium* and *P. striata* has also been found to enhance biological nitrogen fixation in soybean (Dubey, 1996). Enhancement of nodule occupancy of *Bradyrhizobium* in soybean has also been reported by combined inoculation with *P. fluorescens* (Nishijima et al., 1988; Fuhrmann and Wollum, 1989). As the experiments were conducted in black calcareous soil, deficient in iron (5–7 ppm) and the pH of the soil was also conducive for the excretion of siderophores by PGPR isolates for chelating iron and other micronutrients like Zn, Mg, etc. as reported earlier (Leong, 1986), production of siderophore might have played a significant role in enhancing nodule dry weight vis-à-vis biological nitrogen fixation because the nitrogenase enzyme requires a lot of Fe, and the same has also been reported earlier (Catellan et al., 1999). Thus, enhancement in nodule dry weight had a pronounced influence on plant biomass, available soil phosphorus, root length, pod yield, etc. as evident from positive correlation co-efficients between nodule dry weight and those parameters. A similar kind of observation was also noticed with ACC-deaminase activity. Thus, the combined effect of nodulation promotion coupled with ACC-deaminase activity of the inoculant strains played a significant role in growth, yield and nutrient uptake of peanut.

Due to fewer incidences of diseases, plants were healthy. Under natural epiphytotic conditions, the mortality of plants due to incidences of collar and stem rot was well below 10% in the uninoculated control in all the 3 years. Though the disease incidence was low, inoculation of *P. fluorescens* isolates PGPR1, PGPR2 and PGPR4 further reduced the seedling mortality caused by *A. niger*. Inoculation of PGPR4 and PGPR5, which showed strong inhibition to *S. rolfsii*, reduced the incidence of stem rot severity. As all the inoculant strains were rhizosphere competent, if competition and niche

exclusion phenomena were involved in disease suppression, inoculation of all the inoculant strains would have resulted in disease suppression. But it has not happened so. *P. fluorescens* isolates, viz. PGPR1, PGPR2 and PGPR4, also produced siderophore and antifungal metabolites. As soil conditions were conducive for siderophore production, this trait could have been involved in inhibiting pathogens in the rhizosphere and thus, suppressing the incidence of diseases. Production of antifungal metabolites by fluorescent pseudomonads has also been found to suppress soil-borne fungal pathogens on many occasions (Cartwright et al., 1995; Pal et al., 2001). There are some cases where PGPR promoted plant growth in non-sterile soil by controlling fungal diseases (Catellan et al., 1999). The addition of a siderophore-producing *P. putida* converted a Fusarium-conducive soil into a Fusarium-suppressive soil for growth of three different plants (Scher and Baker, 1982). The peanut cultivar GG2 used for the field experiment was susceptible to iron chlorosis. Inoculation of siderophore-producing PGPR gave green foliage as compared to control and non-producing isolate PGPR3, wherein iron chlorosis (pale green) was visualized in the leaf lamina of plant. Thus, the microbial siderophore might have ameliorated the symptoms. Similar observations were reported with the inoculation of fluorescent *Pseudomonas* strain GRP3A in mung bean (Sharma and Johri, 2003).

It has been observed in our experiments that *P. fluorescens* isolates PGPR1, PGPR2 and PGPR4 which consistently enhanced growth, yield and nutrient uptake of peanut, both under potted and field conditions, had multiple plant growth-promoting traits. All the traits of PGPR might not get expressed at a given point of time. Thus, the more the presence of the PGPR traits in an individual organism, the more the chances of the organism being a successful inoculant strain.

ACC-deaminase activity might have produced better root growth in the initial stages of crop growth, but other attributes like siderophore production, phosphate solubilization and nodulation promotion coupled with reduction in plant mortality and disease severity by the PGPR isolates might have helped in better nutrient mobilization, nitrogen fixation, availability of nutrients and thus, uptake by the plants. This resulted in healthy plants due to balanced nutrient availability and uptake, which in turn increased plant height and biomass, N and P contents in shoot and kernels and pod yield. Colonization studies of these PGPR isolates using antibiotic resistance as well as spontaneous rifampicin resistant mutants established the fact that all the isolates were aggressive colonizers of the peanut cultivar, JL24, under potted conditions and maintained a sizeable population density at a given point of time to produce the desired effect.

Thus, involvement of ACC deaminase, siderophore production, phosphate solubilization, nodulation promotion and disease suppression and their coordinated expression were responsible in enhancing plant growth, yield and nutrient uptake of peanut by inoculation of *P. fluorescens* isolates PGPR1, PGPR2 and PGPR4. Mutational analyses of all these PGP traits of *P. fluorescens* and subsequent evaluation only can unravel the exact mechanism(s) of growth promotion of peanut by these PGPR isolates.

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