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Fluorescent *Pseudomonas* influences palisade mesophyll development and spatial root development in *Phaseolus vulgaris*

H.P. Deka Boruah[1,5], B. K. Rabha[2], N. Saikia[3] & B.S. Dileep Kumar[4]
[1,3,4]*Soil Microbiology, Regional Research Laboratory (Council of Scientific and Industrial Research), Jorhat 785 006, Assam, India.* [2] *Crop Physiology, Assam Agricultural University, Jorhat 785 013, India.* [5]*Corresponding author**

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Abstract

Three strains of plant growth promoting fluorescent Pseudomonads (HPR6, RRLJ008 and RRLJ134) were studied for their effect on growth and yield of French bean (*Phaseolus vulgaris* L.) under field conditions. The effect of these strains on nature of root development and leaf palisade tube length were also examined. The strains induced positive response on growth and physiological parameters resulting in higher yield in *P. vulgaris*. Strain HPR6 produced the most promising results in thickening of leaf palisade layer, spreading of lateral roots and production of root hairs. The increase in specific leaf weight (SLW), net assimilation rate (NAR) and relative growth rate (RGR) by these strains were 68%, 152% and 167%, respectively. The growth and yield parameters were also significantly improved compared to the uninoculated control. Antibiotic resistant mutant strains demonstrated that these bacteria effectively colonized the rhizosphere of French bean. The results suggest that the strains could be developed for field application on a large scale.

Introduction

Large scale use of microorganisms in agriculture may be considered as a serious proposition for they are capable of exerting multiple effects of fertilizers, pesticides and plant growth regulators (Deka Boruah and Dileep Kumar, 2002; Dileep Kumar et al., 2001; Duijff et al.,1993; Kloepper, 1993; Ma et al., 2001). The potential benefits of manipulating agricultural systems through modifications of rhizosphere and phyllosphere microflora are recognised (Okon et al., 1999). However, the growth-promoting effect of any strain is dependent on its capability to colonize root systems and compatibility with the existing microflora and environment to which they are introduced (Brown, 1974; Bull et al., 1991; Kloepper et al., 1980; Schippers, 1993).

Yield in most crops, is the ultimate outcome of an integration of processes at the morphological, phenological, physiological and biochemical levels at all stages of plant growth and development (Evans, 1993; Fredrick and Hesketh, 1994). The objective of this study was to evaluate effects of three hydrogen cyanide and siderophore producing fluorescent *Pseudomonas* strains viz., HPR6, RRLJ008 and RRLJ134 on growth and development of French bean (*Phaseolus vulgaris* L). The effect of these strains on pattern of root development and palisade mesophyll dimension were also studied. While development of absorptive root surface plays a dominant role in plant nutrition, with much-reduced intercellular space and increased chloroplast number per cell, the palisade mesophylls largely determine the photosynthetic potential of leaves.

* FAX No.: +91 0376 2370 011.
E-mail: dekaboruah@yahoo.com

Materials and methods

Test plants

Seeds of French bean (*P. vulgaris* cv. *contender*) were supplied by Agrotech Engineering, a certified seed distributor in Jorhat, India.

Soil type

Study was conducted at the field experimental site of the Regional Research Laboratory (RRL), Jorhat. The soil was sandy loam (48.7% silt, 43.6% sand and 7.3% clay) with a pH of 6.4. The total organic carbon (C), nitrogen (N) and phosphorus (P) contents of the soil were 2.4%, 0.75% and 0.48%, respectively that gave a C:N:P ratio of 66:21:13. Nitrogen and total organic carbon of the soil were estimated according to Subbiah and Ashija (1978) and Walkley and Black (1934), respectively and phosphorus estimated according to Jackson (1973). Particle size distribution of the soil was determined by a laser diffraction particle size analyzer (Model CILAS 1180) using sodium carbonate and sodium hexametaphosphate as dispersing agents.

Bacterial strains and culture conditions

Bacterial strains RRLJ134 and RRLJ008 were obtained from Soil Microbiology Division, RRL, Jorhat. Strain HPR6 was isolated from rhizoplane of Kohlrabi (*Brassica oleracea* var. gongylodes) and identified according to Bergy's Manual of Systematic Bacteriology (1984) and Cuppuccino and Sherman (1983). Bacterial inocula of HPR6, RRLJ008 and RRLJ134 were prepared in King's B (KB) (King et al., 1954) broth for 36 h. From the broth culture, a bacterial lawn was prepared on KB agar plate by transferring 1 mL of the culture and incubating for 48 h at 30 °C ± 2 °C. Bacterial cells were harvested in 50 mL of sterile distilled water and shaken in a rotary shaker (at 125 rpm) for 30 min with glass beads. The absorbance of the bacterial cell suspensions was adjusted to optical density 0.5 at 600 nm (in UV-VIS spectrophotometer, Jasco, Japan) by adding sterile distilled water. This gave around 2.5 $\times 10^7$ CFU mL $^{-1}$. This cell suspension was used for seed bacterization.

Siderophore and hydrogen cyanide assay

To detect the production of siderophore and hydrogen cyanide by RRLJ008, HPR6 and RRLJ134, the universal siderophore assay described by Schwyn and Neilands (1987) and hydrogen cyanide detection test described by Bakker and Schippers (1987) were performed. These strains were inoculated on to KB plates containing glycine 4 g L^{-1} and $FeCl_3$ (100 μM) and hydrogen cyanide was detected by colour change in a filter paper impregnated with solution of picric acid (0.5%) and sodium carbonate (2%) dried and attached to the lid of the culture dish.

Seed bacterization and sowing

Seed bacterization was carried out according to Dileep Kumar et al. (2001). Seeds of French bean were surface sterilized in 2.4% sodium hypochlorite solution for 3 min followed by rinsing in aqueous 30% hydrogen peroxide solution for 30 min and dried under sterile condition. The surface sterilized seeds were dipped for 1 h in the bacterial cell suspensions and dried overnight in sterile petri-dishes. Uninoculated controls consisted of seeds treated with sterile distilled water. The treated and control seeds were sown in small field beds (3.0 m × 2.5 m) at a plant to plant and row to row spacings of 20 cm and 25 cm, respectively.

Root colonization study

To monitor the colonization of roots by the introduced rhizobacteria, spontaneous dual antibiotic-resistant mutants were selected according to Dileep Kumar (1999). A high concentration of inoculum of strains HPR6, RRLJ008 and RRLJ134 was streaked on to KB medium, containing ampicillin, penicillin and streptomycin sulphate (100 mg L^{-1}). The strains which grew in the antibiotic amended medium designated as HPR6^{aps}, RRLJ008^{aps} and RRLJ134^{aps} and showed the parental character of production of hydrogen cyanide and siderophore were selected for further study. The total CFU was measured on 7, 14, 21 and 28 day old plants. For this the uprooted plants were shaken gently to remove the loosely adhered soil-particles from the roots and then cut into 2 – 3 mm long pieces. One gram fresh root piece was placed in a sterile conical flask containing 10 mL of sterile distilled water with glass beads and agitated for 5 min to release rhizoplane bacteria into water. Serial dilutions of the root washings were pour-plated on KB media (supplemented with ampicillin, penicillin or streptomycin sulphate 100 mg L^{-1}) to establish the number of introduced bacteria and on nutrient agar media for total aerobic bacteria on the rhizoplane. Total CFU

mg $^{-1}$ of roots (from an average of four readings) were counted after 48 h of incubation at 30 °C ± 2 °C.

Growth analysis

Samplings were done at 7, 14, 21 and 28 days of plant growth for growth analysis. Specific leaf weight (SLW), net assimilation rate (NAR) and relative growth rate (RGR) were determined using the methodology of Gardner et al. (1985).

Root study

For the enumeration of total number of lateral roots and root hairs, the 28 day old plants were uprooted with a shovel. The beds were irrigated the day before sampling. The roots were kept immersed for 15 – 20 min in a bucket of clean water and then the soil particles adhered to the roots were carefully washed-off under a tap. The excess water was removed by pressing the roots between blotting papers. The roots were allowed to dry for 30 – 40 min in the laboratory and then placed on a hard paper and carefully pressed with a scale. With the help of a sharp razor the roots are dissected into three parts of 0.0 – 1.0 cm, 1.0 – 2.5 cm and >2.5 cm to represent the roots of respective soil depth.

Leaf tissue dimensions

Youngest fully expanded leaf from the main axis of the 28 day old plants were sampled for leaf palisade tissue studies. Transverse sections were prepared by hand sectioning with a sharp blade. The sections were stained with safranin and fast green after passing through different grades of alcohol and mounted on clean slides and observed under the compound microscope at 100× (Leitz Wetzler, Germany) and photographed with automatic photographic attachment Orthomate E.

Growth and yield parameters

The measurements of various growth and yield parameters were taken at final harvest (90 days). For root length and root biomass, the roots were removed as described earlier and maximum root length recorded. The root biomass was recorded after drying in an oven at 105 °C to a constant weight. The plant height was recorded from the base of the plant (soil surface) to the apex of the main axis. Number of branches, number of leaves and number of pods per plant were counted and the leaf area was recorded by fractional multiplication by 0.660 (Misra, 1992). The dry weight of pods was taken as economic yield. Total biomass was computed by adding the dry weight of component parts of plants excluding that of the roots.

Statistical analysis

Experiment was performed in a completely randomized block design and each treatment was replicated four times. All the data were subjected to analysis of variance (ANOVA) and treatment means were compared using least significance difference (LSD) at $p<0.05$.

Results and discussion

Siderophore and hydrogen cyanide production

Universal siderophore assay with the strains RRLJ008, HPR6 and RRLJ134 produced an orange halo around the colony when grown in KB agar plate containing chromazurol S indicating siderophore production by these strains. All these bacterial strains also turned picric acid and sodium carbonate amended filter paper brown or orange which confirmed hydrogen cyanide production by these strains. These results were in conformity with the earlier reports of Schwyn and Neilands (1987) and Bakker and Schippers (1987).

Root colonization

Root colonization estimation with antibiotic resistant mutant strains revealed that all the tested fluorescent Pseudomonads successfully colonized root surfaces of *P. vulgaris* (Table 1). The extent of colonization by each strain gradually increased, reaching a peak on 21 day old plants. At peak colonisation the strains produced 42.4% and 29.2% more colony forming units over 7 day and 14 day old plants, respectively. The maximum colony forming units by total aerobic bacteria were enumerated in the roots of relatively younger plants.

Effect of seed bacterization on plant growth

The tested bacterial strains induced a considerable increase in SLW (Figure 1A). The increase signifies consolidation of the photosynthetic apparatus of the

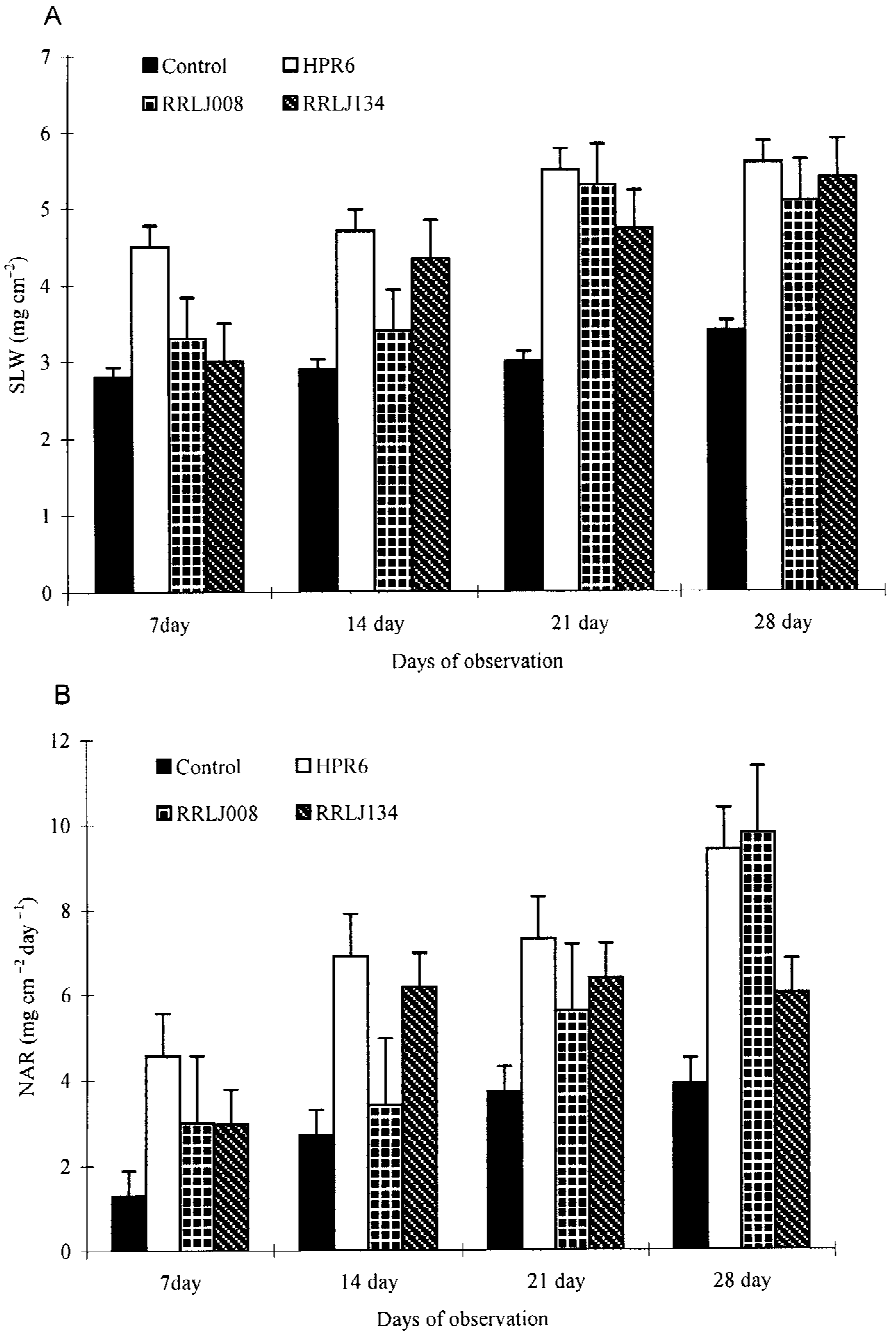

Figure 1. (A) Effect of seed bacterization with fluorescent *Pseudomonas* strains on specific leaf weight (SLW) of *Phaseolus vulgaris.* Error bars represent ±1.0= standard error means of observed value. (B) Effect of seed bacterization with fluorescent *Pseudomonas* strains on net assimilation rate (NAR) of *Phaseolus vulgaris*. Error bars represent ±1.0= standard error means of observed value.

Table 1. Effect of seed bacterization on root colonization by fluorescent *Pseudomonas* strains on *Phaseolus vulgaris* L. over time

Strain	Total aerobic/ introduced bacteria	Total colony forming unit in root (CFU Mg^{-1})			
		7 day	14 day	21 day	28 day
Control	TAB	6.9±1.2	12.7±1.1	11.9±1.3	13.7±1.5
HPR6	IB	1.9±0.8	2.2±0.9	2.8±0.8	2.5±0.5
RRLJ008	IB	2.0±0.7	2.1±0.8	2.7±0.9	2.3±0.4
RRLJ134	IB	2.0±0.7	2.2±0.4	2.9±0.8	2.3±0.4

Data are mean of four replicates, ± 1.0 = Standard deviation of observed values, TAB= Total aerobic bacteria, IB= Introduced bacteria.

leaf. The increase may be primarily attributed to the increase in palisade axial dimension. This was apparent from the corresponding increase in SLW in 28 day old leaves where a 52% increase in palisade layer coincides with 65% increase in SLW under HPR6 and a 43% increase in the palisade thickness with 59% and 50% increase in SLW under RRLJ134 and RRLJ008, respectively. The enhanced NAR (Figure 1B) over the stages with HPR6 may be directly traced to the improved photosynthetic apparatus as indicated by the increased SLW. The other strains RRLJ008 and RRLJ134 also achieved significantly greater NAR probably on similar grounds. The probable relationship of declining rate of respiration with enhanced SLW and amount of carbon per unit leaf area may also contribute to greater NAR with the strains.

The greater RGR with HPR6 (Figure 2A) may have been contributed by the greater NAR over the growth stages. The RGR is the expression of combined effects of NAR and leaf area ratio (LAR). The greater increase in RGR with RRLJ134 over RRLJ008 may be because of greater LAR with RRLJ134 (data not shown). The early enhancement of RGR with the strains signifies the quick establishment of the canopy with greater partitioning to leaf area growth. Subsequent slow leaf growth might have helped the effective partitioning of carbon to developing harvestable sink tissues. Photosynthate accumulation is related to total photosynthesis of the entire plant over the growing season, more than to the instantaneous rates of leaf photosynthesis typically reported (Medrano and Primo-Millo, 1985). Medrano et al. (1995) also concluded that dry matter gain is related to carbon balance rather than to plant photosynthesis. The NAR computations consider these aspects and variations in NAR in the present investigation reflected the differential increment on the growth and yield attributing components. Many workers have shown good correlation between photosynthesis and productivity in *Vigna mungo* (Chandra Babu et al., 1985); maize (Crosbie and Pearce, 1982); soybean (Harrison et al., 1981); sorghum (Peng et al., 1991); cotton (Pettigrew and Meredith, 1994); and in 24 different wild species (Poorter et al., 1990).

Effect of seed bacterization on leaf thickness

The spongy mesophyll layer of leaf was marginally reduced by bacterization, but greater-than-proportional increase in palisade layer more than compensated this reduction resulting in a considerably thicker leaf. More than half of the leaf thickness (52%) was contributed by palisade tube layer under HPR6 (Figure 3C) against one third (33%) under the uninoculated control (Figure 3A). Strain HPR6 induced a 93% increase in palisade tube length over that of control. Both RRLJ134 and RRLJ008 induced a 43% increase in palisade layer thickness as compared to uninoculated control. The contribution of palisade mesophyll layer to leaf thickness were 44% and 43% with RRLJ134 and RRLJ 008, respectively. With increase in axial dimension without associated radial expansion, the palisade tube layer largely determined the leaf thickness. Thus the total number of cells per unit leaf surface might have been maintained or even reduced. Van Volkenburgh and Cleland (1979) observed that cell division in *P. vulgaris* may cease when the leaf is quite small (10% of full size in primary leaves) and cell enlargement alone dominates the expression of the leaf thereafter. Non-expansion of radial dimension of palisade tube cells might have facilitated faster conductance of CO_2 to the carboxylation site of the chloroplast. The importance of cell size and volume in determining the movement of CO_2 to the carboxylation sites has been analysed (Raven and Glidewell,

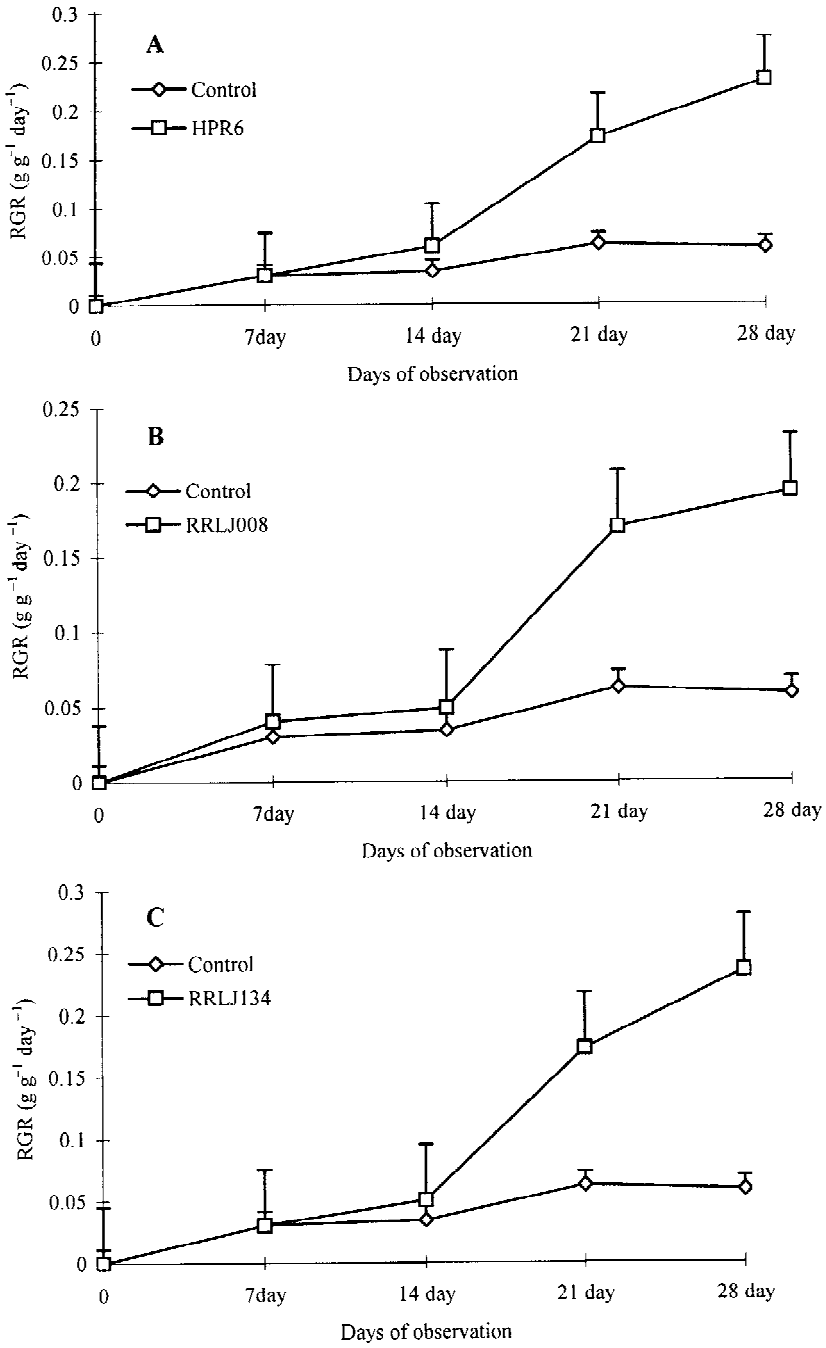

Figure 2. Effect of seed bacterization with fluorescent *Pseudomonas* strains (A. HPR6, B. RRLJ 008 and C. RRLJ134) on relative growth rate (RGR) of *Phaseolus vulgaris.* Error bars represent ±1.0= standard error means of observed value.

Table 2. Effect of seed bacterization with fluorescent *Pseudomonas* strains on growth and yield parameters of *Phaseolus vulgaris* L.

Strain	Root length (cm)	Root mass (g plant^{-1})	Plant height (cm)	Number of leaves plant^{-1}	Leaf area (cm^2 plant^{-1})	Number of branches plant^{-1}	Number of pods plant^{-1}	Pod yield (g plant^{-1})	Biomass (g plant^{-1})
Control	33.3±2.5	1.4±1.2	15.0±3.2	24±1.6	157.7±20.9	6.4±1.1a	7.5±3.3	1.2±2.0	3.3±0.3
HPR 6	37.9±2.6a	2.9±2.2a	25.6±2.7a	37.6±3.1a	211.9±13.3a	8.5±1.2ab	13.2±2.1a	3.9±0.8a	7.2±0.6a
RRLJ008	38.8±3.0a	3.7±0.8a	24.3±2.2a	39.7±2.8a	240.8±10.3	10.6±1.8bc	11.6±3.0a	3.7±0.7a	10.7±0.8
RRLJ134	37.6±2.6a	3.3±2.8a	24.8±2.6a	40.5±3.7a	212.8±19.2a	10.8±1.6c	11.4±3.0a	3.8±1.1a	7.2±0.8a

Data are mean of four replicates; values followed by similar letter are not significantly different at $p < 0.05$.

1981). Studies by Wilson and Cooper (1970) on *Alium* and Dunstone and Evans (1974), Le Cain et al. (1989) and Morgan et al. (1990) on *Triticum* spp. showed an inverse relationship between the size of mesophyll cells and the rate of CO_2 assimilation per unit leaf area. Smaller cells increase the surface:volume ratio and hence enhance the rate of photosynthesis (Wilson and Cooper, 1970).

Number of functional and lateral roots

The distribution of lateral roots and root hairs was significantly influenced by the bacterial strains. HPR6 and RRLJ008 induced 3 fold and 2 fold increase in lateral root production, respectively as compared to the uninoculated control (Figure 4A). The increased number of first order lateral roots progressively reduced the dominance of the tap root towards greater depth, but triggered substantial secondary growth towards the base supporting generation of new shoot tissues. The spatial root development determines the potential absorption capacity of roots and hence assume greater significance. The uninoculated control plants produced 67% of the lateral roots at 1.0 – 2.5 cm soil depth, 33% at greater depth (>2.5 cm) and none at the top layer (0 – 1.0 cm). HPR6 produced laterals in all these layers with greater share (58%) at deeper layer (>2.5 cm). Strains RRLJ134 and RRLJ008 tended to produce more lateral roots in 1.0 – 2.5 cm soil depth. Highly significant increase in root hair production was obtained with HPR6 and RRLJ134 (Figure 4B). The 1.0 – 2.5 cm soil depth recorded greater share of root hair ranging from 70% (HPR6) to 83% (RRLJ008) as against 87% under uninoculated control. The greater depth (>2.5 cm) recorded the highest share of root hairs with HPR6 (24%) and least with the control (10%). The top rhizosphere (0.0 – 1.0 cm) housed nearly 7% of the total root hairs of inoculated plants, against 3% of the uninoculated control. The strains HPR6 and RRLJ134 induced a 2 fold increase and RRLJ008 a 1.7 fold increase in number of root hairs in this rhizosphere in comparison to the uninoculated control. It is evident that HPR6 was most effective in spreading the absorptive system to deeper rhizosphere for efficient soil exploration.

Effect of seed bacterization on growth and yield parameters

Bacterization with fluorescent *Pseudomonas* strains significantly increased the root biomass and length of roots as compared to the control (Table 2). All the bacterial strains produced more than one-fold increase in root length that ranged from 13% (RRLJ134) to 17% (RRLJ008). Leaf area and leaf numbers per plant were also significantly increased by these bacterial strains (Table 2). The highest increase in leaf area (53%) was obtained with RRLJ008. The increase in number of leaves ranged between 69% (RRLJ134) and 57% (HPR6), while the plant height was increased by 62% (RRLJ008) to 71% (HPR6). The increase in branch numbers ranged from 33% (HPR6) to 69% (RRLJ134) in comparison to the unioculated control.

The bacterial strains also stimulated significantly positive response in yield attributing components of French bean (Table 2). Pod numbers per plant were increased by 76%, 55% and 52% with the strains HPR6, RRLJ008 and RRLJ134, respectively. Pod yield was increased by more than 2 fold with all the bacterial strains. The greater increase of 2.25 fold was obtained with strain HPR6. However, the highest increase in plant biomass (2.24 fold) was produced by RRLJ008, while the increase by HPR6 and RRLJ134 was slightly greater than one fold. The data on growth and yield parameters demonstrate that relatively greater dry matter was partitioned for vegetative parts such as root biomass, leaf area and branch numbers under RRLJ008 resulting in greater plant biomass. How-

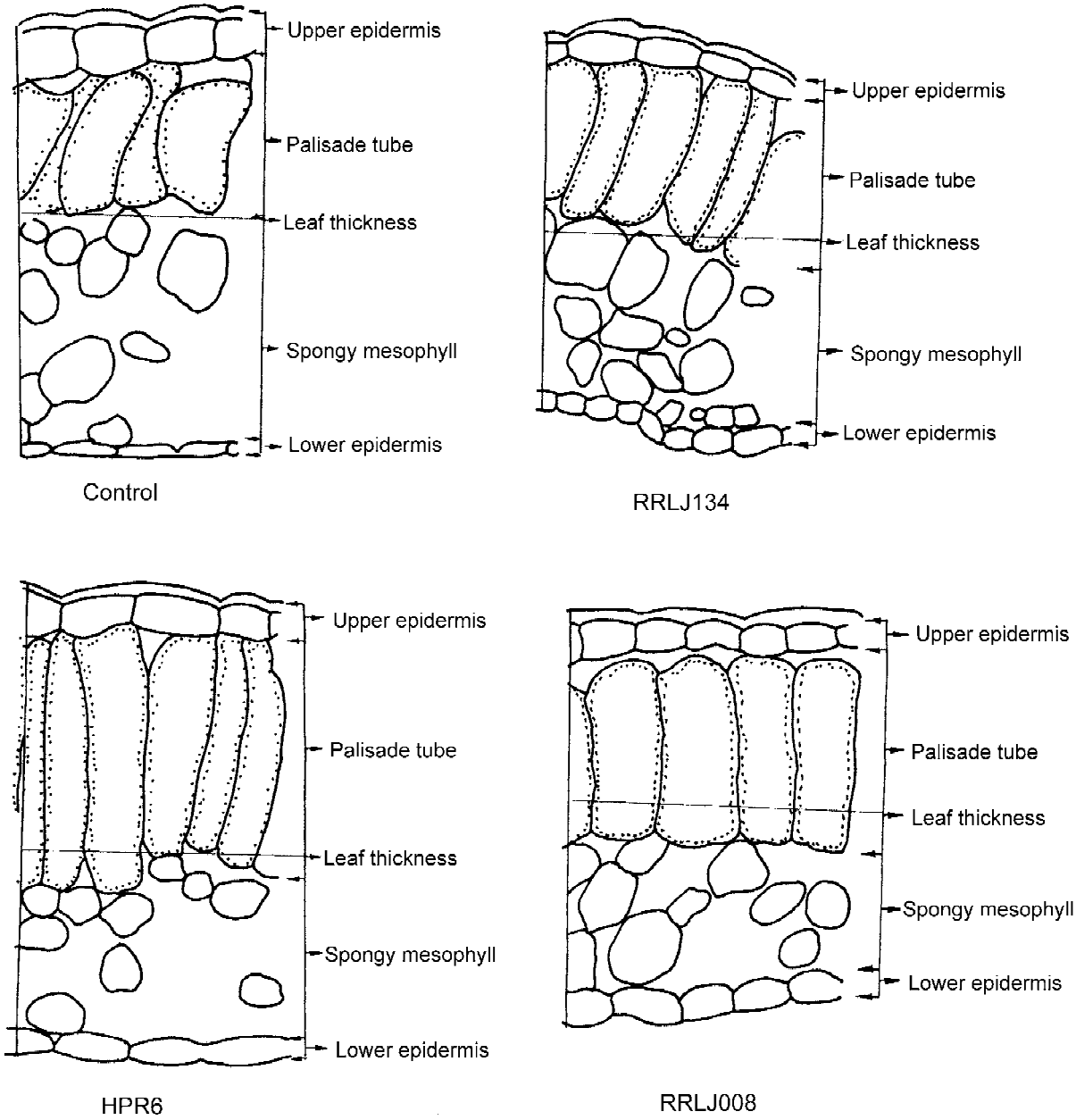

Figure 3. Diagrammatic representation of the palisade axial dimension in leaf thickness of *Phaseolus vulgaris*.

ever, more efficient dry matter partitioning to yield attributing components with HPR6 resulted in highest yield improvement. Dry matter partitioning played a determinant role in pod yield also with RRLJ134. Similar results were reported by Deka Boruah and Dileep Kumar (2002).

The studies have revealed that the fluorescent *Pseudomonas* strains are effective in root colonization in French bean under field condition. The ability to produce hydrogen cyanide and siderophore may be associated for their role as growth promoters (Bakker and Schippers, 1987; Schippers, 1993). The strains showed consistency in promoting plant growth and development through morphological and physiological modifications. This influenced the yield and yield attributing components in French bean. The effects of

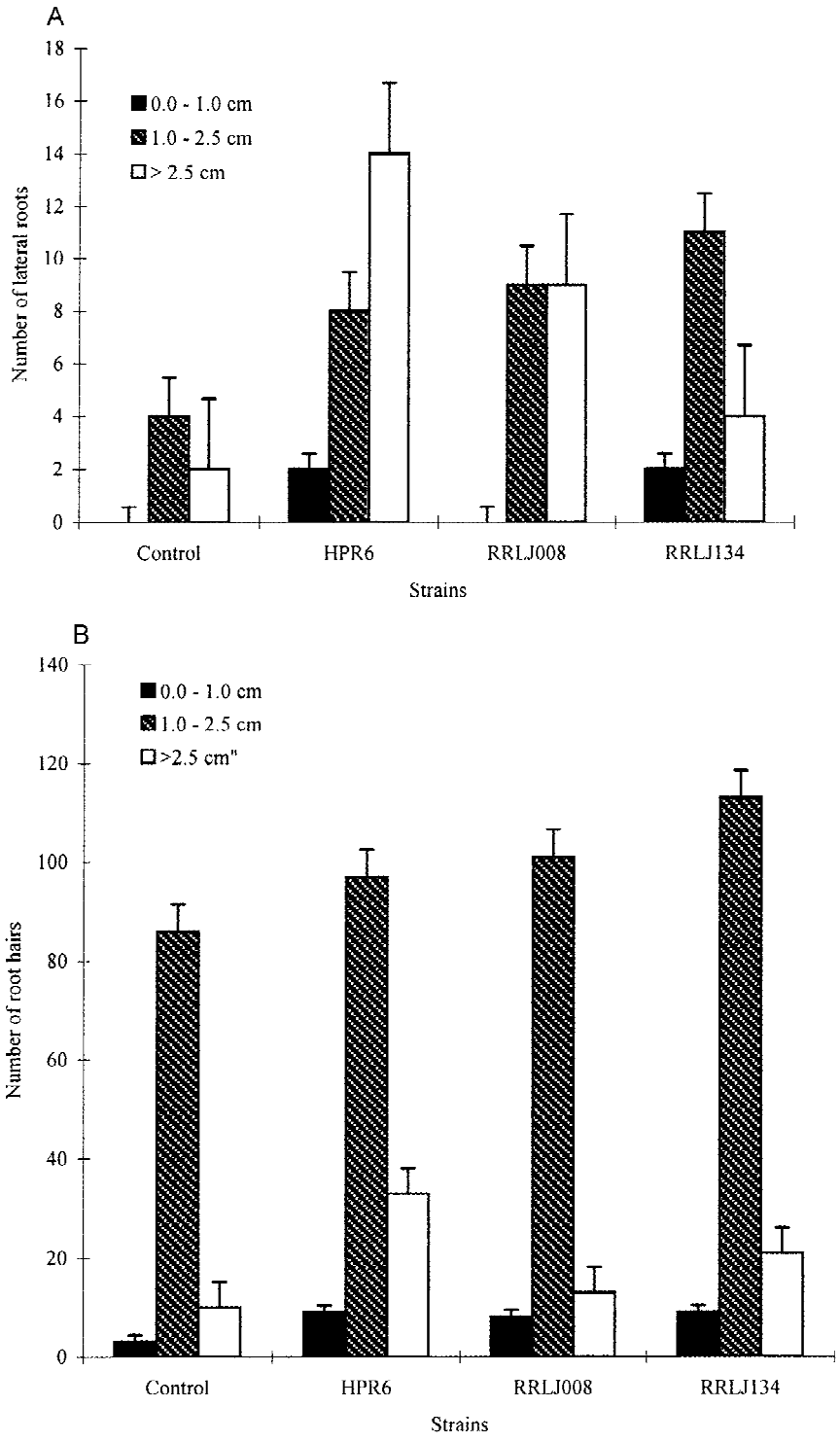

Figure 4. (A) Effect of seed bacterization with fluorescent *Pseudomonas* strains on lateral root development in *Phaseolus vulgaris*. Error bars represent ±1.0= standard error means of observed value. (B) Effect of seed bacterization with fluorescent *Pseudomonas* strains on development root hairs in *Phaseolus vulgaris*. Error bars represent ±1.0= standard error means of observed value.

the strains may be further examined with a wide variety of target species under variable soil conditions to develop these strains for large scale field applications.

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References

Bakker A W and Schippers B 1987 Microbial cyanide production in the rhizosphere in relation to potato yield reduction and *Pseudomonas* spp mediated plant growth stimulation. Soil Biol. Biochem. 19, 451–457.

Brown M E 1974 Seed and root bacterization. Ann. Rev. Phytopathol. 12, 181–197.

Bull T C, Weller D M and Thomashow S L 1991 Relationship between root colonisation and suppression of *Gaeumannomyces graminis* var *tritici* by *Pseudomonas fluorescens* strain 2–79. Phytopathology 81, 954–959.

Bergy's Manual of Systematic Bacteriol 1984 Ed. J G Holt. pp 141. Williams and Wilkins, Baltimore.

Cuppuccino J G and Sherman N 1983 Microbiology: A laboratory manual, pp 131–195. Addition-Wesley, California.

Chandra Babu R, Srinivasan P S, Natarajaratnam N and Sree Rangaswamy S R 1985 Relationship between leaf photosynthetic rate and yield in blackgram (*Vigna mungo* L. Hepper) genotypes. Photosynthetica 19, 159–163.

Crosbie T M and Pearce R B 1982 Effect of recurrent photosynthetic selection for high and low photosynthesis on agronomic trials in two maize populations. Crop Sci. 22, 809–813.

Deka Boruah H P and Dileep Kumar B S 2002 Disease suppression and plant productivity through a plant growth promoting flourescent *Pseudomonas* strain RRLJ008. Folia Microbiol. 47, 137–143.

Dileep Kumar B S 1999 Fusarial wilt suppression and crop improvement through two rhizobacterial strain in chick pea growing in soil infested with *Fusarium oxysporum* f.sp. *ciceris*. Biol. Fert. Soils 29, 87–91.

Dileep Kumar B S, Berggren I and Martensson A M 2001 Potential for improving pea production by co-inoculation with fluorescent *Pseudomonas* and *Rhizobium*. Plant Soil 225, 25–34.

Duijff B J, Meijer J W, Bakker P A H M and Schippers B 1993 Siderophore mediated competition for iron and induced disease resistance in the suppression of Fusarium wilt of carnation by fluorescent *Pseudomonas* spp. Neth. J. Plant Pathol. 99, 277–291.

Dunstone R L and Evans L T 1974 Role of changes in cell size in the evolution of wheat. Aust. J. Plant Physiol. 1, 157–165.

Evans L T 1993 Crop evolution, adaptation and yield. Cambridge University Press. Fredrick J R and Hesketh J D 1994 Genetic improvement in Soybean: Physiological attributes. *In* Genetic Improvement of Field Crops. Ed. G A Slafer. pp. 237–286. Marcel Dekker, Inc. New York.

Gardner F P, Pearce R B and Mitchell R L 1985 Physiology of crop plants. The Iowa State University Press, Ames.

Harrison S A, Boersma H R and Ashley D A 1981 Heritability of canopy apparent photosynthesis and its relationship to seed yield in soybeans. Crop Sci. 21, 222–226.

Jackson M L 1973 Soil chemical analysis, Prentice Hall of India Pvt Ltd., New Delhi.

King E O, Ward M K and Raney D E 1954 Two simple media for the demonstration of pyocyanin and fluorescin. J. Lab. Clin. Med. 44, 301–307.

Kloepper J W, Schroth M N and Miller T .D 1980 Effect of rhizosphere colonisation by plant growth promoting rhizobacteria on potato plant development and yield. Phytopathology 70, 1078–1082.

Kloepper J W 1993 Plant growth promoting rhizobacteria as biological control agent. *In* Soil Microbial Ecology-Applications in Agricultural and Environmental Management Ed. F B Metting. pp. 255–274. Marcel Dekker, New York.

Le Cain D R, Morgan S A and Zerbi G 1989 Leaf anatomy and gas exchange in nearly isogenic semi-dwarf and tall winter wheat. Crop Sci. 29, 1246–1251.

Ma W, Zalec K and Glick B R 2001 Biological activity and colonization pattern of the bioluminescence labeled plant growth promoting bacterium *Kluyvera ascorbata* SUD 165/26. FEMS Microbiol. Ecol. 35, 137–144.

Medrano H and Primo-Millo E 1985 Selection of *Nicotiana tabacum* haploids of high photosynthetic efficiency. Plant Physiol. 79, 505–508.

Medrano H, Keys A J, Lawlor D W, Parry M A J, Azcon-Bieto J and Delgado E 1995 Improving plant production by selection for survival at low CO_2 concentrations. J. Expt. Bot. 46, 1389–1396.

Misra K C 1992 Manual of Plant Ecology. pp. 464. Oxford and IBH publishing Co. Ltd, New Delhi.

Morgan J A, Le Cain D R and Wells R 1990 Semidwarfing genes concentrate photosynthetic machinery and effect leaf gas exchange of wheat. Crop Sci. 30, 602–608.

Okon Y, Bloemberg G V and Lugtenberg B J J 1999 Biotechnology of biofertilization and phytostimulation. *In* Agricultural Biotechnology. Ed. Arie Altman. pp. 327–349. Marcel Dekker, Inc.New York.

Peng S, Krieg D R and Grima F S 1991 Leaf photosynthetic rate is correlated with biomass and grain production in grain sorghum lines. Photosyn. Res. 28, 1–7.

Pettigrew W T and Meredith Jr W R 1994 Leaf gas exchange parameters vary among cotton genotypes. Crop Sci. 34, 700–705.

Poorter H, Remkes C and Lambers H 1990 Carbon and nitrogen of 24 wild species differing in relative growth rate. Plant Physiol. 94, 621–627.

Raven J R and Glidewell S M 1981 Processes limiting photosynthetic conductance. *In* Physiological Processes Limiting Plant Productivity. Ed. Johnson C B. pp. 109–136. Butterworth, London.

Schippers B 1993 Exploration of microbial mechanisms to promote plant health and plant growth. Phytoparasitica 21, 275–279.

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

Subbiah B V and Ashija G L 1978 A rapid procedure for the determination of available nitrogen in soils. Curr. Sci. 25, 259–260.

Van Volkenburgh E and Cleland R E 1979 Separation of cell enlargement and division in bean leaves. Planta 146, 245–247.

Walkley A and Black C A 1934 An examination of Degjareff methods for determining soil organic matter and proposed modification of the chromic acid titration method. Soil Sci. 37, 29–38.

Wilson D and Cooper J P 1970 Effect of selection for mesophyll cell size on growth and assimilation of *Lolium perenne* L. New Phytol. 69, 233–245.

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