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Comparative performance of formulations of plant growth promoting rhizobacteria in growth promotion and suppression of downy mildew in pearl millet

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Abstract

Five plant growth promoting rhizobacterial formulations, each consisting of two *Bacilli* strains with chitosan as a carrier were tested for their capacity to promote growth and induce resistance against downy mildew in pearl millet under both greenhouse and field conditions. Three modes of applications were tested: seed treatment, soil amendment, and seed treatment + soil amendment. In general, irrespective of application method, most of the formulations, in comparison with the control, increased plant growth and vigor as measured by seed germination, seedling vigor, plant height, fresh and dry weight, leaf area, tillering capacity, number of earheads, length and girth of earhead, 1000 seed weight and yield. The time of flowering was also advanced by 4–5 days over the control. Likewise all the formulations significantly reduced downy mildew incidence relative to the nontreated control. However, the rate of growth enhancement and disease suppression varied considerably with the formulations. Formulations LS256 and LS257 besides being the best growth promoters were also the most efficient resistance inducers. None of the formulations matched the level of the fungicide metalaxyl in offering protection against downy mildew. Among the application methods tested, soil amendment was found to be the most suitable and desirable way of delivering the formulations. Combination of seed treatment and soil amendment produced the same effect that was produced by soil amendment alone. The study demonstrates a potential role for plant growth promoting rhizobacterial formulations in downy mildew management.

Keywords: Biofungicides; Plant growth promoting rhizobacteria; Induced systemic resistance; Biofertilizers

1. Introduction

To reduce crop loss, pesticides are generally used. They are cost-effective and thus have become an integral part of modern agriculture. Environmental and human health related concerns associated with use of hazardous chemicals have necessitated the search for eco-friendly alternatives. Such approaches must enhance and sustain agricultural productivity and at the same time be safe from environmental and health perspectives.

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is one of the world's main coarse grain crops. It is an indispensable cereal that has been widely grown in arid and semi-arid regions in Africa and Asia since pre-historic times. It provides staple food for the poor, and is cultivated during a short growing season in relatively dry tracts of land with poor soil fertility. It is also valued for its dry fodder in livestock-based farming systems, which are predominant in these regions. The crop is grown in India as a rain-fed or irrigated crop on 10 million hectares producing 7.01 million tones (Bhatnagar et al., 2002).

Among the diseases caused by fungi, bacteria, viruses and nematodes that reduce pearl millet yield, downy mildew caused by the biotrophic oomycete, *Sclerospora graminicola* (Sacc.) Schroet., is one of the most damaging. It causes annual losses of up to 40%, amounting to 270 million US dollars annually (Shetty

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et al., 1995). Various strategies such as chemical control, development of resistant varieties, and somaclonal variation are employed to manage the disease; however, these strategies each have limitations. Therefore, alternative approaches that are eco-friendly have become necessary. One such potential alternative is inducing resistance in plants. Plants possess an immediate and complex induced defense response against pest and pathogen invaders. This response is activated upon inducement with appropriate biotic and abiotic agents, and is termed systemic acquired resistance (SAR) (Heil and Bostock, 2002). Although a particular agent induces the response, the reaction is nonspecific and can provide resistance to a wide variety of organisms. Such resistance reactions can last for several weeks after activation so that the plant is resistant to future invaders. When resistance is induced by saprophytes, such as Plant Growth Promoting Rhizobacteria (PGPR), it is termed induced systemic resistance (ISR). ISR has been reported to be one of the mechanisms by which PGPR control plant disease through the manipulation of host plant's physical and biochemical properties (Kloepper, 1993; Van Loon et al., 1998). ISR is a simple and chemical-free method that enables the plant to defend itself against attack from multiple pathogens (Wei et al., 1996; Zehnder et al., 1997; Murphy et al., 2000; Ramamoorthy et al., 2001; Conrath et al., 2002). The main objective of this research was to determine if commercial PGPR formulations having growth promoting and ISR activity in other crops could also promote growth of pearl millet and offer protection against downy mildew.

2. Materials and methods

2.1. PGPR formulations and inoculum preparation

Five PGPR formulations were obtained from the Department of Entomology and Plant Pathology, Auburn University, USA for greenhouse tests and field trials. The formulations were are as follows: LS254 (spores of *Bacillus subtilis* strain GBO3 + *B. pumilus* strain SE34); LS255 (*B. subtilis* strain GBO3 + *B. subtilis* strain IN937b); LS256 (*B. subtilis* strain GBO3 + *B. pumilus* strain INR7); LS257 (*B. subtilis* strain GBO3 + *B. pumilus* strain T4); LS213 (*B. subtilis* strain GBO3 + *B. amyloliquefaciens* IN937a). Each formulation contained chitosan as a carrier. These strains have been used in various plants like cucumber, tobacco, ornamentals, vegetables, pepper, cotton, cantaloupe, watermelon, loblolly pine, slash, lodgepole pine, white spurce, and Douglas fir. These formulations are mainly used in the American and European countries (Reddy et al., 1999; Kenney et al., 1999; Kloepper et al., 1999; Martinez-Ochoa et al., 1999; Ryu et al., 1999; Yan et al., 1999; Zhang et al., 1999).

2.2. Host

Seeds of pearl millet cv. HB3 a cultivar highly susceptible to *S. graminicola* were obtained from the International Crop Research Institute for Semi Arid Tropics (ICRISAT), Hyderabad, India and the All India Co-ordinated Pearl Millet Improvement Project, Mandor, Jodhpur, India.

2.3. Source of pathogen and inoculum preparation

S. graminicola was isolated from severely infected pearl millet cv. HB3 grown under field conditions (Safeeulla, 1976). The pathogen was maintained under greenhouse conditions on its susceptible host prior to use. Leaves showing profuse sporulation of *S. graminicola* on the abaxial side were collected in the evening hours, and thoroughly washed under running tap water to remove sporangia. The leaves were then blotted dry, cut into small pieces, and maintained in a moist chamber to promote sporulation. The following morning fresh sporangia were washed into distilled water. For use as inoculum, the resulting zoospore concentration was adjusted to 40,000 zoospores/ml using a haemocytometer.

2.4. Modes of application

PGPR formulations were used as seed treatments, soil amendments and seed treatments + soil amendments. For seed treatment, the seeds of pearl millet were surface-sterilized with 0.02% mercuric chloride for 5 min, and rinsed thoroughly in sterile distilled water. Seeds were coated with 2% gum arabic as an adhesive and rolled into the formulations until uniformly coated. Seeds treated with sterile distilled water amended with gum arabic served as the nontreated control. For soil amendment, the formulations were mixed with the potting mix in the ratio 1:40 (v/v). Combining both seed treatment and soil treatment as described above resulted in the final application treatment.

2.5. Effect of PGPR formulations on seed germination and seedling vigor of pearl millet under laboratory conditions

Germination tests were carried out by the paper towel method (ISTA, 1993). PGPR-treated seeds and controls were seeded onto paper towels. The brown germination paper was soaked in distilled water. Fifty seeds of pearl millet were placed equidistantly on the paper. Another presoaked paper towel was placed on the first one so that the seeds were held in position. The towels were then rolled and wrapped with polythene to prevent

drying. After incubation for 7 days, the towels were unrolled and the number of seeds germinated were counted. Seedling vigor was analyzed at the end of 7 days of incubation by the method of Abdul Baki and Anderson (1973). The length of the root and shoot of individual seedlings was measured to determine the vigor index. The vigor index was calculated using the formula: Vigor index = (mean root length + mean shoot length) × (% germination). The experiment was carried out with four replicates of 100 seeds each and was repeated three times.

2.6. Effect of PGPR formulations on growth promotion of pearl millet under greenhouse and field conditions

For the evaluation of growth promotion under greenhouse conditions there were 15 treatments (1 treatment = formulation × application method), the five PGPR formulations were applied following seed treatment, soil amendment and seed treatment + soil amendment methods as described earlier and seeds were sown in 10 cm diameter plastic pots filled with 250 ml autoclaved soil and sand at the ratio of 2:1. Each treatment (formulation × application method) consisted of 50 plants i.e., five replicates with 10 pots per replication, and a single seed per pot. For seed treatment method seeds treated with distilled water served as control and for soil amendment method untreated seeds sown in sterile soil that did not receive the formulations served as control. Treatments were arranged in a randomized complete block design. Seedlings were maintained at 25–30°C with 95% relative humidity. Seedlings were watered daily, and no artificial fertilization was used. Plant height was measured from the base to the tip of the plant, fresh weight was determined by weighing the uprooted plant, dry weight was determined by drying the plants in an oven at 65°C until the weight remained constant, leaf surface area was measured with the automatic leaf analyzer Licor-2100, and number of tillers per plant were recorded 30 days after seeding (DAS) The experiment was repeated four times.

An experiment was conducted in the field to determine the effect of PGPR formulations on the growth of pearl millet. There were 15 treatments (1 treatment = formulation × application method), the five PGPR formulations were applied following seed treatment, soil amendment and seed treatment + soil amendment. Treatments were the same as described earlier except for the soil amendment, which was conducted using in-furrow application method wherein with 10 g formulation per 5 m row was uniformly applied in the furrow at 4 cm depth with the seeds placed above the formulations. Each treatment was replicated four times. Replications consisted of a single row 5 m long, hand-seeded with 50–100 seeds per row. The field was maintained according to recommended growing conditions (red loamy soil, irrigated once in every 15 days, and manual thinning done after 21 days so as to maintain uniform number of plants per row). The time taken by the plants to attain 50% flowering was recorded. Seedling height, leaf surface area, total number of earheads/plant, length of earheads, and girth of earheads were measured 60 days after seeding. Weight of 1000 seeds was determined according to ISTA (1993) procedure and yield was determined according to the procedure of Williams and Singh (1981) at the time of harvest. The experiment was repeated two times in the same year.

2.7. Screening of PGPR formulations for potential to elicit resistance against downy mildew under greenhouse and field conditions

In the greenhouse PGPR formulations were applied as seed treatment, soil amendment and seed treatment + soil amendment as described earlier for growth promotion studies. Seeds treated with sterile distilled water served as the control. Seeds treated with the systemic fungicide, metalaxyl (Apron 35 SD at 6 g/kg seeds) served as fungicide treated control. Treatments were sown in plastic pots containing a mixture of soil and sand at 2:1 ratio. Each treatment was replicated five times and consisted of 10 pots with a single seedling per pot. Treatments were arranged as a randomized complete block design. Two-day-old seedlings were challenge-inoculated by the whorl inoculation method (Singh and Gopinath, 1985) using the zoospore suspension of *S. graminicola* at a concentration of 4×10^4 zoospores/ml prepared as described earlier. In the whorl inoculation method, droplets of *S. graminicola* zoospores are dropped onto the leaf whorl formed by the emerging seedlings and allowed to flow down to the base. Inoculated plants were maintained under greenhouse conditions (90–95% RH, 20–25°C temperature) and observed for disease development. The plants were rated for disease when they showed any one of the typical downy mildew symptoms such as sporulation on the abaxial leaf surface, chlorosis, stunted growth, or malformation of the earheads. Downy mildew disease incidence was recorded at 30 and 60 DAS.

During 2000, field trials were designed to test the reaction elicited by PGPR in pearl millet against downy mildew. The trials were established at the Mysore University Downy Mildew Nursery where the soil was heavily infested with oospores of *S. graminicola*. PGPR treatments (formulation × application method) and the controls were the same as previously described. Soil-borne oospores of *S. graminicola*, served as the source of primary inoculum. Additional inoculum was provided by infector rows (spreader rows) that were planted 21 days prior to the planting the test rows as described by Williams (1984) which provided a continuous shower of

zoospores to the tester rows. Each treatment consisted of four replications. Each replicated row was hand-seeded with 50–100 seeds per row. The experiment was a randomized complete block design. Normal agronomic practices were followed to raise the crop. Thinning was done after 21 days to maintain uniform number of plants per row and uniform distance between the plants. The crop was irrigated once at 15 days. The plants were rated for disease when they showed any one of the typical downy mildew symptoms described above. Downy mildew disease incidence was recorded at 30 and 60 DAS.

2.8. Data analysis

Data from greenhouse and field experiments were analyzed separately for each experiment and were subjected to arcsine transformation and analysis of variance (JMP Software; SAS Institute Inc., Cary, NC). Significance effects of PGPR treatments were determined by the magnitude of the F value ($P \leqslant 0.05$). Treatment means were separated by Tukey's HSD test.

3. Results

3.1. Effect of PGPR formulations on seed germination and seedling vigor of pearl millet under laboratory conditions

In comparison with the nontreated control, all the formulations of PGPR except for LS255, significantly enhanced seed germination. Seedling vigor of pearl millet was significantly enhanced due to treatments with all formulations over the control (Fig. 1) and the rate of enhancement varied with the formulations used. The highest enhancement rate of germination and vigor index was obtained with the formulations LS257 and LS256, which recorded 92% and 88% germination and a 1138 and 1178 vigor index, respectively, compared to the control with 84% germination and a 746 vigor index (Fig. 1).

3.2. Effect of PGPR formulations on growth promotion of pearl millet under greenhouse and field conditions

In general, all the formulations tested increased growth parameters under both greenhouse and field conditions. However, the degree-of-growth promotion varied among formulations and their mode of application. All the formulations when applied as seed treatment, except for LS255, enhanced the height of the plants when compared to the nontreated control. Specifically, LS256 and LS257 were found to elicit the most growth promotion and showed consistent results under both greenhouse (Table 1) and field conditions (Table 2) with all modes of application. Plant height was the greatest with LS257, the plants being 30 cm taller than the nontreated control, under field conditions. Fresh weight of the plants was enhanced by all the formulations and modes of applications. However fresh weight of the plants due to seed treatment with formulation LS255 did not differ significantly form the

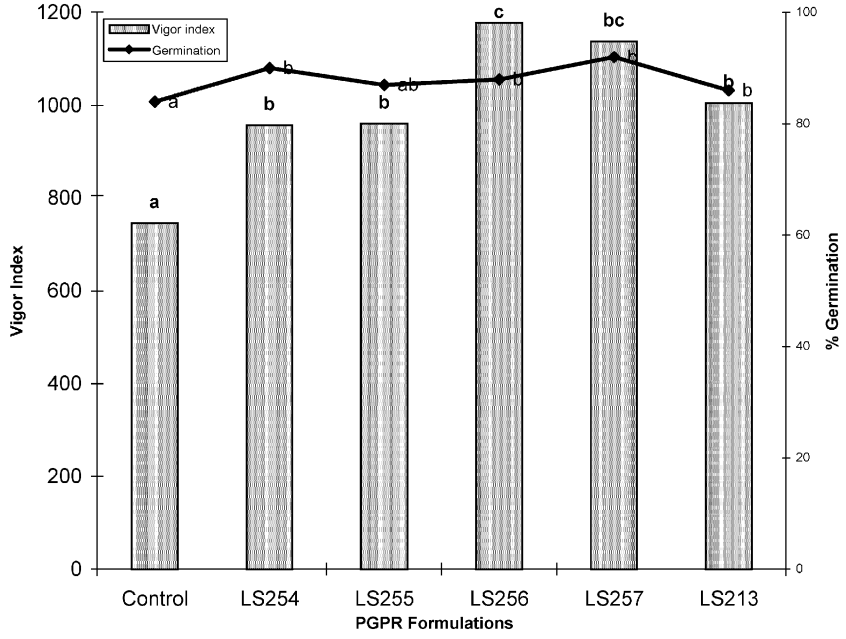

Fig. 1. Effect of seed treatment with PGPR formulations on seed germination and vigor index of seedlings of pearl millet 7 days after seeding under in vitro conditions. Percentage of seed germination and vigor index is mean from three repeated experiments. Vigor index was calculated based on percentage germination and mean root and shoot lengths of the seedlings.

Table 1
Effect of treatment with PGPR formulation on vegetative growth of pearl millet seedlings 30 days after seeding under greenhouse conditions

Treatment[a]	Height (cm)[b]	Fresh eight/seedling (g)[b]	Dry weight/seedling (g)[b]	Leaf surface area (cm^2)[b]	No. of basal tillers/ seedling[b]
Nontreated control	24.7a	7.13a	2.6a	30.5a	2.8a
Seed treatment[a]					
LS254	31.2cd	9.5ab	3.4ab	36.9ab	3.2ab
LS255	27.2ab	9.9bc	3.3ab	36.3ab	3.2ab
LS256	33.4de	11.7bcdef	3.6bcd	35.9ab	3.5ab
LS257	34.8e	12.2cdef	3.8bcd	36.2ab	3.6ab
LS213	29.2bc	10.8bcd	3.2ab	36.5ab	3.2ab
Soil amendment[c]					
LS254	35.3e	11.2bcde	3.5abcd	37.2ab	3.2ab
LS255	28.6b	10.1bc	3.6bcd	36.8ab	3.2ab
LS256	38.1f	12.9def	4.0bcd	35.7ab	3.5ab
LS257	39.1f	13.5ef	4.4d	39.9b	3.6ab
LS213	33.3de	11.4bcdef	3.4ab	37.0ab	3.2ab
Seed treatment + soil amendment					
LS254	34.9e	11.6bcdef	3.4abc	35.8ab	3.6ab
LS255	31.0cd	10.6bcd	3.3ab	38.5b	3.5ab
LS256	38.3f	13.0def	3.9bcd	41.8b	3.4ab
LS257	38.1f	13.7f	4.3cd	40.2b	4.0b
LS213	34.6e	11.7bcdef	3.9bcd	35.9ab	3.2ab

Means with the same letters within a column are not significantly different according to Tukey's HSD test at $P = 0.05$.
[a] Each PGPR applied onto surface gum arabic coated seeds by rolling the seeds in the formulations. (0.5 g/1 g of seeds).
[b] Mean of four repeated experiments with 50 plants per treatment in each experiment.
[c] Each PGPR formulation was mixed with the soil in the ratio 1:40 (v/v).

control. Fresh and dry weight and leaf surface area were significantly enhanced by formulations LS256 and LS257 irrespective of the application modes tested. Among the five formulations tested, LS255 showed the lowest growth promotion effect in comparison with the other formulations except for fresh weight due to seed treatment and leaf area due to combination treatment. Numbers of tillers was significantly enhanced by LS257 when treated as combination of seed treatment + soil amendment. The days required by plants to reach 50% flowering was significantly advanced by four days with seed treatment and by five days with soil amendment and seed treatment + soil amendment treatment. Under field conditions the reproductive parameters of the number of tillers, number of earheads, length and girth of earheads, 1000 seed weight and yield were significantly increased by all the formulations compared with the nontreated control when treated as soil amendment and seed treatment + soil amendment. Application as soil amendment resulted in better reproductive parameters in the plants compared with seed treatment. However, no significant difference was found among plants receiving the soil amendment and combination of seed treatment and soil amendment. The reproductive parameters of plants treated with LS256 and LS257 were the best and remained consistent under greenhouse and field conditions for all methods of application (Table 2).

3.3. Screening of PGPR formulations for potential to elicit systemic protection against downy mildew under greenhouse and under field conditions

In general, all the PGPR formations tested protected pearl millet against downy mildew in greenhouse studies, but the degree of protection offered varied considerably with the formulations. Each treatment resulted in a significant reduction in the number of plants with downy mildew disease in comparison with the nontreated control. A visual assessment of treatments indicated a noticeable difference in growth promotion and disease incidence when compared to the nontreated control pots. The highest protection resulted with LS256 and LS257, which gave 54% and 56% protection when used as seed treatments (Fig. 2). The same formulations resulted in 59% and 62% protection when applied as soil amendments (Fig. 2). LS256 and LS257 resulted in 63% and 66% when applied as both seed treatment and soil amendments (Fig. 2). The lowest level of protection resulted with LS255, which showed 42%, 51%, and 55% protection when applied as seed treatment, soil amendment and seed treatment + soil amendment, respectively. Protection against downy mildew was more effective with use of soil amendment rather than seed treatment. Seed treatment + soil amendment was almost equal in protection to the soil amendment application.

Table 2
Effect of treatment with PGPR on reproductive growth parameters of pearl millet under field conditions

Treatment[a]	Height (cm)[b]	Leaf surface area (cm)[b]	Days to 50% flowering[c]	No. of earheads/plant[d]	Length of earhead (cm)[e]	Girth of earhead (cm)[f]	1000 seed weight (g)[g]	Yield (kg/ha)[h]
Nontreated control	91.8a	103.0a	49a	5.0a	8.5a	4.7a	5.6a	1225a
Seed treatment[a]								
LS254	108.5b	124.2bc	45b	6.1b	11.3bc	5.3b	6.7bc	1353b
LS255	111.0bc	122.6b	45b	6.5bcd	10.5b	5.3b	6.3abc	1429c
LS256	121.3cd	124.4bcd	45b	6.5bcd	12.3bcde	5.5b	6.5abc	1448c
LS257	122.5cde	128.6bcdef	45b	6.6bcde	12.4cde	5.5b	6.5abc	1477c
LS213	104.0b	123.4b	45b	6.2b	10.9bc	5.3b	6.0ab	1436c
Soil amendment[i]								
LS254	121.8cd	123.3b	44b	7.1defg	12.4bcde	5.4b	7.1c	1534d
LS255	121.7cd	129.4bcdefg	44b	6.6bcde	11.5bcd	5.6b	6.8bc	1528d
LS256	134.3efg	130.6cdefg	44b	7.5fg	13.3de	5.4b	7.0c	1554de
LS257	134.6efg	135.6g	44b	7.8g	13.7e	5.6b	7.0c	1585e
LS213	110.8bc	127.0bcde	45bc	6.3bc	11.6bcd	5.5b	6.9bc	1535d
Seed treatment + soil amendment								
LS254	128.6defg	132.0efg	44b	7.3efg	12.4cde	5.3b	6.9bc	1530d
LS255	124.0de	128.0bcdef	44b	7.0cdef	12.6cde	5.5b	6.9bc	1532d
LS256	137.0fg	136.5g	44b	7.5fg	13.6e	5.6b	7.0c	1552de
LS257	138.7g	135.2fg	44b	7.7fg	13.8e	5.6b	7.2c	1587e
LS213	126.1def	131.2defg	44b	6.5bcd	12.3bcde	5.1b	7.0c	1539de

[a] Each PGPR applied onto surface gum arabic coated seeds by rolling the seeds in the formulations. (0.5 g/1 g of seeds).
[b] Mean of two repeated experiments with 50 plants per replication and four replications of each treatment.
[c] Number of days taken by 50% of total number of plants in each replicate for flowering.
[d] Number of earheads produced by the main axis and the basal tillers of the plant.
[e] As measured from the base to the tip of the earhead using a measuring tape.
[f] Measured as the circumference of the earhead at the center using a measuring tape.
[g] Calculated by weighing 100 seeds in 8 replicates (as per the ISTA procedure, 1993).
[h] based on the weight of seeds collected from four replicates and converting it into one hectare as per the procedure of Williams and Singh (1981).
[i] Each PGPR formulation was applied in the furrow at 10 g/5 m row.
Means with the same letters within a column are not significantly different according to Tukey's HSD Test at $P = 0.05$.

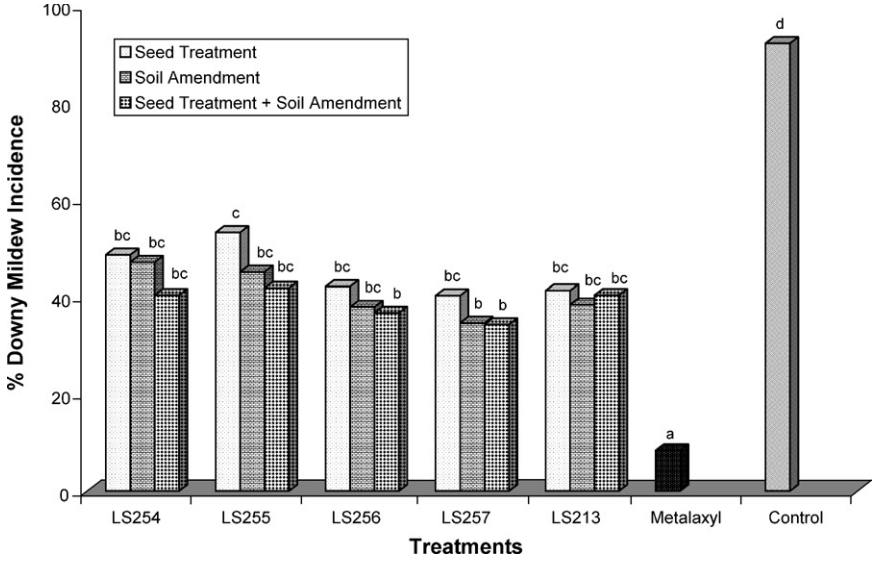

Fig. 2. Effect of treatments with PGPR formulations by seed treatment, soil amendment and seed treatment + soil amendment against pearl millet downy mildew under greenhouse conditions. Percentage of downy mildew is mean from two repeated experiments. Means designated with the same letter are not significantly different at $P = 0.05$.

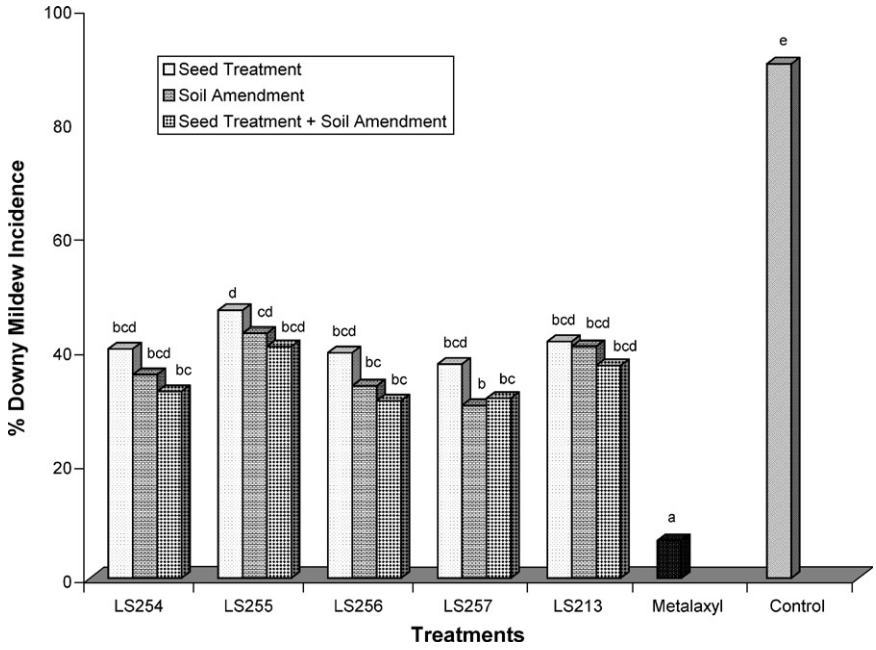

Fig. 3. Effect of treatments with PGPR formulations by seed treatment, soil amendment and seed treatment + soil amendment against pearl millet downy mildew under field conditions. Percentage of downy mildew is mean from two repeated experiments. Means designated with the same letter are not significantly different at $P = 0.05$.

Under, field plot conditions, significant reduction of downy mildew disease was observed in the test rows treated with PGPR formulations compared to the nontreated control rows. As in the greenhouse experiments, LS256, LS257, and LS254 proved very efficient in reducing downy mildew disease incidence (Fig. 3). Both LS256 and LS257 resulted in 65% protection, which was the highest, when applied as seed treatment + soil amendment (Fig. 3). The lowest protection of 55% resulted with LS255 applied as seed treatment + soil amendment. The results also confirmed that the soil amendment application resulted in significantly better protection compared to seed the treatment application. There was no significant differences among soil amendment and seed treatment + soil amendment.

4. Discussion

The ability of PGPR in growth promotion and resistance induction in various crops is well documented (van Loon et al., 1998; Barka et al., 2000; Burdman et al., 2000; Ramamoorthy et al., 2001). The results reported here corroborate earlier studies and indicate a future possibility that PGPR formulations can be used to promote growth and health of crop plants. Treatments with rhizobacterial formulations significantly enhanced the growth of pearl millet plants and also reduced the percentage of downy mildew incidence. These formulations have also promoted growth and also induced resistance against various pathogens in different plants like cucumber, watermelon, squash, ornamentals, vegetables, pepper, tobacco and tree species like loblolly pine, and lodge pine Douglas fir. (Reddy et al., 1999; Kenney et al., 1999; Kloepper et al., 1999; Martinez-Ochoa et al., 1999; Ryu et al., 1999; Yan et al., 1999; Zhang et al., 1999). However, this is the first report that demonstrates the efficiency of such formulations inducing resistance against an obligate pathogen, particularly in a cereal crop.

Our results suggest that PGPR formulation can be used practically in production of pearl millet in the tropics and subtropics where downy mildew disease is a major threat. The practical applications of these formulations were supported by the magnitude of growth promotion recorded by these treatments which was highly significant in comparison with the nontreated control. The most important result was the considerable increase in yield of pearl millet. Another important result was the advancement of flowering date by 4–5 days. Disease protection offered by these formulations with different modes of treatment ranged from 54% to 66% in comparison with the control under greenhouse conditions. Similarly these formulations offered protection up to 65% control under field conditions.

In the present study, five commercial PGPR formulations tested showed their capacity to enhance growth parameters and also to suppress downy mildew disease in pearl millet, under both greenhouse and field conditions and also with all the application methods tested. Earlier studies on PGPR have also reported that rhizobacteria are potential growth enhancers in different

crops like potato, pearl millet, and sorghum, (Lazarovitz and Nowak, 1997; Umesha et al., 1998; Raju et al., 1999). In the present study, all the formulations tested showed their efficacy in enhancing germination and vigor of pearl millet seedlings. The treated plants showed advanced emergence of seedlings in comparison to the control. In general, all the formulations showed a significant enhancement of growth and reproductive parameters such as height, fresh and dry weight, leaf area, number of tillers under greenhouse conditions and number of earheads, length and girth of earheads, and 1000 seed weight and yield of pearl millet under field conditions.

Among the three methods of application tested, seed treatment with the formulations was less effective in growth promotion. The soil amendment method was more effective than seed treatment. Furthermore, the combination of seed treatment + soil amendment performed similarly to soil amendment alone, thus supporting the observation that the soil amendment method was more effective than seed treatment. These results were true for both greenhouse and field conditions.

The ratings of disease incidence among the formulation-treated plants and the control plants were significantly different in that the treated lines showed fewer diseased plants. In general, all the formulations offered protection against downy mildew disease but to varied degrees. Two of the formulations LS256 and LS257, resulted in highly significant suppression of downy mildew disease when compared to the other formulations and to the control. These two formulations consistently elicited protection both under greenhouse and field conditions and with different methods of their application. These two formulations showed equivalent growth promoting capacity as well. However, no formulation was equal to the effectiveness of systemic fungicide metalaxyl (Apron 35SD) in managing downy mildew as it offered the highest protection under both greenhouse and field conditions.

The mechanisms by which PGPR formulations treatment reduced the disease was not determined in the present study, but induction of systemic resistance may have been the cause. This conclusion is based on the in vitro evaluation of the formulations against the zoospores of *S. graminicola,* which did not show any fungitoxic effect (data not shown), and also on the available literature, which suggests the mechanisms of action of these PGPR. Earlier work suggests that some PGPR strains may activate host defense systems however, evidence supporting the conclusion that PGPR which remain on plant roots can induce resistance in plants to foliar or systemic pathogens was published independently for different pathosystems: cucumber and anthracnose (Wei et al., 1991); and bean and halo blight (Alstrom, 1991). Another observation was the extensive rooting of the treated plants in comparison to the control. The rooting may have a role in growth promotion and resistance development. The possible mechanisms could be the larger and healthier root system leading to improved uptake of water and nutrients. Production of phytohormones and enhanced root growth accompanied by increased branching and a higher number of root tips that synthesize cytokinins. Prolonged synthesis of these phytohormones that may be regarded as a cause of improved yields. Culture filtrates of *Bacillus* species have been found to contain zeatin and zeatin riboside, which act as resistance inducers (Steiner, 1990; Kilian et al., 2000).

The beneficial effects produced by these formulations may also be attributed to the carrier material chitosan, which might have played a positive role. Chitosan, a growth promoter, is comprised of bioactive oligosaccharides known for their inhibitory effect on the growth of various fungi (Leuba and Stossel, 1986) and their capacity to be potent elicitors of plant defense reactions (Hadwiger et al., 1988). Recently, chitosan was reported to induce resistance to *Fusarium oxysporum* in susceptible tomato plants when applied as root dressing, foliar spray, seed dressing and soil amendment (Benhamou and Theriault, 1992; Benhamou et al., 1994; Lafontaine and Benhamou, 1996).

Similar formulations have long been used in various countries. In 1985, Gustafson, Inc. (Plano, Texas) introduced the first commercial rhizobacterial biological control product in the US The product contained the *B. subtilis* A-13 strain (Broadbent et al., 1977) and related strains GBO3 and GBO7 (sold under the trade names Quantum, Kodiak and Epic, respectively). In China, PGPR have been in commercial development for over 20 years and are referred to as yield increasing bacteria (YIB) (Tang, 1994) that are applied over 20 million hectares of crops (Chen et al., 1996; Kilian et al., 2000).

These formulations produce multiple beneficial effects, are easy to handle and, most importantly, they are chemical-free. However, cost-effectiveness has to be worked out and if found feasible then these PGPR formulations may effectively integrated into a downy mildew control program.

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