Asian Journal of Plant Pathology

ISSN 1819-1541

Academic Journals Inc.

www.academicjournals.com

Asian Journal of Plant Pathology 1 (1): 1-11, 2007
ISSN 1819-1541

Endophytic Bacteria from Different Plant Origin Enhance Growth and Induce Downy Mildew Resistance in Pearl Millet

Chandrashekhara, Sathyanarayana Niranjanraj, Saligrama A. Deepak,
Kestur N. Amruthesh, Nandini P. Shetty and Hunthrike S. Shetty
Department of Studies in Applied Botany, Seed Pathology and Biotechnology,
Downy Mildew Research Laboratory, University of Mysore,
Manasagangotri Mysore-570 006, Karnataka, India

Abstract: Five medicinal plants, two agricultural crops grown in different agroclimatic conditions and one weed plant were tested for the presence of endophytic bacteria in their root and stem tissues. The plants tested were *Cymbopogon citratus*, *Azadirachta indica*, *Phyllanthus emblica*, *Boerhaavia diffusa*, *Boerhaavia repens*, *Pisum sativum*, *Sorghum bicolor* and *Parthenium hysterophorus*. Sixty different endophytic bacterial isolates belonging to different genera were isolated. Growth promotion ability of these endophytic bacteria was tested on seed germination, seedling vigor and biomass of pearl millet crop. The result indicated that, among sixty isolates tested, ten endophytic bacteria showed significant growth promoting effects on the pearl millet crop plants tested. The same endophytes when coated to seeds of pearl millet, the resulting seedlings showed resistance against downy mildew disease caused by *Sclerospora graminicola*, an oomycetes pathogen. Apron 35 SD and Benzothiadiazole (BTH) seed treatments were compared with endophytic bacterial treatments. Among the different endophytic bacteria, *Pseudomonas fluorescens* ISR 34 and *Bacillus* sp. ISR 37 showed highest protection of 68 and 63%, respectively. Experimental evidences have been provided to show that resistance offered in pearl millet against downy mildew disease is by induced systemic resistance phenomenon.

Key words: Endophytic bacteria, growth promotion, pearl millet, downy mildew

INTRODUCTION

Endophytic microorganisms are ubiquitous in plants and colonize internal tissues without causing any substantive harm or gaining benefit other than securing residency (Kado, 1992; Bell *et al*., 1995; McInroy and Kloepper, 1995). They have been found in numerous plant species with most being members of common soil bacterial genera such as *Pseudomona*s, *Bacillu*s and *Azospirillum* (Chanway, 1998). Several reports indicated that endophytic bacteria are involved in promotion of plant growth (Chanway, 1998; Hallmann, 2001; Gutierrez-Zamora and Martinez-Romero, 2001; Roncato-Maccari *et al*., 2003a,b), accumulation of pathogenesis related proteins, deposition of cell wall barriers, (Benhamou *et al*., 1996), inhibit growth of pathogens by producing antimicrobial compounds like siderophores, (Chen *et al*., 1995). The capability of colonizing internal host tissues of plant has made endophytic bacteria valuable for agriculture as a tool to improve crop performance especially for those bacteria having commercial features such as plant growth promotion and activation of plant defense mechanisms (Hallmann, 2001). Several studies have indicated that treatment with selected

Corresponding Author: H.S. Shetty, Department of Studies in Applied Botany, Seed Pathology and Biotechnology, Downy Mildew Research Laboratory, University of Mysore, Manasagangotri, Mysore-570 006, Karnataka, India Tel: +91-0821-2515126

endophytic bacteria could induce resistance against vascular pathogens and also enhance plant growth promotion (Chen *et al*., 1995; Tuzun and Kloepper, 1995; Hallmann *et al*., 1997; Benhamou *et al*., 1998; Manjula *et al*., 2002).

In the present investigation, pearl millet [*Pennisetum glaucum* (L.) R.Br.] crop was considered for studies because of its increasing importance as a food and forage crop worldwide. The research efforts are intensified to meet the production constraints originating from biotic and abiotic stresses. One such major biotic constraint is the downy mildew disease caused by *Sclerospora graminicola* (Sacc.) Schroeter, which gains prime importance due to limited host resistance and is presently managed by metalaxyl fungicides (Singh and Shetty, 1990). The use of agrochemicals, although controls the attack of phytopathogens, but pose a high risk to human health, development of resistance strains, environmental pollution as well as economical feasibility. The ecofriendly approaches such as biological control and host resistance induction have gained much attention in the past decades as a way of reducing the use of chemical products in agriculture. Hence, the efficiency of endophytic bacteria from different plant origin were tested to promote growth and to control downy mildew disease in pearlmillet.

MATERIALS AND METHODS

Isolation of Endophytes

Endophytic bacteria were isolated from stem and root regions of selected plants. The plant parts were washed thoroughly in running tap water and surface sterilized with sodium hypochlorite (2%) containing 0.1% Tween 20 for 10 and 60 sec for stems and roots, respectively. The disinfectant was removed by rinsing five times each in two washes of sterile distilled water and finally in sterile water and plant parts were dried on sterile paper towels (Hallmann *et al*., 1997; Zinniel *et al*., 2002) dissected into 1 cm pieces and then pressed onto nutrient agar as a disinfestations control. Roots and stems were macerated with sterile mortar and pestle. Tissue extracts were then serially diluted in 12.5 mM potassium phosphate buffer (pH 7.0) and plated in triplicate to recover any bacterial endophytes present in the plant tissue. For further experiments different isolates of endophytic bacteria was selected only those samples for which the disinfestations controls lacked any bacterial growth after incubation at 28°C for 24-48 h. Sixty isolates were recovered and reisolated and subjected to gram staining and were used for seed germination and seedling vigor test. Bacteria isolated were further identified after greenhouse experiments based on morphology characters such as color, form, elevation, margin, diameter, surface, opacity, texture, endospore and size and biochemical tests like catalase test, KOH solubility test and oxidase test (Sneath *et al*., 1986) (Table 1). Bacteria were stored at -40°C until further experiments.

Host and Pathogen

Pearl millet seeds cv. 7042S susceptible to downy mildew were obtained from All India Co-ordinated Pearl Millet Improvement Project (AICPMIP) and used for the studies.

Table 1: Endophytic bacteria recovered from root and stem regions of different plant species

	Gram reaction	
Plant species	Gram positive	Gram negative
Cymbopogon citratus	04	04
Azadirachta indica	02	05
Phyllanthus emblica	03	02
Boerhaavia diffusa	02	03
Boerhaavia repens	01	04
Pisum sativum	04	07
Sorghum bicolor	04	06
Parthenium hysterophorus	04	05
Total	24	36

Sclerospora graminicola isolated and maintained on pearl millet under greenhouse conditions at the Department of Studies in Applied Botany and Biotechnology, University of Mysore, Mysore, India was used for all experiments.

Growth of Bacteria/Seed Treatment

Bacterial cultures were grown in nutrient broth for 48 h to obtain spore\cells for use as seed treatment. Bacterial suspensions were centrifuged at 6,000 revolutions per min (rpm) for 5 min and the pellet obtained was resuspended in sterile distilled water. The optical density of the suspension was spectrophotometrically and at 610 nm (Hitachi U-2000, Japan) and adjusted to obtain density of 1×10^8 cfu mL^{-1} (Niranjanraj *et al*., 2003; Umesha *et al*., 1998). Seeds of pearl millet were surface sterilized with 2% sodium hypochlorite (2 min) and washed in sterile distilled water, dried on sterile blotter paper and soaked in the bacterial suspension (1×10^8 cfu mL^{-1}) at 25±2°C for 6 h at 150 rpm in rotary shaker. Seeds treated with sterile distilled water served as control. Seeds treated with 0.75% BTH (Benzothiadiazole) and Apron 35SD at the rate of 6 g kg^{-1} seeds served as positive controls for greenhouse and field screening.

Effect of Seed Treatment with Endophytic Bacteria on Seed Germination and Seedling Vigor

Germination test was carried out according to International seed testing association (ISTA, 2003) and vigor index (VI = Mean Root Length + Mean Shoot Length × % Germination) was calculated at the end of 7 days (Abdulbaki and Anderson, 1973). Four replicates of 100 seeds were used per treatment. Seeds treated with endophytic bacteria (1×10^8 cfu mL^{-1}) were placed on moist germination paper and incubated at 26±2°C. Seeds treated with sterile distilled water served as control.

Effect of Seed Treatment with Endophytic Bacteria on Growth Parameters of Pearl Millet

Seeds treated with endophytic bacterial isolates as well as sterile distilled water were sown to clay pots containing a sterilized mixture of 5 kg of sand, soil and manure (1:2:1) and maintained under green house conditions for vegetative growth parameter studies. Vegetative growth parameters such as height, fresh weight, dry weight and number of basal tillers per plant were recorded after 30 days of growth. The fresh weights of the plants were determined by weighing the individual plants immediately after harvesting. Dry weight was estimated after drying the plants at 65°C in an oven for 12 h. The treated pearl millet plants were also observed for (a) Total number of productive tillers (b) Number of days required to 50% flowering (c) height of the plant during flowering (d) Length and girth of the ear head and (e) 1000 seed weight and in comparison with untreated plants.

Effect of Seed Treatment with Endophytic Bacteria on Downy Mildew Disease Incidence under Green House Conditions

For inoculum preparation, leaves of infected pearl millet plants were collected in the evening hours and washed in running tap water to remove fungal spores which was then blot dried and placed in a moist chamber for sporulation. Fresh sporangia were collected the next morning and zoospores released by them were adjusted to 4×10^4 zoospores mL^{-1} and used as inoculum. Seeds of pearl millet cv. 7042S were treated with endophytic bacterial isolates, BTH, Apron 35 and sterile distilled water for 6 h and sown into clay pots as explained above and maintained under green house conditions. Coleoptiles of two-day old seedlings were inoculated with a suspension with 4×10^4 zoospores mL^{-1} of *S. graminicola* by whorl inoculation method (Singh and Gopinath, 1985). Each treatment consisted of 25 plants (per treatment) in four replications.

Demonstration of Induced Resistance by Time Gap Studies in Pearl Millet

Seeds treated with endophytic bacterial isolates, BTH (benzothiadiazole) and sterile distilled water for 6 h were sown into clay pots as explained above and maintained under greenhouse conditions.

Coleoptiles of two-day-old seedlings were inoculated with a suspension of 4×10^4 zoospores mL^{-1} of *S. graminicola* as described above. A time gap of 1, 2, 3, 4 and 5 days was maintained between the endophytic bacteria treatment and inoculation with the pathogen *S. graminicola*. Each treatment consisted of 25 plants in four replicates.

Effect of Seed Treatment with Endophytic Bacteria on Downy Mildew Disease Incidence under Field Conditions

Field trial was carried out by seed treatment with different endophytic bacteria and BTH in the pearl millet experimental plot of the Department of Applied Botany and Biotechnology, University of Mysore, Karnataka, India. Apron 35SD was used for comparing the performance of all treatments along with sterile distilled water control (Williams *et al*., 1981). The plot size was 10×5 m and recommended normal agronomic practices were followed during the trial. Plant-to-plant distance of 15 cm and row-to-row distance of 45 cm was maintained. The experiment was designed as a random block design with four replicates.

Disease Assessment

After seven days, the plants were observed daily for expression of downy mildew disease. Plants were rated diseased when they showed typical symptoms of downy mildew, i.e., sporulation, stunting, chlorosis and green ear (Shetty *et al*., 1995). The disease incidence was recorded at 30 and 60 days after sowing.

Data Analysis

All the experimental results were suscitated to Drican's Multiple Range Test (DMRT). The means were compared for significance using DMRT ($p = 0.05$).

RESULTS

Isolation of Endophytic Bacteria

Five medicinal plants, two agricultural crops with two different varieties and one weed plant were tested for the presence of endophytic bacteria. The medicinal plants used were *C. citratus*, *A. indica*, *P.emblica*, *B. diffusa* and *B. repens*, two cultivars of *P. sativum* and *S. bicolor* and *P. hysterophorus*. *C. citratus* is a member of Graminae used for scented oil. *A. indica* is used for wide range of products such as pesticides and human health care. *P. emblica* is a rich source of antioxidants. *B. diffusa* and *B. repens* are used in herbal medicine to correct liver disorders. *P. sativum* and *S. bicolor* are important legume and cereal crop, respectively and harbor wide range of bacteria in root and stems. *P. hysterophorus* is a weed with plenty of allelopathic chemicals secreted in the rhizosphere.

Sixty endophytic bacteria were isolated from all the plant species tested. From each plant species bacteria isolated were different and they were identified based on gram staining and biochemical tests. Out of sixty isolates, ten isolates showed promising effects on pearl millet growth and were used for further experiments. *C. citratus* isolate was identified as *Bacillus* sp. ISR 38, *A. indica* as *P. fluorescens* ISR 36, *P. emblica* as *Bacillus* sp. ISR 42, *B. diffusa* as *Bacillus* sp. ISR 40 and *B. repens* as *P. fluorescens* ISR 34, was observed. In *P. sativum* revealed *Bacillus* sp. ISR 39 and *P. fluorescens* ISR 33. *S. bicolor* revealed *P. fluorescens* ISR 35 and *Bacillus* sp. ISR 41, respectively. *Bacillus* sp. ISR 37 was isolated from *P. hysterophorus*.

Effect of Seed Treatment with Endophytic Bacteria on Seed Germination and Seedling Vigor

In general, all the endophytic bacterial isolates significantly enhanced the seed germination and seedling vigor of pearl millet. However, the degree of enhancement varied between the bacterial

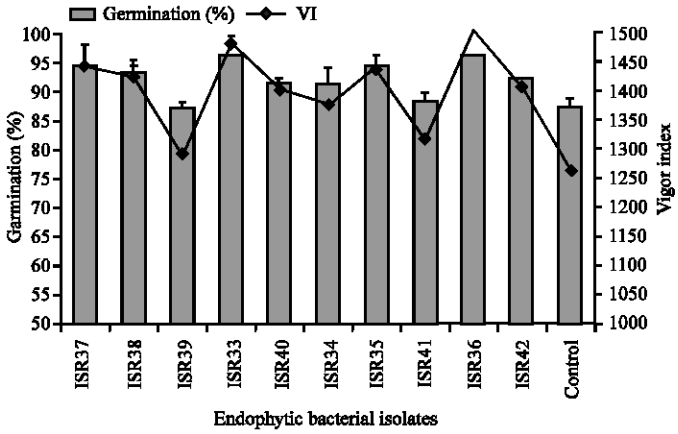

Fig. 1: Effect of seed treatment with promising endophytic bacteria on seed germination and seedling vigor of pearl millet

treatment. The highest germination percentage and vigor index was recorded for the *Pseudomonas* isolates. *P. fluorescens* ISR 33 and *P. fluorescens* ISR 36 achieved 96% germination followed by *Bacillus* sp. ISR 37 and *P. fluorescens* ISR 35 with 94% germination, which was significantly higher when compared with the control (87.5%). The lowest percent germination of 87 was observed in *Bacillus* sp. ISR 39 (Fig. 1). The similar trend was also noticed for the seedling vigor in which all the bacterial isolates enhanced the vigor index to varied degrees. The highest seedling vigor 1507 was recorded for the treatment with *P. fluorescens* ISR36, followed by *P. fluorescens* ISR33, *Bacillus* sp. ISR37, ISR35, ISR 38, ISR 42, ISR 40, ISR34, ISR41 and ISR39 ranged from 1482-1292 while control showed 1263.

Effect of Seed Treatment with Endophytic Bacteria on Growth Parameters of Pearl Millet

All the tested endophytic bacterial isolates were significant in promoting the vegetative growth parameters of pearl millet plants. The vegetative growth parameters such as height of the plant, fresh weight, dry weight and number of basal tillers were significantly increased over control due to seed treatment with endophytic bacteria. Maximum height of 42 cm was recorded in plants treated with *P. fluorescens* ISR 36 compared to 31 cm in control. The *Bacillus* isolates enhanced the plant height significantly, which ranged from 35-40 cm. *P. fluorescens* ISR36 and *Bacillus* sp. ISR 37 recorded the maximum fresh weight of 17 g and 16.8 g, respectively while control plants showed fresh weight of 10 g. Similar trend in improvement was also noticed for dry weight. Further, increase in the number of tillers per plant was evident in all endophytic bacterial treatments. *P. fluorescens* ISR 36 and *Bacillus* sp. ISR 37 recorded maximum of 4.5 tillers in comparison to 2.0 tillers in the control (Table 2).

Similar to the vegetative growth parameters offered by the endophytic bacterial isolates were also enhancing the reproductive growth parameters such as 50% flowering, length and girth of ear heads and 1000 seed weight, plant height, tillering of pearl millet plants (Table 3). The maximum improvement of all reproductive traits was noticed in plants raised from seeds treatment with *P. fluorescens* ISR 36 subsequently, ISR 35, ISR 37, ISR 38 isolates. All the endophytic bacterial isolates reduced the 3-4 days following by compared to untreated control plants.

Table 2: Effect of seed treatment with promising endophytic bacteria on vegetative growth parameters of pearl millet plants cv.7042S

Endophytic bacterial isolates number	Height (cms) of plants	Fresh weight (g) (average per plant)	Dry weight (g) (average per plant)	Number of basal tillers (average per plant)
ISR37	40.5^{ab}	16.75^{a}	6.15^{a}	4.5^{a}
ISR38	38.0^{bc}	16.20^{a}	5.80^{abc}	4.0^{ab}
ISR39	36.0^{c}	12.45^{c}	4.70^{ef}	3.0^{bcd}
ISR33	40.0^{ab}	16.40^{a}	6.05^{a}	4.5^{a}
ISR40	40.0^{ab}	16.10^{a}	6.00^{ab}	4.0^{ab}
ISR34	35.5^{c}	14.80^{b}	5.50^{cd}	2.5^{cd}
ISR35	37.5^{bc}	14.60^{b}	5.55^{bcd}	3.0^{bcd}
ISR41	35.0^{c}	11.70^{c}	4.25^{fg}	2.5^{cd}
ISR36	42.0^{a}	17.00^{a}	6.30^{a}	4.5^{a}
ISR42	35.5^{c}	14.10^{b}	5.15^{de}	3.5^{abc}
SDW control	31.0^{d}	9.95^{d}	4.00^{g}	2.0^{d}

Note: Results were taken 30 days after sowing and are based on the four replicates with 100 plants per treatment; Means followed by the same letter are not significantly different according to DMRT ($p \leq 0.05$); SDW-sterile distilled water

Table 3: Effect of seed treatment with promising endophytic bacteria on reproductive growth parameters of pearl millet cv.7042S

Endophytic bacterial isolates No.	Height (cm) of plants	No. of days required for flowering (50%)	Length of earhead/plant	Girth of earhead/plant	No. of basal tillers/plant	No. of nodal tillers/plant	1000 seed weight
ISR37	128.5^{ab}	40	11.5^{e}	4.9^{ab}	4.5^{a}	3.5	11.9^{c}
ISR38	126.2^{bc}	41	10.8^{de}	4.9^{b}	4.0^{ab}	3.0	11.4^{d}
ISR39	121.5^{de}	42	10.0^{f}	3.1^{fg}	3.0^{bcd}	2.5	10.9^{d}
ISR33	122.7^{de}	40	10.4^{ef}	4.6^{bc}	4.5^{a}	3.5	13.1^{b}
ISR40	123.5^{cd}	40	12.0^{ab}	4.1^{d}	4.0^{ab}	3.0	13.8^{a}
ISR34	126.5^{bc}	41	11.1^{cd}	3.3^{efg}	2.5^{cd}	2.5	10.2^{e}
ISR35	130.0^{a}	40	11.6^{bc}	3.5^{ef}	3.0^{bcd}	2.5	9.8^{e}
ISR41	119.2^{e}	41	10.8^{de}	3.0^{g}	2.5^{cd}	2.0	11.4^{d}
ISR36	131.2^{a}	40	12.3^{a}	5.4^{a}	4.5^{a}	4.0	14.0^{a}
ISR42	121.0^{de}	41	11.2^{cd}	4.8^{b}	3.5^{abc}	3.0	13.2^{b}
SDW control	112.0^{f}	45	9.3^{g}	3.6^{e}	2.0^{d}	2.0	8.6^{f}

Results were taken 60 days after sowing and based on two replicates with 50 plants per treatment; Means followed by the same letter are not significantly different according to DMRT ($p \leq 0.05$); SDW-Sterile distilled water

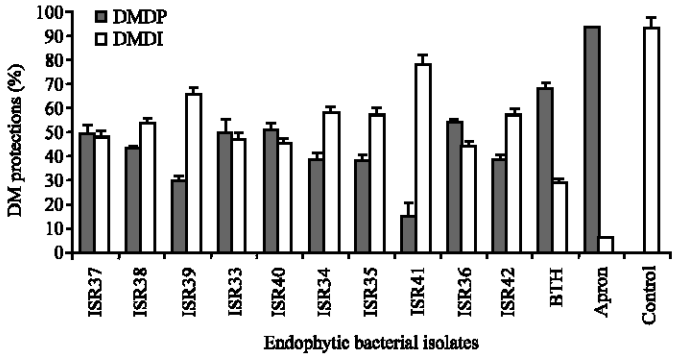

Fig. 2: Effect of seed treatment with endophytic bacteria on downy mildew protection under green house conditions (DMDI - Downy mildew disease incidence, DMDP- Downy mildew disease protection)

Effect of Seed Treatment with Endophytic Bacteria on Pearl Millet Downy Mildew Incidence Under Greenhouse Conditions

All the endophytic bacterial isolates were efficient in reducing the downy mildew incidence compare to control under greenhouse conditions. However, the degree of disease reduction varied with

the isolates tested ranging from 15 to 53%. Maximum protection of 53% was offered by *P. fluorescens* ISR36 followed by 51, 49.5, 49, 43% protection by *Bacillus* sp. ISR40, *P. fluorescens* ISR33, *Bacillus* sp. ISR37, *Bacillus* sp. ISR 38, respectively. However, none of the treatments were on par to BTH (68%) or Apron (92%) treatments in offering protection against downy mildew disease (Fig. 2).

Demonstration of Induced Resistance by Time Gap Studies in Pearl Millet

In order to test the nature of resistance offered by endophytic bacterial isolates, induction studies were conducted by treating seeds with the bacteria and inoculating with the pathogen at different time

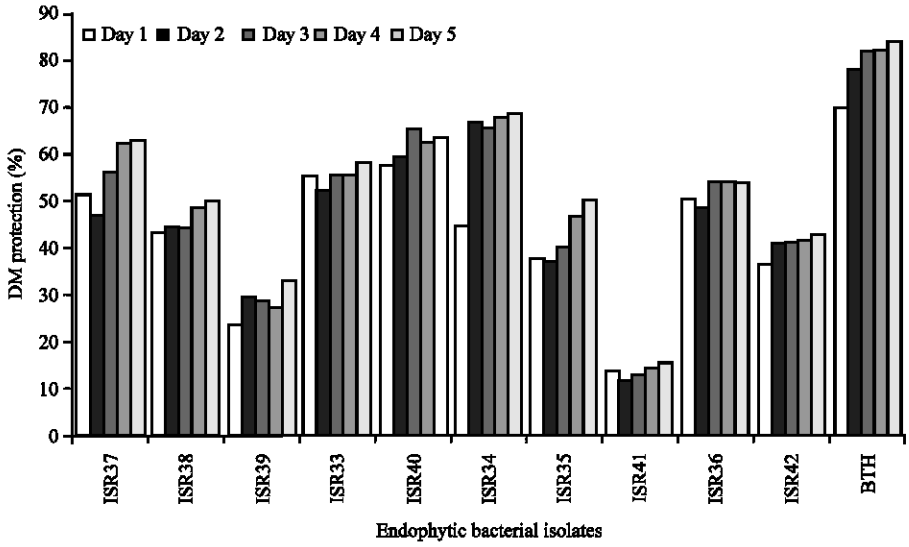

Fig. 3: Effect of seed treatment with endophytic bacterial isolates due to inoculation with *Sclerospora graminicola*, DM-Downy mildew

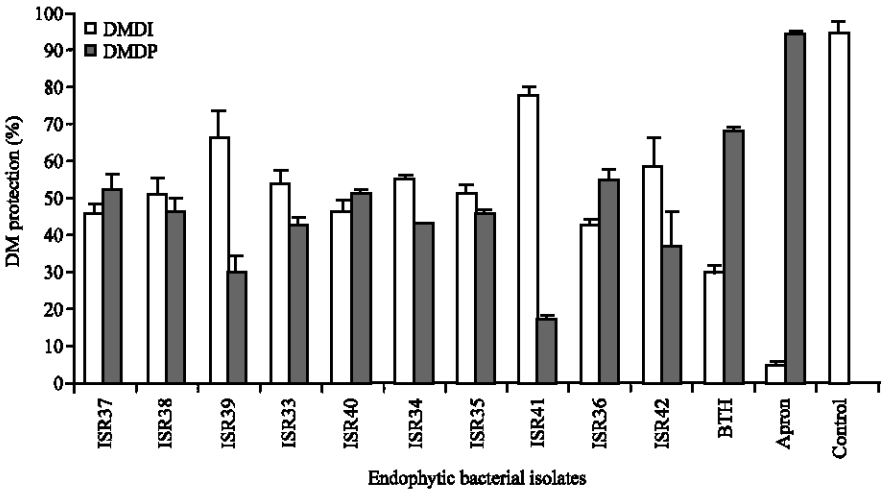

Fig. 4: Effect of seed treatment with endophytic bacteria on downy mildew disease protection under field condition (DMDI-Downy mildew disease incidence, DMDP-Downy mildew disease protection)

intervals. After seed treatment with endophytic bacteria, protection of 14-68% was observed, depending on the time interval between bacteria treatment and inoculation with pathogen. Maximum protection was achieved by the isolate *P. fluorescens* ISR 34, which recorded 68% protection on 4th and 5th day of time gap between seed treatment and pathogen inoculation (Fig. 3). On the first day this isolate recorded 44%, which was raised to 66 and 65% on the 2nd and 3rd day, respectively followed by *Bacillus* sp. ISR 40, ISR 37, *P. fluorescens* ISR 33, *P. fluorescens* ISR 36 showed 63, 58, 54%, respectively. The lowest protection was recorded by *Bacillus* sp. ISR 41, which recorded 14% protection. BTH, which was used as a positive control offered the highest protection of 83.5% in comparison to other treatments and the control.

Effect of Seed Treatment with Endophytic Bacteria on Pearl Millet Downy Mildew Incidence under Field Conditions

Under field conditions, all the endophytic bacterial isolates showed reduced downy mildew incidence, but the degree of protection varied between isolates. The protection offered due to seed treatment ranged from 18 to 55%. Maximum protection by *P. fluorescens* ISR36 followed by *Bacillus* sp. ISR37, ISR 40, ISR 38 with 52.5, 51 and 46.5%, respectively. The lowest protection was recorded for *Bacillus* sp. ISR41 with 18%. However, BTH or Apron provided even better protection, with 68 and 94%, respectively (Fig. 4).

DISCUSSION

Medicinal plants, agriculturally important crops and weed plants harbor different types of microorganisms on the surface as epiphytes or endophytes. Epiphytic microorganisms are associated on the surface of the plants may have beneficial or harmful effects to the plants. However, the endophytic microbes show beneficial effects to the harboring plants. The secondary metabolites in medicinal plants may be elicited due to the presence of endophytes.

Endophytic bacteria isolated in the present study have shown different beneficial effects to the pearl millet plants, which is grown as important cereal crop of the semi-arid tropics. Most of the endophytic bacteria isolated in the study belonged to *Bacillus* and *Pseudomonas* species. Results of the present study evidenced the fact that seed treatment with endophytic bacteria enhanced seed germination and seedling vigor of pearl millet. The germination percentage of 96% and seedling vigor of 1507 was offered by *P. fluorescens* ISR 36. There are no reports of the effect of endophytic bacteria on seed germination and seedling vigor. This study also showed the endophytic bacteria isolated from *A. indica* showed considerable influence on seed germination and seedling vigor enhancement. Similarly, seedling vigor enhancement has been noticed with seed treatment of pearl millet by different endophytic bacteria isolated from medicinal, agricultural and weed plants.

Seeds treated with endophytic bacteria showed increased fresh weight and dry weight of the plant, more number of tillers, early flowering and 1000 seed weight. Maximum plant biomass enhancement was offered by *P. fluorescens* ISR 36. There are a few reports on the increased plant biomass of different crop species such as oilseed rape, tomato, maize, sorghum, wheat and rice when endophytic bacteria used as seed and seedling treatments (Nejad and Johnson, 2000; Gutierrez-Zamora and Martinez-Romero, 2001; Roncato-Maccari *et al*., 2003a,b). This phenomenon has been attributed to microbial processes leading to nutrient solubilization by production of phosphorous and siderophores and plant growth hormones such as auxins, cytokinins, gibberlins, abscisic acid (Sturtz *et al*., 1997).

Significant reduction of downy mildew disease of 53 and 55% were recorded under greenhouse and field conditions, respectively due to seed treatment with endophytic bacteria. The protection offered by the endophytes due to seed treatment was tested for induction of resistance in the plant by

time gap studies between seed treatment and pathogen inoculation. In several case studies, an endophytic bacteria *Bacillus pumilus* SE34 against root rot causing fungus *Fusarium oxysporum* f. sp. *pisi* in *Pisum sativum* and increased resistance against *Fusarium oxysporum* f. sp. *lycopersici* in tomato has been recorded (Benhamou *et al*., 1996; M'Piga *et al*., 1997). Further, in rice against *Rhizoctonia solani* (Krishnamurthy and Gnanamanickam, 1997), cotton against *Verticillium dahliae* (Xia *et al*., 1996) and wilt diseases of oil seed rape and tomato have shown the pretreatment of endophytic bacteria has resulted in increased host defense responses (Nejad and Johnson, 2000). Although, the exact mechanism by which seed treatment with endophytic bacteria reduces disease incidence are not fully understood, it has been reported that endophytic bacteria are known to control plant pathogens by induction of resistance leading to the production of phytoalexins, accumulation of pathogenesis related proteins, deposition of structural barriers in the cell wall of the host plant and by production of antimicrobial compounds (Manjula *et al*., 2002).

Plant Growth Promoting Rhizobacteria (PGPR) have been well documented to improve the growth promotion and host resistance against the downy mildew pathogen in pearl millet (Niranjanraj *et al*., 2003). Endophytic bacteria are often closely related to PGPR/Plant Health Promoting Rhizobacteria (PHPR) and these have lately found to colonize the root internally also indicated modes of action described for PGPR/ PHPR also apply for endophytic bacteria. These endophytic bacteria induced resistance can last longer than PGPR, since they establish a much closer relationship with the host and on the other hand, PGPR may be inhibited by competition with other microorganisms on the root surface (Hallmann, 2001). The penetration of endophytic bacteria into the seeds or sprouting seedlings leading to defense elicitation is most likely the possible explanation.

Seed treatment is the only feasible technology in pearl millet due to economic constraints for crop production. Alternative is the exploitation of biotic and abiotic inducers, which have plant growth promoting effects and also induction of resistance against pests and diseases. The use of endophytes may be preferable to reduce the use of chemical fertilizers and pesticides because of cost and contribution to sustainable agricultural systems.

ACKNOWLEDGMENTS

This study has been carried out in the project on 'Systemic Acquired Resistance' funded by Danish International Development Agency under the Enhancement of Research Capacity Programme (DANIDA ENRECA). We are grateful to Dr. Eigil de Neergaard, the Principal Responsible Party of the DANIDA ENRECA project for his cooperation during the study. The facilities provided by Indian Council of Agricultural Research (ICAR), Government of India through All India Coordinated Pearl Millet Improvement Programme (AICPMIP) is also gratefully acknowledged.

REFERENCES

Abdulbaki, A.A. and J.D. Anderson, 1973. Vigour determination in soybean seed by multiple criteria. Crop Sci., 13: 630-633.

Bell, C.R., G.A. Dickie, W.L.G. Harvey and J.W.Y.F. Chan, 1995. Endophytic bacteria in grapevine. Can. J. Microbiol., 41: 46-53.

Benhamou, N., J.W. Kloepper, A. Quadt-Hallmann and S. Tuzun, 1996. Induction of defense related ultrastructural modifications in pea root tissues inoculated with endophytic bacteria. Plant Physiol., 112: 919-929.

Benhamou, N., J.W. Kloepper and S. Tuzun, 1998. Induction of resistance against *Fusarium* wilt of tomato by combination of chitosan with an endophytic bacterial strain: Ultrastructure and cytochemistry of the host response. Planta, 204: 153-168.

Chanway, C.P., 1998. Bacterial endophytes: Ecological and practical implications. Sydowia, 50: 149-170.

Chen, C., E.M. Bauske, G. Musson R. Rodrigues-Kabana and J.W. Kloepper, 1995. Biological control of *Fusarium* wilt on cotton by use of endophytic bacteria. Biol. Cont., 5: 83-91.

Gutierrez-Zamora, M.L. and E. Martinez-Romero, 2001. Natural endophytic association between *Rhizobium etli* and maize (*Zea mays* L.). J. Biotechnol., 91: 117-126.

Hallmann, J., A. Quadt-Hallmann, W.F. Mahaffee and J.W. Kloepper, 1997. Bacterial endophytes in agricultural crops. Can. J. Microbiol., 43: 895-914.

Hallmann, J., 2001. Plant Interactions with Endophytic Bacteria. Biotic Interactions in Plant-Pathogen Associations. Jeger, M.J. and N.J. Spence (Eds.), CAB International, pp: 87-119.

ISTA, 2003. International rules for seed testing. By: The International Seed Testing Association, P.O. Box 308, 8303 Basserdorf, CH-Switzerland.

Kado, C.I., 1992. Plant Pathogenic Bacteria. In: The Prokaryotes. Balows, A., H.G. Truper, M. Dworkin, W. Harder and K.H. Schleifer (Eds.), Springer-Verlag, New York, pp: 660-662.

Krishnamurthy, K. and S.S. Gnanamanickam, 1997. Biological control of sheath blight of rice: Induction of systemic resistance in rice by plant associated *Pseudomonas* sp. Curr. Sci., 72: 331-334.

M'Piga, P., R.R. Belanger, T.C. Paulitz and N. Benhamou, 1997. Increased resistance to *Fusarium oxysporum* f. sp. *radicis lycopeirsici* in tomato plants treated with endophytic bacterium *Pseudomonas fluorescens* strain 63-28. Physiol. Mol. Plant Pathol., 50: 301-320.

Manjula, K., S.D. Singh and K.G. Kishore, 2002. Role of endophytic bacteria in biological control of plant diseases. Ann. Rev. Plant Pathol., 1: 231-252.

McInroy, J.A. and J.W. Kloepper, 1995. Survey of indigenous bacterial endophytes from cotton and sweet corn. Plant Soil, 173: 337-342.

Nejad, P. and P.A. Johnson, 2000. Endophytic bacteria induce growth promotion and wilt disease suppression in oilseed rape and tomato. Biol. Control, 18: 208-215.

Niranjanraj, S., G. Chaluvarju, K.N. Amruthesh, H.S. Shetty, M.S. Reddy and J.W. Kloepper, 2003. Induction of growth promotion and resistance against downy mildew on pearl millet (*Pennisetum glacum*) by rhizobacteria. Plant Dis., 87: 380-384.

Roncato-Maccari, L.D.B., H.J.O. Ramos, F.O. Pedrosa, Y. Alquini, L.S. Chubatsu, M.G. Yates, L.U. Rigo, M.B.R. Steffens and E.M. Souza, 2003a. Root colonization, systemic spreading and contribution of *Herbaspirillum seropedicae* to growth of rice seedlings. Symbiosis, 35: 261-271.

Roncato-Maccari, L.D.B., H.J.O. Ramos, F.O. Pedrosa, Y. Alquini, L.S. Chubatsu, M.G. Yates, L.U. Rigo, M.B.R. Steffens and E.M. Souza, 2003b. Endophytic *Herbaspirillum seropedicae* expresses *nif* genes in gramineous plants. FEMS Microbiol. Ecol., 45: 39-47.

Shetty, S.A., H.S. Shetty and S.B. Mathur, 1995. Downy mildew of pearl millet. Technical Bulletin, Downy Mildew Research Laboratory. Department of Studies in Applied Botany, University of Mysore, Manasagangotri, Mysore, India.

Singh, S.D. and R.A. Gopinath, 1985. Seedling inoculation technique for detecting downy mildew resistance in pearl millet. Plant Dis., 69: 582-584.

Singh, S.D. and H.S. Shetty, 1990. Efficacy of systemic fungicide metalaxyl for the control of downy mildew (*Sclerospora graminicola*) of pearl millet (*Pennisetum glaucum*). Indian J. Agric. Sci., 60: 575-581.

Sneath, P.H.A., N.S. Mair, M.E. Sharpe and J.G. Holt, 1986. Endospore Forming Gram-Positive Rods and Cocci. In: Bergey's Manual of Systemic Bacteriology. Williams and Wilkins, USA., 2: 1104-1138.

Sturtz, A.V., B.R. Christie, B.G. Matheson and J. Nowak, 1997. Biodiversity of endophytic bacteria which colonize red clover nodules, roots, stems and foliage and their influence on host growth. Biol. Fert. Soils, 25: 13-19.

Tuzun, S. and J.W. Kloepper, 1995. Practical Application and Implementation of Induced Resistance. In: Induced Resistance to Disease in Plants. Hammershchmidt, R. and J. Kuc (Eds.), Kluwer Academic Publishers, Dordrecht, The Netherlands, pp: 152-168.

Umesha, S., S.M. Dharmesh, S.A. Shetty, M. Krishnappa and H.S. Shetty, 1998. Biocontrol of downy mildew disease of pearl millet using *Pseudomonas fluorescens*. Crop Prot., 17: 387-392.

Williams, R.J., S.D. Singh and M.N. Pawar, 1981. An improved field screening technique for downy mildew resistance in pearl millet. Plant Dis., 65: 239-241.

Xia, Z.J., B.K. Gu and A.M. Wu, 1996. Studies on induced resistance of cotton plants against *Verticillium dahliae* by endophytic and rhizosphere bacteria. Chin. J. Biol. Control, 12: 7-10.

Zinniel, D.K., P. Lambrecht, N.B. Harris, Z. Feng, D. Kucznarski, P. Highley, C.A. Ishimaru, A. Arunakumari, R.G. Barletta and A.K. Vidaver, 2002. Isolation of Characterization of Endophytic Colonizing Bacteria from Agronomic Crops and Prairie Plants. Applied Environ. Microbiol., pp: 2198-2208.