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Screening for plant growth promoting fungi and their ability for growth promotion and induction of resistance in pearl millet against downy mildew disease

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Abstract
A total of forty nine plant growth promoting fungi (PGPF) were successfully isolated from the rhizosphere of various grass species in Karnataka State, India. All the PGPF isolates were tested for their ability to enhance pearl millet seed quality parameters and to induce resistance against downy mildew disease in pearl millet. Susceptible pearl millet seeds 7042S were treated with PGPFs conidial suspension (1 x 10^8 cfu ml^{-1}) and barley grain inocula (BGI) at 5%, 10% and 20% concentrations. Only six isolates among the forty nine tested recorded significant ($P < 0.001$) enhancement of seed germination and vigor when compared with the untreated control. Of the PGPF, *Penicillium* sp. (UOM PGPF 27) at 5% (w/w) concentration recorded highest seed germination of 92% and 1701.9 seedling vigor. The *in planta* colonization of the six PGPF isolates determined successfully in re-isolating the fungus from the basal root segments of 6 cm and 4 cm plated on PDA plates and also from the rhizosphere serial dilution of 10^{-3} to 10^{-5}. Among the PGPFs tested in two modes, in BGI treatments, *Penicillium* sp. (UOM PGPF 27) at 5% (w/w) and *Pythium* sp. (UOM PGPF 41) at 10% (w/w) showed maximum disease protection of 67% and 61% respectively against downy mildew disease of pearl millet In case of conidial suspension treatments *Penicillium* sp. (UOM PGPF 27) and *Trichoderma* sp. (UOM PGPF 37) recorded highest disease protection of 71% and 66%, respectively under greenhouse conditions. Thus, the present study suggests that the tested PGPF, both as BGI inocula and conidial suspensions, can be used for pearl millet downy mildew disease management and also for plant growth.

Keywords: PGPF isolates; pearl millet; downy mildew disease; growth promotion, induced systemic resistance.

INTRODUCTION

Chemical fertilizers pose health hazard and affect the microbial population in soil by degrading the physical structure of the soil leading to lack of oxygen in the plant root zone besides being quite expensive and making the cost of production high. Whereas naturally, the majority of the microorganisms distributed around plant root surface have a role in the decomposition of organic matter and some may suppress deleterious microorganisms, which could inhibit plant growth. A few of the root-associated microorganisms can promote plant growth and they have been called “plant growth-promoting fungi” (PGPF) or “plant growth-promoting rhizobacteria” (PGPR). The PGPF and PGPR are known to suppress some plant diseases.

Plants have the ability to defend themselves against most microbial pathogen with a complex array of physical barriers and antimicrobial compounds, which are either performed or inducible. Most, if not all the plants studied in natural ecosystems are infested by fungi that cause no disease symptoms. The existence of induced resistance against a broad range of pathogens in host plants previously infected with a pathogen or non-pathogenic microorganisms is well documented [1]. As we all know biological control is an environment-friendly strategy to reduce crop damage caused by plant pathogens [2]. Biological control of soil-borne pathogens with antagonistic bacteria and fungi has been intensively investigated [3]. Rhizosphere-resident antagonistic microorganisms are ideal biocontrol agents, as the rhizosphere provides frontline defense for roots against infection by the pathogens [4]. Biocontrol research has gained considerable attention and appears promising as a viable alternative to chemical control strategies.

In the past few years, an increasing amount of research was devoted to the study of induced systemic resistance mechanisms. Non-necrotizing mutualistic rhizosphere microorganisms trigger resistance, the best studied of which are several species of plant growth promoting rhizobacteria (PGPR) [5]. While most studies have focused on the interaction between rhizobacteria and plant pathogens and little is known about the plant growth promoting fungi (PGPF) and molecular mechanisms of response of resistance offered by PGPF. The beneficial effects of certain rhizosphere fungi in terms of plant growth promotion and biological control has been reported by many researchers [6, 7, 8 and 9]. PGPF are non-pathogenic saprophytes and are reported to suppress fungal and bacterial diseases of a number of crop plants [10, 11 and 12]. Colonization of roots with PGPF can also lead to systemic resistance in distal parts of the plant [13 and 14]. Only a few studies of signaling pathways during PGPF-mediated induced systemic resistance, using *Trichoderma* sp. have been performed [15]. The PGPF *Phoma* sp., which generally does not sporulate under natural conditions, has been found to improve plant growth, suppress plant pathogens and induce systemic resistance [16].

The PGPF association with roots of various plant species and

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infection has also been shown to modulate growth, morphology, nitrogen assimilation, resource allocation and mineral uptake of the host plant and also improves host reproductive fitness by enhancing plant growth, increase biomass and grain yield of crop plants [17, 18, 19, 20 and 21]. The hosts include the cereal crops rice, wheat, maize, barley as well as *tobacco, bacopa, artemisia, parsley*, poplar including *Arabidopsis* [21, 22 and 23].

Pearl millet is one among the vital crops that feed poor people and provides basic sustainable living in the semi-arid regions of the globe [24]. It is usually cultivated as food, feed/fodder and fuel crop in regions that are too hot, too dry and/or have soil constraints that prevent economic production of other staple food grain crops [25]. Pearl millet accounts for 50% of the total millets in the world and India produces more than half of world's pearl millet in an area of 10 million hectares. 60% of the pearl millet produced in India is with hybrids and it seems to survive anything except Downy mildew.

Downy mildew results in harvest losses up to 80% amounting 800kg/hectare of cultivated land. Translated into economic terms the yield loss realized by the farmers is about rupees 2000-2500/hectare [26]. Considering high disease incidence could inflict irreversible damage if the disease hits epidemic properties and in India alone, the estimated annual yield loss value represents more than €11 million [27].

So far it is clear from the studies that many PGPF isolated from different rhizosphere soils have been used to induce disease resistance and to promote plant growth. No information is also available regarding the use of PGPF to enhance downy mildew disease resistance in pearl millet. So it is interesting to know that PGPF present in different rhizosphere soils of grasses which belong to Poaceae members as that of pearl millet and to use them against induction of downy mildew disease resistance and thus in the present study we have taken PGPF for the induction of downy mildew disease resistance in pearl millet against downy mildew disease.

MATERIALS AND METHODS

Seed material

Pearl millet seeds of highly susceptible (7042S) to downy mildew disease [*Sclerospora graminicola* (Sacc.) Schoreter] were obtained from ICRISAT, Hyderabad, India under material transfer agreement and were used throughout the study.

Source of pathogen and inoculum preparation

Sclerospora graminicola was isolated from pearl millet cv. 7042S grown under heavily infested field conditions. The pathogen was maintained on its susceptible host prior to use. Leaves of pearl millet showing profuse sporulation of *S. graminicola* on the abaxial side were collected in the evening hours from the plants maintained under greenhouse conditions. The collected leaves were thoroughly washed under running tap water to remove sporangia. The leaves were then blot dried, cut into smaller pieces and maintained in humidity chamber prepared by lining the interiors of Petri dishes 50 cm x 30 cm x 12 cm sizes with a wet double layer of blotting paper. These chambers were kept at 20^{o}C and >95% RH in the dark in an incubator for 6–7 h. Sporangia produced on the leaves were harvested into distilled water, the spore load was adjusted to 4 x 10^4 zoospores ml^{-1} using a Haemocytometer and used as a source of inoculum in greenhouse studies [28].

Isolation and identification of Plant Growth Promoting Fungi (PGPF)

Different PGPF isolates from the rhizosphere soils of many grass species (belonging to the Poaceae family) such as *Heteropogon contortus, H. oliganthus, Hackelochola granularis, Imperata cylindrical, Melanocenctrrus jaquemontii, Oropetium thomaecum, Aristida setacea, Aristida hysteric, Paspalidium flavidum and Panicum repeus* were collected from four districts of Karnataka state, India and screened for their effectiveness as inducers of resistance in pearl millet against downy mildew disease. All the PGPF were isolated by serial dilution method on potato dextrose agar (PDA) medium and incubated for 7 days at 25 °C. After 7 days of incubation, each individual fungal colony was picked from the edge with a sterile fine tipped inoculation needle and transferred on to the PDA medium. The fungi were identified based on the morphological, conidial, fruiting bodies and culture characters. Classification of the fungi was carried out based on the standard procedures. All the PGPF isolates were named and maintained in test tubes and Petri plates on PDA media and used for further studies.

Mass multiplication of Plant Growth Promoting Fungi

Mass multiplication of the isolated PGPF were carried out by two methods, which are as follows:-

Potato Dextrose Agar (PDA)

PGPF isolates were mass multiplied on PDA plates and incubated at 23±2 °C under 12/12 h alternate cycles of NUV light and darkness for 7 days. After 7 days of incubation, culture broth was centrifuged at 10,000 rpm for 10 min. The pellets were resuspended in sterile distilled water and washing was repeated thrice. The washed fungal pellet was made into a turbid solution with sterile distilled water. The OD of the solution was adjusted to 0.45 (*A*610nm) to obtain 1 x 10^8 cfu ml^{-1} [29].

Barley Grain Inocula (BGI)

Autoclaved barley grains (100 g in 100 ml of distilled water in 500 ml Erlenmeyer flask) were inoculated with 10-15 disks (5 mm) obtained from the actively growing margins of 7-21 day-old PGPF cultures in 500 ml Erlenmeyer's flask. After 10-15 days of incubation at 25 °C, completely colonized barley grains were air dried at laboratory temperature (23-25 °C). The dried BGI was ground to a 1-2 mm particle size in a blender and stored at 4 °C until used for further studies.

Seed treatment with Plant Growth Promoting Fungi

Seeds of pearl millet highly susceptible (7042S) to downy mildew disease were treated with conidial suspension of PGPFs at the rate of 1 x 10^8 cfu ml^{-1} by mixing 400 seeds with 5 ml conidial suspension. In another set of experiment 400 seeds (7042S) were seed coated with different concentrations of BGI viz., 5%, 10%, 20% w/w. Treated seeds were kept at 25±2 °C in a rotary shaker for 6 h to facilitate the penetration of the inducer inside the seeds. Seeds treated with sterile distilled water were served as untreated control.

Inoculation technique

Emerging seedlings (2-day-old) at coleoptile stage were challenge inoculated by the whorl inoculation method with 4 x 10^4 zoospores ml^{-1} concentration of *S. graminicola*. The plants were maintained under greenhouse conditions at 22±2 ºC with 80% relative humidity and observed for disease development. The plants are rated diseased 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. Percent downy mildew disease incidence is recorded at 30 days after sowing (DAS) and final counts were made at 60 DAS. This experiment was repeated three times.

Effect of seed treatment with Plant Growth Promoting Fungi on pearl millet seed germination and seedling vigor

PGPFs treated and untreated control seeds (four replicates of 100 seeds) were plated equidistantly on three layers of moistened blotter discs placed in Petri plates to evaluate percent germination [30] and another set of treated seeds were subjected to between paper method to record seedling vigor [31]. The experiment consisted of four replications of 100 seeds (50 seeds in eight towels). After seven days, percent germination, root length and shoot length was recorded and vigor index was calculated as follows.

Vigor index = Seed germination (%) × [Mean Root Length + Mean Shoot Length]

Greenhouse conditions

Treatment of Plant Growth Promoting Fungi for in-planta colonization

PGPFs isolates which stood superior in enhancing seed quality parameters were selected for *in planta* colonization studies. PGPF isolates viz., *Penicillium* sp. (UOM PGPF 27), *Trichoderma* sp. (UOM PGPF 37), *Rhizoctonia* sp. (UOM PGPF 48), *Fusarium* sp. (UOM PGPF 20) and *Pythium* sp. (UOM PGPF 41) suspension of (100 ml) containing (1 x 10^8 cfu ml^{-1}) was thoroughly mixed with potting medium consisting of red soil, sand (red sandy soil) and farmyard manure (FYM) (2:1:1 by weight) which was autoclaved at 121 °C for 1 h earlier. Then the mixture was transferred into the earthen pots (12 x 13 cm diameter) and left overnight. Highly susceptible seeds of pearl millet (7042S) were then sown into these pots.

In another set of experiment the dried BGI (completely colonized with isolated PGPF) was ground to 1-2 mm particle size and was mixed with potting medium which was autoclaved at 121 °C for 1 h earlier consisting of red soil, sand (red sandy soil) and farmyard manure (FYM) (2:1:1 by weight) at different concentrations (5%, 10%, 20% w/w). Then the mixture was transferred into the pots (9 x 9 inch diameter) and left overnight. Highly susceptible seeds of pearl millet (7042S) were then sown into the pots. Each treatment consisted of four replications, i.e. 20 pots/replication and 10 seedlings/pot. The treatments were arranged in an RBD (Random Block Design).

Colonization of PGPFs was measured by uprooting the 15 day old pearl millet plants. Measured from the radicals, only the first 6 cm of roots were retained; these were aseptically cut into 2 cm segments, washed with 5 ml of sterile distilled water and sequentially numbered and plated onto PDA agar plates. The samples of soil particles were numbered according to the root segments from which they were recovered. Serial dilutions were prepared (10^{-3} to 10^{-5}), from each of which a 0.2 ml aliquot was inoculated onto PDA agar plates. All plates were incubated for 7 days at 23±2°C. Frequency of occurrence of PGPF isolates was assessed from each root segments and corresponding soil particles.

Effect of Plant Growth Promoting Fungi seed treatment on pearl millet downy mildew disease incidence

PGPFs treated and untreated control seeds were sown in earthen pots (9 x 9 inch diameter) containing 2:1:1 red soil, sand (red sandy soil) and farmyard manure (FYM) which was autoclaved at 121 °C for 1 h earlier under greenhouse conditions. Two-day-old-seedlings were whorl inoculated with *S. graminicola* zoospore suspension (4 x 10^4 zoospores ml^{-1}). The challenge-inoculated plants were arranged in a randomized complete block design and maintained under greenhouse conditions (25±2° C, 95% relative humidity). Plants were observed daily and the progression of disease was recorded. Plants were rated diseased when they showed any one of the typical symptoms of downy mildew, i.e. chlorosis, stunting, sporulation or 'green ear'. At the end of 60 days, disease incidence was recorded as the percentage of plants showing symptoms of downy mildew disease and percentage protection was calculated using the formula;

Percent protection:

$$\frac{\text{Percent downy mildew in untreated plant - Percent downy mildew in PGPF-treated plants}}{\text{Percent downy mildew in untreated plants}} \times 100$$

RESULTS

Isolation of Plant Growth Promoting Fungi

A total of forty nine PGPFs were isolated from rhizosphere soil of different grass species from four districts of Karnataka, India (Table 1).

Table 1. Plant Growth Promoting Fungi isolated from the rhizosphere soil of the grass plants

PGPF Isolates	Place of collection							No. of Isolates
	H. D.Kote	Mandya	C. Nagara	Mysore	Hassan	Periyapatna	K.R. Nagara	
Fusarium sp.	-	2	-	1	1	1	2	07
Trichoderma sp.	3	1	1	2	5	-	1	13
Apergillus sp.	1	-	-	-	-	3	-	04
Penicillium sp.	-	1	3	1	-	1	1	07
Phytopthora sp.	-	-	-	-	-	1	-	01
Rhizoctonia sp.	4	-	1	-	2	-	1	08
Phoma sp.	1	1	-	2	1	-	-	05
Pythium sp.	-	1	2	-	-	-	-	03

Figures inside the column represent the total number of PGPF isolates.

Effect of seed treatment with Plant Growth Promoting Fungi on seed germination and seedling vigor

Among forty nine PGPF isolates screened, only six PGPFs isolates revealed significantly ($P < 0.001$) enhanced germination and seedling vigor to varying degrees (Table 2). Among the BGI PGPF treatments, *Penicillium* sp. (UOM PGPF 27) at 5% (w/w) concentration recorded highest seed germination of 92% and 1701.9 seedling vigor, followed by *Rhizoctonia* sp. (UOM PGPF 48) and *Pythium* sp. (UOM PGPF 41) at 10% concentration both recorded 89% germination and 1479 and 1357 seedling vigor respectively. In case of PGPFs conidial suspension (1 x 10^8 cfu ml^{-1}), *Trichoderma* sp. (UOM PGPF 37) offered maximum germination of 91% and 1765 seedling vigor, followed by *Penicillium* sp. (UOM PGPF 27) with 90% germination and 1673 seedling vigor. A lower percent seed germination of 82% was recorded both in *Fusarium* sp. (UOM PGPF 20) and *Pythium* sp. (UOM PGPF 41) and seedling vigor were noticed 1115 and 1091 respectively which stood still significant when compared to untreated control which recorded seed germination of 82% and 991 seedling vigor. The PGPFs treatment in the form of conidial suspension treatment recorded early emergence compared to BGI treatment (data not shown) under green house conditions.

Table 2. Effect of Plant Growth Promoting Fungi isolates on seed germination and vigor of pearl millet

Treatments	% Germination				Vigor Index			
	(1 x 10^8 cfu ml^{-1})	5%	10%	20%	(1 x 10^8 cfu ml^{-1})	5%	10%	20%
Fusarium sp. (UOM PGPF 20)	82±0.2c	82±0.5c	82±0.7c	80±1.2d	1115±8.0c	1207±7.5d	1295±7.2d	1208±10.9c
Trichoderma sp. (UOM PGPF 37)	91±0.7a	84±0.3b	89±0.5b	89±0.7b	1765±3.2c	1428±9.0b	1244±7.5d	1263±11.6c
Rhizoctonia sp. (UOM PGPF 48)	85±0.2b	85±0.6b	89±0.5b	87±0.5c	1381±9.5b	1319±9.0c	1479±7.1b	1383±7.3b
Penicillium sp. (UOM PGPF 27)	90±0.5a	92±0.7a	92±0.2a	91±1.0a	1673±6.5a	1701.9±4.7a	1644±9.3a	1623±9.2a
Pythium sp. (UOM PGPF 41)	82±0.2c	83±0.2c	89±0.5b	86±0.7c	1091±7.3c	1228±8.1d	1357±6.1c	1079±10.2d
Phoma sp. (UOM PGPF 04)	86±0.8b	84±0.1b	82±0.4d	82±0.7d	1317±9.1b	1080±8.2e	1110±4.7e	1157±5.3e
Untreated control	82±0.4c	81±1.1d	83±0.7c	82±0.3d	991±5.5d	958±7.3e	995±9.4e	1009±8.7e
Degrees of freedom	21	21	21	21	21	21	21	21
F value	21.13	24.5	29.0	27.6	469.0	413.7	410.0	396.5

[1] Values are means of four independent replications.

[2] Means followed by the same letter(s) within the same column are not significantly different according to Tukey's HSD at $P < 0.001$.

In Planta colonization of Plant Growth Promoting Fungi

A fair to good response of PGPFs colonization was noticed in the treated plant roots. The colonization frequency of the PGPF isolates were significantly greater in the first 2 cm [upper] [1 x 10^3 cfu/ gm soil/ cm root bit] of treated root and remained constant in the next 2 cm [middle] [1 x 10^3 cfu/ gm soil/ cm root bit], but a few PGPFs was not detected in the rhizosphere soil in the last 2 cm [lower] of the root bit (Table 3). The PGPF isolates were re-isolated from basal root segments of 6 and 4 cm plated on PDA plates and also from the rhizosphere serial dilution of 10^{-3} to 10^{-5}. In control plants there was no colonization of the PGPFs isolates.

Table 3. Root colonization of Plant Growth Promoting Fungi isolates

Treatments	Root Segments			Serial Dilution		
	6 cm	4 cm	2 cm	10^{-3}	10^{-4}	10^{-5}
Fusarium sp. (UOM PGPF 20)	--	++	--	--	--	++
Trichoderma sp. (UOM PGPF 37)	++	++	++	++	++	++
Rhizoctonia sp. (UOM PGPF 48)	--	--	++	--	++	++
Penicillium sp. (UOM PGPF 27)	++	++	++	++	++	++
Pythium sp. (UOM PGPF 41)	--	--	--	--	++	++
Phoma sp. (UOM PGPF 04)	++	++	--	++	++	++
Untreated control	--	--	--	--	--	--

Effect of Plant Growth Promoting Fungi seed treatment on disease protection

The efficacy of the fungi isolated from different grass species as mentioned above were tested under green house conditions with two modes of treatment. Among the PGPFs tested, the species of *Penicillium* (UOM PGPF 27), *Trichoderma* (UOM PGPF 37) and *Pythium* (UOM PGPF 41) were found to be promising and recorded atleast more than 35% disease protection in both conidial suspension and BGI treatments (as mentioned above) and *Rhizoctonia* sp. (UOM PGPF 48) and *Phoma* sp. (UOM PGPF 04) recorded more than 35% protection in conidial suspension treatment which is detailed in this paper (Table 4).

Table 4. Effects of Plant Growth Promoting Fungi seed treatment on downy mildew disease incidence in pearl millet under greenhouse conditions

Treatments	% plants with downy mildew symptoms ± SE				Disease protection (%) ± SE			
	(1 x 10^8 cfu ml^{-1})	5%	10%	20%	(1 x 10^8 cfu ml^{-1})	5%	10%	20%
Fusarium sp. (UOM PGPF 20)	77±0.2b	94±0.5a	87±0.7b	70±1.2c	22±0.4e	05±0.2d	12±0.4c	19±0.7d
Trichoderma sp. (UOM PGPF 37)	33±0.7d	51±0.5c	46±0.3c	49±0.7d	66±0.6b	48±0.7b	53±0.1c	50±0.2b
Rhizoctonia sp. (UOM PGPF 48)	49±0.2c	86±0.6b	85±0.5b	86±0.5b	50±0.2c	13±0.6d	14±0.4c	13±0.8e
Penicillium sp. (UOM PGPF 27)	28±0.5e	32±0.7d	30±0.2d	31±1.0e	71±0.7a	67±0.7a	69±0.1a	69±0.5a
Pythium sp. (UOM PGPF 41)	58±0.2c	52±0.5c	38±0.2d	66±0.7c	41±0.3d	47±0.7b	61±0.9b	33±0.1c
Phoma sp. (UOM PGPF 04)	40±0.8d	91±0.1a	78±0.4d	82±0.7b	59±0.4c	08±0.3d	21±0.4c	17±0.3d
Untreated control	97±0.4a	98±1.1a	98±0.7a	98±0.3a	00	00	00	00
Degrees of freedom	12	12	12	12	12	12	12	12

[1] Values are means of four independent replications.

[2] Means followed by the same letter(s) within the same column are not significantly different according to Tukey's HSD at P < 0.001.

In general all the isolated PGPFs treatment recorded a considerable disease protection. In BGI treatments, *Penicillium* sp. (UOM PGPF 27) at 5% (w/w) and *Pythium* sp. (UOM PGPF 41) at 10% (w/w) recorded maximum disease protection of 67% and 61% respectively, followed by *Trichoderma* sp. (UOM PGPF 37) at 10% (w/w) recorded 53% protection. Lowest disease protection of 5% was recorded by *Fusarium* sp. (UOM PGPF 20) at 5% (w/w). With respect to conidial suspension treatments (1 x 10^8 cfu ml^{-1}), significant (P < 0.001) disease protection was observed in all the tested PGPFs when compared to untreated control. Seed treatment with *Penicillium* sp. (UOM PGPF 27) and *Trichoderma* sp. (UOM PGPF 37) recorded highest disease protection of 71% and 66% respectively. Further disease protection of 59%, 50% and 41% was offered by *Phoma* sp. (UOM PGPF 04), *Rhizoctonia* sp. (UOM PGPF 48) and *Pythium* sp.(UOM PGPF 41) respectively. The least disease protection of 22% was recorded in *Fusarium* sp. (UOM PGPF 20), whereas the untreated control plants recorded 97% disease incidence.

DISCUSSION

Certain PGPF are reported to suppress disease effectively [32] and induction of systemic resistance is reported in cucumber [33]. PGPF isolates have known to effectively control soil-borne diseases (damping-off caused by species of *Fusarium, Rhizoctonia* and *Sclerotium)* and take-all caused by *Gaeumannomyces graminis* of a number of crop plants [34]. Hence the effect of these PGPF was tested as inducers of systemic resistance using the classic model of pearl millet host-pathogen system. The purpose of this paper is to present the dual role of PGPF in inducing resistance as well as in promoting growth of pearl millet plants. The present study revealed that seed quality parameters of pearl millet were enhanced in all the tested PGPF isolates and maximum germination of 92% and seedling vigor of 1701.9 was observed in BGI of *Penicillium* sp. (UOM PGPF 27) at 5% (w/w) concentration followed by conidial suspension of *Trichoderma* sp. (UOM PGPF 37) with 91% germination and 1765 seedling vigor. Various other reports suggested that PGPF improve the growth of plants and affect the expression of plant defense responses [35, 36, 37 and 38]. PGPF may also improve plant growth indirectly, via alterations to the structure of rhizosphere soil, which benefit the plant. The present study revealed that disease caused by *S. graminicola* can be suppressed by using PGPF isolates viz., *Penicillium* sp. (UOM PGPF 27) *Trichoderma* sp. (UOM PGPF 37), *Rhizoctonia* sp. (UOM PGPF 48) and *Pythium* sp. (UOM PGPF 41). Pathogen control by PGPF may also occur via niche exclusion, antibiosis, predation, mycoparasitism and ISR induction [10, 39 and 40]. The isolates afforded better protection when they were challenge inoculated. This observation corroborates with that of Meera *et al*., [35] wherein cucumber plants were protected from *Colletotrichum orbiculare* by induction with PGPF isolates. Earlier reports have shown that *Trichoderma* isolates, known to act directly on pathogens as biocontrol agents, have been also found capable of inducing systemic resistance [41]. *Penicillium chrysogenum* (PEN) induces significant resistance against *Fusarium oxysporum* f. sp. *vasinfectum* (Fov) and *Verticillium dahliae* (Vd) in potted cotton plants under glasshouse conditions [42 and 43]. Pearl millet seedlings treated with conidial suspension enhanced resistance against challenge-inoculation with *S. graminicola*. Suppression of disease appeared to be systemic, as roots were treated with PGPF isolates and the pathogen was challenge inoculated on leaves, thereby separating the two spatially. Plant growth promotion by fungi has been demonstrated in a few crops [32, 33 and 44] compared with a large number of reports on growth promotion by rhizobacteria [45 and 46]. In our study, PGPF isolates caused growth promotion of plants independent of the root colonization ability. Seed treatment with certain PGPF isolates for 6 h resulted in a significant enhancement of plant growth compared with that of untreated ones and these isolates lacked colonization ability. These results suggest that such isolates might produce certain metabolites that induce growth promotion. The growth promotion ability of PGPF has also been largely attributed to the production of growth-regulating substances [47]. One mechanism of growth promotion could be the ability of certain isolates to colonize roots and provide minerals to plants in a more available form. This study sheds light on the potential of some saprophytic, sterile fungi as plant growth promoters as well as biocontrol agents. A detailed investigation is under way to understand the exact mechanism of growth promotion and systemic resistance in pearl millet using plant growth promoting isolates.

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