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Short Research Article

Efficiency of Different *Trichoderma* Isolates on Plant Growth Promoting Activity in Rice (*Oryza sativa* L.)

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Abstract

The application of *Trichoderma* strains with biocontrol and plant growth-promoting capacities to plant substrates can help reduce the input of chemical pesticides and fertilizers in agriculture. Some *Trichoderma* isolates can directly affect plant pathogens, but they also are known to influence the phytohormonal network of their host plant. In the current study, we evaluated the production of potential growth-promoting metabolites (IAA and phosphate) for eight isolates of *Trichoderma* collected from different geographical locations of Chhattisgarh and some isolates were procured from STRASA-BMGE using 83 elite rice lines. All the eight isolates assessed their growth response onrice. All the *Trichoderma* isolates were able to release inorganic phosphorus from tri-calcium phosphate and showed consistent ability to produce indole-3-acetic acid (IAA). The production of these metabolites varied greatly within species. Confrontation assays of *Trichoderma* isolates against two soil borne plant pathogens (*Scelrotium rolfsii* and *Rhizoctonia solani*) expressed varying degrees of antagonistic responses, *In-vitro* antagonism being more effective against *R. solani* than *S. rolfsii*. The production of metabolites in all the *Trichoderma* isolates did not correlate with enhanced root growth in rice lines and bio control efficacy. However, one of the *Trichoderma viride* isolate (T_{14}) was identified as highest producer of inorganic phosphate, IAA exhibited high antagonistic and plant growth promoting ability. A characteristic aromatic odor resembling coconut in T_{14} isolate was observed which we speculate is due to 6-Pentylpyrone (one of the best studied secondary metabolites having both antifungal and plant growth-promoting activities. Besides the T_{14} isolate isolates designated as IRRI-2, IRRI-3 and IRRI-4 were the promising inducer of plant growth.

1. Introduction

In the biological system the soil can contaminated with different microbes, there are some root beneficial microbes present in soil that modulate plant physiology when interact with plant roots and affect plant response to different environmental conditions.The need for increasing agricultural productivity andquality has led to an excessive use of chemical fertilizers, creating serious environmental pollution. The use of biofertilizers and biopesticides is an alternative for sustaining high production with low ecological impact. Besides the classic mycorrhizal fungi and *Rhizobium* bacteria, other plant-growth-promoting *Rhizobacteria* (PGPR) and fungi such as *Trichoderma* spp. can stimulate plant growth by suppressing plant diseases (Van Wees et al., 2008). These micro-organisms can form endophytic associations and interact with other microbes in the rhizosphere, thereby influencing disease protection, plant growth and yield. The *Trichoderma* spp. are one that can used extensively as a biocontrol agent that colonize the rhizosphere of plants (Saxena et al., 2005) and promote growth of the plants through various direct and indirect mechanisms such as nutrient competition, antibiosis, mycoparasitism, induction of systemic resistance, increased plant-nutrient availability. This all mechanisms were show the resistance to various stresses conditions. Due to this *Trichoderma* is gaining importance to the involvement in the Plant Growth Promoting Response (PGPR). The term Induced Systemic Tolerance (IST) has been proposed for PGPR-induced physical and chemical changes that result in

enhancement of plant tolerance (Venkateswarlu et al., 2008; Yang et al., 2009). The plant–microbe association involves molecular recognition between the two partners through a signaling network mediated by the plant hormones salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) (Contreras-Cornejo et al., 2011). JA and ET have been described as signal transduction molecules for induced systemic resistance (ISR) due to the effect of beneficial microbes, and the signal transduction pathway through SA accumulation is found in the systemic acquired resistance (SAR) induced by attack by pathogens. A common feature of ISR responses to beneficial microbes is priming for enhanced defence. In primed plants, defence responses are not activated directly, but are accelerated upon attack by pathogens or insects, resulting in faster and stronger resistance to the attacker encountered (Van Wees et al., 2008).

Trichoderma species are highly opportunistic and have been isolated from a diverse range of natural and artificial substrata, which shows their adaptability to various ecological conditions (Druzhinina et al., 2011, 2012). As it was known that *Trichoderma* affects the root morphology thus a technique described as Root Pulling Resistance (RPR) was used to evaluate differences in root growth of different rice cultivars after seed treatment with *Trichoderma* isolates. The method appears sensible for root depth phenol typing in rice, especially when other direct field methods for root selection are not very forthcoming. Several root characteristics in rice are associated with drought tolerance and other biotic-abiotic stresses and avoidance capability of plants. The RPR measurements showed a significant positive correlation with maximum root length, root thickness, branching number, and root dry weight, number of tips and forks. Rice genotypes that had a high RPR value were identified as having longer, thicker, and denser root systems. Further more, the data demonstrated that the RPR technique is ideal for selecting superior root systems and potential drought tolerant rice cultivars.

The objective of this study was to evaluate isolates of *Trichoderma* collected from different geographical locations of Chhattisgarh for *in vitro* antagonistic activity against two phytopathogens, *S. rolfsii* and *R. solani*. Screening of *Trichoderma* isolates for their ability to stimulate plant growth promotion in selected rice lines and traits or parameters associated to growth (such as indole-3-acetic acid, IAA production and phosphate solubilisation ability).

2. Material and Methods

The study was conducted in molecular plant pathology laboratory at department of plant molecular biology and biotechnology as well as field study was done during wet season of 2013−2014 at research come experimental field of Directorate of Research Station, Indira Gandhi Agricultural University, Raipur, Chhattisgarh, India. For field studies each rice line/variety was planted out in a field during wet season of *Kharif*-2014 at the experimental farm.

2.2.1. Morphological characterization of the isolates of Trichoderma spp.

For morphological characterization we used eight different isolates of *Trichoderma* spp. isolated from Chhattisgarh region and some isolates were procured from STRASA-BMGE namely as T-14 (*Trichoderma viride*), 94a (not defined), IRRI-Tv (*Trichoderma viride*), IRRI-TH-3 (*Trichoderma harzianum*), IRRI-1 (*Trichoderma viride*), IRRI-2 (*Trichoderma viride*), IRRI-3 (*Trichoderma viride*) and IRRI-4 (*Trichoderma viride*). Cultural characters of all the *Trichoderma* isolates under investigation were studied on 20 ml sterilized PDA in petri plates. Mycelia disc of approximately 5 mm diameter were transferred aseptically from culture slants using inoculation needle at the center of each petridish and incubated at 28±2 °C for 4 days. The cultured petri dishes were observed for growth pattern (presence or absence of aerial mycelium or subdued growth etc.), pigmentation of varying shades of green of the vegetative growth and pigmentation of the secreted metabolite in the substrate medium against white background under sunlight. The cultural characteristics were photographed using digital camera.

2.2.2. Microscopic characterization of the isolates of trichoderma

Conidiophores and conidial morphology, branching pattern, critical for identification to the species level are best observed before the conidia are completely mature. Mounts from the actively growing (fungal growth from the growing colony margin) all the eight isolates of *Trichoderma viride*, *Trichoderma harzianum* and other *Trichoderma* spp. were prepared in lacto phenol cotton blue on glass microscopic slide (preferably young tufts where the conidia just begins to develop pigment in actively growing cultures). Mounts were prepared using a cello tape. A strip of cellotape was held in thumb and forefinger and the gum coated surface was impressed against the sporulating growth of the isolate. The cellotape thus lifted the intact sporulating growth (including conidiophores, philides, and sporulation) without forming any clump of mycelium and injuring the substrate. Microscopic morphological features of all the selected isolates were observed using a Leica binocular microscope and were micro photographed digitally.

2.2.3. In vitro fungal growth inhibition assays

Dual culture was performed on PDA plates as described

by Dennis and Webster (1971) to assess the ability of *Trichoderma* isolates to over-grow and lyse the mycelia of two test plant pathogens, *S. rolfsii* and *R. solani*. Five mm agar plugs from the edge of actively growing colonies of *Trichoderma* and the pathogen respectively were placed approximately 7 cm opposite to each other and incubated at 28±2 °C for 5 days. *In vitro* antagonistic potential of different isolates of *Trichoderma* was assessed by calculating percentage inhibition growth of pathogens in presence of *Trichoderma* using the formula: (C-T)/C×100 where C and T are radial growth of pathogen in control and in presence of *Trichoderma* spp. respectively. Isolates were also scored for degree of antagonism as per a scale of classes 1–5 designated by Bell et al. (1982): 1=*Trichoderma* completely overgrew the pathogen and covered the entire medium surface (>75% inhibition); 2=*Trichoderma* over-grew at least 2/3 of the medium surface(50–75% inhibition); 3=*Trichoderma* and pathogen each colonized approximately ½ of the medium surface (more than 1/3 and less than 2/3), and neither organism appeared to dominate the other (25–50% inhibition); 4=the pathogen colonized at least 2/3 of the medium surface and appeared to with stand encroachment by *Trichoderma* (<25% inhibition); 5=the pathogen completely overgrew the *Trichoderma* and covered the entire medium surface.

2.2.4. Quantitative estimation of inorganic phosphate for phosphate solubilisation ability of Trichoderma spp.

Quantitative estimation of phosphate solubilisation in Pikovskaya broth (Himedia) containing tricalcium phosphate as a phosphate source was performed for all the eight isolates according to the procedure of Jackson (1973). Freshly grown *Trichoderma* isolates were inoculated to 50 ml of Pikovskaya's broth and incubated at 28±2 °C and 100 rpm. The amount of inorganic phosphate (Pi) released in the broth was estimated after 5 days of incubation in comparison with uninoculated control. The broth culture was centrifuged at 10,000 rpm for 10 min to separate the supernatant from the mycelial growth and insoluble phosphate. To the 0.5 ml of the culture supernatant, 5 ml of chloromolybdic acid was added and mixed thoroughly. Volume was made up to 10 ml with distilled water and 125 µl chlorostannous acid was added to it. Immediately, the final volume was made-up to 25 ml with distilled water and mixed thoroughly. After 15 min, the blue color developed was read in a spectrophotometer at 610 nm using are agent blank. Corresponding amount of soluble phosphorous was calculated from a standard curve of potassium dihydrogen phosphate (KH_2PO_4). Phosphate solubilizing activity was expressed in terms of tricalcium phosphate solubilization whichin turn was measured by µg ml^{-1} of available orthophosphate as calibrated from the standard curveof KH_2PO_4.

2.2.5. Quantification of indole acetic acid (auxin) production by Trichoderma spp.

For the quantitative estimation of IAA, four agar plugs (5 mm) from the edge of actively growing colonies of *Trichoderma* were inoculated to 20 ml DF salts minimal media (Dworkin and Foster, 1958) in 100 ml conical flasks and incubated for 3 days at 28±2 °C. The medium was supplemented with L-tryptophan at a concentration of 1.02 g l^{-1}. After incubation for 72 h, the mycelia were removed from the culture medium by centrifugation at 5,000 rpm for 5 min. One ml aliquot of the supernatant was mixed vigorously with 4 ml of salkowski's reagent (Gordon and Weber, 1951) and allowed to stand at room temperature for 20 min. The absorbance at 535 nm was measured with DF salts minimal media (plusSalkowski's reagent) as blank. The concentration of IAA in each culture supernatant was determined using an IAA (Himedia) standard curve.

2.2.6. Evaluation for plant growth promoting response

For field studies total 83 elite rice lines were planted in field. Each rice line/variety was planted out in a field during wet season of *kharif*-2014 at the experimental farm of the College of Agriculture. IGKV, Raipur (Chhattisgarh, India). There were two replicates for each rice germplasm line/varieties in each phenotypic evaluation experiment. The single seedling from each rice variety was transplanted in the field of plot size 30×54 m^2 area and within plant distance was 15×15 cm^2 in two replicates. At maximum tillering stage, random five plants of each line/variety were sampled in order to examine root pulling strength using root pulling machine (Table 1). On the basis of root pulling strength and root length the 10 rice genotypes (Table 2) having lower root pulling strength (LRS) and higher root pulling strength (HRS) were selected. These selected rice genotypes were used for screening the effect of *Trichoderma* isolates on plant growth promotion activity.

For seed treatment, *Trichoderma* spores were harvested from 7-day old sporulating cultures of different isolates and re-suspended in 20 ml of sterile distilled water containing 0.5% (w/v) carboxy methyl cellulose such that the spore concentration was maintained to 10^8 conidia ml^{-1}. Seeds of each of selected rice lines (Table 2) i.e. 10 lines having lower root pulling strength and 10 having higher root pulling strength (HRS and LRS) were treated with spore suspension for one hour. Treated seeds were sown in pots containing autoclaved soil condition with regular irrigation as per requirement (soil was not amended with any fertilizer). Control seeds were treated with 0.5% (w/v) carboxyl methyl cellulose prepared in sterile distilled water. The pots were kept in green house condition with the temperature ranging from 28 to 32 °C and

Table 1: Total phenotypic data for Root Length (RL) and Root Pulling Strength (RPS) of 83 rice genotypes/lines used in study

Sl. no.	Line	Root length (RL)	Root Pulling Strength (RPS)	Shoot length (SL)
1.	R-RF-69	14.33±4.04	27.00±1.41	26.33±5.53
2.	R-RF-84	15.00±4.27	31.00±1.41	30.17±0.76
3.	R-RF-75	14.53±1.05	32.00±0.00	30.57±4.76
4.	R-RF-95	16.77±1.94	31.00±1.41	36.43±7.10
5.	R-RF-78	14.67±2.08	27.50±0.71	26.73±3.27
6.	R-RF-81	23.10±5.91	30.00±2.83	43.73±5.43
7.	R-RF-65	16.83±2.02	32.50±3.54	37.33±1.89
8.	R-RF-82	10.00±2.00	34.00±2.83	38.50±1.80
9.	IR 83376-B-B-150-3	13.37±2.87	26.50±0.71	28.70±0.96
10.	IR 84882-B-120	13.20±1.59	32.00±0.00	29.83±1.04
11.	IR 84887-B-15	18.73±1.67	23.50±2.12	34.70±2.71
12.	IR 33929-B-B-132-2	15.00±0.00	37.00±1.41	39.53±2.16
13.	IR 86857-46-1-1-2	11.53±1.36	27.00±1.41	31.80±4.20
14.	IR 86781-3-3-1-1	16.50±1.32	38.00±0.00	34.50±4.77
15.	IR 83372-B-B-133-2	11.70±2.21	29.50±0.71	21.60±3.11
16.	R-RF-45	16.17±0.29	33.00±1.41	30.50±6.95
17.	IR 83381-B-B-55-4	14.17±2.29	31.50±3.54	25.03±1.94
18.	IR 83381-B-B-6-2	13.23±2.42	32.00±5.66	32.67±1.44
19.	IR 83383-B-B-140-2	13.60±0.44	29.50±2.12	26.03±1.18
20.	IR 83383-B-B-129-3	21.00±8.23	32.00±0.00	26.90±3.48
21.	CB-09-510	14.27±2.02	37.50±2.12	32.33±3.35
22.	PM 6004	11.53±0.90	35.00±1.41	27.87±7.94
23.	CR 2642-52	15.03±5.56	37.00±1.41	32.00±9.51
24.	IR 83381-B-B-137-3	9.67±0.29	33.00±1.41	32.17±3.88
25.	Sahbhagi dhan (CH)	14.60±4.65	39.00±4.24	29.33±1.61
26.	IR 88287-677-60-3	16.57±0.64	29.00±1.41	31.73±4.75
27.	IR 84878-B-60-4-1	14.20±1.47	33.00±1.41	38.37±2.32
28.	IR 84852-B-12-1-4	12.20±2.86	35.00±1.41	29.50±2.75
29.	IR 88287-677-53-7	13.63±2.83	30.00±5.66	35.10±2.60
30.	IR 83381-B-B-38-3	14.00±0.50	28.00±8.49	31.97±3.16
31.	IR 83383-B-B-129-4	18.10±4.37	32.00±0.00	39.07±6.93
32.	IR 84859-B-41-1-2	23.83±1.04	29.00±1.41	35.17±1.53
33.	IR 83383-B-B-140-3	18.20±2.82	31.00±1.41	27.90±2.98
34.	IR 83372-B-B-133-2	15.20±5.63	34.00±2.83	21.00±9.26
35.	IR 84882-B-120-CRA-6	16.53±1.76	37.50±3.54	26.57±6.02
36.	Annnada	18.30±2.15	27.00±1.41	41.00±0.78
37.	MTU 1010	12.57±1.21	32.50±4.95	26.33±1.89
38.	IR 64	13.27±2.74	29.00±4.24	38.63±9.84
39.	Mahamaya	17.47±1.42	31.00±1.41	32.90±5.15
40.	Poornima	15.80±1.47	32.50±0.71	33.97±0.64

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Sl. no.	Line	Root length (RL)	Root Pulling Strength (RPS)	Shoot length (SL)
41.	Samleshwari	17.27±2.93	33.00±1.41	34.50±5.41
42.	Vandana	23.20±1.99	29.00±1.41	36.30±0.98
43.	Danteshwari	16.50±2.18	23.00±1.41	24.17±3.18
44.	Swarna	12.53±2.00	37.00±4.24	23.80±3.36
45.	Swarna sub 1	17.83±1.61	34.00±2.83	25.17±1.61
46.	ARB8	15.83±2.02	31.00±1.41	34.33±5.80
47.	Abhaya	9.90±4.60	33.00±4.24	25.60±3.65
48.	Azucina	14.07±2.29	35.00±7.07	40.20±0.20
49.	ARB6	13.83±2.25	29.00±4.24	22.50±16.48
50.	Bamleshwari	12.70±0.82	28.00±2.83	30.93±3.86
51.	Budda	18.23±7.60	36.00±2.83	51.67±2.75
52.	Bakal	16.37±0.15	37.00±7.07	42.17±2.15
53.	Bas 370	16.33±1.15	35.00±1.41	37.33±2.36
54.	Bhata phool	15.67±3.67	40.00±2.83	42.63±1.17
55.	Batroo	13.33±2.89	33.00±1.41	43.50±3.04
56.	Bhata jhooli	14.00±3.04	33.00±1.41	43.73±4.24
57.	CT 9993	10.67±0.76	31.50±2.12	33.33±5.69
58.	Cross116	15.67±1.26	40.00±2.83	45.30±14.41
59.	Chapti Gurmatiya	19.47±4.50	38.00±8.49	53.33±5.53
60.	Desi Lal dhan	19.30±3.80	23.00±4.24	35.40±5.39
61.	Desi no.17	11.17±2.02	34.00±11.31	51.40±2.25
62.	Dagad Desi	14.47±1.00	31.00±1.41	40.67±2.08
63.	IC267982	14.83±1.15	26.50±0.71	32.77±5.06
64.	IR36	23.00±5.07	32.00±2.83	38.00±6.00
65.	IR42253	13.83±2.57	32.50±0.71	39.57±3.40
66.	IR55419	13.20±2.52	27.00±1.41	40.73±3.65
67.	Kranti	21.37±4.01	28.00±2.83	46.67±5.84
68.	Laloo 14	12.77±1.36	27.50±0.71	39.00±1.99
69.	Ramjiyawan	15.70±0.44	26.00±5.66	46.37±1.65
70.	Safri 17	11.13±2.44	35.00±1.41	35.50±5.96
71.	Shennong89366	14.33±2.31	28.00±2.83	45.17±2.89
72.	IBD 1	13.37±2.90	32.50±3.54	33.67±1.68
73.	SLO 16	16.83±2.93	21.50±2.12	44.50±3.61
74.	Kalo kuchi 223	14.77±0.64	25.50±4.95	38.17±1.37
75.	Kalia	10.40±3.38	29.00±1.41	30.33±9.07
76.	PRATAO	12.87±2.12	32.50±0.71	44.43±6.99
77.	Chua Dau130	14.50±0.87	32.00±2.83	53.67±4.01
78.	IR 55419	14.77±3.01	32.00±2.83	32.13±0.29
79.	CHENGRI 2	12.50±2.50	31.00±1.41	39.67±5.03
80.	CR5272	16.23±0.55	27.50±0.71	32.80±0.44
81.	EPAGRI 109	13.57±2.80	29.50±0.71	32.17±1.04
82.	PIN KAEO	11.20±2.25	32.00±5.66	31.30±1.90
83.	DJOGOLON-DJOGOL	14.50±3.50	26.00±0.00	26.83±5.51

Table 2: Selected rice genotypes/lines used to evaluation effect of *Trichoderma*

Sl. no.	Genotypes/lines	Maximum RPS	S. no.	Genotypes/ lines	Minimum RPS
1.	RRF-82	34	1	RRF-69	27
2.	IR 83929-B-B-132-2	37	2	RRF-78	27.5
3.	IR 86781-3-3-1-1	38	3	IR83376-B-B-150-3	26.5
4.	CB-09-510	37.5	4	IR86857-46-1-1-2	27
5.	IR 84882-B-120-CRA-6	37.5	5	Annanada	27
6.	IR 86857-46-1-1-2	34	6	Danteshwari	23
7.	Swarna	37	7	Bamleshwari	28
8.	Bakal	37	8	IR-64	29
9.	Chapti Gurmatiya	38	9	Kalo kuchi-223	25.5
10.	Safri 17	35	10	CR-5272	27.5

the plants were irrigated every day. From different treatment combinations, seedlings were harvested 13 days after sowing. The roots of seedling were removed by submerging the pots in water for an hour so that the soil in pots was loose and roots were easily separated. The increase in root length was recorded using standard scaling method.

3. Results and Discussion

3.1. Morphological and microscopic characterization of the isolates of Trichoderma spp.

Trichoderma spp. is among the most widely used organisms for biological disease control and for plant growth promotion (Harman et al., 2004). So it was necessary to differentiate such isolates from one another for their easy identification in future. Cultural characteristics comprising culture color, odor and colony appearance were examined (Table 3). These characteristics were regarded as taxonomically useful characteristics for *Trichoderma* (Samuels et al., 2006). In present investigation, the three different species of *Trichoderma* show different cultural characteristics and exhibited different growth rate (Figure 1). Members of the genus *Trichoderma* (where known, the teleomorphs belong to Hypocrea) are *Sordariomycetes*, like the model filamentous fungus *Neurosporacrassa*. *Trichoderma's* variations on the Neurospora themes of morphogenesis and photobiology have been studied for several decades, pointing to those molecular details that are conserved and those that vary between species. On PDA after 4−5 days of inoculation, TH-3 (*T. harzianum*) formed green conidial production. The conidia production was denser and uniform throughout the culture. *T. viride* isolate #IRRI-4 showed irregular yellow zone without conidia was present around the inoculum. Some green/yellow pustules were also found growing on the green mat of conidia and cottony white and light yellowish mycelium of floppy growth concentric rings were formed by *T. harzianum* (Tv, IRRI-2, IRRI-3 and IRRI-4). *T. viride* appears to be a bit granular

Table 3: Morphological characteristics of isolates based on key characters described for the identification of *Trichoderma* species

Iso#	Colony colour	Growth pattern	Appearance	Pustule	Different pigments	Odor
94a	Dark green	Aereial and subdued	Uniform velvety	Absent	Yellow pigment	Absent
T_{14}	Dark green	Aereial and subdued	Uniform velvety	Absent	Absent	Coconut
TH-3	Dark green	Subdued	Uniform slightly cottony	Absent	Feint yellow crystals at the centre	Absent
Tv	Dark green	Subdued	Uniform velvety,slightly cottony	Absent	Feint yellow pigment spots	Coconut
IRRI-1	Feint green	Subdued	Uniform cottony	Absent	Absent	Fruity
IRRI-2	Dark green	Subdued	Uniform slightly cottony	Present	Feint yellow	Fruity
IRRI-3	Dark green+White	Subdued	Uniform slightly cottony	Present	Feint yellow	Fruity
IRRI-4	Feint green	Aereial and subdued	Uniform velvety	Present	Feint yellow crystals at the centre	Fruity

on PDA, with green conidia distributed throughout. An irregular yellow zone without conidia was present around the inoculums (Figure 1). Some white pustules were also found growing on the green mat of conidia. These morphological features *Trichoderma* isolates help when you isolate the new *Trichoderma* strain, this characteristic features allow a relatively easy identification of *Trichoderma* as a genus. This

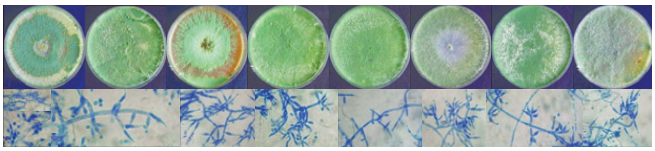

Figure 1: Cultural and phialid morphology of different *Trichoderma* isolates

gave us the first initial information regarding classification of *Trichoderma* strain.

For more defined identification of second step we used was light microscopy to observe the typical characteristics features of the phialides and conidia. Harman and Kubicek had given the characteristics key feature of the sections to classify the isolates into different species. This helped us to classify allocate the *Trichoderma* isolates collected from different geographical locations to different sections as per the characteristics key feature of the sections (Harman and Kubicek, 1998). Isolates that show phialides cylindrical, often sinuous or hooked and fully mature conidia more or less roughened or warted, globose to subglobose were categorized as *T. viride*. Fully mature conidia sub globose (not quite round or spherical) to obovoid, pale green were classified into *T. harzianum isolates*.

3.2. In-vitro antagonistic reactions of Trichoderma spp.

Dual culture confrontation assay was performed on PDA plates as described by Dennis and Webster (1971) to assess the ability of *Trichoderma* isolates to overgrow and lyse the mycelia of two test plant pathogens, *S. rolfsii* and *R. solani*. The present study determined the potential antagonistic variation of isolates of *Trichoderma* to the two pathogens. Similar results were reported by Bell et al. (1982) in their antagonistic study of *Trichoderma* spp. against six phytopathogens. In this investigation screening of *Trichoderma* isolates for antagonism showed diversify results (Figure 2) from scale 1-5 against the two rice phytopathogens. The percent of inhibition were varied from 55.5% to 65.55% for *S. rolfsii* and 55.55% to 100% for *R. solani* (Table 4). More effective antagonistic response of *Trichoderma* isolates was observed against *R. solani* than *S. rolfsii* on agar plates. An isolate of *T.viride* i.e. #IRRI-1, #IRRI-2 and #IRRI-3 were identified as strong antagonist with as it completely overgrew *R. solani* as it shows class 1 type reaction. Another isolate 94a (class

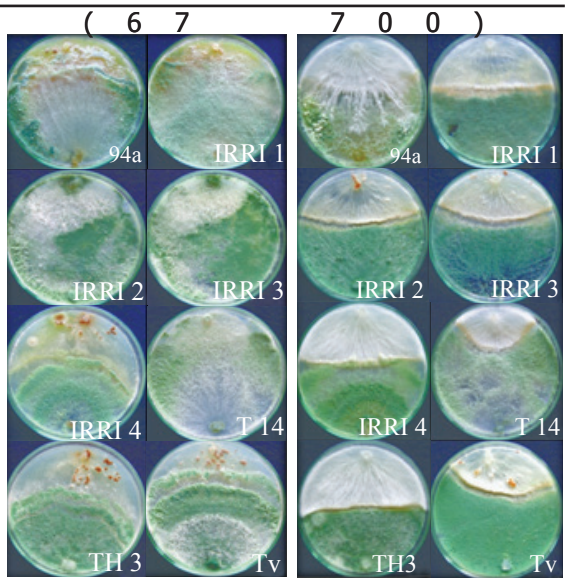

Figure 2: *In-vitro* mycoparasitic ability of different *Trichoderma* isolates against *S. rolfsii* and *R. solani*.

Table 4: *In vitro* mycoparasitic activity of different *Trichoderma* spp. against rice pathogen (s) *R. solani* and *S. rolfsii* following dual culture

Species of *Trichoderma* isolate (s)	% inhibition by *Trichoderma s*pp. over control		Reaction type	
	S. rolfsii	*R. solani*	*S. rolfsii*	*R. solani*
Trichoderma spp.				
94a	61.11	100	2	1
Trichoderma viride				
T_{14}	65.55	55.55	2	2
Tv	55.55	70	2	2
IRRI-1	57.77	100	2	1
IRRI-2	61.11	100	2	1
IRRI-3	60.00	100	2	1
IRRI-4	58.33	64.44	2	2
Trichoderma harzianum				
TH-3	52.22	53.33	3	3

1 type) also shows strong antagonistic property against *R. solani*. Rest of the isolates T_{14}, Tv, IRRI-4 were came under class 2 reaction type.

As a consequence, plants treated with beneficial fungi may be larger and healthier and have greater yields than plants without them. Mechanisms by which these changes occur are becoming known and it help in increased in PGPR activity of plant and it was known that BCF also increase percentages of germination and rates of germination of seeds (Barazani et al., 2005; Bjorkman et al., 1998; Chang et al., 1986; Mastouri et al., 2010). More importantly, the effect of BCF on plant growth has a long duration and even lasts for the entire life of annual plants (Barazani et al., 2005; Harman, 2000, Harman

et al., 2004, Waller et al., 2005). The many scientist worked on this objective and most of these studied show the positive results that *Trichoderma* suppressed the growth of plant pathogens present in soil.

The degree of antagonismwas not the same for particular *Trichoderma* isolate with both the phytopathogens. For example, isolates 94a, IRRI-1, IRRI-2 and IRRI-3 showing 100% apparent inhibition against *R. solani* was poor against *S. rolfsii*. All of the eight isolates are not very good antagonism against *S. rolfsii* as compared to *R. solani*, all of the isolates except TH-3 shows class 2 type reaction, was poor antagonists for both the phytopathogens.

Trichoderma species are biocontrol fungi (BCF) are beneficial organisms that reduce the negative effects of plant pathogens and promote positive responses in the plant. Recent data indicate that their abilities to control plant diseases are only a subset of their capabilities. They do control diseases and in addition have other benefits, including amelioration of intrinsic physiological stresses in seeds and alleviation of abiotic stresses. As a consequence, plants treated with beneficial fungi may be larger and healthier and have greater yields than plants without them. The PGPR are known to participate in many important ecosystem processes, such as the biological control of plant pathogens, nutrient cycling, and/or seedling growth (Persello-Cartieaux et al., 2003; Barea et al., 2004; Zahir et al., 2004).

Mechanisms of mycoparasitism have been studied with several modelspecies of *Trichoderma* which involvelysis of host's cell wall due to both physical as well as enzymatic activity (Benitez et al., 1998). It was observed that the mycoparasite drained out the host mycelia contentsthus leaving behind the empty host carcass (Agrawal and Kotasthane 2010a, b). Mycelial growthof *Sclerotinia sclerotiorum* (Kapil and Kapoor., 2005; Lee and Wu 1984) was inhibited by metabolites produced by *T. viride*. Mukherjee et al., (2003) and Rawat and Tewari (2011) reported that in contrast to hyperparasitic coiling in *R. solani*, hyphal overgrowth occurs during *S. rolfsii* interaction by *Trichoderma* spp. Molecular mechanisms involving expression of different enzymes, metabolites and differentiation betweenself- versus non-self-fungal cell-wall degradation has been well documented and reviewed (Mukherjee et al., 2012a, b; Baker et al., 2012; Gruber and Seidl-Seiboth, 2012). This opens new avenues towards understanding of the use and careful choice of the senecrotrophs as potential biocontrol agents to replacechemical control agents.

3.3. Screening isolates of Trichoderma spp. for in vitro phosphate solubilization ability

Each of the 8 isolates was capable of differentially utilizing tri-calcium phosphate in broth assays. Quantitative estimation of soluble phosphate concentrations in Pikovskaya's broth was expressed as µg ml^{-1} and it varied from 74 to 365.5 µgmL-1. All the rhizosphericisolates of *Trichoderma* showed variable phosphate solubilizingpotential with T_{14}, IRRI-4, TV and TH3 being the best Psolubilizers (Figure 3 and Table 5).

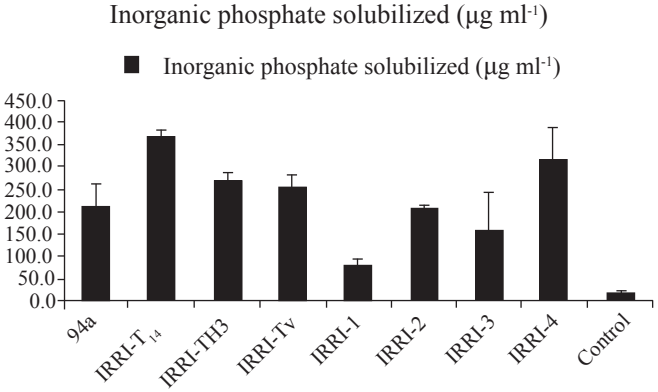

Figure 3: Quantitative estimation of inorganic phosphate concentration (µg ml^{-1}) produced by different isolates of *Trichoderma* spp. in colorimeter (OD-610 nm)

Table 5: Screening isolates of *Trichoderma* spp. *in vitro* for phosphate solubilizing ability

Sl.no.	Isolates	pH of the culture media	Inorganic phosphate solubilized (µg ml^{-1})
Trichoderma spp.			
1.	94a	4.718±0.180	209.5±54.44
Trichoderma viride			
2.	T_{14}	4.073±0.067	365.5±16.263
3.	Tv	6.1475±0.018	250.5±2.121
4.	IRRI-1	6.0675±0.760	74±21.213
5.	IRRI-2	6.16±0.085	205±11.314
6.	IRRI-3	5.6375±0.803	155.5±88.388
7.	IRRI-4	6.0025±0.046	315±73.539
Trichoderma harzianum			
8.	TH3	5.773±0.293	266±22.627
9.	Control	6.64±0.014	14±7.071

Phosphorus is the second most important micro nutrient in the soil and is essentially unavailable to the plants and use of plant associated organisms may help in solubilization of mineral Phosphate (P) for easy uptake by the plants andtheir mobilization(Rudresh et al. (2005). Fungi are reported to solubilize P by production of organic acids and are known to have a higher efficiency of solubilization than bacteria. It was observed that *Trichoderma* viride isolates showed higher ability to solubilize the phosphate as they also exhibited good responses to PGPR activity after direct seed treatments.

• *Trichoderma viride* (#T_{14}) exhibited higher phosphate solubilizing ability in pikovaskaya broth media used for screening and therefore can be considered as promising inducer of phosphate mobilization. The amount of inorganic phosphate solubilized was 365.5 µg mL^{-1}.

• Other *Trichoderma viride* isolates i.e. #IRRI-2, #IRRI-3, #IRRI-4 and #Tv also a good phosphate solubilizer that they solubilized 205 µg mL^{-1}, 155 µg mL^{-1}, 315 µg mL^{-1}, 266 µg mL^{-1}, 250.5 µg mL^{-1} respectively.

• *Trichoderma harzianum* (#TH-3) showed good phosphate solubilising ability (266 µg mL^{-1}) in pikovaskaya broth media as compared to control. Isolate #94a produced 209.5 µg mL^{-1} phosphate in media µg mL^{-1} (Table 5).

Several metabolic factors such as phosphatesolubilisation, siderophore and auxin production maybe responsible for growth regulation in different agricultural and vegetable crops. Dunaitsev et al. (2008) demonstrated *Trichoderma* spp. as promising inducers of phosphate mobilization. The ability to release phosphorus from mineral raw materials can be considered as promising producers of a biological preparation of combined an operating bio-fungicide and inducer of phosphate mobilization.

3.5. Quantification of indole acetic acid (auxin) production by Trichoderma spp.

Production of IAA was evaluated forplaceµ eight isolates of *Trichoderma* in culture medium amended with 1.02 g l^{-1} of L-tryptophan as precursor molecule. *Trichoderma* spp. produces auxins that are able to stimulate plant growth and root development. Auxins are key hormones effecting plant growth and development that can be produced by fungi in both symbiotic and pathogenic interactions with plants (Gravel et al., 2007; Losane and Kumar, 1992; Shayakhmetov, 2001). It was known that IAA (auxin) helps in plant growth and potentially increases the root length as well. Potential indole acetic acid-producing isolate (T_{14}) was identified in the current study and it also shows the significant increase in root length after seed treatment this shows that T_{14} was the promising isolate to produce IAA. Laboratory studies have emphasized on use of (plant growth promoting fungi) PGPF as biocontrol agents (Hossain et al., 2007) and the role of auxin (IAA) production (Contreras-Contreras-Cornejo et al., 2009) in plant growth promotion.

Interpolation of the colorimeter readings with standard curve were used to quantify the amount of IAA produced by different isolates of *Trichoderma* in the media which ranged from 1.09 to 35.54 µg ml^{-1} (Figure 4 and Table 6). The highest IAA was produced by *Trichoderma viride* isolate #T_{14} (35.54 µg ml^{-1}) which is significantly high whereas isolate #IRRI-4 (1.09 µg ml^{-1}) was the lowest producer. Other isolates such as 94a, Tv, IRRI-1, IRRI-2, IRRI-3 and TH-3 produces 8.68 µg ml^{-1}, 12.1368 µg ml^{-1}, 4.545 µg ml^{-1}, 4.40 µg ml^{-1}, 8.72 µg ml^{-1} and 11.63 µg ml^{-1} Indol acetic acid (IAA). After T_{14} the promising IAA producer was Tv (*Trichoderma viride*). Several auxin-like secondary metabolites produced by *Trichoderma* strains were able to induce plant growth and are required for development of lateral roots in Arabidopsis (Contreras et al., 2009; Vinale et al., 2008) that enhances the water holding capacity of plant.

3.6. Evaluation for plant growth promoting response

The root pulling strength (RPS) of 83 different rice cultivars/ lines were recorded in field condition using root puller machine according to technique describe by O'Toole (1987) to identify the cultivar with deep and denser root system. Because if the force required to pull the complete hill of plant is greater means that the cultivar is having the deep or denser

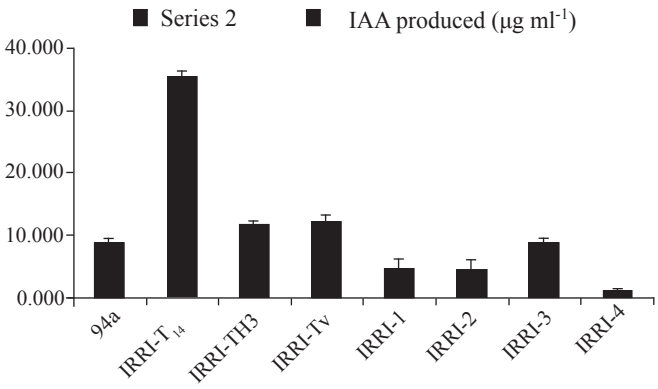

Figure 4: Quantitative estimation of IAA (µg ml^{-1}) produced by different isolates of *Trichoderma* spp. in colorimeter (OD-535 nm)

Table 6: Efficacy of different *Trichoderma* spp. for IAA production

Sl. No.	Isolates	pH of the culture media	IAA produced (µg ml^{-1})
Trichoderma spp.			
1.	94a	6.5	8.682±0.450
Trichoderma viride			
2.	T_{14}	6.34	35.545±1.029
3.	Tv	6.38	12.136±1.350
4.	IRRI-1	6.57	4.545±1.671
5.	IRRI-2	6.45	4.409±1.607
6.	IRRI-3	6.43	8.727±0.900
7.	IRRI-4	6.76	1.091±0.643
Trichoderma harzianum			
8.	TH3	6.67	11.636±0.900

root morphology. Ekanayake et al. (1985) found a significant positive correlation across diverse rice genetic materials between root pulling force and dehydration avoidance as expressed in leaf water status maintenance and visual scored of drought resistance under severe drought stress in the field. The observation on root pulling force formed the basis of selection (Table 2) to evaluate the effect of seed treatment with different isolates of Trichoderma on different parameters of root which has a direct effect on alleviating biotic, abiotic and physiological stress responses.

The root pulling strength was recorded at maximum tillering stage. Root Pulling Strength (RPS) varied from 21.5 (minimum) for rice line SLO-16 to 40 for cross 116 and bhataphool (maximum). Out of 83 rice lines/genotype, each of 10 rice lines/cultivar has been selected on the basis of Root Pulling Strength (RPS). The standard scaling method used to evaluate the increase in root length (cm) as compare to control.

The observations were recorded for root length (RL), the mean root length varied from 9.67 cm for a rice line IR83381-B-B-137-3 (minimum RL) to 23.83 for IR84859-B-41-1-2 (maximum RL) after treatment with different *Trichoderma* isolates. *Trichoderma viride* isolate T_{14} produced significantly higher increase in root length as compared to control and other treatments. Besides this isolates designated as IRRI-1, IRRI-2, IRRI-3 and IRRI-4 (*Trichoderma viride*) were observed to be more effective to promote plant growth and modulating plant physiology (Figure 5).

It was observed that both positive and negative inducers for root and shoot length can be found in the isolates of single species. The present investigation shows that use of any particular *Trichoderma* spp. as a plant growth promoter cannot be generalised for all the test materials. Tucci et al. (2011) demonstrated the effects of the plant genetic background on the outcome of the interaction between different tomato lines and two biocontrol strains of *T. atroviride* and *T. harzianum*. They observed that in at least one tomato cultivar the *Trichoderma* treatment did not exert any plant growth promotion effect and was even seen to be detrimental. However, *Trichoderma* are multifunctional plant symbionts responsible for enhanced nutrient uptake, increased root and shoot growth, improved plant vigour and biotic/abiotic stress tolerance (Inbar et al., 1994; Yedidia et al., 2001; Harman, 2011). Several lines of evidence indicate that *Trichoderma* induce phytohormones-like effect in the treated plants which is responsible for root and shoot development. Several auxin-like secondary metabolites produced by *Trichoderma* strains were able to induce plant growth and are required for development of lateral roots in Arabidopsis (Contreras-Cornejo et al., 2009;

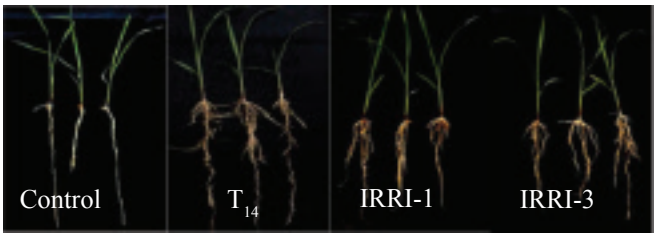

Figure 5: Difference as compared to control in root morphology of elite rice line after seed treatment with different *Trichoderma* isolates.

Vinale et al., 2008).

Seed treatment with *T. viride* T_{14} induced comparatively larger effects similar to hormonal application forplant growth promotion as compared to other isolates used in the present investigation which was measurable in terms ofindole acetic acid production (35.54 µg ml^{-1}) and phosphate solubilisation (365.5 µg ml^{-1}). This result reveled that these two activities of plant *Trichoderma* interaction help to increase plant growth in term of root and shoot. Apparent correlation between plant growth and metabolite production was observed for *T. viride* isolate T_{14}. *T. viride* isolate T_{14} in the present study had a characteristic aromatic odor resembling coconut reported to be produced commonly by strains of *T. viride*. 6-Pentypyrone (6-PP), the 'coconut aroma'volatile compound produced by *Trichoderma* spp., is one of the best-studied secondary metabolites from a biocontrol perspective having both antifungal and plant growth-promoting activities (Mukherjee et al., 2012a).

4. Conclusion

Trichoderma genomes have revealed mycotrophy and mycoparasitism as ancestral lifestyles of species of this genus. Some *Trichoderma* strains have become established in the plant rhizosphere and evolved as intercellular root colonizers. As a result, they stimulate plant growth and defences against pathogens like *Rhizoctonia solani* and *Sclerotinia rolfsii*. It also has the phosphate solubalization ability that can help the plant to utilized phosphate for soil rocks. Production of IAA helps in plant growth with increases the biomass of plant and ultimately the yield.

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6. References

Agrawal, T., Kotasthane, A.S., 2010a. A simplified method

for extraction of genomic DNA from filamentous fungus belonging to the genus *Trichoderma*. International Journal of Biotech and Bioengineering Resources 1, 47–52.

Agrawal, T., Kotasthane, A.S., 2010b. Mycoparasitism and AFLP characterization of *Trichoderma* spp. isolated from Chhattisgarh in Central India effective against *Rhizoctonia solani* infecting rice. Journal of Mycology and Plant Pathology 40, 532–539.

Barazani, O., Benderoth, M., Groten, K., Kuhlemeier, C, Baldwin, I.T., 2005. *Piriformospora indica* and *Sebacina vermifera* increase growth performance at the expense of herbivore resistance in *Nicotiana attenuata*. Oecologia (Berlin) 43,146−234.

Barea, J.M., Azcon, R., Azcon-Aguilar, C., 2004. Mycorrhizal fungi and plant growth promoting rhizobacteria. In: Varma A, Abbott L, Werner D, Hampp R, eds. Plant surface microbiology. Heidelberg, Germany: Springer-Verlag, 351–371.

Bell, D.K., Wells, H.D., Markham, C.R.,1982. *In vitro* antagonism of *Trichoderma* species against six fungal plant pathogens. Phytopathology 72, 379–382.

Benitez, T., Delgado-Jarana, J., Rincon, A.M., Rey, M., Limon, M. C.,1998. Biofungicides: *Trichoderma* as a biocontrol agent against phytopathogenic fungi. In S.G. Pandalai (Ed.), Recent research developments in microbiology 2, 129–150.

Chang, Y.C., Baker, R., Kleifeld, O., Chet, I.,1986. Increased growth of plants in the presence of the biological control agent *Trichoderma harzianum*. Plant Disease70, 145–148.

Contreras-Cornejo, H.A., Macias-Rodriguez, L., Cortés-Penagos, C., Lopez-Bucio J.,2009. *Trichoderma virens*, a plant beneficial fungus, enhances biomass production and promotes lateral root growth through an auxin-dependent mechanism in Arabidopsis. Plant Physiology149,1579–1592.

Contreras-Cornejo, H.A., Macias-Rodríguez, L., Beltrán-Pena, E., Herrera- Estrella, A., Lopez-Bucio, J., 2011. *Trichoderma* induced plant immunity likely involves both hormonal- and camalexin dependent mechanisms in *Arabidopsis thaliana* and confers resistance against necrotrophic fungus *Botrytis cinerea*. Plant Signal Behavior 6,1554–1563.

Dennis, C.J., Webster, J.,1971. Antagonism properties of species groups of *Trichoderma*, III hyphal interaction. Transactions of the British Mycological Society.57, 363–369.

Dworkin, M., Foster, J., 1958. Experiments with some microorganisms, which utilize ethane and hydrogen. Journal of Bacteriology 75, 592–601.

Dunaitsev, I.A., Kolombet, L.V., Zhigletsova, S. K., Bystrova, E.V., Besaeva, S.G., Klykova, M.V., Kondrashenko, T.N., 2008. Phosphate releasing microorganisms with antagonisticactivity against phytopathogenic microorganisms. Mikologiya i Fitopatologiya, 42, 264–269.

Ekanayake, I.J., Garrity, D.P., O'TOOLE, J.C.,1985. Influence of Deep Root Density of Root Pulling Resistance in Rice. Crop Science 26(1), 181-1186.

Gordon, S.A.,Weber, R.P., 1951. Colorimetric estimation of indole-acetic acid. Plant Physiology 26, 192–195.

Gravel, V., Antoun, H., Tweddell, R.J., 2007. Growth stimulation and fruit yield improvement of greenhouse tomato plants by inoculation with *Pseudomonas putida* or *Trichoderma atroviride* : possible role of indole acetic acid (IAA). Soil Biology and Biochemistry 39,1968–1977.

Gruber, S., SeidlSeiboth,V., 2012. Self-versus non-self: fungal cell wall degradation in *Trichoderma*. Microbiology 158,26–34.

Harman, G.E., 2011. Multifunctional fungal plant symbionts: new tools to enhance plant growth and productivity. New Phytologist 189, 647–649.

Harman, G.E., Howell, C.R., Viterbo, A., Chet, I., Lorito, M., 2004. Trichoderma species–opportunistic, avirulent plant symbionts. Nature Reviews Microbiology 2, 43–56.

Harman, G.E., Kubicek, P.K., 1998. *Trichoderma* and *Gliocladium* Vol 2. Enzymes, biological control and commercial applications. Taylor and Francis, London, 1−393.

Harman, G.E., 2000. Myths and dogmas of biocontrol. Changes in perception derived from reaserch on *Trichoderma herzanum* T22. Plant Diseases 84, 377−393.

Hossain, M., Sultana, F., Kubota, M., Koyama, H., Hyakumachi, M.,2007. The plant growth-promoting fungus *Penicillium simplicissimum* GP17-2 induces resistance in *Arabidopsis thaliana* by activation of multiple defense signals. Plant Cell Physiology 48,1724−1736.

Inbar, J., Abramsky, M., Cohen, D., Chet, I.,1994. Plant growth enhancement and disease control by *Trichoderma harzianum* in vegetable seedlings grown under commercial conditions. European Journal of Plant Pathology 100, 337–346.

Jackson, M.L.,1973. Soil chemical analysis (pp 392). New Delhi: Printice hall of India.

Kapil, R., Kapoor, A.S., 2005. Management of white rot of pea incited by *Sclerotinia sclerotiorum* using *Trichoderma* spp. and biopesticides. Indian Phytopathology 58,10–16.

Lee, Y.,Wu, W., 1984. The antagonisms of *Trichoderma* spp. and *Gliocladium virens* against *Sclerotinia sclerotiorum*. Plant Protection Bulletin (Taiwan ROC), 26, 293–304.

Losane, B.K., Kumar, P K.R.,1992. Fungal plant growth regulators. In: Arora, D.K., Elander, K.G., Mujerji, K.G. (Eds.), Handbook of Applied Mycology. Fungal Biotechnology 4, 565–602.

Mastouri, F., Bjorkman, T., Harman, G.E., 2010. Seed treatment with *Trichoderma harzianum* alleviates biotic, abiotic, and physiological stresses in germinating seeds and seedlings. Phytopathology 100, 1213−1221.

Mukherjee, P. K., Latha, J., Hadar, R., Horwitz, B. A. 2003. TmkA, a mitogen-activated protein kinase of *Trichoderma virens*, is involved in biocontrol properties and repression of conidiation in the dark. Eukaryotic Cell2, 446–455.

Mukherjee, P.K., Buensanteai, N., Moran-Diez, M.E., Druzhinina, I.S., and Kenerley, C.M., 2012a. Functional analysis of non-ribosomal peptide synthetases (NRPSs) in *Trichoderma virens* reveals a polyketide synthase (PKS)/ NRPS hybrid enzyme involved in the induced systemic resistance response in maize. Microbiology 158, 155–165.

Mukherjee, P.K., Horwitz, B.A., Kenerley, C.M., 2012b. Secondary metabolism in *Trichoderma*–a genomic perspective. Microbiology 158, 35–45.

O'Toole, J.C., Bland, W.L., 1987. Genotypic variation in crop plant root systems. Advances in Agronomy 41, 91–143.

Persello-Cartieaux, F., Nussaume, L., Robaglia, C., 2003. Tales from the underground: molecular plant–rhizobacteria interactions. Plant, Cell and Environment 26,189–199.

Pikovskaya, R.I.,1948. Mobilization of phosphorus in soil connection with the vital activity of some microbial species. Microbiologiya 17, 362–370.

Rawat, R., Tewari, L. 2011. Effect of abiotic stress on phosphate solubilization by biocontrol fungus *Trichoderma* spp. Current Microbiology 62, 1521–1526.

Rudresh, D.L., Shivaprakash, M.K., Prasad, R.D., 2005. Tricalcium phosphate solubilizing abilities of *Trichoderma* spp. in relation to P uptake and growth and yield parameters of chickpea (*Cicer arietinum* L.). Canadian Journal of Microbiology 51, 217–222.

Samuels, G.J., Dodd, S., Lu, B., Petrini, O., Schroers, H.J., Druzhinina, I. S.,2006. The *Trichoderma* koningii morphological species. Studies in Mycology 56,67–133.

Saxena, A.K., Lata,Shende R., Pandey, A.K., 2005, Culturing of plant growth promoting rhizobacteria. In: Gopi, K.P., Varma, A. (Eds.), Basic research applications of mycorrhizae. I K International Pvt Ltd, New Delhi, 453–474.

Shayakhmetov, I. F., 2001. Biological activity of metabolites from culture filtrate of *Cochliobolus sativus* and *Fusarium oxysporum* in connection with in vitro cellular selection of crop plants for resistance to phytopathogens. Mycology and Phytopathology 35, 66–71.

Tucci, M., Ruocco, M., De Masi, L., De Palma, M.,Lorito, M., 2011. The beneficial effect of *Trichoderma* spp. on tomato is modulated by the plant genotype. Molecular Plant Pathology 12, 341–354.

Van Wees, S.C.M., vanderEnt, S., Pieterse, C.M.J.,2008. Plant immune responses triggered by beneficial microbes. Current Opinion in Plant Biology 11, 443–448.

Venkateswarlu, B., Desai, S., Prasad, Y.G., 2008. Agriculturally important microorganisms for stressed ecosystems: Challenges in technology development and application". In: Khachatourians, G.G., Arora, D.K., Rajendran, T.P., Srivastava, A.K. (Eds.), Agriculturally important Microorganisms, vol 1. Academic World, Bhopal, 225–246.

Vinale, F., Sivasithamparam, K., Ghisalberti, E.L., Marra, R., Barbetti, M.J., 2008. A novel role for *Trichoderma* secondary metabolites in the interactions with plants. Physiological and Molecular Plant Pathology 72, 80–86.

Waller, F., Achatz, B., Baltruschat, H., Fodor, J., Becker, K., 2005. The endophytic fungus *Piriformospora indicare* programs barley to salt-stress tolerance, disease resistance, and higher yield. In: Proceedings of the National Academy of Sciences of the United States of America 102, 13386–91.

Yang, J., Kloepper, J.W., Ryu, C.M., 2009. Rhizosphere bacteria help plants tolerate abiotic stress. Trends Plant Sciences 14, 1–4.

Yedidia, I., Srivastva, A.K., Kapulnik,Y., Chet, I., 2001. Effect of *Trichoderma harzianum* on microelement concentrations and increased growth of cucumber plants. Plant and Soil 235, 235–242.

Zahir, Z.A., Arshad, M., Frankenberger, W.T., 2004. Plant growth promoting rhizobacteria: applications and perspectives in agriculture. Advances in Agronomy 8, 97–168.