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ROOT GROWTH STIMULATION IN RICE (*Oryza sativa* L.) BY SEED BIO-PRIMING WITH *Trichoderma* sp.

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ABSTRACT

***Trichoderma* species are commonly used as biological control agents against phytopathogenic fungi and some of its strains are able to produce metabolites that enhance plant growth. In current study the elite rice germplasm population was used for the assessment of root pulling strength (RPS). RSP showed positive correlation with root length and other root parameters. On the basis of RSP, 10 lines each with higher root pulling strength (HRPS) and minimum root pulling strength (MRPS) were selected to check the effect of different *Trichoderma* spp. on root traits by seed biopriming. RPS varied from 21.5 for rice genotype 'SLO-16' to 40 for 'Cross 116' and 'Bhata Phool'. Greater RPS reflected dense root system or greater root length/volume. This analysis revealed that the response of *Trichoderma* spp. varied with different rice cultivars. Roots were analyzed using Epson perfection v700/v750 3.81 version scanner with WinRhizoReg software. All the isolates of IRRI showed positive response on increase in root length. The growth was enhanced in presence of rhizosphere-competent endophytic strains of *Trichoderma,* and these characteristics were strain-specific and not characteristic for species. These endophytic plant symbionts can be widely used as seed treatment to control diseases and enhance plant growth and yield.**

Key words: Plant growth promoting response (PGPR), rice, root pulling strength, root scanning, *Trichoderma*

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important food crops grown worldwide. To boost rice yield it is imperative to broaden the genetic variation base in germplasm. The demand for more agricultural productivity and quality has led to the excessive use of chemical fertilizers which, in turn, has serious environmental consequences. The use of biofertilizers and biopesticides is an alternative sustainable technology to enhance productivity without affecting ecology. Different soil-borne bacteria and fungi are able to colonize plant roots and show beneficial impact on plants. Besides the classic mycorrhizal fungi and *Rhizobium* bacteria, other plant-growth-promoting rhizobacteria (PGPR) and fungi such as *Trichoderma* spp. and *Piriformospora indica* reportedly stimulate plant growth by suppressing plant diseases (Hermosa *et al.*, 2012).

Trichoderma species are highly opportunistic fungi isolated from diverse range of natural and artificial substrata and show adaptability to various ecological conditions (Druzhinina *et al*., 2012). In rice, several root characteristics *viz.*, root length, rooting depth, root thickness, root to shoot ratio, etc. play an important role in resisting water deficit environmental stress. Rice cultivars with longer, thicker and bigger root systems are considered drought resistant and are associated, in some cases, with higher grain yield under drought. O'Toole and Bland (1987) reported that greater rooting depth and density results in more available soil water and hence better resistance to moisture stress. The single application of *Trichoderma* as seed treatment is considered beneficial for the enhancement of root-related traits inducing vigorous plant root system. Some strains of *Trichoderma* have the ability to reduce the severity of plant diseases by inhibiting plant pathogens, mainly in the soil or on plant roots, through their high antagonistic and mycoparasitic potential (Viterbo and Horwitz, 2010; Grover *et al.*, 2011). Some *Trichoderma* rhizosphere-competent strains reportedly have direct effects on plants by increasing growth potential, nutrient uptake, fertilizer use efficiency and seed germination and stimulation of plant defenses against biotic and abiotic damage (Shoresh *et al*., 2010). *Trichoderma* is gaining importance because of its involvement in plant growth promoting response (PGPR) to elicit tolerance in plants for various stresses. 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). Growth stimulation is evidenced by increases in biomass, productivity, stress resistance and increased nutrient absorption. Increased crop productivity associated with *Trichoderma* has been observed in a broad range of plant species such as carnation, chrysanthemum, petunia, cucumber, eggplant, pea, pepper, radish, tobacco, tomato, lettuce, carrot, corn, and ornamental grasses (Mackenzie *et al*., 1995; Yedidia *et al*., 1999; Harman *et al*., 2004; Gravel *et al*., 2007; De Souza *et al*., 2008). *Trichoderma* species play important role in three-way interaction with plant and pathogen (Shoresh *et al*., 2010; Hermosa *et al*., 2012). *Trichoderma* species are endophytic plant symbionts that may establish themselves in rhizosphere and are widely used as seed treatments to control diseases and to enhance plant growth and yield (Mastouri *et al*., 2010; Harman 2011). Hence the present study was aimed to is to evaluate the response of different *Trichoderma* isolates on plant root growth stimulation.

MATERIALS AND METHODS

***Plant material*:** In this study the experimental population consisted of 83 elite rice lines/cultivars collection from undivided Madhya Pradesh and Chhattisgarh which include popular rice varieties, and advanced breeding lines.

***Fungal isolates*:** Eight genetically purified isolates of *Trichoderma* were isolated from Chhattisgarh region (India) and some isolates procured from STRASA- BMGF (Stress Tolerant Rice for Africa and South Asia- Bill and Melinda Gates Foundation) project were T-14 (*Trichoderma viride)*, 94a (not defined), IRRI-Tv (*Trichoderma viride)*, IRRI-TH-3 (*Trichoderma harzianum)*, IRRI-1 (*T. viride)*, IRRI-2 (*T. viride)*, IRRI-3 (*T. viride)* and IRRI-4 (*T. viride)*.

Assessment of root pulling strength of elite rice lines

Eighty three rice cultivars were planted out in a field at the experimental farm of College of Agriculture, IGKV, Raipur (India). Two replications for each rice germplasm line/varieties were maintained in each phenotypic evaluation experiment conducted during 2013-2014. The seeds of each line were sown in nursery and a single seedling from each rice variety was transplanted in the field of plot size 30 m width and 54 m length area of fields and plant to plant spacing of 15 x 15 cm in two replicates. At maximum tillering stage randomly 5 plants of each line/variety was sampled to

examine root pulling strength using root pulling machine methods used by IRRI (Philippines). On the basis of root pulling strength 10 rice genotypes having lower root pulling strength (LRS) and higher root pulling strength (HRS) were selected. The selected rice genotypes were used for screening the effect of *Trichoderma* isolates on plant growth promotion activity (Table 1).

Analyzing root system morphology in response to Trichoderma isolates for plant growth promoting activity on selected rice lines

For seed treatment, *Trichoderma* spores were harvested from 7-day old sporulating cultures of different isolates and re-suspended in 20 mL sterile distilled water containing 0.5% (w/v) carboxy methyl cellulose. The spore concentration was maintained to 10^8 conidia mL^{-1}. Seeds of selected 10 rice lines having lower root pulling strength (LRS) and 10 lines having higher root pulling strength (HRS) were treated with spore suspension for one hour. Treated seeds were sown in pots containing autoclaved soil and regularly irrigation every day as per the need. The soil was not amended with any fertilizer. Control seeds were treated with 0.5% (w/v) carboxy methyl cellulose prepared in sterile distilled water. The pots were kept in a green house with temperature 28-32°C. The seedlings were harvested 13 days after sowing. The roots of seedling were removed by submerging the pots in water for 1 h so that the soil in pots was loosened and roots could be easily separated. The increase in root length was recorded using standard scaling method and the roots were preserver in 25% ethanol for root scanning.

Root scanning of selected rice lines

The root scanning was done by using root scanner machine Epson Perfection V700/V750, 3.81 version, and the data was analyzed using WinRhizoReg 2009 software (Regent Instruments Inc., Quebec, Canada) [source: *http://regent.qc.ca/assets/winrhizo_about.html*]. The data for various root parameters including root length, average root diameter, root volume, number of tips, forks, surface area, etc. was recorded. Generally the roots were scanned at 600 dpi in 10 x15 cm trays and the optimum scanning resolution depends on the type of samples.

RESULTS AND DISCUSSION

Root system morphology and plant growth promoting response against Trichoderma isolates

The root pulling strength (RPS) of 83 different rice cultivars/lines were recorded in field using root puller machine to identify the cultivar with deep and denser root system. If the force required to pull a complete hill of plant is greater it means that the cultivar has deep or denser root morphology. Ekanayake *et al.* (1985) in diverse rice genetic materials found a significant positive correlation 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 field. The root pulling force formed the basis for genotype/variety selection for experiment on evaluating the effect of seed treatment with various *Trichoderma* isolates on root parameters which influence biotic, abiotic and physiological stress responses. RSP varied from 21.5 (for rice line SLO-16) to 40 (for Cross 116 and Bhata Phool). The standard scaling method was used to evaluate the increase in root length as compare to control. The mean root length varied from 9.67 cm for rice line IR83381-B-B-137-3 to 23.83 for IR84859-B-41-1-2 after treatment with *Trichoderma*. Isolates IRRI-1, IRRI-2 and IRRI-3 of *Trichoderma viride* were observed to be more effective in promoting plant growth and modulating plant physiology (Fig. 1 and 2). The *Trichoderma* isolates showed differential response to the ten selected rice genotypes with maximum and minimum root pulling strength (Table 1). Enhanced root growth due to *Trichoderma* isolates was observed in some rice lines while other rice

Table 1: Root pulling strength (RPS) of selected rice genotypes/lines used for evaluating the effect of *Trichoderma*

S. No.	Genotypes /lines	Maximum RPS	S. No.	Genotypes /lines	Minimum RPS
1	RRF-82	34.0	1	RRF-69	27.0
2	IR 83929-B-B-132-2	37.0	2	RRF-78	27.5
3	IR 86781-3-3-1-1	38.0	3	IR83376-B-B-150-3	26.5
4	CB-09-510	37.5	4	IR86857-46-1-1-2	27.0
5	IR 84882-B-120-CRA-6	37.5	5	Annanada	27.0
6	IR 86857-46-1-1-2	34.0	6	Danteshwari	23.0
7	Swarna	37.0	7	Bamleshwari	28.0
8	Bakal	37.0	8	IR-64	29.0
9	ChaptiGurmatiya	38.0	9	Kalo kuchi-223	25.5
10	Safri 17	35.0	10	CR-5272	27.5

lines showed no increase in root length. The results suggested that *Trichoderma* could play a significant role in stress management, once their unique properties of tolerance to extremities, their ubiquity, genetic diversity are well-understood and the methods for their successful deployment in agriculture production are developed (Grover *et al.*, 2011). The enhancement in root growth due to *Trichoderma* may be attributed to the production of auxin and other phytoharmones in root zone which helps in root growth promotion. Auxin deprivation keeps pericycle cells in G1 phase while re-addition promotes G1-S transition of cell cycle, thus promoting lateral root initiation (Himanen

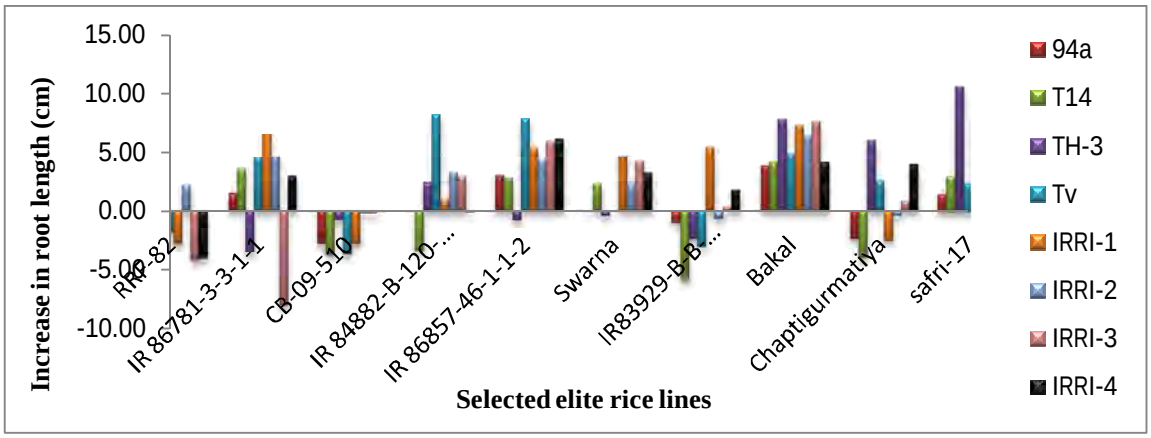

Fig. 1: Effect of seed treatment with various *Trichoderma* isolates on root length of selected rice genotypes with maximum root pulling strength

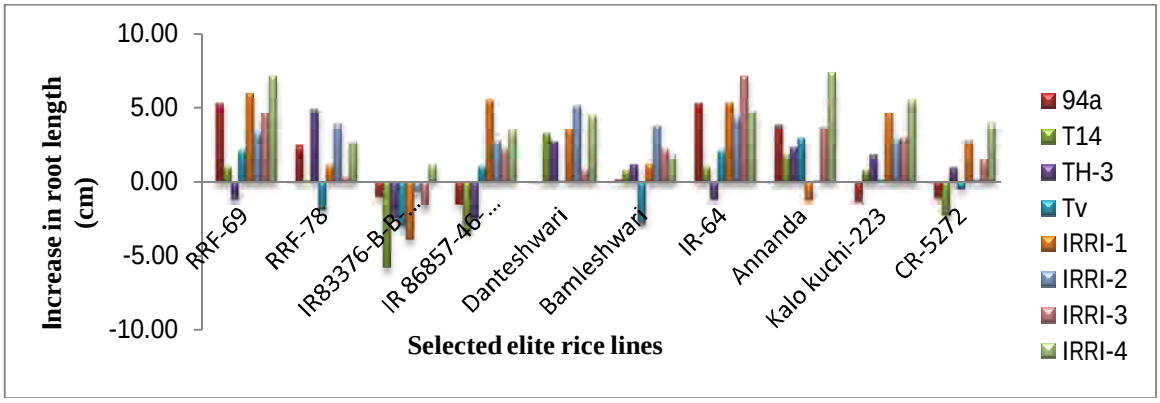

Fig. 2: Effect of seed treatment with various *Trichoderma* isolates on root length of selected rice genotypes with minimum root pulling strength

Table 2: Effect of seed treatment with various *Trichoderma* isolates on root parameters of selected rice genotypes with maximum root pulling strength

Total length (cm)		Root volume (cm^3)	
94a	IR83372-B-B-132-2 (175.33)	94a	Swarna (0.02)
TH-3	Swarna (157.00) IR86781-3-3-1-1 (156.35)	T14	IR 86857-46-1-1-2(0.02)
Tv	IR84882-B-120-CRA-6 (135.97)	Tv	IR86781-3-3-1-1(0.02), CB-09-510(0.02)
IRRI-1	Bakal (172.81) RRF-82(172.11) CB-09-510(137.41)	IRRI-1	IR 86857-46-1-1-2(0.02), Bakal (0.03) RRF-82(0.02), IR 84882-B-120-CRA-6(0.02), IR
IRRI-2	IR 86857-46-1-1-2 (206.13)	IRRI-2	86857-46-1-1-2(0.02), Swarna (0.02) CB-09-5100.02, IR 86857-46-1-1-2(0.02), Safri-
IRRI-3	Safri-17(176.37)	IRRI-3	17(0.02) RRF-82(0.02), IR 86857-46-1-1-2(0.02),
IRRI-4	Chapti Gurmatiya (197.89)	IRRI-4	IR83372-B-B-132-2(0.03), Safri-17(0.02)
Total surface area (cm^2)		Root tips (No.)	
TH-3	Chapti Gurmatiya (17.59), Safri-17(18.09) IR86781-3-3-1-1(14.55), IR 84882-		
Tv	B-120-CRA-6(15.00)	IRRI-1	Bakal (668.00), Safri-17(993.00) RRF-82(970.33), IR86781-3-3-1-1(827.00),CB-
IRRI-1	Swarna(15.56), Bakal (13.70) RRF-82(15.63), IR 86857-46-1-1-	IRRI-2	09-510(832.33), IR84882-B-120-CRA6(713.67), Swarna (929.67)
IRRI-2	2(15.73)	IRRI-3	IR83372-B-B-132-2(836.33) IR86857-46-1-1-2(925.00), Chapti Gurmatiya
IRRI-3	CB-09-510(13.19)	IRRI-4	(986.67)
IRRI-4	IR83372-B-B-132-2(15.28)		
Average diameter (mm)		Forks (No.)	
94a	Swarna (0.24), Safri-17(0.22) RRF-82(0.27), IR 84882-B-120-CRA-6(0.26), IR83372-B-B-132-2(0.26), Bakal (0.32), Chapti	Tv	Chapti Gurmatiya (376.33)
T14	Gurmatiya (0.23), Safri-17(0.22) IR 86857-46-1-1-2(0.29), Safri-	IRRI-1	Bakal (271.00) RRF-82(476.67), IR86781-3-3-1-1(320.00), IR
TH-3	17(0.22)	IRRI-2	84882-B-120-CRA-6(326.00), Swarna (292.00) CB-09-510(241.33), IR86857-46-1-1-2(350.33),
Tv	CB-09-510(0.32)	IRRI-3	Safri-17(348.00)
		IRRI-4	IR83372-B-B-132-2(282.33)

*Digit in parenthesis indicates increase in respective trait value as compared to control; 94a, T14, TH-3, Tv, IRRI-1, IRRI-2, IRRI-3 and IRRI-4 are *Trichoderma* isolates

et al., 2002). *Trichoderma* inoculation resulted in increased number of lateral roots which could be attributed to the *de novo* formation of lateral root primordial by activation of pericycle cells.

Effect on maximum root pulling strength (MRPS) rice lines/genotypes

To analyze closely and precisely assess the effects of *Trichoderma* on plant development, the roots derived from all the treatment combination were scanned for various root parameters like total length, total surface area, average diameter, root volume, number of root tips, number of forks were determined in 15 day old seedlings grown in pots. Following standard scaling method (with maximum root pulling strength) various treatment combination of *Trichoderma* v/s selected rice lines showed increase in different root parameters. Similar results have been obtained after root scanning. The rice line IR83372-B-B-132-2 (94a), IR86781-3-3-1-1 (Tv), Bakal (IRRI-1), RRF-82 (IRRI-2), Safri-17 (IRRI-3) and Chapti Gurmatiya (IRRI-4) exhibited remarkable increase in total

length with the respective isolates. Also, these genotypes had more root volume, total surface area of roots, more number of tips, root diameter and number of forks (Table 2). These root characteristics render the plant more efficient to absorb water and nutrients that ultimately enhances plant biomass. *Trichoderma* operates through a variety of mechanisms and appears to induce novel genes in plants contributing towards enhanced root growth. Kotasthane *et al.* (2015) have reported that *Trichoderma* isolates have the ability to stimulate growth in seeds of cucumber, bottle gourd and bitter gourd.

Effect on minimum root pulling strength (MRPS) of rice lines/genotypes

The observations recorded following root scanning method for *Trichoderma* v/s selected rice lines (with minimum root pulling strength) treatment-combination revealed increases in various root parameters. Some scanned images showed significant increase in root length, secondary roots and root branching (Fig. 4) for minimum RSP of rice genotypes. In present study the prominent rice genotypes like Annanda (94a), RRF-69, Danteshwari (IRRI-1) and Bamleshwari (IRRI-2) showed significant increase in all the root parameters (Table 3). 6-pentyl pyrone (6-PP), the 'coconut

Table 3: Effect of seed treatment with various *Trichoderma* isolates on root parameters of selected rice genotypes with minimum root pulling strength

Total length (cm)		Root volume (cm^3)	
94a	Annanda (175.33)	IRRI-1	Kalo kuchi-223(0.02)
IRRI-1	RRF-69V (166.07), IR 86857-46-1-1-2V (155.59)	IRRI-2	RRF-69 (0.02), RRF-78 (0.02), Danteshwari (0.02), Bamleshwari (0.02), IR64 (0.02)
IRRI-2	RRF-78 (172.1), IR-64 (177.09)	IRRI-3	Danteshwari (0.02), Bamleshwari (0.02), IR64 (0.02)
IRRI-3	Bamleshwari (170.20)	IRRI-4	RRF-78 (00.02), IR83376-B-B-150-3 (0.02), Bamleshwari (0.02), IR-64 (0.02), Annanda (0.03), Kalo kuchi-223 (00.02)
IRRI-4	IR83376-B-B-150-3 (139.38), Danteshwari (196.47),Kalo kuchi-223 (192.54), CR5272 (211.84)		
Total surface area (cm^2)		Root tips (NO.)	
Tv	Danteshwari (15.41)	TH-3	CR5272 (753.00)
IRRI-1	IR 86857-46-1-1-2 (12.53)	Tv	Bamleshwari (694.00), Kalo kuchi-223 (1065.33)
IRRI-2	RRF-69 (14.80), RRF-78 (15.63), Bamleshwari (14.80)	IRRI-1	RRF-69 (865.33)
IRRI-3	IR-64 (14.70)	IRRI-2	RRF-78 (970.33)
IRRI-4	IR83376-B-B-150-3 (12.78), Annanda (15.28), Kalo kuchi-223 (16.46), CR5272 (16.48)	IRRI-3	Annanda (836.33)
		IRRI-4	IR83376-B-B-150-3(930.67), IR86857-46-1-1-2 (699.0), Danteshwari (1174.33), IR-64 (1298.0)
Average diameter (mm)		Forks (No.)	
T14	Danteshwari (0.20), IR-64 (0.20), Annanda (0.26), CR5272 (0.21)	Tv	CR5272 (260.00)
Tv	IR 86857-46-1-1-2 (0.22)	IRRI-2	RRF-69 (251.67), RRF 78 (476.67), IR 86857-46-1-1-2 (246.33)
IRRI-1	Bamleshwari (0.22)	IRRI-4	IR83376-B-B-150-3 (338.67), Danteshwari (464.67), Bamleshwari (380.67), IR-64 (414.67), Annanda (282.33), Kalo kuchi-223 (293.33)
IRRI-3	RRF-69 (0.22), Bamleshwari (0.22), Kalo kuchi-223 (0.21)		
IRRI-4	RRF-78 (0.24)		

*Digit in parenthesis indicates increase in respective trait value as compared to control; 94a, T14, TH-3, Tv, IRRI-1, IRRI-2, IRRI-3 and IRRI-4 are *Trichoderma* isolates

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*., 2012). Vinale *et al.* (2008) reported an auxin-like effect in etiolated pea stems when treated with harzianolide and 6-pentyl pyrone, the major secondary metabolites produced by different *Trichoderma* strains. Above results of root scanning revealed that 4 rice lines *viz*., IR 86781-3-3-1-1, IR 86857-46-1-1-2, Bakal and Safri-17 (Fig. 3) were categorized as high responsive rice lines after treatment with *Trichoderma* isolates under maximum root pulling strength while RRF-69, Danteshwari, IR-64, Annanda were categorized as high responsive rice genotypes under minimum root pulling strength (Fig. 4) after treatment with *Trichoderma* isolates. The positive response of *Trichoderma* is due to the root-microorganism associations that cause substantial changes to plant proteome and metabolism. Plants are protected from a wide range of plant pathogen by responses that are similar to systemic acquired resistance and rhizobacteria-induced systemic resistance (Harman *et al.,* 2004).

The colonization of *Trichoderma* spp. in rhizosphere promote plant root growth by either controlling the population of pathogenic microorganisms in rhizosphere (Benítez *et al*. 2004) and

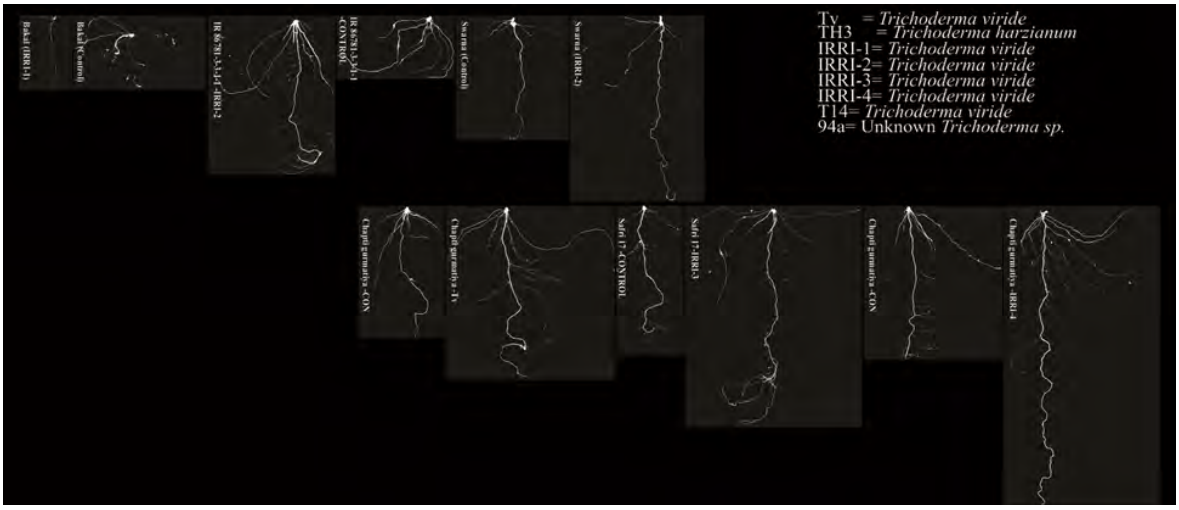

Fig. 3: Difference as compared to control in root morphology (scanning images) of elite rice line (with maximum root pulling strength) after seed treatment with different *Trichoderma* isolates

Fig. 4: Difference as compared to control in root morphology (scanning images) of elite rice line (with minimum root pulling strength) after seed treatment with different *Trichoderma* isolates

by influencing plant physiology through mineral solubilization (Altomare *et al*., 1999; Harman *et al.*, 2004) or hormone secretion (Blanchard and Bjorkman, 1996). Further, the plant-cell-wall degrading enzymes produced by *Trichoderma* such as xylanases and cellulases are able to induce ethylene biosynthesis in plants, a well-known response to the presence of pathogens (Kubicek and Harman, 1998). There are extensive evidences for the induction of plant defense systems by *Trichoderma* species in a number of plants (Howell *et al.*, 2000; Hanson and Howell, 2004).The present study revealed that *Trichoderma* is able to colonize plant roots, inducing changes in plant defense systems and plant physiology without destroying plant tissues (Yedidia *et al.*, 2000; Mariola *et al.*, 2007).

Conclusion: Root pulling strength showed a remarkable relation with root length, root thickness, roots branching and root number with most of the rice lines/cultivars. Differential response of *Trichoderma* seed treatment was observed for root parameters in a set of rice lines with minimum and maximum root pulling strength. Isolates of *Trichoderma viride* showed more increase in root length, secondary branching of roots as compared to other isolates.

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