J. Mycopathol. Res. **42(1)** : 9-13 *(2004)*

Influence of plant growth promoting fungi on growth and yield of chickpea, pea and mustard

BHARAT RAI AND T. SANTAVIR SINGH
Department of Botany, Banaras Hindu University, Varanasi 221005, India

The influence of some plant growth promoting fungi (PGPF), isolated from rhizospheres and rhizoplanes of chikpea, pea and mustard, has been studied. The PGPF were screened for their plant growth promoting activity through seed treatment with their culture filtrates on increase in per cent seed germination and seedling growth. *Cladosporium cladosporioides. Curvularia lunata. Fusarium moniliforme. Trichoderma harzianum* and *Trichoderma virens* (*Gliocladium virens)* exhibited stimulatory effect on seed germination and seedling growth and therefore these fungi were considered as PGPF.

Thereafter the PGPF were mass cultured on sterilized healthy boild wheat grains and were amended in soil. Seeds of each test crop were sown in polythene bags containing well mixed soil inocula of individual PGPF in different ratios and were incubatee on roof top open place protected all round with net. Increase in plant growth time of flowering and fruiting increase in yield and total dry biomass of each host were recorded.

All the PGPF increased the growth and yield of the test crops in *in vitro* and *in vivo* but more in case of mustard. Early flowering was seen in case of treated chickea and pea. *T harzianum* BS-2, *T. harzianum* BS-3 and *T. virens* were idenified as most potent plant growth promoting fungi of chickpea pea and mustard respectively.It is concluded that the PGPF have great potential to use them as biofertilizers by improving their strains through biotechnological aids which is a challenge to microbial biotechnologists.

Key words : Plant growth promoting fungi (PGPF), growth promotion and increase in yield of chickpea pea and mustard

INTRODUCTION

The beneficial effect of some non-pathogenic fungi in terms of plant growth promotion and biological control of plant diseases has recently drawn attention of microbiologists and plant pathologists. These fungi have been designated as plant growth promoting fungi (PGPF) like plant growth promoting rhizobacteria (PGPR).

Although several studies have been conducted on PGPR. only a few have dealt with PGPF. The potential of *Trichoderma harzianum* to increased growth of various floricultutral and horticultural crops was studied by Chang *et al. (*1986). Among fungi species of *Trichoderma* and *Rhizoctonia* have been found to promote plant growth and as to suppress plant pathogens (Sneh *et al.,* 1986 ; Windham *et al.* 1986 : Baker. 1988, 1991: Kleifeld and Chet, 1992). Certain isolates of *Trichoderma, Fusarium, Penicillium* and *Mucor*, isolated from the rhizoshere of turfgrass and some cultivated crops. displayed the activity of plant growth promotion as well as disease suppression (Hyakumachi. 1994).

During the present study, we have screened root region mycoflora of pea, chickpea and mustard for PGPF and have studied their activity to promote growth and yield of these crops *in vivo*.

MATERIALS AND METHODS

Isolation of root region mycoflora

The test plants were grown in field at the Agriculture Research Farm, Banaras Hindu

University (BHU), Varanasi. Rhizosphere and rhizoplane fungi were isolated from the healthy roots of the test plants by conventional soil dilution plate method and the method of Harley and Waid (1955). Soil dilution series of 1 : 100 and 1: 1000 were prepared in sterile distilled water for the isolation of rhizosphere fungi. Root bits in the flask were washed 10 times serially with sterile water and used for the isolation of rhizoplane fungi on Martin's Agar and Czapek Dox Agar media. After incubation at 25 ± 2°C for a week the fungi were identified and were maintained for the screening of their activity to promote growth and increase in yield of the selected crops.

Screening of growth promoting fungi in vitro

The isolated rhizosphere and rhizoplane fungi (RF) were screened *in vitro* through their effects on seed germination and seedling growth of the selected crops by seed treatment with their culture filtrates. Surface sterilized seeds of each plant were soaked for 24 h separately in culture filtrates tobtained by 10 days growth of the fungus in potato dextrose broth at 25 ± 2°C of each fungus in Erlenymeyer flasks.Seeds soaked for the same time in potato destrose broth medium and in sterilized water separately served as controls. Two layers of sterilized blotting paper moistened with sterillized distilled water were placed in sterilized petri plates.Five soaked seeds of pea, chickpea and 12 of mustard were placed in each petri plate and incubated for a week at 25 ± 2°C in NUV light for 12 h cycles of light and darkness. The culture filtrates of RF which showed higher per cent seed germination and more seedling growth in comparison to controls were considered as PGPF and were maintained for further trials *in vivo.*

Preparation of mass inocula of PGPF

PGPF were mass cultured on healthy wheat grains boiled for 40-45 min. These grains were spread over blotting papers for 20 min. to break the lumps and to reduce excess moisture. The grains were mixed with gypsum (Calcium sulphate) and chalk powder (Calcium carbonate) @ 2 and 1.5 % respectively on dry weight basis to check the pH of the medium and to prevent the grains from sticking each other. The grains were filled in flasks (500 ml), plugged with cotton and steam sterilized for 1 hr at 15 lb pressure. On cooling, 5 discs of 5 mm diameter from 7-day-old culture of PGPF were inoculated in each flask and incubated at 25 ± 2°C for 10-15 days. During incubation the flasks were shaken twice to ensure rapid and uniform colonized wheat grains were airdried in shade and wheat grain inoculum (WGI) was pulverized to 1-2 mm particle size with mortar and pestle and stored at 5°C.

Effect of soil amendment with PGPF on growth and yield of the crops

Dried brown loam virgin soil (pH 7.3) was used for pot experiment. The soil was air dried ground sieved and sterilized. Wheat grain inoculum of PGPF was mixed well with the soil @ 1% (w/W) and filled in polythene bags (2 kg per bag). The inoculted bags were kept at room temperature (27 ± 2°C) for a week and adequately watered. Soil amended with unifested and autoclaved wheat grains served as a control.Six healthy and surface sterilized seeds of pea. Chickpea and 12 seeds of mustard per bag were sown. Plant growth in terms of plant height early flowering and fruiting increase in yield and total dry biomass were recorded.

RESULTS

Isolation of rhizosphere and rhizoplane fungi and their screening for plant growth promoting activity in vitro

Total 38 fungal species from chickpea. 41 from pea and 35 from mustard plants were isolated and were screened for their plant growth promoting activity. Total 9 fungi namely. *Cladosporium cladosporioides. Curvularia lunata, Fusarium moniliforme, Trichoderma harzianum* BS-2, *T. harziamum* BS-2, *T. harzianum* BS-4, *T. harzianum* BS-8 and *T. virens* were recorded as plant growth promoting fungi (PGPF) (Table 1).

Effect of soil amendment with PGPF on growth and yield of the crops

All the test PGPF showed stimulatory effect on the growth and yield of selected crops (Tables 2). The continuity of plant growth promotion by PGPF was not similar for all the test crops. In case of mustard

remarkable increase in growth was observed from early stage of vegetative growth. There was almost no difference in plant height upto four weeks of seed sowing between treated and control plants of chickpea and pea, but after that considerable difference in growth occurred. Early flowering was found in PGPF treated chickpea and pea plants.

Almost all the PGPF which increased the plant height also enhanced the number of pods, total dry biomass and yield of the plants. The mean values of top length, total dry biomass, seed pod numbers showed significant increase ($P < 0.001$) in comparison to their respective controls. For chickpea and pea both *Cladosporium cladosporioides* and *C. lunata* enhanced the pod numbers significantly ($P < 0.01$) but other PGPF increased the same more significantly ($P < 0.001$) in comparison to control. In chickpea, *Trichoderma harzianum* BS-2 maximally enhanced the top length, pod numbers, total dry biomass and yield than the control (Table 2). In pea, maximum increase in top length, pod numbers, total dry biomass and yield was observed by *T. virens (*Table 2). All the PGPF showed more enhancement in terms of yield in case of mustard as compared to chickpea and pea plants (Table 2). The maximum enhancement was shown by *T. harzianum* BS-3 and the minimum by *C lunata.*

DISCUSSION

In the present study all the selected PGPF stimulated growth and yield of the test crops in *in vitro* and *in vivo.* The plant growth promotion of some cultivated crops by certain fungi have been reported by other workers also (Chang *et. al.,* 1986 ; Windham *et. al.,* 1986 ; Baker, 1991; Kleifeld and Chet, 1992 ; Hyakumachi *et. al.,* 1992, 1993a ; Hayakawa *et. al.* 1993; Hyakumachi, 1994). All the PGPF increases the growth of mustard from seedling to maturity stage whereas in case of chickpea and pea the growth enhancement was found after 4 weeks of sowing. It suggests that more prolonged association of PGPF with plants will result more apparent beneficial effect.

The mechanisms of growth enhancement by inoculation with PGPF are not well understood.

Table 1: Effect of culture filtrates of effective fungi per cent seed germination and seedling growth of chickpea. pea and mustard *in viro*

Name of fungi	Seedling growth (cm)*			
	Chickpea	Pea	Mustard	
			Root length(cm)	Soot length (cm)
Cladosporium cladospoioides	5.51**	5.38**	3.86	2.08
Curvularia lunata	5.50**	5.42**	3.90	2.07
Fusarium moniliforme	5.75**	5.63**	4.51	2.30
Trichoderma harzianum BS-2	6.39	6.31	4.75	2.68
T. harzianum BS-3	6.36	6.38	4.87	2.82
T. harzianum BS-1	6.01**	6.19**	4.34	2.55
T. virens	6.55	6.39	4.61	2.37
T. viride BS-7	5.81**	6.02**	4.27	2.41
T.viride BS-8	6.45**	5.87	4.43	2.49
Control in distilled water	5.37	5.09	2.51	1.13
Control in liquid medium	5.41	5.12	2.52	1.18
CD (P=0.001)	0.9191	1.0813	0.6044	0.5970

*Average of three replicates

Note: 100% germination of healthy seeds was obseved of the test plants with the treatments of all the test fungi.

**Mean values seedling growth are not significant (P=0.001) in comparison to controls.

One of the hypotheses is that PGPF suppress deleterious soil microbes by competing for active sites. Some evidence of this hypothesis concerns with the role of these isolates in reducing the soil-borne diseases of crop plants (Hyakumachi *et. al.,* 1993b ; Singh, 1997). Further, the plant growth promotion by microbes have been related with their capability of production of growth regulating substances (Arshad and Frankenberger, 1991) or increasing the absorption of mineral nutrients by plants (Barber and Lynch, 1977). Association of PGPF isolates with roots might help plants to derive mineral nutrients in the readily available form. This might be closely related to the root colonization ability of PGPF isolates. Dewan and Sivasithamparam (1989a, b) demonstrated that the sterile red fungus invaded the inner root regions which helped plants in deriving nutrients from the soil and in protecting root regions which helped plants in deriving nutrients from the soil and in protecting roots against pathogens. The possibility of some growth promoting isolates of *Phialophora graminicola* to increase the mineral uptake by plants in the same way as do mycorrhizal fungi was reported by Cowan (1979). A significant correlation between the cellulose and starch degrading activity of certain sterile PGPF isolates and plant growth promotion was reported by Kohra *et al.,* (1993).

Table 2 : Effect of soil amedment with PGPF inocula (1%W/W) on growth and yield of chickpea. pea and mustard in potted natural soil round figures).

Name of PGPF	Toplength* after 12 weeks (cm)			Pod numbers*			Total dry biomass* (g/plant)			Seed numbers*		
	CP	P	M	CP	P	M	CP	P	M	CP	P	M
Cladosporium cladospoioides	37	33	7	3**	2**	12	3	3	788	3	7	162
Curvularia lunata	38	33	8	3**	2**	10	2	3	757	3	5	138
Fusarium moniliforme	40	37	7	4	2	13	4	3	920	3	7	176
Trichoderma harzianum BS-2	41	36	10	5	2	17	4	4	1343	4	9	265
T. harzianum BS-3	38	36	10	4	2	18	4	3	1204	4	8	328
T. harzianum BS-1	38	34	7	4	2	14	3	3	934	3	8	185
T. virens	40	40	9	4	3	16	3	4	1058	4	10	212
T. viride BS-7	38	33	9	3	2	16	3	3	982	3	6	207
T. virede BS-8	39	34	7	3	2	11	3	3	834	3	9	148
Control	30	26	4	2	1	3	2	1	476	2	2	26

CP-chickpea. P-pea. M- mustard

Note: The mean valuesw in each column showed significant increase (P=0.001) except those followed by ** (P=0.01) compared with their respective controls.

* Average of three replicates.

CD (P=0.001)	Chickpea	Pea	Mustard
Top length	3.1456	5.6358	2.3917
Pod numbers	1.2377	1.0302	5.7627
Total dry biomass	0.3569	0.3062	0.0605
Seed numbers	1.0744	2.1239	0.0350

Table 3 : Chemical analysis of soil samples amended with *Trichoderma* speceis before and after growth of chickpea plants in pots for phosphorus, potassium and nitrogen contents.

Speceis of *Trichoderma*	Contents (mg/Kg)					
	Available phosphorus (P_2O_5)		Available potassium (K_2O)		Available nitrogen (NH_3N)	
	BS	AH	BS	AH	BS	AH
Trichoderma harzianum BS-3	13.5	16.1	81.5	84.0	109.2	114.8
T.virens	13.3	15.0	80.1	82.5	107.4	116.2
T.viride BS-7	13.0	14.8	80.5	83.2	106.8	112.3
Control	12.5	14.0	79.5	77.5	106.4	106.3

BS-Before sowing. AH-After harvest

Analysis of some soil samples amended with some PGPF before and after growth of chickpea plants in pots (Table 3) during the present study revealed that the PGPF treated soil was richer in available phosphorus (P_2O_5), potassium (K_2O) and nitrogen (NH_3N) content than the control. The garden soil which was nutrient deficient supported the poor growth of controlled plants resulting in reduced grain yield.This suggests that the PGPF increase the nutrient status of soil in the root region. The mineralization of the substrate of organic matter in soil by PGPF may be related to the plant growth

Table 4 : Chlorophyll content of treated and untreated plants of chickpea. pea and mustard with PGPF after 12 after 12 weeks, 8 weeks and 5 weeks respectively.

Plant growth promoting fungi (PGPF)	Chlorophyll a (mg g^{-1} dry leaf)			Chlorophyll b (mg g^{-1} dry leaf)			Total Chlorophyll (Chl a + Chl b) (mg g^{-1} dry leaf)		
	Chickpea	Pea	Mustard	Chickpea	Pea	Mustard	Chickpea	Pea	Mustard
Cladosporium cladospoioides	1.245	1.340	0.853	0.291	0.510*	0.253*	1.636	1.850	1.106
Curvularia lunata	1.265	1.310	0.876	0.423	0.492*	0.283	1.688	1.802	1.159
Fusarium moniliforme	1.413	1.343	0.882	0.513	0.513*	0.311	1.926	1.856	1.193
Trichoderma harzianum BS-2	1.905	1.550	0.942	0.718	0.575	0.375	2.623	2.125	1.317
T. harzianum BS-3	1.632	1.420	1.132	0.575	0.593	0.414	2.207	2.013	1.546
T. harzianum BS-1	1.296	1.370	0.901	0.476	0.539	0.355	1.772	1.909	1.256
T. virens	1.721	1.560	0.943	0.585	0.590	0.380	2.306	2.150	1.323
T. viride BS-7	1.362	1.346	0.890	0.463	0.518*	0.341	1.825	1.864	1.231
T. virede BS-8	1.639	1.372	0.919	0.596	0.535	0.363	2.235	1.907	1.282
Control	1.089	1.229	0.702	0.346	0.460	0.210	1.435	1.689	0.912
CD (P = 0.001)	0.0490	0.0481	0.0429	0.0380	0.0622	0.0678			

* Mean values are not significant (P = 0.0001) in comparison to control

promoting effect of PGPF. It was also observed that chlorophyll content increased in the test crops due to soil amendment with PGPF (Table 4). The increase in chlorophyll content might be due to increase of nitrogen in soil by the activity of PGPF which is absorbed by the plant. Thus another mechanism for increasing plant growth and yield could be due to higher rate of photosynthesis due to higher chorophyll content. However, intensive researches are needed to see the direct and indirect mechanisms of plants growth promotion and yield by PGPF.

ACKNOWLEDGEMENTS

The authors are thankful to the Head. Department of Botany. Banaras Hindu University for providing necessary facilities and to Mr. B. K. Singh for his suggestions and help.The financial assistance from U. G. C., New Delhi to one of us (TSS) is gratefully acknowledged.

REFERENCS

Arshad, M. and Frankenberger, W. T. (1991). Microbial production of plant hormones. In: *The Rhizosphere and Plant Growth*. (Eds. Keister. D. L. and Cregan. P. B.). Kluwer Academic Publishers. Dordrecht. The Netherlands, 327-334.

Baker, R. (1988). *Trichoderma* spp. as plant growth regulators. *CRC Critical Review in Biotechnology.* **7** : 97-106.

Baker, R. (1991). Induction of rhizosphere competence inthe biocontrol fungus *Trichoderma.* In : *The Rhizosphere and Plant Growth.* (Eds. Keister. D. L. and Cregan. P. B.). Kluwer Academic Publishers. Dordrecht. The Netherlands. 221-228.

Barber, D. A. and Lynch, J. M. (1977). Microbial growth in the rhizosphere. *Soil Biol. Biochem.* **9** : 305-308.

Chang, Y. C., Chang, Y. C. and Baker, R. (1986). Increased growth of plants in the presence of the biological control agent *Trichoderma harxianum. Plant Disease.* **70** : 145-148.

Cowan, M. C. (1979). Water use and phosphorus and potassium status of wheat seedling colonized by *Gaeumanomyces graminis* and *Phialophora radicicola. Plant and Soil.* **52** : 1-8.

Dewan, M. M. and Sivasithamparam, K. (1989a). Growth promotion of rotation crop science by a sterile fungus from wheat and the effect of soil temperature and moisture on its suppression of take-all. *Mycol. Res.* **93** : 155-160.

Dewan, M. M. and Sivasithamparam, K. (1989b). Behaviour of a plant growth promoting sterile fungus on agar and roots of rye-grass and wheat. *Mycol. Soc.* **93** : 161-166.

Harley, J. L. and Waid, J. S. (1955). A method of studying active mycelia on living roots and other surfaces in he soil. *Trans. Brit. Mycol. Soc.* **38** : 104-118.

Hayakawa, T., Kageyama, K. and Hyakumachi, M. (1993). Isolation of plant growth promoting fungi from rhizoplane of several plants. *Ann. Phytopathol. Soc. Jpn.,* **59** : 72.

Hyakumachi, M. (1994). Plant growth promoting fungi from Turfgrass rhizosphere with potential for disease suppression. *Soil microorganisms.* **44** : 53-68.

Hyakumachi, M. ; Ichikawa, M. ; Hayakawa, T. ; Kohara, E. and Kageyama, K. (1993a). Identity and frequency of occurrence of plant growth promoting fungi from rhizosphere of turfgrass and cultivated crops. *Sixth International Congress of Plant Pathology.* Montreal. Que.. July-Aug. 270 (Abstr.).

Hyakumachi, M. ; Ichikawa, M. and Kageyama, K. (1992). Plant growth promoting fungi isolated from rhizoshpere of *Zoysia japonica. Ann. Phytopathol. Soc. Jpn..* **59** : 72.

Hyakumachi, M., Ichikawa, M. and Kageyama, K. (1993b). Potentiality of plant growth promoting fungi in disease suppression. *Sixth International Congress of Plant Pathology.* Montreal. Que.. July-Aug.. 270 (Abstr.).

Kleifeld, O. and Chet, I. (1992). *Trichoderma harzianum:* Interaction with plants and effect on growth response. *Plant and Soil.* **144** : 267-272.

Kohara, E., Kageyama, K. and Hyakumachi, M. (1993). Investigation on the plant growth enhancing mechanism by plant growth promoting fungi. *Ann. Phytopathol. Soc. Jpn..* **59** : 73.

Singh, T. S. (1997). Influence of non-pathogenic microfungi on growth and yield of crop plants. *Ph.D. Thesis.* Banaras Hindu University.

Sneh, B. ; Zeidan, M. ; Ichielevich-Auster. M. ; Barash, I. and Koltin, Y. (1986). Increased growth responses induced by a non-pathogenic *Rhizoctonia solani. Can. J. Bot..* **64** : 2372-2378.

Windham, M. T. ; Elad. Y. and Baker, R. (1986). A mechanism for increased plant growth induced by *Trichoderma* spp. *Phytopathology.* **76** : 518-521.

(Accepted for publication October 04 2003)