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Induction of defense responses in blackgram by *Trichoderma viride*

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ABSTRACT: Induced systemic resistance (ISR) in blackgram using organic amendments and biocontrol agents against *Macrophomina phaseolina* was studied under pot culture conditions. Soil application of FYM 12t ha^{-1} combined with seed treatment (4g kg^{-1}) and soil application (5kg ha^{-1} on 30 DAS) of *T. viride* significantly increased defense related proteins, *viz*., peroxidase (14.34 units min^{-1} g^{-1}) and polyphenoloxidase (3.96 units min^{-1} g^{-1}) activities as a sign of systemic resistance coupled with reduction in root rot incidence from 99.6 to 13.8 per cent and also increased the yield and yield attributes of blackgram.

KEY WORDS: Black gram, defense related proteins, *Macrophomina phaseolina, Trichoderma viride*

INTRODUCTION

Blackgram (*Vigna mungo* L. Hepper) is one of the important pulse crops grown throughout India and is affected by several diseases. Dry root rot incited by *Macrophomina phaseolina* (Tassi) Goid. is an important disease of blackgram causing significant loss in yield. There are no effective chemical measures, however, biocontrol and organic amendments may serve as promising alternatives for managing this disease. Induced Systemic Resistance (ISR) by *Trichoderma* spp. one of the important mechanisms for biological control of plant diseases (Yedida *et al*., 1999) The present investigation was undertaken to study the ISR against root rot in blackgram, when organic amendments were applied along with *Trichoderma viride*.

MATERIALS AND METHODS

Green house study

A pot culture study was conducted with unsterilized garden soil in completely randomized block design with 12 treatments and 3 replications, at Department of Plant Pathology, Faculty of Agriculture, Annamalai University. The variety used was CO 5 of blackgram. Inoculum multiplied in sand maize medium (Riker and Riker, 1936) was mixed in soil @ 50g kg^{-1}and pots were filled @ 6 kg soil pot^{-1}. FYM (farm yard manure), decomposed coconut coir pith (DCCP) and pressmud @ 12t ha^{-1} were applied 10 days before sowing. Seed treatment with *T. viride* (commercial formulation, 4g talc formulation per kg of seed and its combination with organic amendments were done. The amendments were tested individually and in combination with seed (4g kg^{-1} talc based formulation of *T. viride*) and soil application of *T. viride* 5 kg ha^{-1} at on 30 DAS. Seed treatment with carbendazim (Bavistin) was done for comparison @ 2g kg^{-1}. Untreated seeds in pots having no organic amendments served as control. Seeds were sown @ 12 seeds pot^{-1}. For assaying induced proteins in roots samples collected at 0, 1, 3, 6 and 8 days after challenge inoculation with *M. phaseolina*. Observations on root incidence and yield and yield attributes were recorded.

Assay for Peroxidase (PO)

Peroxidase activity was determined according to the procedure given by Hammerschmidt and Kuc (1982). Blackgram root tissues (1g) were homogenized with 2ml of 0.1M sodium phosphate buffer (pH 7) in a chilled mortar and centrifuged at 10000 rpm for 10 mins at 4^{0}c and the supernant served as the enzyme source. The (PO) activity was expressed on a fresh weight basis as changes in absorbance min^{-1}g^{-1}.

Assay for Poly Phenol Oxidase (PPO)

Polyphenoloxidase was determined from root tissues according to Mayer *et al*. (1965). The absorbance was determined at 250nm. The rate of reaction was expressed as min^{-1} g^{-1} fresh weight basis.

Table 1. Changes in peroxidase activity in roots of blackgram treated with *Trichoderma viride* and organic amendments and challenge inoculated with *Macrophomina phaseolina*

Treatment	*$ Peroxidase activity					
	0DAI	1DAI	3DAI	6DAI	9DAI	Mean
T_1 - Control	3.39	3.52	4.46	5.32	4.89	4.32
T_2 - DCCP (SA)	5.29	4.86	4.90	4.92	4.86	4.97
T_3 - FYM (SA)	5.30	4.89	5.02	5.12	4.93	5.05
T_4- Pressmud (SA)	4.31	4.72	5.01	4.92	4.86	4.76
T_5 - T. viride (SA)	5.60	6.31	9.68	12.24	10.92	8.95
T_6 - T_5 + T_2	6.00	8.83	10.92	14.12	13.32	10.64
T_7 - T_5 + T_3	5.62	6.59	9.01	13.92	12.36	9.30
T_8 - T_5 + T_4	4.42	5.01	5.26	4.89	4.81	4.88
T_9 - T_6 + *T. viride* (SA30DAS)	6.01	8.82	10.19	13.32	13.39	10.35
T_{10} - T_7 + *T.viride* (SA30DAS)	6.20	9.01	11.12	15.76	14.34	11.29
T_{11} - T_8 + *T.viride* (SA30DAS)	4.48	5.12	5.36	4.82	4.73	4.90
T_{12} - Bavistin 1% (ST)	5.59	6.29	9.62	12.29	10.98	8.60
Mean	5.18	6.16	7.55	9.30	8.70	

ST- Seed treatment, SA– soil application; *values are means of three replications; factorial completely randomized block design (FCBD) $ Changes in absorbance / min / fresh weight at 420nm, (P = 0.05); Treatments = 0.0621; Days = 0.0401; Interaction 0.1389

Table 1. Changes in peroxidase activity in roots of blackgram treated with *Trichoderma viride* and organic amendments and challenge inoculated with *Macrophomina phaseolina*

Treatment	*$ Peroxidase activity					
	0DAI	1DAI	DAI	DAI	DAI	Mean
T_1- control	0.42	0.93	0.72	1.42	1.20	0.94
T_2 - DCCP (SA)	0.59	0.98	0.78	1.52	20.1	1.18
T_3 - FYM (SA)	0.60	0.98	0.79	1.52	1.89	1.18
T_4 - Pressmud (SA)	0.59	0.98	0.77	1.59	1.89	1.17
T_5 - T. viride (ST)	0.63	1.11	1.82	1.98	2.51	1.61
T_6 - T_5 + T_2	0.82	0.98	1.12	1.83	2.01	1.35
T_7 - T_5 + T_3	0.79	0.92	1.24	1.89	1.98	1.36
T_8 - T_5 + T_4	0.80	0.99	1.02	1.93	2.02	1.32
T_9 - T_6 + *T. viride* (SA30DAS)	0.92	1.01	20.02	3.16	2.24	1.87
T_{10} - T_7 + *T.viride* (SA30DAS)	1.03	1.20	2.98	5.53	3.96	2.94
T_{11} - T_8 + *T.viride* (SA30DAS)	0.91	0.98	2.12	2.24	2.19	1.69
T_{12} - Bavistin 1% (ST)	0.64	1.09	1.83	2.01	2.52	1.62
Mean	0.73	1.01	2.93	2.22	3.71	

ST- seed treatment; SA- soil application; *values are mean of there replications; factorial randomized block design (FRBD), $ Changes in absorbance / min / I fresh weight at 420 nm, (P = 0.05); treatments = 0.0154; days = 0.0099; interaction = 0.0345

Table 3. Effect of *T. viride* and organic amendments on root rot incidence and yield attributes in blackgram

Treatment	Yield and yield attributes				
	Root % rot 60 DAS	No of nodules plant	No of seeds / pod	Total biomass (g)	Yield kg ha^{-1}
T_1 - Control	91.6	34	4.7	8.5	630
T_2 - DCCP (SA)	47.2	35	6.6	8.9	711
T_3 – FYM (SA)	44.4	34	7.0	8.9	713
T_4- Pressmud (SA)	52.7	35	6.1	8.9	708
T_5 - T. viride (SA)	27.7	38	7.9	9.4	758
T_6 - T_5 + T_2	24.9	40	9.7	8.9	746
T_7 - T_5 + T_3	22.2	39	9.8	8.9	742
T_8 - T_5 + T_4	25.0	40	8.7	8.9	789
T_9 - T_6 + *T. viride* (SA30DAS)	16.6	41	11.5	9.9	802
T_{10} - T_7 + *T.viride* (SA30DAS)	13.8	42	11.5	10.1	819
T_{11} -T_8 + *T.viride* (SA30DAS)	19.4	41	10.3	9.8	764
T_{12} - Bavistin 1% (ST)	30.5	34	7.3	9.0	761
S.Ed	1.01	0.18	0.23	0.12	1.47
CD(P=0.05)	2.02	0.38	0.47	0.26	3.20

ST – seed treatment; SA - soil application; DAS – days after sowing; *values are mean of three replications.

RESULTS AND DISCUSSION

The green house study clearly indicated that organic amendments and antagonist mediated ISR was operative under pot culture. *T. viride* and FYM induced PO and PPO activity in blackgram root challenged with root rot pathogen. Higher amounts of PO (15.76 unit min^{-1} g^{-1} and PPO (5.53 unit min^{-1} g^{-1}) were observed in basal application of FYM and *T. viride* as seed treatment along with soil application 5kg ha^{-1} on 30 DAS, as against control which recorded 5.32 and 1.42 unit / min / g, respectively. This was followed by seed treatment and soil application of *T. viride* along with application of pressmud. The induction of PO and PPO gradually increased for up to six days of inoculation and for decreased on 9th day of inoculation (Table 1 and 2). Earlier, Cheriff *et al*. (1994) found that application of silicon significantly stimulated the activity of PO and PPO after infection with *Pythium* spp.

Effect of *T. viride* and organic amendments on root rot incidence and yield attributes

In pot culture studies, soil application of FYM (12 t ha^{-1}) combined with seed (4g kg^{-1}) and soil (5 kg ha^{-1} on 30 DAS) application of *T. viride* apart from inducing the defense related proteins reduced the root rot incidence significantly from 91.6 to 13.8 % (control). It increased the yield attributes, *viz*., number of pods / plant (42.2), number of seeds/pod (11.5) and biomass (10.1g). Ultimately the yield increased from 630 to 819 kg ha^{-1} (Table 3). This was followed by *T. viride* seed treatment and soil application on 30 DAS along with pressmud as basal application. This observation is in accordance with the results reported by Dinakaran *et al*. (1995) and Usharani (1999).

The results reported here indicated that the organic amendment and antagonists mediated ISR holds promise for practical disease management. This study will allow a full assessment of the potential applicability of organic amendments and antagonists mediated ISR in integrated pest management strategies.

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