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Resistance to Wheat Spot Blotch induced by crude extract of *Chaetomium globosum* and mild strain of *Drechslera sorokiniana*

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Pre-treatment with crude extract of *Chaetomium globosum* and pre- inoculation with a mildly virulent strain of *Drechslera sorokiniana* induced resistance in wheat seedlings against spot blotch (*D. sorokiniana*) with reduction of disease severity by 94.9% and 49.9% respectively. Both the inducing agents were applied as foliar spray, Chaetomium crude extract as 0.2% solution in water and *D. sorokiniana* mildly virulent strain @ 10^4 conidia/ml. Both the treatments increased phenolic content and soluble protein content in wheat leaves. Per cent disease index (PDI) showed highly negative correlation with phenol content (r = 0.96) as well as with soluble protein content (r = -0.99).

Key words : Induced resistance, *Drechslera*, wheat, *Chaetomium*

INTRODUCTION

Spot blotch of wheat caused by *Drechslera sorokiniana* (Sacc.) Subram & Jain is an important disease of global concern which causes about 3-36% yield losses. (Anonymous 1995) High temperature and high relative humidity favour the outbreak of the disease, particularly in South Asia's intensive 'irrigated rice-wheat' production systems. Although biological control of the disease has been attempted as a promising supplement or substitute of chemicals, work on induced resistance against the pathogen is very rare. Pre inoculations with avirulent or virulent pathogens induced local resistance against powdery mildew in barley and wheat (Sahashi and Shishiyama, 1986, Schweizer *et al.*, 1989). Resistance in rice plants against *Helminthosporium oryzae* has been reported to be induced by pre-inoculation with the spore suspension of an avirulent race (Sinha and Trivedi, 1969) as well as a mildly virulent race (Sinha and Das, 1972) of the pathogen. Recently some biocontrol agents are also known to induce resistance in plants in addition to their antimicrobial activities. Nzojiyobiri *et al.* (2003) have reported that rice seedlings pretreated with *Trichoderma harzianum* exhibited moderate resistance against blast and bacterial leaf blight. Pathak *et al.* (2004) have reported that *Pseudomonas* strain GRP3 induced systemic resistance to sheath blight in rice. Biocontrol (antagonistic) potential of *Chaetomium globosum* against the spot blotch pathogen of wheat, *Drechslera sorokiniana* has been reported by Mandal *et al.* (1999). In addition to this Biswas *et al.* (2000) have observed growth stimulation of wheat seedlings by *C. globosum.* In the present investigation attempts are made to induce resistance in wheat seedlings against spot blotch caused by *D. sorokiniana* by applying spore suspension of a mildly virulent strain of *D.sorokiniana.*

MATERIALS AND METHODS

The present study was conducted during 1997-98 to 2000-01 at the Division of Plant Pathology, I.A.R.I., New Delhi. Isolates of virulent strain (DS-141) and mildly virulent strain (DS-68) of *D. sorokiniana* were obtained from Wheat Pathology Laboratory, Division of Plant Pathology, I.A.R.I. *Chaetomium globosum* showing antagonistic tendency towards *D. sorokiniana* which was isolated from wheat leaf surface (Mandal *et al.*, 1999) was used in the present study. All the isolates were maintained on PDA separately.

Virulent and mildly virulent strains of *D. sorokiniana* were applied as conidial suspension @ 10^4 conidia/ml. *Chaetominum globosum* was applied as crude

extract. The crude extract was obtained from the culture filtrate through extraction with ethyl acetate in 1:2 ratio.To ascertain whether *C. globosum* and mildly virulent strain of *D. sorokiniana* can induce resistance in wheat against the spot blotch pathogen a pot experiment was conducted in the glass house. Plastic pots of 4" size were filled with sterilized soil and ten seeds of susceptible cultivar, Arnez were sown in each pot. The experiment comprised of eight treatments *viz.* pre-inoculation with Chaetomium crude (T_1, post-inoculation with Chaetomium crude (T_2), pre inoculation with mildly virulent *D. sorokiniana* (T_3), post inoculation with mildy virulent *D. sorokiniana* (T_4), only *Chaetomium* crude (T_5), only mildly virulent *D. sorokiniana* (T_6), only virulent strain of *D. sorokiniana* (check-I) (T_7), and healthy, without any treatment (Check-II) (T_8). Four pots served as one treatment and each treatment was replicated three times. The pots were kept in completely randomized block (CRD) design and watered as required.

Foliar applications of all the treatments were made on 21 days old wheat seedlings. The crude extract (0.2% water dissolved) of C. *globosum* was sprayed 48 h before (T_1 and after (T_2) pathogenic inoculation. Likewise, conidial suspension (10^4 conidia/ml) of mildly virulent isolate of *D. sorokiniana* was sprayed 48 h before (T_3) and after (T_4) pathogenic inoculation.The pathogenic inoculation was made with the virulent strain, DS-141 @ 10^4 conidia/ml by an automizer as per the treatments'(T_1,T_2,T_3,T_4 & T_7). All these plants were incubated in humidity chamber for 48 h after inoculation. The plants were observed regularly for appearance of disease and disease observations were recorded seven days after inoculation. Ten leaves were randomly selected from each treatment. Rating of spot blotch was done using 0-4 scale (Chenulu and Singh, 1964) and per cent disease index (PDI) was calculated. Average number of lesions per leaf were also calculated.

Wheat leaves from all the treatments were collected 12 h and 24 h after concerned treatment and biochemical analysis was carried out to find out total phenol and soluble protein contents. Phenol content was estimated following Brar and Thorpe (1954) procedure. The absorbance at 650 nm against a reagent blank was measured using ultraviolet (UV-VIS) spectra and the standard curve using different concentrations of catechol (as blank) was prepared. From the standard curve the concentrations of phenol in the test sample was determined and expressed as mg/g of sample material. Soluble proteins were extracted from the samples of all the treatments and estimated following Lowry *et al.* (1951).

RESULTS AND DISCUSSION

After all the treatments were imposed the plants were kept in the humidity chamber for appearance of disease. In treatments T_2, T_4, T_6 and T_7 initial spot blotch symptom was noticed on the very next day (after 24 hrs) of pathogenic inoculation. However, in treatments Ti (pre-inoculation with Chaetomium crude) and T_3 (pre-inoculation with mildly virulent *D. sorokiniana)* initial disease symptom appeared 48 hrs after pathogenic inoculation. Disease rating of all the treatments were done after five days of inoculation. In diseased check (T_7) average number of lesions per leaf were 13.3 and PDI was as high as 41.54. Minimum number of lesions per leaf (2.5) and lowest PDI (2.11) were recorded in Ti (pre inoculation with Chaetomium crude), whereas in

Table 1 : Effect of different treatments on the incidence of spot blotch disease in wheat.

Treatment		Av. no. of lesions/leaf	Per cent disease Index	% reduction in PDI over disease & check
T_1:	Pre inoculation Chaetomium crude	2.5	2.11	94.9
T_2:	Post inoculation Chaetomium Crude	7.5	10.04	75.8
T_3:	Pre inoculation mild *D. sorokiniana*	9.6	21.23	49.9
T_4:	Post inoculation mild *D. sorokiniana*	10.1	28.74	30.8
T_5:	Only Chaetomium crude			-
T_6:	Only mild *D. sorokiniana*	8.4	15.36	63.0
T_7 :	Only virulent *D. sorokiniana* (diseased check)	13.3	41.54	
T :	Healthy check (no treatment)			

case of post inoculation (T_2) the respective values were 7.5 and 10.04 (Table 1). Pre-inoculation with Chaetomium crude extract (T_1) could reduce the disease severity by 94.9% where as its post inoculation could reduce the disease severity by 75.8% as compared to the diseased check. Pre inoculation and post-inoculation with mildly virulent strain of *D. sorokiniana* reduced the per cent disease index (PDI) by *49.9%* and 30.8%, respectively.

From these results it is clear that pre inoculation with mildly virulent strain of *D. sorokiniana* and pre-treatment with crude extract of *Chaetomium globosum* both induced resistance in wheat against spot blotch caused by *D. sorokiniana*. However, *C. globosum* performed much better than mild strain of *D. sorokiniana*. Even post-inoculation application of crude extract of *C. globosum* provided considerable protection against the disease.

To understand the biochemical basis of the resistance induced, phenolic compound and soluble protein content of wheat leaves were estimated for each treatment 12 and 24 hrs after their application. The correlations of PDI with phenol and soluble protein content were also calculated.

Table 2 : Effect of foliar spray with crude extract of *Chaetomium globosum* and conidial) suspension of mildly virulent strain of *Drechslera sorokiniana* on total phenol content of wheat leaves after 12 & 24 h of application.

Treatment	Total phenol content (mg/g of fresh leaves)		% increase in Phenol contents			
			Over disease & check		Over healthy check	
	12 h.	24 h.	12 h.	24 h.	12 h.	24 h.
T_1: Pre inoculation Chaetomium crude	1.99	2.19	50.76	63.43	68.64	79.51
T_2: Post inoculation Chaetomium crude	1.59	1.73	20.45	29.10	34.75	41.80
T_3: Pre inoculation mild *D. sorokiniana*	1.42	1.48	7.58	10.45	20.34	21.31
T_4: Post inoculation mild *D. sorokiniana*	1.35	1.39	2.27	3.73	14.71	13.93
T_5: Only Chaetomium crude	2.24	2.39	69.70	78.36	89.83	95.90
T_6: Only mild *D. sorokiniana*	1.37	1.44	3.79	7.46	16.10	18.03
T_7: Only virulent D. sorokiniana (diseased check)	1.32	1.34	-	-	11.86	9.84
T_8: Healthy check (no treatment)	1.18	1.22	-10.61	-8.96		
CD. at 1%	0.20	0.07				

Total phenol contents recorded after 12 and 24 hrs of application of all treatments have been presented in Table 2. The results indicated that both pre-inoculation and post inoculation foliar spray of Chaetomium crude extract increased total phenol content in wheat leaves. However, phenol accumulation in case of pre-inoculation application (2.19

Table 3 : Effect of foliar spray with crude extract of *Chaetomium globosum* and mildly virulent strain of *Drechslera sorokiniana* on total soluble protein content of wheat leaves after 12 & 24 h of application

Treatment	Total soluble protein content (mg/g of leaves)		% increase in protein contents			
			Over disease check		Over healthy check	
	12 h.	24 h.	12 h.	24 h.	12 h.	24h.
T_1 : Pre inoculation Chaetomium crude	32.24	33.31	35.12	43.02	29.17	32.50
T_2: Post inoculation Chaetomium crude	28.24	29.02	18.36	24.60	13.14	15.43
T_3: Pre inoculation mild *D. sorokiniana*	25.81	25.47	8.17	9.36	3.41	1.31
T_4: Post inoculation mild *D. sorokiniana*	24.40	24.10	2.26	3.48	-2.24	-4.14
T_5: Only Chaetomium crude	33.20	34.82	39.15	49.51	33.01	38.50
T_6: Only mild *D. sorokiniana*	26.47	27.01	10.94	15.97	6.05	7.44
T_7 : Only virulent *D. sorokiniana* (diseased check)	23.86	23.29	-	-	- 4.41	-7.36
T_8 : Healthy check (no treatment)	24.96	25.14	4.61	7.94	-	-
CD. at 1%	0.17	0.28				

mg/g) was higher than post inoculation application (1.73 mg/g). Pre inoculation treatment with the mildly virulent strain of *D. sorokiniana* also registered increased phenol content (1.48 mg/g). Accumulation of phenols is considered the expression of defence response in plants (Matern and Kneusal, 1988). Vidyasekaran (1974) reported enhanced quantity of phenols in ragi plants resistant to *Helminthosorium tetramera*. The findings in the present study are in conformity with the observations of Sivi Kumar and Sharma (2003) who also reported increase in phenol content in maize plants due to seed treatment with *Pseudomonas fluorescens*.

The estimation of soluble protein revealed that its content varied from 23.29 to 33.31 mg/g of fresh wheat leaves (Table 3). Pre-inoculation application of both Chaetomium crude extract and mild strain of *D. sorokiniana* increased the soluble protein content. In the first case soluble protein content (33.31 mg/g) was much higher than the latter (25.47 mg/g).

Table 4 : Correlation of PDI with total phenol and soluble protein content of wheat leaves

Parameters	Correlation coefficient (r)	Regression equation
Total phenol content		Y = 5.1417-4.5517 X,
After 12 h.of treatment	-0.953	Y = 4.1215-3.66 X,
After 24 h. of treatment	-0.965	
Soluble protein content		
After 12 h. of treatment	-0.994	Y = 8.8754-0.3947 X_2
After 24 h. of treatment	-0.994	Y = 7.1397-0.3276 X_2

Y - PDI
Xi - Phenol content
X_2 - protein content

Considerable accumulation of soluble protein (29.02 mg/g) was also recorded in post inoculation foliar spray of Chaetomium crude extract. Boiler (1985) reported association of protein with plant defense against fungi and bacteria. Earlier investigations also indicated marked increase in protein content in graminaceous hosts, such as wheat (Sock *et al* 1990), oat (Fink *et al,* 1988), maize (Nasser *et al,* 1990) and barley (Hoj *et al,* 1989) due to activity of β-1, 3 glucanase etc. Babu *et al.* (2003) reported that induction of systemic resistance in rice to *Xanthomonas oryzae* pv. *oryzae* by salicylic acid was associated with increased phenolics content and enhanced activities of some PR-proteins. *Pseudomonas* strain GRP 3 was reported to induce systemic resistance to sheath blight in rice with concomitant increase in peroxidase and phenol synthesis (Pathak *et al,* 2004).

Table 5 : Multiple regression equation of spot blotch PDI

Time interval after treatment	R^2	Multiple regression equation
12h	0.991	Y = 9.475 + 0.905 X, - 0.468 X_2
24 h	0.988	Y = 6.9527- 0.276 X, - 0.304 X_2

Xi - Phenol content
X_2 - Soluble protein content
Y - PDI

The per cent disease index (PDI) of spot blotch showed highly negative correlation with both phenolic content (r= -0.96) and soluble protein content (r=0.99) of wheat leaves estimated after 12 hrs as well as 24 hrs of treatment with the inducing agents (Table 4).

The results are in conformity with what reported by Kumawat (2006) in rice against brown spot. The regression analysis indicates that both the variables, protein and phenolic content influenced the disease severity levels (Table 5). However, further study is needed with greater number of vaiables such as peroxidase, polyphenol oxidase, phenyl alanine ammonia-lyase etc. to under stand the mechanism of induced resistance against spot blotch of wheat.

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