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Resistance to Helminthosporium leaf blight and biochemical responses of wheat genotypes of diverse origins

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Helminthosporium leaf blight (HLB) is one of the most serious disease constraints to wheat (*Triticum aestivum* L.) in the Gangetic plains of India, particularly in rice-wheat system. The disease occurs as a complex of spot blotch (*Bipolaris sorokiniana*), leaf blight (*Alternaria triticina*), zonate eye spot (*Drechslera gigantea*) and tan spot (*Pyrenophora tritici-repentis*) ; of which spot blotch causes significant yield losses. Yield losses up to 43% were recorded in the terai zone of West Bengal. The relative dominance of *B. sorokiniana, A. triticina* and *D. gigantea* was studied at different growth stages of wheat. It was observed that *Alternaria* was dominant pathogen in the early growth stages whereas *Bipolaris* and *Drechslera* appered after flag leaf sheath opening stage of wheat and caused significant yield losses. A field study was conducted using 300 genotypes of Indian and CIMMYT origin during 2003-04 and 2004-05 crop seasons for their resistance to HLB disease. The biochemical parameters associated with disease resistance i.e., phenolics, proteins, polyphenoloxidase and peroxidase activities were measured in two resistant wheat genotypes (Nepal 1 and RWP 40) and two susceptible cultivars (Sonalika and HUW 234). The resistant genotypes always had higher levels of phenol and OD-phenol and enhanced PPO and PO enzyme activities than the susceptible genotypes, Due to complexity of the disease, a holistic approach, using varietal resistance, induced resistance by plant growth promoting rhizobacteria, bio-control agents and chemical control, was attempted. An Integrated Disease Management (IDM) had been formulated for the terai zone of West Bengal.

Key words : Foliar blight, wheat, disease resistance, biochemical changes, disease management

INTRODUCTION

Helminthosporium leaf blight (HLB) caused by *Bipolaris sorokiniana* (Sacc. in Sorok) Shoem. has been a major disease of wheat (*Triticum aestivum* L.) grown under humid subtropical climates (Duveiller, 2002). Currently it has become a major limitation for sustainable production of wheat in rice based cropping system in eastern Gangetic plains of India. Substanital economic losses are caused by HLB in this region (Singh *et al.*,1998). The yield losses due to HLB may be aggravated as a result of increased severity when abiotic stresses such as residual soil moisture, nutrient deficiency and high temperature occur (Sentelhans *et al.*, 1993). Although significant progress has been made in the breeding programmes, most of the cultivars grown in the warmer areas still posses relatively low levels of resistance against this disease (Sharma and Duveiller, 2004). The objectives of this report are to determine the causal organisms of foliar blight disease complex, its occurrence and management and also the biochemical mechanisms of disease resistance.

MATERIALS AND METHODS

Yield loss assessment

Nine different cultivars viz. HD 2329, Sonalika, Raj 4015, HW 2004, K 9107, PBW 343, Raj 3765, HUW 234 and K 8027 were sown in the field and exposed to natural infection and also artificially inoculated with *Bipolaris sorokiniana* (10^6 conidia/ml) at flag

leaf sheath opening stage. Similar sets of varieties were sown in controlled environment to assess the yield loss. The experiment was continued during 2002-04 crop seasons.

Distribution of foliar blight pathogens

Large number of blight infected wheat samples were collected from different locations of terai zone of West Bengal. The leaf samples were washed with mercuric chloride (0.1%) solution and dried. The leaf segments having typically blight symptoms were cut into small pieces (4 mm^2). These leaf pieces were placed on wheat bran extract sucrose agar medium in 10 cm diameter Petri dishes and incubated at 25° C ± 1°C for 7 days. The pathogens were isolated and identified by comparing the respective isolate maintained in the Department of Plant Pathology of Uttar Banga Krishi Viswavidyalya. The pathogenecity was confirmed by detached leaf assy.

Relative dominance of the pathogens

Newly infected wheat leaf samples of five varieties (HD 2329, Sonalika, Raj 4015, HW 2004 and K 9107) were collected from naturally infected plants at different growth stages of wheat (Zadok's scale), viz., 4th node (Stage 34), flag leaf sheath opening (Stage 47), one half of year emerged (Stage 55), Flowering half way complete (Stage 65), early milk (Stage 73) and early dough (Stage 83). The pathogens were isolated by following the methods as described earlier. Per cent recovery of different pathogens was calculated.

Disease assessment

The disease was visually scored using the double digit scale (00-09) developed by Eyal *et al.* (1987). AUDPC (area under disease progress curve) was calculated using the formula given by Das *et al.* (1992).

Biochemical estimation

The different biochemical parameters were estimated by using the followin methods :

i) Total phenol : Mahadevan and Sridhar (1982)
ii) Orthodihydroxyphenol : Mahadevan and Sridhar (1982)
iii) Polyphenoloxidase (PPO) activity : Jennings *et al.* (1969)
iv) Peroxidase (PO) activity : Addy and Goodman (1972)

Integrated disease management

The field experiment was laid out in a randomized block design by using the following treatments :

i) Fertilizer dose (120 : 60 : 40 and 120 : 60 : 60 kg/ha N : P: K)
ii) Seed treatment (Carboxin @ 4g/kg of seed)
iii) Three foliar sprays with :
   a) *Tricohderma harzianum* (10^6 conidia/ml)
   b) Econeem (2ml/l)
   c) Propiconazole [Tilt] (0.1%)
iv) Combined treatment of 120 : 60 : 60 kg/ha N : P : K + seed treatment (Carboxin) + single spray (Propiconazole)

RESULTS AND DISCUSSION

Yield loss assessment

It appears that all nine varieties showed significant yield losses due to foliar blight infection ranging from 19-43% (Table 1). Highest yield loss (43%) was recorded in cv. Sonalika and least in cv. K 8027 (19%).

Table 1 : Yield loss assessment due to foliar blight disease (2002-2004)

Cultivars	Grain Yield (Kg/ha)	Yield Loss (%)	AUDPC
HD 2329	2075	34.3	326
Sonalika	1770	43.8	888
Raj 4015	1825	37.5	604
HW 2004	2145	24.5	456
K 9107	1660	40.4	518
PBW 343	2460	28.6	308
RAJ 3765	1670	43.3	561
HUW 234	2105	32.2	308
K 8027	1480	19.1	265
Mean	1885.6	33.9	489.2
CD (P=0.05)	596	11.1	261

Distribution of foliar blight pathogens

A large numer of blight affected wheat samples were isolated starting from seedling stage to harvesting. After repeated isolations, *Bipolaris sorokiniana, Alternaria triticina, Drechslera gigantea, Curvularia lunata, Alternaria alternata, and Pyrenophora tritici-repentis* were found to be associated with foliar blight. Of these, *B. sorokiniana, A. triticina* and *D. gigantea* were found to be pathogenic.

Relative dominance of pathogens in growth stages of wheat

Maximum population of *Alternaria triticina* was ob-

tained in 47th growth stage i.e. during flag leaf stage opening whereas *Bipolaris sorokiniana* and *Drechslera gigantea* showed significant continuous increase with the advancement of growth stages starting from stage 55 (Table 3). Overall recovery of *B. sorokiniana* population (73%) was significantly higher than *A. triticina* population (31.3%). Among five different varieties tested, all showed almost similar results.

Table 2 : Pathogenecity of fungi associated with foliar blight

Fungi isolated	Pathogenecity
Bipolaris sorokiniana	++
Alternaria triticina	++
Drechslera gigantea	++
Curvularia lunata	–
Alternaria alternata	–
Pyrenophora tritici - repentis	NT

++ : Pathogenic ; – : Non pathogenic ; NT : Not tested

Table 3 : Occurence of *Alternaria, Bipolaris* and *Drechslera* in different growth stages of wheat

Growth Stage	HD 2329	Sonalika	Raj 4015	HW 2004	K 9107
4th node (Stage 34)	*A.t.*(+)	*A.t.*(++)	*A.t.*(+)	*A.t.*(+)	*A.t.*(+)
Flag leaf sheath opening (Stage 47)	*A.t.*(++)	*A.t.*(+++)	*A.t.*(+++) *B.s.* (+)	*A.t.*(++)	*A.t.*(+)
One half of ear emerged (Stage 55)	*A.t.*(++) *B.s.*(+)	*A.t.*(+++) *B.s.*(++)	*A.t.*(+++) *B.s.*(+)	*A.t.*(++)	*A.t.*(++)
Flowering halfway complete (Stage 65)	*B.s.*(++) *D.g.*(++)	*A.t.*(++) *B.s.*(+++)	*B.s.*(++) *D.g.*(++)	*B.s.* (++) *D.g.* (+)	*B.s.*(++) *D.g.*(++)
Early milk (Stage 73)	*B.s.*(++) *D.g.*(++)	*B.s.*(+++) *D.g*(++)	*B.s.*(++) *D.g.*(++)	*B.s.* (++) *D.g.*(++)	*B.s.*(++) *D.g.*(++)
Early dough (Stage 83)	*B.s.*(++) *D.g.*(++)	*B.s.*(+++) *D.g*(++)	*B.s.*(++) *D.g.*(++)	*B.s.* (++) *D.g.*(+)	*B.s.*(++) *D.g.*(+)

A.t. : *Alternaria triticina* ; *B.s.* : *Bipolaris sorokiniana* ; *D.g.* : *Drechslera gigantea* ; +++ : High recoverable ; ++ : Moderate recoverable ; + : Trace recoverable

Table 4 : Foliar blight severity and climatic conditions during 2001-2003

Year	Meteorological parameters				Disease severity (00–99)				
	Max. Temp. (°C)	Min. Temp. (°C)	RH (%)	Rainfall (mm)	HD 2329	Sonalika	Raj 4015	HW 2004	K 9107
2003	23.1	9.8	93.0	39.1	79	99	99	77	77
2002	25.7	10.9	92.0	29.7	77	99	97	75	75
2001	26.7	10.4	91.0	31.2	77	97	97	75	75

Table 5 : Levels of total phenol and O – dihydroxyphenol in healthy and infected leaves of resistant and susceptible germplasms of wheat

Treatment	Total phenol (mg/g fresh weight)				O – dihydroxyphenol (mg/g fresh weight)			
	Resistant		Susceptible		Resistant		Susceptible	
	Nepal 1	RWP 40	Sonalika	HUW 234	Nepal 1	RWP 40	Sonalika	HUW 234
Healthy	3.54 ± 0.04	3.40 ± 0.01	3.02 ± 0.03	3.15 ± 0.04	0.29 ± 0.02	0.30 ± 0.04	0.28 ± 0.02	0.24 ± 0.02
Infected*	3.93 ± 0.03	3.81 ± 0.04	2.88 ± 0.02	3.16 ± 0.02	0.33 ± 0.04	0.36 ± 0.01	0.25 ± 0.03	0.25 ± 0.01

* Levels estimated after 48 hours of inoculation ; ± : SEM value

Table 6 : Polyphenol oxidase and peroxidase activity in healthy and infected leaves of resistant and susceptible germplasms of wheat

Treatment	Polyphenol oxidase activity*				Peroxidase activity*			
	Resistant		Susceptible		Resistant		Susceptible	
	Nepal 1	RWP 40	Sonalika	HUW 234	Nepal 1	RWP 40	Sonalika	HUW 234
Healthy	13.01 ± 1.1	13.20 ± 0.9	12.20 ± 0.7	13.10 ± 1.1	14.40 ± 0.8	14.21 ± 0.9	14.17 ± 0.9	14.16 ± 1.2
Infected**	16.60 ± 0.9	15.65 ± 0.7	13.56 ± 0.9	14.21 ± 0.7	17.50 ± 0.7	18.40 ± 1.1	15.25 ± 0.8	15.91 ± 0.9

* Data is expressed as Δ OD/g tissue / minute ; ** Activity was estimated after 48 hours of inoculation ; ± : SEM value

Table 7 : Integrated control of leaf blight of wheat

Treatment	Seedling blight	Disease appearance date (DAS)	Disease record (00-99)		Yield (q/ha)
			Flowering stage	7 days after flowering	
Control (untreated) – K 40 Kg/ha + N + P	12	45	57	79	30.71
K 60 Kg/ha + N + P	12	48	37	77	31.07
Seed treatmen (Carboxin)	00	55	37	77	31.42
Seed treatment (Carboxin) + Spray (*Trichoderma harzianum*)	00	55	35	77	33.92
Seed treatment (Carboxin) + Spray (Econeem)	00	59	35	77	33.14
Seed treatment (Carboxin) + Spray (*Trichoderma harzianum*) + Econeem	00	61	57	77	33.00
Seed treatment (Carboxin) + Spray (Tilt)	00	63	35	57	36.78
Spray (Tilt)	00	65	37	57	35.71
K 60 Kg/ha + Seed treatment (Carboxin) + Spray (Tilt)	00	63	24	35	39.28

Foliar blight epidemiology

Based on three years observations, the disease on five wheat varieties (HD 2329, Sonalika, Raj 4015, HW 2004 and K 9107) was found to correlate to meteorological parameters for the month of February. Foliar blight initiation was found to begin as early as the second fortnight of December, but severity increased during heading and flowering stages. Results presented in Table 4 showed that a maximum temperature of about 25°C and a minimum temperature of about 10°C and relative humidity (RH) of 92 % promoted maximum foliar blight development. Rainfall directly increased disease intensity.

Biochemical parameters associated with disease resistance

Three hundred germplasms were evaluated for their resistance against foliar blight disease. Of these two germplasms viz. Nepal 1 and RWP 40 were selected and their AUDPC ranged from 124 to 155 which suggested these genotypes are resistant to foliar blight. The biochemical changes usually associated with defense responses of plants like phenolics, polyphenoloxidase and peroxidase activities were measured in resistant germplasms viz. Nepal 1 and RWP 40 and also in susceptible germplasms viz. Sonalika and HUW 234.

In respect of total phenol, infection resulted moderate increase (11%) in resistant germplasm whereas in susceptible germplasm the level fell slightly short of the level in comparable with the healthy plants. The trend of orthodihydroxyphenol content showed the same way as that of the phenol content (Table 5). Stimulated PPO and PO enzyme activities were recorded in resistant germplasm following infection as comparable to susceptible germplasms (Table 6).

Integrated disease management

Results presented in Table 7 revealed that a fertilizer dose of 120 : 60 : 60 Kg/ha (N : P : K) and seed treatment with carboxin followed by single Tilt spray at panicle initiation stage significantly reduced the disease symptoms followed by spray of Tilt thrice. The effective treatment also delayed the disease incidence by 18 days as compared to the check. Application of *Trichoderma harzianum* and Econeem had no effect on disease reduction. The yield also showed the same trend as recorded with disease reduction in different treatments.

The results of the present study demonstrate that foliar blight is the most serious disease of wheat in terai zone of West Bengal causing 43 % yield reduction. The disease is a complex one and caused by *Bipolaris sorokiniana, Alternaria triticina* and *Drechslera gigantea*. It was also observed that *A. triticina* appears at early growth stage whereas *B. sorokiniana* and *D. gigantea* appear late and cause considerable damage. Two germplasms collected from CIMMYT International programme, viz. Nepal 1 and RWP 40 are identified as resistant source of foliar blight disease. The disease can be managed by combined application of 120 : 60 : 60 Kg/ha (N : P : K fertilizer) in soil followed by seed treatment with carboxin (4g/Kg seed) and single foliar spray with propiconazole (0.01%) at panicle initiation stage.

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