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Cultural and morphological variability of different isolates of *Bipolaris sorokiniana* infecting wheat in the eastern alluvial plains of India

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Spot blotch of wheat caused by *Bipolaris sorokiniana* is a major disease of wheat growing in the warmer and humid regions of the world. In West Bengal the disease has emerged as a major yield limiting factor. The different isolates of *Bipolaris sorokiniana* collected from different wheat growing zones of West Bengal indicated a wide variation in respect of morphology and physiology among the three isolates. Higher conidial length and more septation was associated with aggressiveness of the pathogen. A strong correlation was observed between toxin production and aggressiveness of the isolates.

Key words: Spot blotch, *B.sorokiniana*, virulence morphology, toxin production

INTRODUCTION

There are numerous foliar blights either of seed borne and/or soil borne diseases reported on wheat. The three blight diseases (spot blotch, tan spot and *Alternaria* blight) have been recorded in most wheat growing areas of India, Bangladesh, Nepal and Pakistan. In Eastern India, the leaf blight diseases represent a complex, and are collectively referred to as Helminthosporium leaf blight (HLB) (Chowdhury *et al*, 2013). Two of the most common diseases, Spot blotch and Tan spot, are caused by the fungi *Bipolaris sorokiniana* (Sacc. in Sorok.) Shoem; and *Pyrenophora tritici-repentis* (Died) Drechs, respectively. Another leaf blight fungus is *Alternaria triticina* Pras. and Prab. In recent surveys, the relative frequency of *A. triticina* seems to be declining, possibly due to availability of more resistant varieties. Besides, another leaf spot disease, zonate eye-spot (c.o. *Drechslera gigantea*) has also been reported from *terai* zone of West Bengal (Chowdhury *et al,* 2005). Now Spot blotch of wheat is considered as one of the most important diseases in the environment of South Asia that is characterized by high temperature (coolest month greater than 17°C) and high humidity. It is also gradually instigating serious concerns among places with irrigated, low rainfall and temperate growing condition. In the present investigation the morphological and cultural variability of different isolates of *B.sorokiniana*, collected from alluvial areas of West Bengal has been reported.

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MATERIALS AND METHODS

Large number of blight infected wheat leaf samples were collected from different locations of

Teesta and Gangetic alluvial plains of West Bengal. The infected leaf samples were washed with Mercuric chloride (0.1%) solution and again rewashed three times with sterile distilled water. The leaf samples having typical blight symptoms were cut into 4 mm2 small pieces. The leaf bits were placed in Petri dishes containing wheat decoction dextrose medium and incubated at 22^{0}C for 7 days. The fungus *B.sorokiniana* was isolated from single discrete lesion from the infected leaf tissue following the standard procedure and the purified monoconidial cultures were maintained at 4^{0}C. The pathogenicity was confirmed on susceptible variety Sonalika.

Three isolates of *Bipolaris sorokiniana* (Isolate 1 = highly virulent; Isolate 2= moderately virulent and Isolate 3= less virulent) were tested for their ability of toxin production. The isolates were inoculated on Potato Dextrose Broth and incubated for 21 days at 25^{0}C with constant light and toxins were isolated following the method of Bach and Kimati (1999).

After 21 days, mycelium was removed by vacuum filtration with Whatman No. 1 to remove the remaining hyphae. The culture filtrates were concentrated under partial vacuum at 40^{0}C to one fifth of the original volume. Equal volumes of cold (4^{0}C) methanol were then added. The mixture was refrigerated overnight and any subsequent precipitate discarded. Methanol was removed from the water phase by rotary vacuum evaporation and vacuum at 40^{0}C, and an equal volume of Chloroform was added. The water phase was separated from the Chloroform, and an equal volume of ether was added. The ether was removed and the water phase was evaporated under vacuum at 40^{0}C for the remaining ether. The water phase was analyzed by TLC. The purified toxic material (200µl) was inoculated to resistant (Chirya 3) and susceptible (Sonalika) genotypes to produce disease symptoms by detached leaf assay..

RESULTS AND DISCUSSION

The artificial inoculation of *Bipolaris sorokiniana* isolates were successful in reproducing the disease symptoms on the susceptible variety Sonalika however, a considerable variability in pathogenicity among the isolates was observed (Table 1). The size of conidia differs and higher in B-1 isolate followed by B-2 and B-3. The colour of colony was dark black in isolate B-1 and dark grey and grey in colour in B-2 and B-3 respectively (Plate 1). The isolate B-1 was highly virulent as compared to B-2 and B-3. The isolate B-1 could induce the typical symptoms and larger lesions after 72 hours of inoculation while B-2 and B-3 took 80 hrs. to develop comparable symptoms. B-1 also produced 93 lesions per leaf as compared to 79 and 68 lesions per leaf by B-2 and B-3 isolates respectively after 7 days of inoculation. Highest disease intensity was recorded in isolate B-1 (76.9%) followed by in isolate B-2 (68.9%) and B-3 (51.8%).

The growth and sporulation of the three isolates on five different media viz. Potato Dextrose agar, Oat Meal agar, Carrot Dextrose agar, Czapek's Dox agar and Richard's agar were also assessed and results obtained are given in Table 2.

The data in Table 2 indicate that the isolates vary in nutrient utilization among themselves. Oat Meal Agar was best medium followed by Potato Dextrose Agar and Carrot Dextrose Agar. Isolate B-1 had significantly superior mean radial growth followed by isolate B-2 and B-3.Sporulation of B-1 isolate was good in Oat Meal and Potato Dextrose Agar, fair in Carrot Dextrose Agar and poor in rest of the media. The sporulation of Isolate B-2 was fair on Oat Meal, Potato Dextrose and Carrot Dextrose Agar and poor in remaining media. Isolate B-3 sporulated fairly on Oat Meal and Potato Dextrose Agar but poor in rest media.

To determine the effect of pH on growth and sporulation, isolates were grown in Oat meal broth adjusted to pH 5.0, 5.5, 6.5 and 7.0 by adding 0.1N HCl or NaOH. The dry weight of the mycelium at each pH level was recorded after 15 days of incubation at 25±1^{0}C. Results are presented in Table 3.

Isolate B-1 gave significantly superior mean growth at various pH levels evaluated, followed by isolate B-2 and B-3. Among the interaction of pH and isolate, significant superior growth of isolate B-1 was recorded at pH 6.0, which remained superior at pH 5.5, pH 6.5 and pH 5.0. It was followed by the growth of isolate B-2 and B-3 at pH 6.0. It was also observed that pH 6.0 proved optimum for maximum growth and sporulation of all the isolates of *B. sorokiniana* and increase or decrease in pH levels decreased the growth and sporulation progressively (Table 3).

Table 1 : Size of conidia and number and size of lesions produced by different *Bipolaris sorokiniana* isolates

Isolate	Conidial size (µm)				No. of septa in conidia	Size of lesions (mm)	Average number of lesions/plant at 66DAS	Percent Disease Index
	Length	Mean	Width	Mean				
B-1	42.5-84.3	65.70±2.7	11.2-19.4	17.3±1.3	8-11	0.7-10.0	93	76.9
B-2	35.1-53.2	48.1±3.9	8.5-18.2	10.1±1.1	6-8	0.7-9.0	79	68.9
B-3	21.4-35.6	27.7±2.9	6.9-11.3	9.3±0.6	3-5	0.7-4.0	68	51.8
SEM±								1.81
CD at 5%								5.29

Table 2 : Growth and sporulation of *Bipolaris sorokiniana* isolates at different solid media

	Radial Growth on culture media (mm) after 7 days					
Isolate	Potato Dextrose Agar	Oat Meal Agar	Carrot Dextrose Agar	Czapeks Dox Agar	Richards Agar	Mean
B – 1	74.22 *(+++)	75.12 (+++)	68.17 (++)	50.33 (+)	49.67 (+)	63.50
B – 2	65.16 (++)	68.60 (++)	54.25 (++)	48.23 (+)	47.70 (+)	56.80
B – 3	49.21 (++)	50.20 (++)	43.20 (+)	45.50 (+)	41.70 (+)	45.96
Mean	62.86	64.64	55.21	48.02	46.35	
SEm±	Media 0.326		Isolate 0.211		Media ×Isolate 0.550	
CD at 5%	0.95		0.62		1.64	

*Number of conidia / microscopic field : + = 1-10 ; ++ = 11-20 ; +++ = 21-30

Table 3 : Mean growth and sporulation of *Bipolaris sorokiniana* isolates at different pH levels at 25 ± 1°C

	Growth at pH levels (mg) after 15 days					
Isolate	5.0	5.5	6.0	6.5	7.0	Mean
B – 1	116.66 * (++)	120.66 (+++)	124.33 (++++)	117.33 (+++)	104.0 (+)	116.60
B – 2	108.66 (+)	113.00 (++)	116.33 (+++)	110.33 (++)	101.33 (+)	109.93
B – 3	102.66 (+)	107.0 (++)	110.22 (+++)	107.3 (++)	97.33 (+)	104.90
Mean	109.33	113.55	116.96	111.66	100.88	
SEm±	pH 0.177		Isolate 0.159		pH ×Isolate 0.391	
$CD_{at\ 5\%}$	0.52		0.47		1.15	

*Number of conidia / microscopic field : + = 1-10 ; ++ = 11-20 ; +++ = 21-30 ; ++++ = > 31

The three isolates exhibited a significant variability in their virulence and pathogenesis along with the considerable variability in their cultural characteristics. Earlier morphological and cultural variability in Indian isolates of *B. sorokiniana* has been reported by various workers (Jaiswal *et al*,2007 and, Aggarwal *et al,* 2011)

Variability among the isolates of *B. sorokiniana* was further studied by characterizing the toxin secretion of the isolates by the methods described earlier and observations were recorded. The purified toxins of three isolates (B-1, B-2, B-3) were inoculated on susceptible (cv.Sonalika) and resistant (cv.Chirya 3) plants of wheat. The results show that the size of the lesions were 8-11 mm in susceptible leaves and 3-5 mm in resistant leaves after 96 hours of inoculation in case of Isolate B-1. the corresponding values were 7-9 mm and 3-4 mm in case of Isolate B-2 and 4-6 mm and 1-2 mm in case of Isolate B-3.

It was observed that a wide variation was recorded among the isolates regarding toxin production as

indicated Rf values of 0.25, 0.37 and 0.87 for isolate B-1, Rf value of 0.13 and 0.25 for isolate B-2 and Rf value of 0.13 for isolate B-3.

There are many early reports on the presence of phytotoxic substances in cultural filtrates of *B. sorokiniana*. Toxins produced by *B. sorokiniana* are non specific and not directly associated with aggressiveness. Helminthosporal, helminthosporol and prehelminthosporal were isolated from the culture filtrates and of these, pre-helminthosporol is the most active and is produced in abundance. Although pre-helminthosporol sensitivity is not correlated with *B. sorokiniana* resistance in barley cultivars, it is supposed to play an important role in pathogenesis by killing or weakening plant cells in advance of the growing hyphae. 1, 3- ²À-glucan synthase is activated by Ca^2+ and can operate in the presence of low concentrations of pre-helminthosporol (which may induce Ca^2+ leakage into the cytoplasm and hence activate the enzyme), to produce callose to seal leaky membranes. Briquette *et al,* (1998) observed that helminthosporol, which is one of the natural sesquiterpenoid toxins, drastically affects the membrane permeability of host mitochondria, chloroplast and microsomes. As a result, there is inhibition of mitochondrial oxidative phosphorylation, the photophosphorylation in chloroplast and the proton pumping across the cell plasma membrane. Low temperature preparation technique in combination with immunogold-labeling were used for localization of pre-helminthosporol in hyphae and germinated conidia of *B. sorokiniana* by electron microscopy. A low level of labeling was obtained throughout the cytoplasm, and the main labeling was seen in membrane-bound organelle identified as Woronin bodies.

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