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PATHOGENIC DIVERSITY IN *BIPOLARIS SOROKINIANA* ISOLATES COLLECTED FROM DIFFERENT WHEAT GROWING AREAS OF THE PUNJAB AND NWFP OF PAKISTAN

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Abstract

Leaf blight of wheat caused by *Bipolaris sorokiniana* (*Cochliobolus sativus*) is a world wide economically important foliar disease. It mainly occurs in warm, humid wheat growing areas. In Pakistan *Helminthosporium* leaf blight (spot blotch) has been noted in southern province of Sindh, where winter temperatures are warmer. During a survey conducted in different agro-ecological zones of Pakistan from 2004 to 2005, *Bipolaris sorokiniana* was found to be a predominant pathogen. Eighty-seven isolates of *B. sorokinaina* isolated from 6 different zones were tested for their aggressiveness on cv. Inqliab-91, Wafaq-2001 and Bhakhar-2001. The majority of the isolates were found more aggressive on cv. Wafaq-2001 than Inqliab-91 and Bhakkar-2001 whereas, two isolates were categorized as least aggressive, 57 slightly aggressive and 27 were moderately aggressive while one isolate P_2 9 collected from Khanewal (zone 5) exhibited most aggressive reaction.

Introduction

Leaf blight caused by *Bipolaris sorokiniana* is gaining importance due to changes in cropping system in many wheat growing areas. The disease is recorded in most growing areas of India (Sharma *et al*., 1996), Bangladesh (Alam *et al*., 1994), and Nepal (Karke & Karke, 1996; Sharma, 1996). In the Southern province of Sindh, where winter temperatures are warmer, *Helminthosporium* leaf blight has been noted (Bhatti & Ilyas, 1986; Hafiz, 1986) where the significant yield losses (Aftabuddin *et al*., 1991). High temperature and high relative humidity favour the outbreak of the disease (Aggarwal *et al*., 2000). Due to this producing on the yield loss of 18-22% in India (Singh *et al*., 1997) and 23.8% in Nepal (Shrestha *et al*., 1997).

The assessment of the aggressiveness of the pathogen is the frontier at which host and pathogen interact. It is the site of pathogen's initial contact with the host's defense systems, successful circumvention of host defenses may lead to colonization, infection pathogen replication and transmission to another host. It is an obvious benefit in developing a better understanding of the host pathogen relationship (Jain & Prabhu, 1976). The extent of variability in natural populations of *B. sorokiniana* of wheat has been investigated worldwide. The aggressive type of *B. sorokiniana* remains potentially viable in the shriveled seeds (Rashid, 2005). Clear understanding of the extent of variation in virulence would be helpful in developing wheat cultivars with stable resistance. The present study was therefore undertaken to analyze the aggressiveness of *B. sorokiniana* among isolates collected from different agro ecological zones and to select the most aggressive one for further studies like screening of wheat genotypes against this pathogen.

Materials and Methods

Isolation and multiplication of fungus: For aggressiveness analysis of *Bipolaris sorokiniana*, blighted leaf samples of wheat were collected from different agro-ecological zones of wheat production during 2004 & 2005. Diseased leaves samples were cut into small pieces. Leaf sections (1cm) were surface sterilized with 1% Clorox for 1 minute, rinsed thrice with sterilized distilled water and placed on moistened blotting paper in 9 cm diameter sterilized Petri-plates. The plates were incubated for 24 hours at 25ºC under light and then for 24 hours at 18ºC in dark. After continuous light and dark period, the fungal growth was observed and then identified (Barnett, 1960; Domsch *et al*., 1980). A single spore culture of *B. sorokiniana* was prepared and transferred in Petri plates containing PDA (potato dextrose agar), then incubated for 5-6 days at 25ºC. Upon the completion of fungal growth, the cultures were transferred to refrigerator for further use.

Aggressiveness analysis: Test tubes (29cm x 3cm) were filled with one inch cotton swab from the bottom. Ten ml distilled water was added to each test tube to moist cotton and were covered with aluminum foil. The test tubes were autoclaved and upon cooling were used for analysis.

Three commercial wheat varieties including Wafaq-2001, Bakkhar- 2001 and Inqliab-91 were used for testing the aggressiveness of the isolates of *B. sorokiniana*. Seeds of each variety were surface disinfected with 1% Clorox solution for one minute and rinsed thrice with sterilized distilled water. Three seeds of each variety were placed in test tube on cotton swab in triplicate along with one disc of 5 mm culture of each isolate. The inoculum potential 3.2×10^{4} conidia/ disc was measured. After inoculations all the tubes were arranged in randomized design (RCD) in the steel test tube stands, after inoculation, covered with aluminum foil and were incubated at 25ºC in growth room.

Data Observation and Analysis: Data was recorded after 30 days. Symptoms on foliar parts were recorded on 0-5 scale, where 0 = no symptoms, 1 = 1-5% spots on leaves, 2 = 6-20% spots on leaves, 3 = 21 – 40% spots on leaves, 4 = 41 - 60, 5 = more than 60% spots on leaves (Anon., 1996).

Results

Of a total of 87 isolates of *B. sorokiniana* tested for aggressiveness, all were found pathogenic to three varieties of wheat; however the reaction varied in terms of aggressiveness. The aggressive reaction of isolates of *B. sorokiniana* on these wheat varieties was subjected to Analysis of Variance (ANOVA). There was no significant effect of replications; however there was highly significant effect of isolates among themselves and their reaction on different varieties on which they were tested (Table 1). The mean value of three varieties showed that isolates were more aggressive on Wafaq-2001 than Inqliab-91 and Bhakkar-2001 (Table 2). All the means of 87 isolates on three commercial varieties were worked out and combined means on three varieties of each isolate was subjected to dendrogram construction for further classification (Fig. 1).

Table 1. Analysis of variance of *Bipolaris sorokiniana* isolates on three commercial wheat varieties.

Sources of variation	Degree of freedom	Mean square	F-value	Probability
Replication	2	0.034 NS	0.2441	NS
Isolates	86	1.643**	11.7259	0.0000
Rep. x solates	172	O.129 NS	0.9227	NS
Varieties	2	38.248**	271.0160	0.0000
Rep. x varieties	4	0.060 NS	0.4271	NS
Isolate x varieties	172	1..375**	9.8167	0.0000
Error	**356**	**0.140**		

** Mean squares of isolates, verities and isolate x verities interaction are highly significant at 1%.
Coefficient of varaince13.13%

Table 2. Mean value of aggressiveness of *B. sorokiniana* on three wheat varieties.

S. No.	Varieties	Mean value
1.	Wafaq 2001	3.282 a
2.	Inqilab 91	2.663 b
3.	Bhakkar 2001	2.608 b
	LSD value	0.0629

Column means followed by a common letter are not statistically different at 5 %

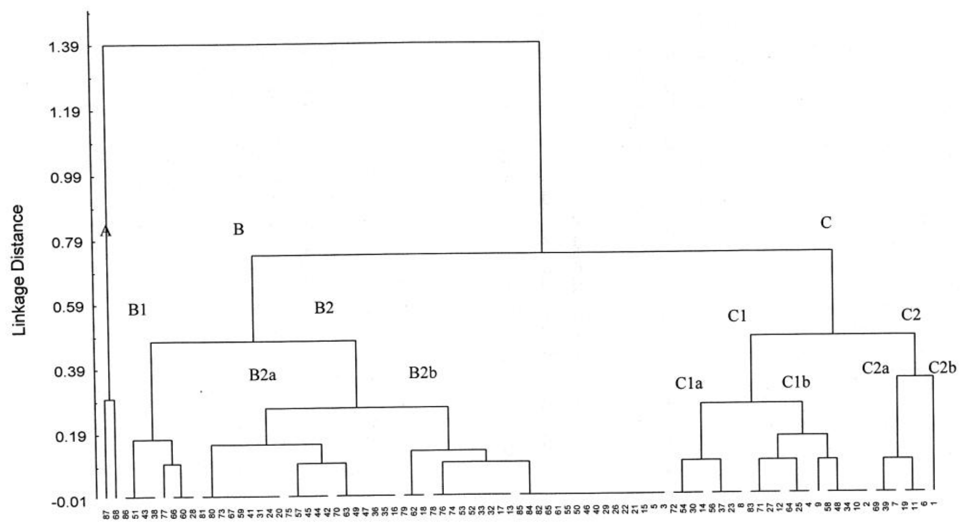

Fig. 1. Dendrogram showing different groups of 87 isolates of *B. sorokiniana*.

These isolates are classified into three major groups and some of which further classified into sub-groups, group A, group B (sub-group B1 and B2; sub group B2 has further mini groups B2a and B2b) and group C.(sub groupC1 and C2; both divided into mini groups C1(C1a and C1b) and C2(C2a and C2b). Group A consisted of six isolates, all belonged to least aggressive isolates. Group B comprised of 53 isolates, out of which

4 isolates belonged to sub-group B1 and 49 belonged to sub group B2, the sub group further sub divided into mini group B2a and B2b of which 21 isolates belonged to B2a and 28 belonged to B2b.All the isolates in these groups consisted of slightly aggressive but due to slight difference in aggressiveness were further sub divided. Group C comprised of 28 isolates out of which 21 isolates belonged to sub group C1 which was further sub divided into C1a and C1b, C1a comprised of 8 isolates and C1b comprised of 13 isolates all belonged to the moderately aggressive isolates. The sub group C2 comprised of 7 isolates and was further sub divided into two mini groups C2a and C2b out of which 6 isolates belonged to C2a and one isolate belonged to C2b.The C2a isolates are categorized to moderately aggressive while C2b one isolate belonged to aggressive group. The details of all the groups are given in Table 3.

The isolates from different zones for both the years of collection 2004-2005 were analyzed for their distribution among the four aggressiveness classes: aggressive, moderately aggressive, slightly aggressive and least aggressive (Table 3).

The isolate P2-9 of zone 5 was the only isolate found aggressive among the 87 isolates. The other isolates of zone 5 (Punjab) were moderately aggressive (9 in number) and 2 slightly aggressive. In zone 6 of Punjab, out of 16 isolates 5 isolates were moderately aggressive while 11 isolates were Slightly aggressive. In zone 7 of Punjab 4 and 13 isolates were moderately aggressive and slightly aggressive respectively (Fig. 2).

Out of 9, 10 & 11 zones of NWFP, six isolates exhibited moderately aggressive reaction while 17 and one isolate showed slightly and least aggressiveness in zone 9. In zone 10 two isolates exhibited moderately and 7 slightly aggressive reaction. As regard zone 11 one isolate each exhibited moderately and one least aggressiveness while 7 isolates exhibited slightly aggressive reaction.

Discussion

Different aggressiveness behaviour of the pathogen to the host was observed during aggressiveness analysis. In our study majority of the isolates exhibited slightly aggressiveness which was collected from different ecologies while the others showed moderately aggressiveness and least aggressiveness. One isolate showed aggressiveness behaviour as observed by Mikhailova *et al*., (2002) who studied the aggressiveness behaviour of 11 isolates of *B. sorokiniana* collected from different geographical location in Russia and checked on 10 varieties of wheat and found significant difference in fungal strains behaviour. Whereas Duveiller & Altamirano (2000) isolated 27 isolates of *B. sorokiniana* (from roots, leaves and grains of spring wheat) collected from a single site in Mexico and found no clear difference between groups of isolates. They reported that this behavior of the fungus appeared as a continuum of isolates differing in aggressiveness. Similar observations were recorded by Rasmussen *et al.*, (2003) that the stability of resistant genetic strains remained essential considering that *B. sorokiniana*, the principal pathogen forms a continuum of strains differing in aggressiveness.

In another perspective if we compare the severity of isolates exhibiting aggressive reaction it varied *In-vitro* and field conditions. Like P2-9 isolate showed 2 severity (0-5 scale) in the farmer’s field as compared to *In-vitro* study where it exhibited aggressive reaction at 4, which reflects the variable conditions provided to the pathogen or for the success of the isolate there is a need of its interaction with the environment and the inoculum pressure (Jain & Prabhu, 1976). During current study under controlled conditions the provision of maximum inoculum led the isolate to deliver its maximum aggressiveness.

Table 3. Grouping of *Bipolaris sorokiniana* isolates, based on cluster analysis (dendrogram) of aggressiveness tests.

Group	Sub-group	Category	No. of isolates	Isolates
A	-	Least Aggressive	2	NP3-29, G5-3,
B	**B1**	Slightly Aggressive	8	P4-40, P2-28, P4-1, NP-9, NP3-12, NP3-27, SWT1-26, S-K-1.
	B2			
	B2a	Slightly Aggressive	21	P2-18, P4-12, P4-31, P1-10, P2-24, P2-25, P3-15, P3-16, P4-5, P4-9, NP1-3, NP6, NP3-9, NP3-10, NP3-16, NP3-28, NP7, NP3-6, G1-6, G1-8, SWT1-7
	B2b	Slightly Aggressive	28	P4-2, P4-17, P1-6, P4-14, P2-22, P2-23, P4-13, P4-14, P4-33, P1-4, P2-3, P2-4, P3-14, NP1-2, NP-8, NP-10, NP-11, NP3-3, NP3-13, NP3-15, NP3-21, SWT1-2, SWT1-22, G1-18 G1-2, G1-9, S-Shing-7, S-G-10
C	**C1**			
	C1a	Moderately aggressive	8	P4-22, P2-7, P4-30, P1-9, P2-26, NP-20, NP3-7,NP3-5
	C1b	Moderately aggressive	13	P2-15, P4-16, P4-27, P4-24, P4-29, P4-32, P4-34, P2-5, NP4, NP3-10, NP3-18, NP3-4, S-Shing-5
	C2a	Moderately aggressive	6	P4-18, P4-20, P4-28, P4-11, P3-5, NP3-31
	C2b	Aggressive	1	P2-9

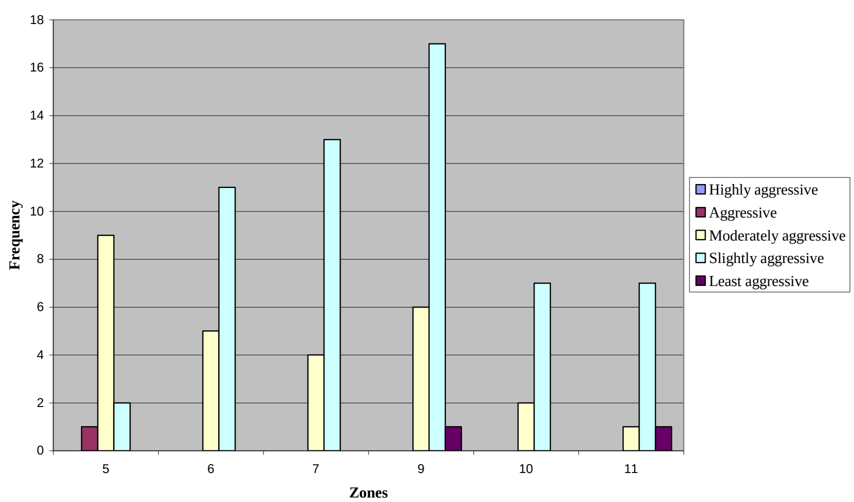

Fig. 2. Zone wise frequency of different groups of *Bipolaris sorokiniana*.

The aggressive isolate (P2-9) collected from one genotype showed different reactions on different genotypes when tested under *In-vitro* conditions confirming the findings of Maraite *et al.*, (1997) who collected the isolates of *B. sorokiniana* from background of one genotype and when inoculated these isolates on a wide range of genotypes under controlled conditions found also different results. Nevertheless there appears to be no specific link between the aggressiveness and the genotypes from which the strain was isolated.

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