

**PREVALENCE AND DISTRIBUTION OF FOLIAR BLIGHT
PATHOGENS OF WHEAT IN DIFFERENT AGRO ECOLOGICAL
ZONES OF PAKISTAN WITH SPECIAL REFERENCE
TO *BIPOLARIS SOROKINIANA***

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Abstract

During previous years blight diseases of wheat were considered to be of minor importance in Pakistan. However in Southern province of Sindh, where winter temperatures are warmer, *Helminthosporium* leaf spots (spot blotch) has been noted. During this study, surveys were conducted at seedling and booting stage of wheat during cropping season of 2004 to assess the foliar spot diseases in different agro-ecological zones of Pakistan including 55 locations (zone 6, 7, 9 & 10) at seedling stage and 101 locations (zone 5, 6, 7, 9, 10 and 11) at booting stage. The fungi isolated from the samples were identified as *Alternaria alternata*, *Bipolaris sorokiniana*, *Curvularia lunata*, and *Drechslera spicifer*. The most prominent fungus was *Bipolaris sorokiniana*, the cause of leaf blotch in wheat. A total of 37 isolates of *Bipolaris sorokiniana* were collected and identified as pathogenic to wheat. Tan spot causing fungus, *Pyrenophora tritici-repentis* was isolated only from the barley leaves but not from wheat.

Introduction

In Pakistan the wheat crop is cultivated over an area of 8.033 million hectares with an annual production of 19 million tons (Anon., 2003). This yield is low as compared to the other important wheat producing countries of the world. Diseases are considered as one of the major yield constraints, yields are lowered due to the reduction in emergence, plant vigor and tillering. These diseases are caused by fungi, bacteria, viruses and nematodes. Among fungal diseases, foliar pathogens are considered to contribute significantly to reduce average yields of cereal crops in many developing countries. The reduction in yield due to leaf blight recorded in India during 1998 and 1999 was varied with the cultivars used, crop year and location; when the 1000 grain weight decreased significantly from 1.2 to 26.1% due to this disease (Singh *et al.*, 2002). Although major fungal diseases of wheat in Pakistan are the rusts and smuts but altogether 50 diseases of wheat have been reported (Joshi *et al.*, 1970) and some of them are important economically. The foliar blight diseases have been recorded in most growing areas of India (Sharma *et al.*, 1996), Bangladesh (Alam *et al.*, 1994) and Nepal (Karki & Karki, 1996; Sharma, 1996). However, in Pakistan Bhatti & Ilyas (1986) and Hafiz (1986) considered the blight disease to be of minor importance. In spring 2000, during a survey of wheat fields in the surroundings of Islamabad, tan spot was found as frequent as spot blotch (Ali, unpublished). Similarly, in the same year different foliar spots of wheat were observed in different frequencies in various districts of Punjab. The fungi isolated were *Alternaria alternata*, *Bipolaris sorokiniana*, *Cladosporium* sp., *Helminthosporium* spp., and *Stemphylium* spp. Tan spot caused by *Pyrenophora tritici-repentis* was also observed at 13 different locations (Ali *et al.*, 2001).



Fig. 1. Distribution of *Bipolaris sorokiniana*, the cause of leaf blotch of wheat in different wheat growing zones of Pakistan.

Occurrence of foliar blights in major wheat areas of Pakistan calls for detailed analysis and improved understanding of patho-biology of foliar blights for better crop productivity. This study was therefore carried out to understand the distribution of foliar spots of wheat crop in Pakistan for the management of the disease.

Materials and Methods

Survey: The surveys for foliar spot of wheat were conducted at two growth stages of the crop. First survey was conducted at seedling stage and second survey was conducted at booting stage. Out of 12 wheat growing zones of Pakistan (Fig. 1), zones 5, 6 and 7 in Punjab, 9 and 10 in NWFP and 11 in Northern Area were surveyed during wheat growing season of 2004.

Seedling stage: A 50 cm² quadrat was used for sampling at seedling stage. The plants within the quadrat were assessed for foliar spots and leaf samples were collected at ten locations within same field randomly and brought to laboratory for isolation and subsequent identification and purification of the fungi associated with the foliage spots. Fifty five locations were surveyed at seedling stage in zone 6, 7, 9 and 10 (Table 1).

Table 1. Prevalence and incidence of leaf blotch (*Bipolaris sorokiniana*) of wheat in different agro-ecological zones of wheat during 2004.

Zones/Area	Total no. of locations surveyed	Crop stage	No. of locations (<i>B. sorokiniana</i> isolated)	% Incidence
5/Punjab	8	Booting	2	25
6/	10	Seedling	2	20
Punjab	12	Booting	7	58.3
7/	36	Seedling	3	8.3
Punjab	17	Booting	5	29.41
9 /	4	Seedling	1	25
NWFP	8	Booting	7	87.5
10/	5	Seedling	-	-
NWFP	32	Booting	6	18.75
11/	24	Booting	4	17.39
Skardu & Gilgit				
Total	55	Seedling	6	
	101	Booting	31	

Booting stage: Samples at booting stage were taken from ten points in a diagonally transect from each field. The leaf samples were collected and brought to laboratory for isolation and identification of the fungi associated with spots. First survey was conducted in the second week of March 2004 in zone 5, 6 and 7 where samples were taken from 37 locations. Second survey was conducted during the second week of April 2004 in zone 9 (NWFP) where 8 locations were visited. In zone 10 the survey was conducted in the last week of April 2004 and 32 locations were visited. Fourth survey was conducted during the first two weeks of June, 2004 in zone 11 (Gilgit and Skardu area) and 24 locations were visited.

Foliar blight assessment: The prevalence of the disease was assessed by noting the presence or absence of foliar spots in each field. Ten plants were selected to assess the disease incidence and severity. Percentage of prevalence and incidence was calculated according following formulae:

$$\text{Prevalence \%} = \frac{\text{Locations showing foliar spots}}{\text{Total locations}} \times 100$$

$$\text{Incidence \%} = \frac{\text{No. of samples showing foliar spots}}{\text{Total No. of samples}} \times 100$$

Disease severity was assessed on a 0-5 visual rating scale, where 0= no symptom, 1=1-5% few spots on < 50% of leaves, 2 = 5-20% spots on < 50% of leaves, 3= 5-20% spots on > 50% of leaves 4= 20-50% spots on < 50% leaves and 5= > 50% on > 50% leaves (Anon., 1996).

Isolation and identification of fungi from foliage: The foliar samples showing diseased spots were cut into small pieces and 4-5 pieces having spots were taken. The leaf sections were first surface sterilized by dipping in 1% Sodium hypochlorite solution for a minute and later rinsed with sterilized distilled water. The leaf pieces were then plated on sterilized wet filter papers in sterilized Petri plates and incubated for 24 hours at 20-22°C under florescent light and then 24 hours at 18°C in dark (De Wolf *et al.*, 1998).

Table 2. Percent incidence of fungi isolated from wheat leaves at seedling and booting stages from different wheat growing agro-ecological zones during 2004.

S. No.	Fungi isolated	Percent incidence		
		Seedling stage	Booting stage	Total
1.	<i>Alternaria alternata</i>	-	16.83	16.83
2.	<i>Alternaria</i> sp.	18.18	30.69	26.28
3.	<i>Bipolaris sorokiniana</i>	10.90	30.69	23.71
4.	<i>Cochliobolus</i> sp.	-	1.98	1.98
5.	<i>Curvularia lunata</i>	1.81	-	1.81
6.	<i>Drechslera spicifer</i>	3.63	-	3.63

After incubation of 48 hours the morphology of the fungal colonies was observed. Slides were prepared by mounting the fungal portion in cotton blue stain and observed under light microscope at 100X and 400X magnifications. Identification was done after reference to Gilman (1945), Barnett (1960) and Domsch *et al.*, (1980).

Fungi isolated were purified by single spore technique on potato dextrose agar (Usmani & Ghaffar, 1982). The plates were incubated at 27°C for 3-4 days. After the complete growth of fungi, the fungal cultures were sealed and preserved in refrigerator for further use.

Results

Fungi isolated at seedling stage from zone 6, 7, 9 and 10: Out of 46 samples collected from zone 6, 7, 9 and 10, *Bipolaris sorokiniana* was isolated from 3 samples in zone 6, from 2 in zone 7 and from one location of Kohat in zone 9, where leaf spots severity was observed in traces. None of the sample from zone 10 was found infested with *B. sorokiniana*. The total incidence of the pathogen at this stage in these zones was 10.90% (Table 2). Number of saprophytic fungi were also isolated which include *Alternaria* sp., with incidence of 18.18%, *Alternaria alternata* 16.83%, *Drechslera spicifer* of 3.63% and *Curvularia lunata* from one location having the incidence 1.81% (Table 2), whereas, *Pyrenophora tritici-repentis*, the pathogen of tan spot in wheat was isolated from barley samples of two locations Akora Khattak and Kohat but not from wheat.

Fungi isolated at booting stage from zone 5, 6, 7, 9, 10 and 11: The leaf blotch causing fungus, *Bipolaris sorokiniana* was most prevalent in zone 5, 6 and 7 in Punjab with incidence of 25, 58.3 and 29.41%, respectively (Table 1). Disease severity was observed in traces during disease assessment. Out of 8 locations of NWFP in zone 9 and 32 locations in zone 10, *Bipolaris sorokiniana* was isolated from 7 and 6 locations with the incidence of 87.5 and 18.75%, respectively (Table 1). During disease assessment in the fields of these locations crop was found with heavy foliar spots with severity of 3-4 on rating scale. Out of 24 locations visited in zone 11 of Gilgit and Skardu Area, *Bipolaris sorokiniana* was found associated with foliar spots at 4 locations with an incidence of 17.39% (Table 1), the severity was observed in traces.

Among the saprophytic fungi at booting stage, *Alternaria* sp., was the most prevalent with an incidence of 30.69% followed by *Alternaria alternata* (16.83%) and *Cochliobolus* sp., (1.98%) (Table 2). *Pyrenophora tritici-repentis* was isolated from the samples of 3 locations from barely foliage not from wheat.

Discussion

Two surveys at different growth stages have been done. The purpose of the survey at seedling stage was to have the disease assessment that will provide the information about the symptom production at the early stage of growth as previous studies on foliar blight of wheat indicates that disease can produce post damping off and seedling blight (Gilchrist *et al.*, 1991). Secondly the isolation of pathogens from the collected samples will intimate the transfer of disease through seeds at this stage. Foliar spots have been observed in traces at seedling stage in few fields whereas, majority of the fields had found with healthy crop. The frequency of isolation of *Bipolaris sorokiniana* along with the saprophytic fungi *Alternaria alternata*, *Drechslera spicifer* and *Curvularia lunata* from zone 6, 7, 9 and 10 was low. *Pyrenophora tritici-repentis* was isolated only from the samples of barley from Kohat (zone 9). Although barley is considered as collateral host of *Pyrenophora tritici-repentis* which promote genetic variation in the fungal population and serve as a reservoir of local primary inoculum for the wheat crop in the next season (Ali & Francl, 2001). It did not produced symptoms on wheat during pathogenicity test, confirming the findings of Ali & Francl (2001) that barley does not play a significant role in tan spot epidemiology on wheat or promote variation in the fungal population.

At booting stage *Bipolaris sorokiniana* was the most prevalent and frequent pathogen isolated from spotted wheat leaves from zone 5, 6 and 7 followed by zone 9 and 10. Fungus is among five pathogenic fungi involved in the seedling blight and root rot of wheat in Pakistan (Bhatti & Ilyes, 1986; Hafiz, 1986; Kishwar *et al.*, 1992). The fungus is one of the most serious foliar disease constraint for wheat crop in warmer growing areas and causes significant yield losses (Jagdish-Kumar *et al.*, 2002) as high temperature and high relative humidity favours the out break of the disease, particularly in South Asia's intensive 'irrigated wheat-rice' system. During the current study heavily infected fields with foliar spots were observed in zone 9 (NWFP) and generally it was observed that the disease mostly affect Inqilab-91 which is grown in 80% area of Punjab and about 70% in NWFP followed by Bakhtawar-92. The Second most infested area with *B. sorokiniana* was zone 6 (58.3%) which is the main rice-wheat growing area of Pakistan. It was also observed that at both of these locations the incidence at seedling stage was 20 and 25%, respectively, indicating that the disease may be masked and appear late in the growth stage of the wheat. Total 37 isolates of *Bipolaris sorokiniana* including six isolates at seedling and thirty one isolates at booting stage have been collected during this season and found pathogenic under artificial inoculation test on wheat cv. Wafaq 2001. It is thus very important to introduce varieties with genetic resistance against the blight disease as disease can come to epidemic level as recorded on wheat in Punjab (India) and adjoining areas during 1995–96, where dominant pathogen involved was *Drechslera sorokiniana* (Satvinder-Kaur *et al.*, 2001).

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