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CHARACTERIZATION OF *BIPOLARIS SOROKINIANA* ISOLATED FROM DIFFERENT AGRO-ECOLOGICAL ZONES OF WHEAT PRODUCTION IN PAKISTAN

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Abstract

Bipolaris sorokiniana is a known cause of foliar blight, seedling blight, head blight and common root rot of wheat worldwide. It causes significant yield losses in South Asian countries and considered as a serious foliar disease constraints in warmer growing areas. The pathogen collected during 2004 and 2005 from foliar samples of wheat of different agro ecological zones was characterized on the basis of culture/colony colour and texture, conidial morphology and pathogenic nature. They were grouped in 4 classes having black, grayish black, brown and albino (whitish) colony color with profusely sporulated and suppressed type of growth to fluffy and less sporulated type. The conidial average size ranged from 38.3–65.8 µm x 12.3–25 µm with slightly curved, brown to olivaceous brown with 2–13 septa. Some isolates had relatively long and broad slender conidia, while some were uniformly straight and cylindrical and light brown in colour. All the isolates did not show difference in pathogenicity test by producing the symptoms on leaves but their reaction varied in terms of aggressiveness.

Introduction

Bipolaris sorokiniana (Sacc.) Shoemaker (Sivanesan, 1990) (teleomorph *Cochliobolus sativus*) is the causal agent of common root rot, leaf spot, seedling blight, head blight of wheat and barley and black point of grains. Several synonyms of the anamorph have been used like *Helminthosporium sorokiniana*, *Drechslera sorokiniana* and *Helminthosporium sativum* (Maraite *et al*., 1998). Previously its generic name *Bipolaris* was proposed by Shoemaker (1959) having the fusoid, straight or curved conidia with bipolar germination and characterized by thick-walled, elliptical conidia (60-120um x 12-20 um) with 4–8 septa. The colony of the fungus has interwoven hyphae as a loose cottony mass white or light to grey color depending on the isolates. The fungus is differentiated from other members of the genus *Bipolaris* on the basis of morphological characters of conidiospores and conidiophores (Kumar *et al*., 2002). *Bipolaris sorokiniana* develops dark brown necrotic lesions on roots, crown, leaves and lower leaf sheaths. It develops oval to elongated light to dark brown blotches on leaf blades and sheaths, when it severely infect the roots and crown portions, the plants dry out without producing any seed. Similarly infected spikelets under favourable conditions produces shriveled grains. The conducive weather conditions i.e., continuous rain for 5-6 days followed by warmer temperatures (day average of 20–30^{o}C), spot blotch epidemic can develop very rapidly (Mehta, 1998). The pathogen is distributed worldwide and has become a major production constraint in South Asia's intensive cropping systems, where more or less 12000,000 ha area is affected (Nagarajan & Kumar, 1998; Ruckstuhl, 1998; and Singh *et al*., 1998). Due to this destructive pathogen, the yield loss was estimated at 18-22% in India (Singh & Srivastava, 1997) and 23.8% in Nepal (Shrestha *et al*., 1997). In Pakistan previously the spot blotch was considered to be of minor importance (Bhatti & Ilyas, 1986; Hafiz, 1986). However in 2000 during a survey of wheat fields in various

districts of Punjab, foliar spots were observed in different frequencies (Ali *et al*., 2001). Later *B. sorokiniana* was found the predominant pathogen of foliar spot in all wheat growing areas of Pakistan (Iftikhar *et al*., 2006). This latest situation calls for detailed study of the pathogen for improved understanding of pathobiology of foliar blights for better crop productivity. During current study *B. sorokiniana* was characterized by microscopy, colony characteristic and its pathogenicity. Colours of the colonies on minimal medium varied from white to light pink and dark green. The black and dark coloured colony showed strong correlation with aggressiveness of the pathogen.

Materials and methods

Phenotypic studies: Blighted/spotted leaf samples of wheat were collected from different ecologies of wheat producing areas. Samples were brought in the CDRP pathology lab at NARC, Islamabad for isolation of *Bipolaris sorokiniana*. On isolation culture was purified by single spore culture technique on potato dextrose agar medium (Usmani & Ghaffar, 1982). Slides were prepared of each isolate and were observed under light microscope at 40x and 100x magnifications, the spore shape and size was noted and measured according to Sivanesan & Holliday (1981). The cultural characteristics were noted when grown on PDA which includes the color and growth pattern of fungal isolate and then pathogenicity was conducted.

Pathogenicity test: The pathogenic nature of all 87 isolates was tested on susceptible variety Wafaq-2001 (Iftikhar *et al*., 2008) by test tube method. The test tubes (20cm x 3cm) were prepared by filling 1/4th of cotton in the bottom of the tubes. Sterilized distilled water (20 ml) was added in each tube and lids were covered with aluminum foil and then autoclaved. Wheat seeds surface disinfected with Clorox (1%) and rinsed thrice with sterilized distilled water, were placed on the moist cotton swab in the test tube @ 3 seeds/tube. One disk of 5 mm of fungal isolate containing 3.2×10^4 spores/disk was placed adjacent to the seeds (Giri *et al*., 2001). The tubes were arranged in randomized (RCD) in the steel racks, after inoculation the tubes were again sealed with aluminum foil and were placed in growth room at 25^oC for incubation. The data was recorded upon the appearance of spots on the leaves by 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% spots on leaves and 5=61% and above spots on leaves (Anon., 1996). The pathogen was re-isolated from the spots of leaves and Koch's postulate was confirmed by comparing these isolates with the mother culture.

Results

Morphological characterization

Colony color and growth pattern: The colony colors of 87 isolates of *B. sorokiniana* collected from different agro-ecological zones during 2004 and 2005 were observed. Four different types of colors of isolates were found on PDA medium. Thirteen isolates exhibited black colony color, 19 grayish black, 2 brownish while 3-showed albino color colony (Fig. 1a, b, c and d). All of the black isolates had suppressed type of colony while the rest showed fluffy type of growth on the medium. The maximum isolates exhibited grayish black color followed by black, albino and brown. Out of 50 isolates 27 isolates exhibited black color, 15 grayish black; three exhibited brownish appearance while five isolates showed albino appearance (Table 1).

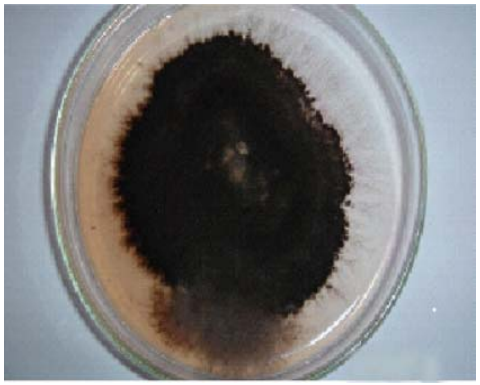
Table 1. Cultural characteristics of *Bipolaris sorokiniana* isolates collected from different agro ecological zones of wheat production during 2004 and 2005.

Zone	Location	Year 2004				Year 2005			
		No. of isolates	Colony colour	Type of growth	Pathogenicity/ aggressive ness (0-5)	No. of isolates	Colony colour	Type of growth	Pathogenicity/ aggressive ness (0-5)
5	Punjab	1	B	S	5	9	B	S	3.3-5
		1	GB	S	2.6	1	GB	S	3
6	Punjab	5	B	S	2.3-3.6	7	B	S	3-4
		1	GB	S	2.3-3.6	2	GB	S	4
		-	-	-		1	BR	S	3
7	Punjab	5	B	S	3-4.3	1	B	S	3.3
		2	GB	S	3	5	GB	S	1.6-4
		2	BR	S	2.6-3	-	-	-	-
		1	AL	F	2.6	1	BR	S	3
9	NWFP	2	B	S	3.3-4.3	6	B	S	3-5
		6	GB	S	2-3.6	6	GB	S	3-4
		-	-	-	-	1	BR	S	2
		1	AL	F	2	2	AL	F	2.3-2.6
10	NWFP	-	-	-	-	2	B	S	3.6-4
		4	GB	S	2-3.3	1	GB	S	3.6
		1	AL	F	2	-	-	-	-
11	Northern areas	-	-	-	-	2	B	S	3-3.6
		5	GB	S	2-3.3	-	-	-	-
		-	-	-	-	3	AL	F	1-2.6

B = Black, GB =Grayish Black, Br = Brown, AL = Albino, S =Suppressed, F = Fluffy, - = nil

Conidial morphology of *Bipolaris sorokiniana*: The measurements of the conidia varied from 35-65µm x 13-25µm with 2-13 septa (Table 2). The conidia of all isolates collected during 2004 were slightly curved with brown to olivaceous brown color. During 2005, the measurement of the conidia varied from 41.6-66.6 µm (mean range) to 11.6-25 µm having 2-10 numbers of septa (Table 2). There were few isolates having conidia of relatively long and broad with dark brown color, slender and slightly curved, while in most of the isolates conidia were uniformly straight and cylindrical, light brown to brown in color (Fig. 2).

Pathogenicity: The pathogenicity of all the isolates collected during 2004 and 2005 from different agro-ecological zones confirms the Koch's postulate by using cv. Wafaq-2001 through cotton swab method technique (Fig. 3a, 3b and 3c), however their reaction to the pathogen varied in terms of aggressiveness.



Error! Fig. 1a. Black colored colony of *Bipolaris sorokiniana*.

Fig. 1b. Grayish black colored colony of *Bipolaris sorokiniana*.

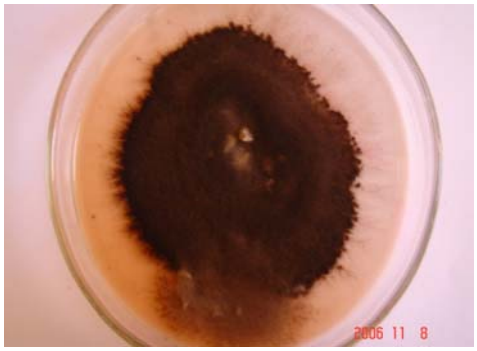


Fig.1c. Brown colored colony of *Bipolaris sorokiniana*.

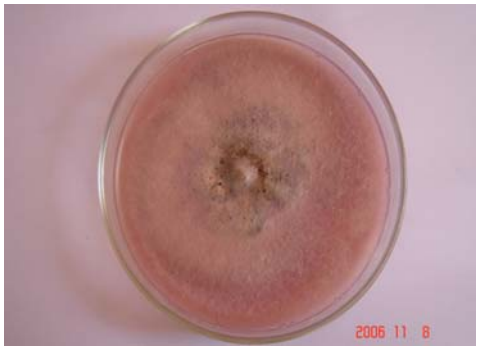


Fig. 1d. Albino colored colony of *Bipolaris sorokiniana*.

Table 2. Dimension of conidia of *Bipolaris sorokiniana* isolated from different agro ecological zones during 2004 and 2005.

Zone	Location	Year 2004				Year 2005			
		No. of isolates	Length of conidia (µ)	Width of conidia (µ)	No. of septa	No. of isolates	Length of conidia (µ)	Width of conidia (µ)	No. of septa
5	Punjab	2	35-65	15-18.3	2-6	10	46.6-73.3	18.3-21.6	2-10
6	Punjab	6	40-63.3	15-25	2-7	10	41.6-66.6	11.6-21.6	2-9
7	Punjab	10	41.6-65	15-25	2-7	7	36.6-60	11.6-18.3	2-9
9	NWFP	9	40-65	15-20	2-13	15	33.3-58.3	13.3-21.6	2-9
10	NWFP	6	48.3-56.6	15-18.3	2-8	3	58.3-65	18.3-25	2-8
11	Northern areas	4	40-56.6	13.3-18.3	2-8	5	35-60	11.6-18.3	2-7

Mean range

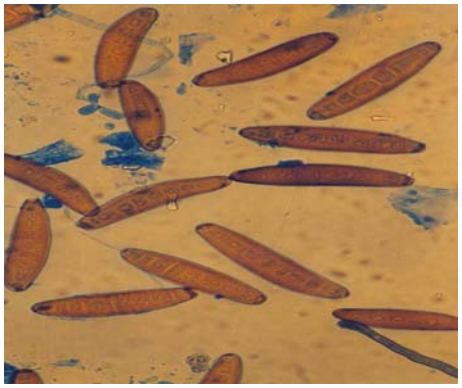


Fig. 2. Conidia of *Bipolaris sorokiniana*.

Fig. 3a. Pathogenicity test by test tube moist cotton swab method.

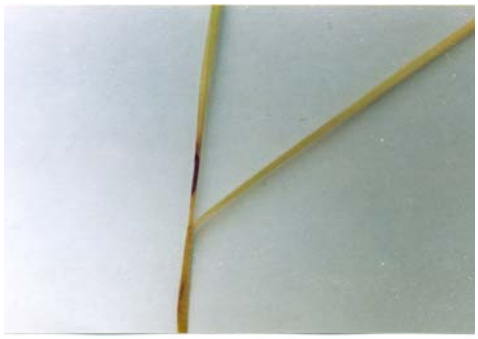


Fig. 3b. Symptom on wheat leaf during pathogenicity.

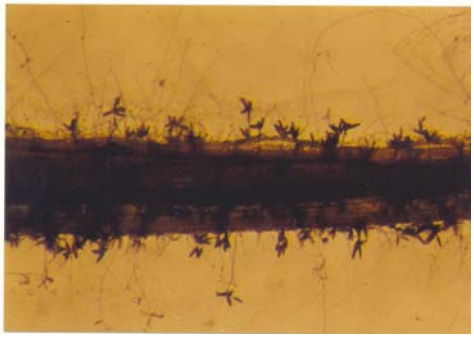


Fig. 3c. Conidia of *Bipolaris sorokiniana* on surface of wheat leaf.

Discussion

The colony/culture colour of both the years (2004 and 2005) exhibited four distinct colors. Among these the black cultures sporulate profusely and had suppressed type of growth. The others were showing grayish to brownish color and few were of albino (whitish) type having less sporulation. Maraite *et al.*, (1997) had also same observation while studying the 27 isolates of *Bipolaris sorokiniana* and found different colours of the colonies on minimal medium varied from white to light pink and dark green. The dark coloured colony showed a strong correlation with aggressiveness of the pathogen as Chand *et al*., (2003) studied the variability in natural populations of the spot blotch pathogen (*B. sorokiniana*) and classified the isolates into 5 groups on the basis of colony morphology, he found that the majority (44.63%) of the isolates of black suppressed type in the natural population were of most aggressive and was identified as the epidemic population as compared to the lowest frequency of the isolates (4.96%) of white coloured having very few conidia. In majority of the zones during second year study, the black coloured cultures were found more as compared to previous year may be due to continuous practicing of same susceptible variety and vigorous establishment of this seed and soil borne pathogen.

A very little difference was observed in dimensions of both the year's collection However the dimension of the conidia of some of the isolates during (2004) having more width and length with less number of septa as compare to the results of 2005. Luttrell (1955) found different measurements of conidia in different isolates of *B. sorokiniana*. The conidia of isolates of 2004 were slightly curved with brown to olivaceous brown while in 2005 collection the conidia were dark brown, slender and gently curved, few were straight and light brown to brown. Sivanesan & Holliday (1981) also reported more or less of same type of conidia having straight to curved, 3-12 septa with olive brown colour.

The pathogenic nature of predominant isolates of *Bipolaris sorokiniana* which were collected from different agro ecological zones was confirmed on cv. Wafaq-2001. This pathogen was also found as the major pathogen of spot blotch in various agro climatic regions of India (Mahto *et al*., 2002). Similar studies were of Singh *et al*., (1998) who found *B. sorokiniana* as one of the pathogenic fungus among the number of the fungi isolated from blighted wheat leaves.

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