

CHARACTERIZATION AND PATHOGENICITY OF *BIPOLOARIS SOROKINIANA* CAUSED SPOT BLOTCH OF WHEAT IN PAKISTAN

MUBASHAR RAZA*¹, MUZAMMIL HUSSAIN¹, M. USMAN GHAZANFER¹, M. IMRAN HAMID¹, SHAHZAD ASAD² AND HASNAIN NANGYAL³

¹Department of Plant Pathology, University College of Agriculture, University of Sargodha, Pakistan.

²Crop Diseases Research Program, National Agricultural Research Centre, Islamabad, Pakistan.

³Department of Botany Hazara University Mansehra, Pakistan

Corresponding author e-mail: mubasharraza73@yahoo.com

Abstract

Bipolaris sorokiniana is a hemibiotroph pathogen (teleomorph: *Cochliobolus sativus*) causes spot blotch of wheat. It is considered as severe pathogen of foliar disease in warm and humid areas and also causes significant losses in South Asian Countries. The pathogen collected from foliar samples of wheat field for identification of fungus associated with symptoms on foliage that characterized on the basis of colony color, texture and conidial morphology. Color and growth of colony was grayish black and suppressed type respectively. Conidiophores were unbranched, septate and conidia brown to olivaceous brown in color. Complete diagnosis is done by performing Koch's postulates. Characteristic symptoms expressed by plant were brown to dark brown spots on leaves, also on roots and there was no chlorotic margin at initial stage of spot formation. After performing Koch's postulates it was confirmed that *Bipolaris sorokiniana* was causal agent spot blotch or *Helminthosporium* Leaf Blight of wheat.

Introduction

Bipolaris sorokiniana (Sacc.) Shoemaker (Sivanesan, 1990) is a soil and seed borne pathogen of wheat, barley and other small cereal grains and grasses that causes head blight, foliar blight/ spot blotch, seedling blight, common root rot, black point diseases and yield losses (Iram & Iftikhar, 2004; Wiese, 1998 ; Aggarwal *et al.*, 2000, Hussain *et al.*, 2013). It is considered most important pathogen of wheat in warmer areas of world (Dubin and ; Ginkel, 1991). In southern province of Sindh, *Helminthosporium* leaf blight has been reported where winter temperature is warm (Hafiz, 1986; Bhatti & Ilyas, 1986). *B. sorokiniana* survive in moderate to warm temperatures (18°C to 32°C) and its infection is more rapid and severe at 28°C than at lower temperatures (Nema and Joshi, 1973 and Singh *et al.*, 1998). Infected plant with *B. sorokiniana* shows greatest yield losses before the emergence of head (Acharya *et al.*, 2011). Yield losses due to destructive pathogen have been reported 18-22 % and 23.8% in India and Nepal respectively (Singh *et al.*, 1997 ; Shrestha *et al.*, 1997). Many species has been reported as the host of *B. sorokiniana* world wide other than wheat and barley. Some of them are identified in Pakistan named as *Avena sativa* (Oat), *Hordeum vulgare* (Barley), *Brassica campestris* (Tulip), *Glycine max* (Soybean), *Lens culinaris* (Lentil), *Vigna radiate* (Mung bean), *Sesamum indicum* (Sesame), *Vigna mungo* (Urad), *Sorghum bicolor* (Sorghum), *Zea mays* (Maize) and *Pennisetum amaranum* (Pearl Millet) (Iftikhar *et al.*, 2009). In Pakistan, *B. sorokiniana* reported to be found to be one of the principal fungus involved in the seedling blight and root rot diseases of wheat (Hafiz, 1986; Kishwar *et al.*, 1992) and also predominant pathogen of foliar spot of wheat Wafaq-2001, Inqliab-91 and Bhakkar-2001 are more susceptible varieties of wheat (Asad *et al.*, 2007).

Bipolaris sorokiniana have thick-walled, elliptical, fusoid, straight or curved conidia with bipolar germination and 4-8 septa. The colony of fungus has interwoven hyphae characteristic by white or light grey color depending on isolates and differentiated on basis of morphological characters of conidiospores and conidiophores (Kumar *et al.*, 2002). It infect spikelets, crown portion and roots that produces shriveled grain, dark brown blotches on leaf blade and sheat or plant dry out without producing seed (Asad *et al.*, 2009). The theme of study was to isolate parasitic mycoflora from wheat for purpose of pathogenicity by applying Koch's postulates and identify it by studying the morphological characteristics of pathogen.

Materials and Methods

Phenotypic studies: Survey of wheat fields of CDRP at NARC was done to observe the symptoms of spot blotch. Wheat fields exhibited the symptoms of spot blotch along with Rust. Spotted leaf samples of wheat varieties such as Wafaq-2001, Inqliab-91 and Bhakkar-2001 were collected by using diagonal transects method and labeled.

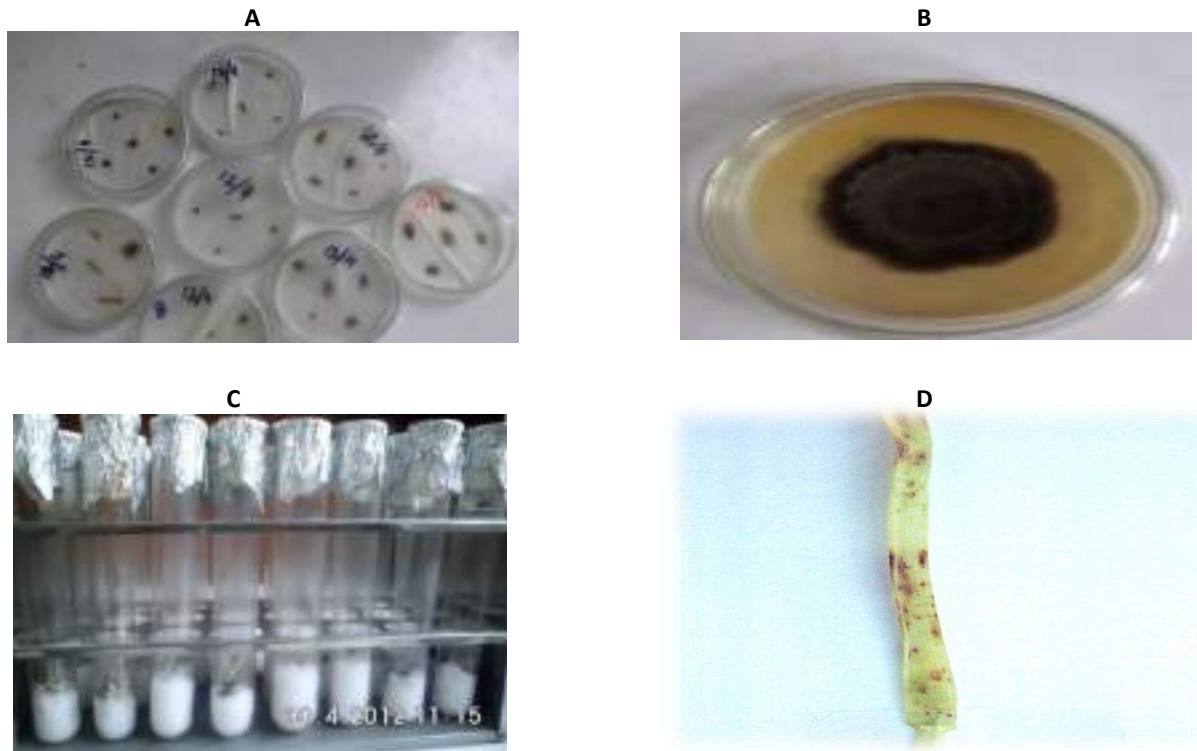


Fig. 1. Pathogenicity test by using Koch's postulates (A) Isolation of fungus on blotter paper (B) Colony morphology of *Bipolaris sorokiniana* on PDA for 5 days at 28°C (C) Inoculation of *Bipolaris sorokiniana* on wheat plants in test tubes to perform Koch's postulates (D) Symptoms on Bhakkar-2001 variety of wheat that confirms pathogenicity.

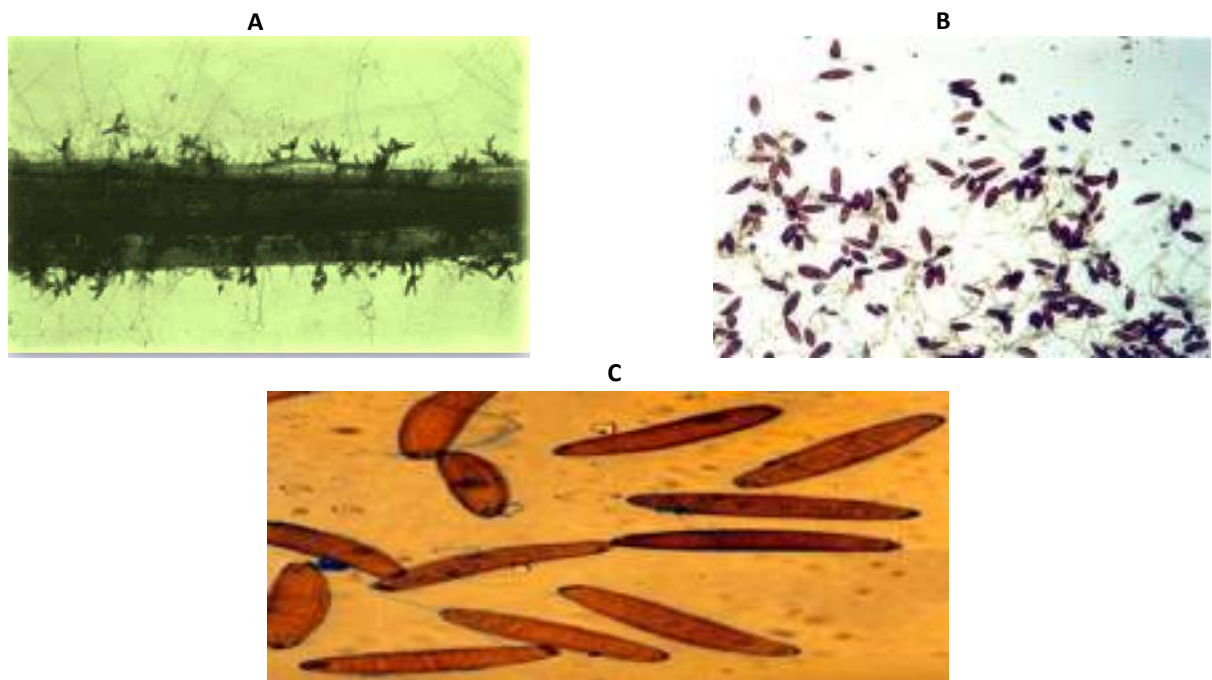


Fig. 2. Morphological characteristics of *Bipolaris sorokiniana* under scanning microscope (A) Conidia of *Bipolaris sorokiniana* on surface of wheat leaf (B) Brown to olivaceous brown color Conidia *Bipolaris sorokiniana* (C) Conidia of *Bipolaris sorokiniana*

Disinfection and rinsing: Firstly, samples washed with running water in order to remove dust particles and adhering contaminants. Foliar samples showing diseased spots were cut into small pieces (approx 4-5 pieces) with some healthy portion. 1% chlorox was used for surface disinfection and diseased sample were rinsed twice or thrice instantly with distilled water (Asad *et al.*, 2009).

Plating and observations: Samples were dried and placed on double layer of blotter paper in petri plates (Figure 1, A). Petri plates were wrapped with paraffin and incubate in light at 20-22°C for light period. After 24 hours petri plates were wrapped in double layer of newspaper and incubate for dark period at 18°C for 24 hrs (Asad *et al.*, 2009). Petri plates were visually observed under the stereoscope after continuous light and dark period for seven days. It observed that blackish growth was visible on surface of wheat leafs and spores of specific shape look like *Bipolaris sorokiniana*.

Use of single spore culture technique: Single spore of *Bipolaris sorokiniana* was transferred to potato dextrose agar medium under the stereoscope with the help of inoculating needle for the purification of fungus (Usmani & Ghaffar, 1982). Slides were prepared and observed under light microscope at 40x and 100x magnifications and spore shape and size was measured (Sivanesan & Holliday, 1981). Fungal culture color, growth pattern and characteristics were noted when it was grown on PDA and conduct pathogenicity.

Pathogenicity test:The pathogenic nature of *Bipolaris sorokiana* was tested on susceptible variety Bhakkar-2001 by test tube method (Iftikhar *et al.*, 2008). The 1/4th portion of test tubes (20cm x 3cm) filled by cotton and 20 ml sterilized distilled water was added. Lid of tubes were covered by aluminum foil and then autoclaved. Surface disinfected wheat seeds were placed on the moist cotton swab in the test tube at the rate of 3-4 seeds per tube. Tubes were again sealed with aluminium foil and arrange in steel rack after inoculation (Figure 1: C). Pathogen was re-isolated after incubation at 25 °C from spots of leaves after few days when seedlings appears and Koch's postulates was confirmed by comparing with mother culture (Asad *et al.*, 2009).

Results and Discussion

The pathogen isolated from foliar samples of Wafaq-2001, Inqliab-91 and Bhakkar-2001 varieties was *Bipolaris sarokiana*. Morphological characterization of *B. sarokiana* was studied by visual observation of colony color, spores shape, hyphal structure, conidia color and size. The confirmation of pathogen with relation to spot blotch disease were carried out by applying Koch's postulate.

Morphological characterization: *B. sorokiniana* isolated from samples of wheat variety were first observed through visual observation by observing colony growth and color. The colony was mature within 4-6 days and colony was grayish black. The colony had suppressed type of growth. Conidial observation of *Bipolaris sarokiana* was carried out under microscope. Conidiophores were unbranched, brown to dark brown, erect, single or clustered, septate and conidia were brown to olivaceous brown color, straight or slightly curved 50-70µ long 15-20µ wide and variation in septation from 3-7 (Figure 2: A,B,C).The same characteristic has been reported by Acharya *et al.* (2011). The colony color of *Bipolaris sarokiana* was of grayish brown color. The culture produces abundant amount of conidia and had obscured growth. It is also reported same observation while studying on characterization of *Bipolaris sarokiana*.

Pathogenicity: The pathogenicity which was tested on Bhakkar-2001 through cotton swab method technique by applying Koch's postulate confirmed by producing different symptoms (Figure 1: C). The symptoms were 1-2 mm long, small and dark brown spots on leaves blade and leaf sheath. There is no sign of chlorotic margin at initial stage but later lesion extend very rapidly and ultimately reaches to several centimeters. Roots have brown to black discoloration and plant has stunted growth. These symptoms also reported by (Kumar *et al.*, 2002). It was confirmed through pathogenicity test that *Bipolaris sorokiniana* cause very dire disease foliar blight/spot blotch of wheat and was able to reduce yield losses upto 16-23% (Dubin and Ginkel, 1991; Saari, 1998). Acharya *et al.* (2011) had reported same spot blotch.

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