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RESEARCH ARTICLE

Bipolaris sorokiniana of barley: infection behaviour in different members of Poaceae

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ABSTRACT: Host range and infection behaviour of *Bipolaris sorokiniana* of barley was studied in wheat, barley, rice, phalaris, maize, pearlmillet and sugarcane. *B. sorokiniana* of barley varied in percent germination, germination behaviour, germ tube length and number of appressoria formation in different hosts. Growth of pathogen was arrested at appressoria formation stage in sugarcane and it could not infect the host. Further accumulation of less cell wall bound phenolics (4-hydroxybenzoic acid, vanillic acid, 4-hydroxybenzaldehyde, vanillin and 4-coumaric acid) and phenylalanine ammonia lyase (PAL) in barley and wheat indicated the role of these compounds in conferring resistance against *B. sorokiniana* of barley.

Key words: Appressoria, barley, *Bipolaris sorokiniana*, host range, phenolic compounds

Bipolaris sorokiniana (Sacc.) Shoemaker (syn. *Helminthosporium sativum* teleomorph: *Cochliobolus sativus*), a hemibiotrophic phytopathogenic fungus is a well-known cause of spot blotch disease in barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.). *B. sorokiniana* usually induces symptoms on the leaf, sheath and stem. Yield losses due to spot blotch vary from 16 to 33% in barley (Clark, 1979).

B. sorokiniana is widely distributed in the areas where cereals are grown. *B. sorokiniana* forms a continuous genetic pool of isolates varying in virulence and aggressiveness to various cereals and grasses (Duveiller and Altamirano, 2000). The infection process on the leaves usually occurs through natural wounding, stomata or with the use of an appresorium-like structure through the cell wall (Yadav, 1981). The presence of other hosts plays an important role in disease epidemic. The primary inoculum of *B. sorokiniana* comes from several sources such as weed hosts, soil, crop debris which enhances the disease level. The grass weeds as collateral hosts of *B. sorokiniana* in rice–wheat system are considered as a possible reason for perceived increase in *Helminthosporium* leaf blight and cause major losses to the crop (Hobbs and Morris, 1996).

Phenolic compounds are formed in response to the ingress of the pathogens and their production is considered as part of active defence response (Nicholson, 1992). Numerous studies suggest that low molecular weight phenols, such as benzoic acids and the phenylpropanoids, are formed in the host at initial response of infection. Rapid accumulation of phenols at the infection site function to slow the growth of the pathogen and allow activation of phytoalexins or other stress related substances (Matern and Kneusel, 1988). However, these are not studied against the *Bipolaris sorokiniana* in different host systems.

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Present study was undertaken to find the role of weed and other hosts which are grown with barley and provide shelter to pathogen during the season. Role of phenolic compounds against spot blotch pathogen was also explored to find out the level of resistance.This would help in developing management strategies in future.

MATERIALS AND METHODS

Fungal isolates and culture conditions

B. sorokiniana infected leaves were collected from different germplasm lines of barley grown in the Agricultural Research Farm of Banaras Hindu University, Varanasi. Isolation and purification of isolates was done according to Kumar *et al.* (2007) and maintained at 25 ^{0}C by transfer on PDA slants and revived at monthly intervals.

Plant material and inoculation of host plants

Leaves were collected from the adult plants of wheat, barley, rice, phalaris, maize, pearlmillet and sugarcane grown in the Agricultural Research Farm of Banaras Hindu University, Varanasi. About 6 cm long leaf segments of different plants were placed on moist chamber and sprayed with spore suspension of isolate B29 (Bashyal *et al.,* 2009) @ 1×10^7 spores/ml and leaf segments were placed on 0.5% water agar amended with 40 mg benzimidazole /l in a Petri dish (>95% RH). Plates were incubated at 25^{0}C for 6 hrs, 12 hrs, 18hrs and 24 hrs for microscopic studies. Lesion size (mm^2) was determined by measuring length and width of lesion produced by the pathogen. Experiment was conducted in randomized block design.

Microscopic studies

After 6 hours interval inoculated leaves were placed on whatman filter paper dipped in clearing solution consisting

Table 1. Infection behaviour of *Bipolaris sorokiniana* of barley in different hosts

Host	Germination*%	Germination behaviour	Length of germtube (ìm)	No. of appressoria formed*
Barley	57.14 (SD ± 2.0)	bipolar	15.60 (SD ±4.5)	7.00(SD ± 2.0)
Pearlmillet	15.00 (SD ± 1.5)	unipolar	12.50 (SD ±2.8)	6.00 (SD ± 2.5)
Rice	33.33 (SD ± 2.0)	bipolar and unipolar	8.60 (SD ±2.8)	3.00 (SD ± 1.5)
Maize	25.00 (SD ± 1.5)	unipolar	14.20 (SD ±4.2)	7.00 (SD ± 2.0)
Sugarcane	4.34 (SD ± 2.2)	unipolar	7.20 (SD ±2.8)	0.00 (SD ± 0.0)
Wheat	54.54 (SD ± 2.4)	bipolar	9.50 (SD ±2.2)	5.00 (SD ± 2.0)
Phalaris	37.14 (SD ± 4.0)	unipolar	13.70 (SD ±3.8)	10.00 (SD ± 3.0)

*observations from 4.54 mm^2
SD = standard deviation

of acetic acid and alcohol (1:1). After 24 hrs leaves were transferred in fresh clearing solution. Process was repeated until the leaf pieces were devoid of chlorophyll. Germination behaviour of *B. sorokiniana* in terms of length of germ tube formed and formation of appressoria were observed under the light microscope at 10 × after stained in lactophenol and cotton blue.

Extraction and determination of phenolics

Method of Kofalvi and Nassuth (1995) was followed for the extraction cell wall bound phenolics. Fresh leaves (0.5 g) were extracted in 50% methanol for 90 min at 80ºC. The extract was centrifuged at 14000g for 15 minutes. The pellet was saponified with 2ml of 0.5N NaOH for 24h at room temperature to release the bound phenolics, neutralized with 0.5 ml 2N HCl and centrifuged at 14000g for 15minutes. The supernatant was taken for bound phenolic determination using the Folin-Ciocalteu's assay. One hundred microlitre of the methanol and NaOH extracts were diluted to 1cm^3 with water and mixed with 0.5 ml 2.0 N Folin-Ciocalteu's reagent and 2-5 ml of 20% Na_2CO_3. After 20 minutes at room temperature, absorbance of sample was measured at 725 nm. Phenolic concentration in the extracts was determined from standard curve prepared with Gallic acid.

Extraction and determination of phenylalanine ammonia-lyase (PAL)

One gram of inoculated leaf tissue was taken and crushed in 10 ml of borate buffer with the help of chilled pestle and mortar. The tissue pulp was then centrifuged at 12000 rpm at 4ºC for 10 minutes and the supernatant collected and used as enzyme extract. Enzyme extract of 0.2 ml (supernatant) from each treatment, was transferred in a separate tube containing 2.5 ml of borate buffer and 1ml of 0.1mM phenylalanine (pH 8.8) and incubated for 30 min at 32±2 ºC. Enzyme reaction was stopped by addition of 0.5 ml 1 M Tri chloro acetic acid. Observations were taken by measuring absorbance at 290 nm with the help of spectrophotometer.

RESULTS AND DISCUSSION

Significant differences were observed for the germ tube length in different plants except rice and sugarcane. Largest germ tube was produced in barley (15.60 µm) followed by maize (14.20 µm). Smallest germtube was produced on sugarcane (7.20 µm) (Table 1).

Conidia germination was highest in barley (57.14%) followed by wheat (54.54%). Lowest number of conidia germinated in sugarcane (4.34%). Bipolar germination was observed in barley and wheat, while, germination was mostly unipolar on pearlmillet, maize, sugarcane and phalaris but germination behaviour was both unipolar and bipolar on rice. Hosts varied significantly for the number of appressoria developed. Maximum number of appressoria were produced in phalaris (10/4.54 mm^2). Appresoria were absent in sugarcane (Table 1, Fig. 2). Size of lesion significantly varied in different host plants. Significant differences were also observed for size of lesion produced by different isolates. Largest lesion was produced in barley (0.81 mm^2) which was followed by wheat (0.75 mm^2). Isolate B29 was observed as a most aggressive isolate (Fig.1).

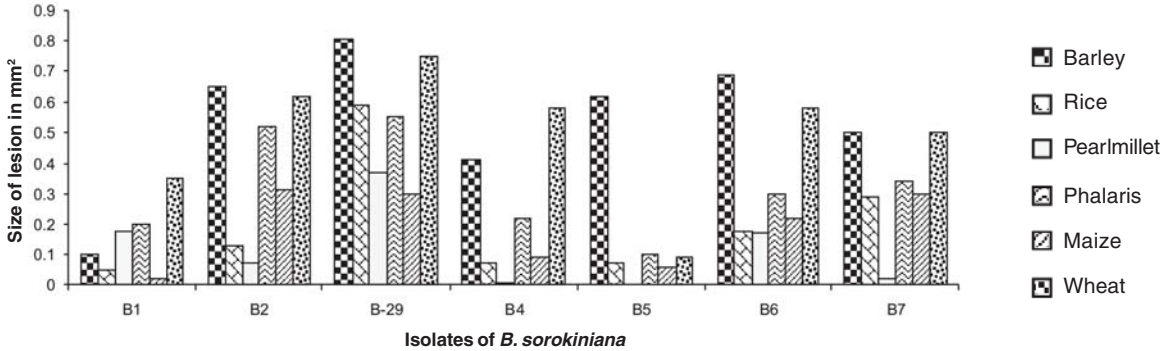

Fig. 1. Lesion size on the different hosts caused by *Bipolaris sorokiniana* of barley

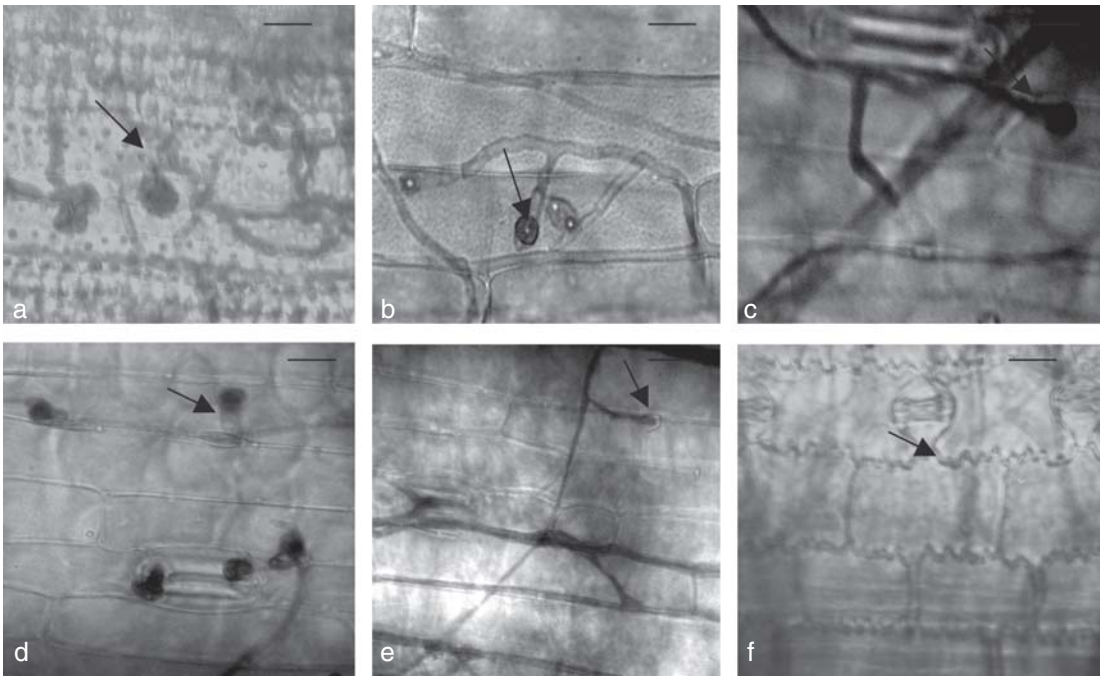

Fig. 2. Appressorium produced by *Bipolaris sorokiniana* of barley in different hosts a. rice bar = 20µm, b.wheat, bar = 20 µm, c. pearlmillet, bar = 20 µm, d. phalaris, bar = 20 µm, e. barley, bar = 12 µm and f. maize, bar = 20 µm

Growth of the germ tubes is known to be influenced by variety of stimuli (Wynn and Staples 1981). Chemical stimuli implicated the directed growth of the germ tubes include H^+ and H_2O gradients as well as various plant exudates and leachates (Hochs and Stapples, 1991). Appressorium formation by the germ tube is also influenced by chemical signals such as K^+, Ca^{++}, sucrose, phenolics and plant extracts (Hoch and Staples, 1991). Podila *et al.* (1993) reported that plant surface lipids also contain chemicals that inhibit germination and appressorium formation, in addition to those which stimulate appressorium formation, and concluded that balance between the two types of components determine whether to promote or inhibit germination and appressorium formation by fungal conidia. Such type of mechanisms could inhibit the appressorium formation in sugarcane and could have induced the formation of more number of appressoria in other hosts.

Cell wall bound phenolics showed increasing tendency upto 72 hrs of inoculation of pathogen and thereafter it decreased. Cell wall bound phenolics were maximum for maize (0.70 µg/g of leaves) followed by sugarcane (0.60 µg/g of leaves) after 72hrs of inoculation (Fig.3). Phenolic compounds were lowest for barley (0.01 µg/g of leaves). Phenylalanine ammonia-lyase (PAL) activity was maximum after 48 hrs of inoculation of the *B. sorokiniana* of barley then it decreased upto 72hrs and remained constant thereafter. PAL activity was maximum in maize followed by sugarcane. It was lowest in barley (Fig.3). PAL activity and phenolic compounds were maximum in maize. Accumulation of phenolics as an initial response to infection may reflect a general increase in host metabolism as well as an accumulation of relatively non toxic secondary metabolites which could ultimately serve as precursors for compounds essential for the expression of resistance

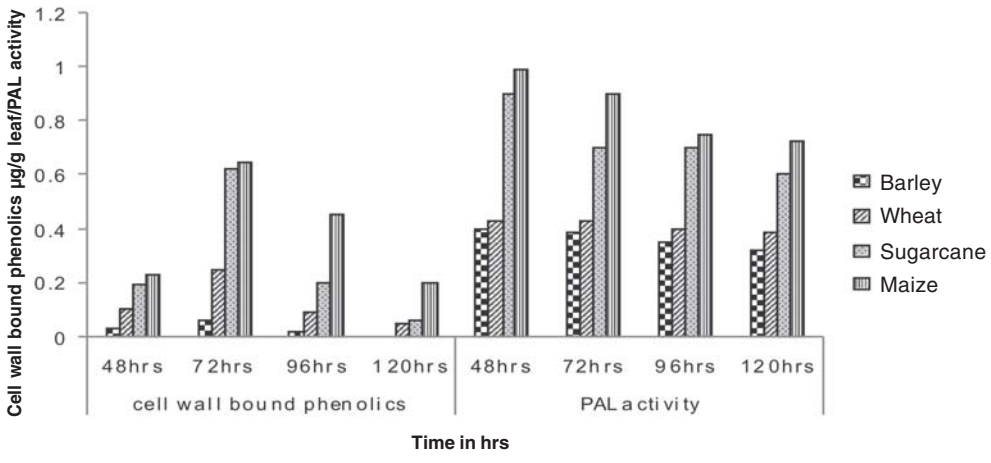

Fig. 3. Cell wall bound phenolics and PAL activity at different time interval in different host

(Perumalla and Heath, 1991). Plants need phenolic compounds for pigmentation,growth, reproduction, resistance to pathogens (Lattanzio *et al.,* 2006). A pattern of rapid accumulation followed by sharp decrease in the amount of phenolics and PAL activity in the tissue suggested that they may serve as a pool required for diversion to other products (Rhodes and Wooltorton, 1978).

Present study indicated that *Bipolaris sorokiniana* of barley could be pathogenic to wheat, rice, maize, bajra, phalaris under *in vitro*. Isolates differed for the lesion size which shows that significant variability existed in the population and virulent isolates were continuously emerging. An intensive survey is needed for study on the survival of *B. sorokinina* on these hosts under natural conditions.

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