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RESEARCH ARTICLE

Variability characterization of *Bipolaris sorokiniana* populations causing black point disease in wheat

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ABSTRACT: Ten monoconidial isolates of *Bipolaris sorokiniana* from wheat seed of distant localities were tested for their variability. In pathogenic capabilities, the isolates differed strikingly irrespective of the host. The isolate from Sabour, Bihar was found to be most virulent followed by Kumarganj, UP; Hissar, Haryana and Ludhiana, Punjab. Colony characters of Kumarganj and Sabour isolates showed fastest growth, wavy with raised central zone, grayish black colour and irregular margin whereas Kalyani and Durgapura were slow growing isolates. Sporulation varied from fair to excellent with different isolates, but no definite relation was established. In conidiophore and conidial characters, the average length of conidiophore of Kumarganj isolate was maximum (143.32µ) as against the Dharwad, Karnataka isolate which had the minimum (132.52µ) average length. The average length of conidia of Kumarganj isolate was maximum (70.99µ) against the minimum (55.33µ) in Kalyani isolate. Maximum average width of conidia and conidiophore was associated with Sabour and Srinagar isolates, respectively. Regarding conidial germination percentage and its pattern, Sabour isolate resulted in the maximum (99%) germination with bipolar germination pattern followed by Kumarganj, Rewa, Ludhiana, Srinagar, Dharwad, Hissar, Mahabaleshwar, Kalyani and Durgapura isolates.

Key words: *Bipolaris sorokiniana*, black point, variability, Wheat

Block point in wheat is an important seed-borne disease in all wheat growing regions of the world including India (Hasabnis *et al*., 2006; Solanki *et al*., 2006). The disease is mainly caused by *Bipolaris sorokiniana* (Sacc.) Shoeam. (Syn. *Helminthosporium sativum* teleomorph (*Cochliobolus Sativus*) and *Alternaria* spp. Athough many other organisms have been isolated from the affected grain (Zishan *et al*., 2005), in case of severe infection, particularly when *B. Sarokiniana* is involved, the grain may be completely discoloured and shrivelled. Impaired seed germination, reduced germination rate, number of embryonic roots and coleoptile length, delayed seedling emergence, significant reduction in seedling vigour and grain/seed yield to black point infection have been reported (Malaker and Mian, 2002; Ozer, 2005). The disease is also reported to affect luster and plumpness of grain and reduce its market value (Lehmensiek *et al*., 2004; Solanki *et al*., 2006). Severity of black point disease is largely dependent on environmental conditions *viz*., low temperature, frequent rainfall during kernel development/grain filling (Wang *et al*., 2002). The pre dominant black point fungus, *B. Sorokiniana* is seed-borne, seed transmitted and even more than 80% seed to plant transmission of this pathogen have been established in wheat (Bazlur Rashid, 1998; Reis *et al*., 1998). Seed abnormality due to influence of seed-borne fungi is very common and often accounts for a large percentage of crop losses (Varshney, 1990). However, a first step towards the attainment of optimal crop yield is the use of high quality seeds (Venter, 2000). Seed with black point disease is more likely to have seedling blight, head blight, leaf blotch, leaf spot, leaf blight, foot rot, discoloured grain, black pointed grain and also may result in sterile spikes if the infection is severe. In most of the countries where cereals are commonly grown, black point can result in reduced seed as well as grain quality and value (Wang *et al*., 2003). In the past decade, there has been an increase in the incidence of foliar blight resulting a serious problem to the wheat cultivation all over the world (Duveiller and Gilchrist, 1994). Pathogenic variability is of crucial significance in disease management where host resistance in the major component. Resistant genotypes are known to show significant reduction in disease as compared to the susceptible cultivars (Siddique *et al*., 2002). The objective of this study was to find out, the extent of variability in the natural population of the black point pathogen *B. Sorokiniana*.

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MATERIALS AND METHODS

Variability amongst the *Bipolaris sorokiniana* isolates cultured from the seed samples of distant localities viz. HS 1 (Kalyani, W.B.), HS 2 (Hissar Haryana) , HS 3 (Kumarganj, U.P.), HS 4 (Ludhiana, Punjab), HS 5 (Mahabaleshwar, M.S.), HS 6 (Dharwad, Karnataka), HS 7 (Sabour, Bihar), HS 8 (Durgapura, Rajasthan), HS 9 (Srinagar, J.&K.), HS 10 (Rewa, M.P.) were studied in the Directorate of Seed Research, Kaithauli, Kushmaur, Mau during 2008-09, 2009-10 and 2010-11 crop seasons. Pathogenicity test of all *B. sorokiniana* isolates was conducted on variety-Sonalika and Koch's postulates were proved. Pathogenic variability tests of different isolates were conducted on the differential lines comprising of fifteen wheat genotypes viz. PATO(R) TZ PPSN 64 NAR, PUSA T 3336, HDR 180, VL 738, ISWYT, PBW 343, Bob white, RL 10-12, Raj 6516, T 3105, K 9408, C 306, UP 2338, WH 542 and Agra Local with three replication under glass house conditions. The healthy seeds of each genotype were surface sterilized with sodium hypochloride solution (2%) for two minutes and thereafter washed thrice with sterilized distilled water. Five seeds were

sown in each sterilized earthen pot in last week of November. Recommended agronomic practices were adopted for raising healthy plants. Caryopsis (green kernel) of wheat differentials were taken in the soft dough stage of development for the screening against black point infection caused by *Bipolaris sorokiniana*. The caryopsis/berry/green kernel is the appropriate stage because if kernels inoculated prior to the dough stage incorrectly may be rated susceptible, and if inoculated after the green colour has disappeared may be rated incorrectly as resistant. Standard blotter paper methods were used in which kernels were placed in crease up position. Spore suspension having concentration of 10^4 spores/ml was prepared with the addition of eight drops of 25% lactic acid @ of one liter to check the bacterial growth. One drop of spore suspension in a 0.1% aqueous solution of tween 20 was transferred aseptically on the crease of each kernel. Kernel plated petri dishes were enclosed by aluminium foil and incubated for five days at 23°C temperature. After incubation, kernels were evaluated for healthy and infected seeds. Healthy seeds were those which were not having any visible symptoms of discolouration or damage. Seeds having slightly discoloured embryo tip, severely discoloured embryo tip, slight discolouration on crease, moderate discolouration on crease and seeds discoloured and damaged along the entire crease were considered as infected seed (Statler *et al*., 1975; Singh *et al*., 2003; Enikuomehin, 2005). All the morphological studies were carried out on potato dextrose agar medium under completely randomized design. To study the important colony characters (colour, size, shape, margin), conidiophore characters (colour, size, number of septa, septa apart) and conidial characters (colour, size, number of septa, shape) of the test isolates, the sterilized petridishes containing potato dextrose agar medium were inoculated with the test isolates. All the observations were made up to 8 days of incubation (28 ± 1°C) and final observations were recorded on 9th day on randomly selected cultures of test isolates. For conducting studies on sporulation, potato dextrose broth medium was used. A randomly selected flask from each isolate, containing the fungal growth was thoroughly shaken. One ml of fungal suspension was taken and diluted. The suspension was divided into five equal drops on glass slides for counting the spores under microscope. The number of spores was counted in each drop. Finally, the average number of spores was calculated. On the basis of spore counts under low power (10 x) of microscope the degree of sporulation was graded in five categories viz. 1 = no spore (nil: -), 2 = 1-5 spores (poor: +), 3 = 6-10 spores (fair: ++), 4 = 11-15 spores (good: +++) and 5 = ≥ 16 spores (excellent: ++++). Conidial germination pattern was studied by hanging drop method as described by Sharma and Mohanan (1990), using 12 day old culture on PDA. One drop of dextrose solution (5%) was placed on the slide and dried. One drop of spore suspension (counted number of spores) was placed on that place of slide where dextrose solution was dried. These slides were kept in moist chamber and incubated at 30 ± 1°C temp for 48 hours. The spores with germ tubes longer than the spore width were considered as germinated. Percentages of germination as well as polar germination of conidia were observed after 48 hours by taking the counts of 200 conidia from three replicated slides.

RESULTS AND DISCUSSION

Pathogenic variability

The observation recorded revealed that each variety got infection, but the degree of infection varied with the test isolates (Table 1). On the basis of overall average per cent seed infection on all the fifteen differential varieties, isolate HS 3 (54.24 %), and HS 7 (54.72 %) were found to be more or less similar, and most virulent. Some isolates were moderately virulent *viz*., HS 2, HS 4, HS 10, HS 5, HS 9 and HS 6. Isolate HS 1 with average score 27.24 % was least virulent and more or less similar to the isolate HS 8 (29.15 %). These findings are in agreement with the finding of Clark (1962), who studied pathogenicity of four isolates of *Bipolaris sorokiniana* on wheat, barley and oat for leaf spot and suggested that if more isolates were screened, several pathogenic groups could be recognized. Similar results have also been discussed by Aggarwal *et al*. (2009) and Chand *et al*. (2003).

Colony characters

The cultural characters exhibited by different test isolates of *B. sorokiniana* were recorded by visual observations (Table 2). The isolate HS 7 was recorded as fastest growing attaining the colony diameter of 87.86 mm with wavy raised central zone mycelium, greyish black colour and irregular margin whereas HS 1 was comparatively slow growing isolate with the colony diameter of 74.64 mm, wavy with scanty aerial mycelium, olivaceous brown colour and irregular margin and similar to HS 8 Isolate. Isolates HS 7 and HS 3 exhibited the same characters that is wavy with raised central zone, grayish black colour and irregular margin. Isolates HS 2, HS 4, HS 5, HS 6, HS 9 and HS 10 recorded as circular with moderate aerial mycelium, grayish olive-green or bottle-green or grayish brown colour with regular margin. These findings were in accordance with the observation of Aggarwal *et al*. (2009) and Bashyal *et al*. (2010).

Conidiophore and conidial characters

The microscopic studies revealed that the average length of conidiophore and conidia of HS 3 was maximum measuring 143.32µm and 70.99µm, respectively as against the isolate HS 6 (132.52µm conidiophore) and HS 1 (55.33µm conidia), respectively which had the least measurement (Table 3). The other isolates were intermediate between the two. Maximum average width of conidiophore and conidia was associated with HS 7 which was 7.63µ and with HS 9 which was 19.44µ, respectively followed by HS 2 (7.45µ) and HS 7 (19.33µ). Isolate HS 1 and HS 8 had maximum variation in number of septa associated with conidiophore which was 2-11 and 3-12, respectively followed by HS 2 (4-11) which was almost similar with HS 5 (3-10) and HS 9 (2-9), HS 4 and HS 6 (5-11) was similar with HS 10 (4-10) and HS 3 (3-8) was similar with HS 7 (4-9). However, regarding conidial variation, isolates HS 3 and HS 7 had maximum variation in number of septa which was 2-9 followed by HS 2 which was similar with HS 9 (4-9 and 3-8, respectively), HS 4 similar with HS 8 and HS 10 (2-6, 2-6 and 4-8, respectively), HS 1 similar with HS 5 (3-6 and 4-7, respectively) and HS 6 (4-6). Dark greyish

Table 1. Variation in pathogenicity of *Bipolaris sorokiniana* isolates on green kernel of wheat differentials for black point disease

Genotype	*Average black point percentage on green kernel									
	HS 1	HS 2	HS 3	HS 4	HS 5	HS 6	HS 7	HS 8	HS 9	HS 10
PATO(R) TZ PPSN 64 NAR	18.33	10	53.33	8.33	6.66	6.66	61.66	25	16.66	3.33
PUSA T 3336	8.33	33.33	68.33	21.66	58.33	33.33	68.33	11.66	43.33	33.33
HDR 180	11.66	23.33	61.66	13.33	18.33	11.66	71.66	13.33	33.33	18.33
VL 738	43.33	6.66	53.33	16.66	4.33	8.33	43.33	18.33	8.33	3.66
ISWYT	3.66	43.33	23.33	50.00	43.33	31.66	36.66	6.86	33.33	41.66
PBW 343	16.66	33.33	15.00	68.33	21.66	56.66	13.33	16.66	41.66	61.66
Bob White	6.66	44.66	76.66	51.66	58.33	41.66	68.33	11.66	10.00	48.33
RL 10-12	13.33	31.66	78.33	33.33	33.33	23.33	78.33	13.33	36.66	38.33
Raj 6516	21.66	66.66	16.66	68.33	53.33	58.33	23.33	25.00	43.33	68.33
T 3105	68.33	43.33	36.66	53.33	23.33	48.33	26.66	61.66	23.33	56.66
K 9408	8.33	68.33	43.33	66.66	80.00	56.66	61.66	11.66	68.33	78.33
C 306	36.66	80.00	80.00	53.33	76.66	58.33	80.00	68.38	66.66	51.66
UP 2338	43.33	68.33	61.66	58.33	58.33	68.33	71.66	46.66	71.66	58.33
WH 542	33.33	51.66	80.00	51.66	75.00	56.66	78.33	36.66	76.66	53.33
Agra Local	78.33	43.33	63.33	33.33	55.00	43.33	36.66	78.33	51.66	48.33
	Isolates	varieties								
SEm±	1.78	2.18								
CD $_{0.05}$	4.95	6.06								

*Average based on three replications

Table 2. Cultural Characters of different test isolates of *Bipolaris sorokiniana*

Isolate	Colony Characters			
	Size in mm	Shape	Colour	Margin
HS 1	74.64	Wavy with scanty aerial mycelium	Olivaceous brown	Irregular
HS 2	84.30	Circular with moderate aerial mycelium	Bottle green	Regular
HS 3	87.35	Wavy with a raised central zone	Greyish black	Irregular
HS 4	85.74	Circular with moderate aerial mycelium	Greyish brown	Regular
HS 5	85.45	Circular with moderate aerial mycelium	Greyish brown	Regular
HS 6	86.23	Circular with moderate aerial mycelium	Greyish olive green	Regular
HS 7	87.86	Wavy with raised central zone	Greyish black	Irregular
HS 8	75.28	Wavy with scanty aerial mycelium	Olivaceous brown	Irregular
HS 9	85.55	Circular with moderate aerial mycelium	Greyish olive green	Regular
HS 10	85.80	Circular with moderate aerial mycelium	Greyish olive green	Regular
Mean = 83.82				

olivaceous colour was recorded with isolate HS 1 and HS 8 of conidiophores and had solitary emergence. Olive to dark olivaceous colour of conidiophores was with HS 2 and HS 6 isolates, brown to dark brown with HS 3, dark olivaceous with HS 4, HS 5, HS 9 and HS 10 and dark brown with HS 7. The remaining *B. sorokiniana* isolates had fascicles to solitary emergence of conidiophore. Regarding colour of conidia, dark olivaceous colour was recorded with isolates HS 1, HS 4, HS 5, HS 7 & HS 10 whereas olive to dark olivaceous colour was found with isolate HS 2 and HS 6. The rest of isolates had light to dark brown coloured conidia. The isolates HS 1, HS 5 and HS 10 had elliptical conidia whereas isolates HS 2, HS 4, HS 6 and HS 9 had slightly curved conidia. HS 3 and HS 7 had ovate to pipe shaped conidia while HS 8, had bent shaped conidia. Study of conidiophores and conidial characters reveals that there were three categorizes. Some isolates were found on top position, others on the bottom while a few existed in between these two. Such variation of characters in conidiophore and conidia were also reported by Christensen and Grahan (1934) and Bashyal *et al*. (2010) in species of *Helminthosporium*.

Sporulation

Sporulation of different isolates varied from fair to excellent with different isolates, but no definite relation was established on the overall. Excellent sporulation was recorded with HS 3, HS 7 and HS 10 isolates. Good sporulation was observed

Table 3. Comparative measurement of conidiophore and conidia of different test isolates of *Bipolaris sorokiniana*

Isolate	Colour		Size				Number of Septa		Conidiophore	Shape of
	Conidiophore	Conidia	Length (µ)		Width (µ)		Conidio-	Conidia	Emergence	Conidia
			Conidiophore	Conidia	Conidiophore	Conidia	phore			
HS 1	Olive to dark olivaceous	Dark olivaceous	67.45-205.8 (136.63)	20.35-90.30 (55.33)	6.40-7.52 (6.96)	11.95-23.43 (17.69)	2-11	3-6	Solitary	Elliptical
HS 2	Olive to dark olivaceous	Olive to dark olivaceous	76.30-189.46 (132.88)	43.00-88.32 (65.66)	6.40-8.50 (7.45)	15.45-22.46 (18.96)	4-11	4-9	Fascicles to solitary	Slightly curved
HS 3	Brown to dark brown	Light to dark brown	94.44-192.20 (143.32)	56.35-85.64 (70.99)	6.36-8.52 (7.44)	16.13-21.83 (18.98)	3-8	2-9	Fascicles to solitary	Ovate to pipe
HS 4	Dark olivaceous	Dark olivaceous	81.81-193.95 (137.88)	43.00-87.32 (65.16)	1.36-6.40 (3.88)	14.64-20.46 (17.55)	5-11	3-7	Fascicles to solitary	Slightly curved
HS 5	Dark olivaceous	Dark olivaceous	84.50-196.73 (140.62)	41.73-91.50 (66.62)	4.38-6.50 (5.44)	13.73-20.46 (17.09)	3-10	4-7	Fascicles to solitary	Elliptical
HS 6	Olive to dark olivaceous	Olive to dark olivaceous	76.30-188.73 (132.52)	42.42-89.30 (65.86)	6.40-8.50 (7.45)	14.64-20.46 (17.55)	5-11	4-6	Fascicles to solitary	Slightly curved
HS 7	Dark brown	Dark brown	90.36-189.21 (139.79)	55.43-85.64 (70.54)	6.86-8.40 (7.63)	16.13-22.53 (19.33)	4-9	2-9	Fascicles to solitary	Ovate to pipe
HS 8	Dark grayish olivaceous	Brown to olivaceous	72.64-209.14 (140.89)	22.64-88.32 (55.48)	5.46-6.40 (5.93)	12.78-21.80 (17.29)	3-12	2-6	Solitary	Bent
HS 9	Dark olivaceous	Dark olivaceous	78.00-190.30 (134.15)	47.20-89.30 (68.25)	5.46-7.52 (6.49)	15.45-23.43 (19.44)	2-9	3-8	Fascicles to solitary	Slightly curved
HS 10	Dark olivaceous	Dark olivaceous	84.50-195.20 (139.85)	44.64-78.40 (61.52)	3.60-5.74 (4.67)	13.73-22.46 (18.09)	4-10	4-8	Fascicles to solitary	Elliptical

*Values in parentheses are average values of three replications

Table 4. Sporulation and conidial germination of *Bipolaris sorokiniana* isolates

Isolate	Sporulation	Conidial germination	
		Germination Pattern	Germination percentage
HS 1	+ + +	Bipolar, Unipolar	71.50 (57.73)
HS 2	+ + +	Bipolar	83.75 (66.23)
HS 3	+ + + +	Bipolar, Interseptal	98.45 (82.85)
HS 4	+ + +	Bipolar	89.50 (71.09)
HS 5	+ + +	Bipolar, Nominal unipolar	79.75 (63.26)
HS 6	+ + +	Bipolar, Nominal unipolar	85.45 (67.58)
HS 7	+ + + +	Bipolar	99.00 (84.26)
HS 8	+ +	Bipolar, Unipolar	68.35 (55.76)
HS 9	+ + +	Bipolar	89.50 (71.09)
HS 10	+ + + +	Bipolar, Nominal Unipolar & Interseptal	90.25 (71.81)

Mean = 85.55(69.16)

$CD_{0.05}$ = 5.79

Figures in parentheses are angular transformed values.

with isolates HS 1, HS 2, HS 4, HS 5, HS 6 and HS 9 while it was fair with HS 8 isolate (Table 4). The results reported is some what similar with the work done by Aggarwal *et al*. (2009) and Bashyal *et al*. (2010) who reported marked differences in sporulation of the pathogen *B. sorokiniana*.

The maximum conidial germination percentage (99%) was observed in HS 7 isolate followed by HS 3 (98.45%), HS 10 (90.25%), HS 4 (89.50%), HS 9 (89.50%), HS 6 (85.45%), HS 2 (83.75%), HS 5 (79.75%), HS 1 (75.50%) and HS 8 (68.35%). Bipolar germination pattern was found with HS 2, HS 4, HS 7, and HS 9 while both bipolar as well as unipolar patterns were associated with HS 1, HS 5, HS 6 and HS 8. Isolate HS 3 had bipolar and interseptal conidial germination pattern whereas bipolar, unipolar and interseptal pattern of germination was associated with HS 10 isolate (Table 4). Hundred per cent conidial germination has been reported in *H. sativum* (Bidari and Gobindu, 1975).

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