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Variability in pathological characters in *Gloeocercospora sorghi* isolates from sorghum

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ABSTRACT

Zonate leaf spot caused by *Gloeocercospora sorghi* Bain and Edgerton (1943) is one of the most destructive diseases of sorghum in India and Uttarakhand is considered as a hot spot for this disease. The present investigation was carried out to record the pathogenic variability of thirty isolates of *Gloeocercospora sorghi* on five different lines of sorghum. The *G. sorghi* isolates differed significantly from each other on the basis of pathological attributes *viz.*, latent period, aggressiveness and virulence index and thus, grouped into three virulence categories. The findings suggest that analysis of variance for latent, aggressiveness, per cent disease intensity (PDI) and virulence index showed that the variations in latent period and virulence disease reaction were attributed more to the isolates and aggressiveness to the host lines than to the isolate × host line interactions.

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INTRODUCTION

Zonate leaf spot incited by *Gloeocercospora sorghi* is an important disease of sorghum. It caused damage upto 85 per cent of photosynthetic area under humid and cloudy weather conditions (Agnihotri and Pandey, 1977). The estimated yield losses due to zonate disease in Asia, Africa and America range from 32 to 60 per cent (Sharma, 1980 and Frederiksen, 2000). In India, zonate leaf spot disease is severe in the states of Andhra Pradesh, Tamil Nadu and Uttar Pradesh (Ravindranath, 1980). The loss caused by zonate leaf spot disease varies from one part of region to another. The overall estimated losses due to zonate disease ranges between 1.2-16.4 per cent in India (Mishra and Siradhana, 1979). Apart from zonate, *Gloeocercospora* species also cause spot disease, blight and damping-off on infected plants (Nicholson and Epstein, 1991). It has been found to be a highly variable pathogen. In the *G. sorghi* system the identity and number of genes governing resistance are not yet well known and so the pathogenic race differentiation was not possible through virulence analysis on different host lines. The variability on zonate leaf spot

is not known. Therefore, study was done on the pathological diversity among the *G. sorghi* populations on sorghum lines.

MATERIAL AND METHODS

Isolation of *G. sorghi* :

Zonate leaf spot which is caused by *G. sorghi*. The isolation of fungus was carried out from infected samples of the sorghum leaves. Leaves of infected plant were cut into small pieces of 2-3 mm size with the help of sterilized blade with half healthy and half diseased tissues. The small pieces were sterilized with $HgCl_2$ solution (1:1000) for 60 seconds and washed properly in sterilized distilled water for 3 times. Then the sample pieces were kept between two layers of sterilized blotter paper to remove excess of water. These pieces were then put into slants and Petri plates having Oat meal agar (OMA) medium inside an inoculation chamber under aseptic conditions, followed by incubation at 28^0C. After incubation of 72-96 hours, the superficial growth was sub-cultured on fresh OMA slants.

Purification and maintenance of the culture:

The hyphal tip method was used to purify the fungus. The pure culture was maintained by sub-culturing it every fifteenth day on OMA medium and then preserved it in refrigerator at 10^0C.

As, no differentials were known for *G. sorghi* isolate variability studies in sorghum, all available 5 sorghum lines were used for assessing the pathogenic variability of thirty isolates. Pathogenic variability among different isolates was recorded using two host lines in the glasshouse with the help of methodology given by Mathur *et al*. (2001). Seeds of 5 different host (SPH1794, CSV1955, CSH13, SPH1752 and PC4) were surface sterilized and sown in 20 cm square plastic pots containing sterilized soil in the glasshouse (25±2 ^{0}C, RH <90%). Isolates were spray inoculated on 21 day old plants (5-6 leaf stage) on each host differential by using atomizers containing spore suspension (1×10^5 conidia ml $^{-1}$) having Tween-20 (1ml/l) with each of the 30 isolates. Thereafter, plants were covered with polythene sheets to separate them from other isolates for the prevention of inoculum drift during inoculation. The treated plants were transferred to humidity chamber (>95% RH) for 24 h for air drying, and then shifted to the glasshouse (25± 2^0C) and then arranged in a Completely Randomized Design on the benches.

Latent period:

The observations regarding latent were noted beginning at 48 hours from inoculation till appearance of visible symptoms of disease development were recorded 10 days after inoculation. Data were recorded for the latent period time in hrs from inoculation to appearance

Table A : Description of *Gloeocercospora sorghi* isolates collected from different locations

Isolate designation	Location	Isolate designation	Location
Gs01	Kanakpur-1	Gs16	Bahadarabad-3
Gs02	Kanakpur-2	Gs17	Patanjali-1
Gs03	Narayanpur	Gs18	Patanjali-2
Gs04	Misarkala	Gs19	Chiddarkala
Gs05	Kilakheda	Gs20	Bhaniyawala
Gs06	Doraha	Gs21	Rashem-manjari
Gs07	Dhampur	Gs22	Funwalley-park
Gs08	Sultanpur	Gs23	Doiwala
Gs09	Kashipur	Gs24	Harawala
Gs10	Surajpur	Gs25	Sahaspur
Gs11	Jaspur	Gs26	Sherkot
Gs12	Haridwar	Gs27	Nagina
Gs13	Jwalapur	Gs28	Aphjhalgarh-1
Gs14	Bahadarabad-1	Gs29	Aphjhalgarh-2
Gs15	Bahadarabad-2	Gs30	Pantnagar

Thirty isolates obtained of sorghum were coded as Gs01, Gs02, Gs03, Gs04, Gs05, Gs06, Gs07, Gs08, Gs09, Gs10, Gs11, Gs12, Gs13, Gs14, Gs15, Gs16, Gs17, Gs18, Gs19, Gs20, Gs 21, Gs22, Gs23, Gs24, Gs25, Gs26, Gs27, Gs28, Gs29 and Gs30

of first/necrotic lesions from 48 hours after inoculation at 8:30 AM onward every day, starting from isolate Gs01 to Gs30 on all sorghum lines in sequence to avoid the time differences as inoculation had also been done in same sequence.

Virulence (disease reaction):

Plants were noticed after 14 days of inoculation to take reading of per cent disease index PDI and disease reaction. Disease reaction was recorded and expressed numerically *i.e.* R (1) = resistant (no symptom present); MR (2) = moderately resistant (necrotic lesions without sporodochia formation) and S (3) = susceptible (necrotic lesion with presence of sporodochia).

Disease observation:

Observations on disease intensity was recorded by using 1-9 scale proposed by All India Co-ordinated Sorghum Improvement Project, as follow:

1= Highly resistant (0 - <1%), 2= Resistant (upto 5% disease intensity), 3= Resistant (6-10% disease intensity), 4= Moderately resistant (11-20% disease intensity), 5= Moderately resistant (21-30% disease intensity), 6= Susceptible (31-40% disease intensity), 7= Susceptible (41-50% disease intensity), 8= Highly susceptible (51-75% disease intensity), 9= Highly susceptible (above 75% disease intensity).

Virulence index (VI):

The overall disease producing ability of each isolate was expressed as 'Virulence index' which was calculated by the formula as suggested by Mathur *et al*. (2001) with slight modifications where needed.

$$VI = [1 + (V \times A) \times L^{-1}]$$

where, VI= Virulence index, V= Virulence *i.e.* disease reaction, A= Aggressiveness *i.e.* disease intensity and L= Latent period in days.

RESULTS AND DISCUSSION

The findings of the present study as well as relevant discussion have been presented under the following heads:

Pathogenic variability:

Symptoms appeared on both the surfaces of leaves which varied from resistant (no lesion) to susceptible (semi-circular lesion). The lesions ranged from straw color to orange-red to dark brown depending on the sorghum varieties. Among semi-circular lesions in some host-isolate interactions, sporulation (sporochodia) was observed at the centre of lesions showing susceptible reaction. The hypersensitive activity was characterized by small necrotic spots without sporulation (sporodochia). Isolates varied with respect to virulence disease reaction, aggressiveness (disease severity) and latent period.

Latent period:

The latent period of isolates varied from 2.19 to 7.99 days (Table 1) in all host-isolate interactions. The isolate Gs 06 and Gs 23 had the shortest mean latent period of 3.17 days followed by 3.29 days across five lines, while Gs 07 had the longest (4.66 days). Among the sorghum lines, longest mean latent period (6.61 days) across the isolates was found in SPH 1794 and the shortest was on PC4 (2.76 days).

Virulence:

All the isolates were avirulent on SPH 1794, but 27 of them produced chlorotic flecks (Table 2 and Fig. 1). PC4 variety showed susceptible reaction for all 30 isolates. CSV 1955 was avirulent to isolate Gs 25 while, moderately resistant to remaining all isolates. CSH13 showed moderately resistant reaction to Gs 08, Gs 09, Gs 10, Gs23, Gs24 and Gs25, whereas it was susceptible to rest all other isolates. Majority of isolates were virulent on SPH 1752 except Gs 25 for which the variety was moderately resistant. Variety PC4 showed susceptible reaction to all the isolates.

Considering the virulence of various host-isolate interactions, isolates of *G. sorghi* were categorized into three different groups- GRI (Gs 01, Gs 02, Gs 03, Gs 04, Gs 05,Gs 06, Gs 07, Gs 11, Gs 12, Gs 13, Gs 14, Gs 15, Gs 16, Gs 17, Gs 18, Gs 19, Gs 20, Gs 21, Gs 22, Gs 26, Gs 27, Gs 28, Gs 29 and Gs 30), GRII (Gs 08, Gs 09, Gs 10, Gs 23, Gs 24) and GRIII (Gs 25). Among these three groups, GRIII was the least virulent and caused infection on one of five sorghum lines, whereas GRI was the most virulent infecting three of the five sorghum lines.

Aggressiveness:

Aggressiveness varied from 0.99 to 7.69 (Table 3). Highest aggressiveness was found in isolate Gs 25 (7.69) on lines SPH 1752. Mean aggressiveness of the isolates

Table 1 : Latent period (days) of *Gloeocercospora sorghi* on five sorghum lines

Isolates	Sorghum lines					Mean
	SPH 1794	CSV 1955	CSH 13	SPH 1752	PC4	
Gs01	7.50	5.48	3.27	2.79	2.79	4.37
Gs02	7.54	5.53	3.32	2.84	2.85	4.42
Gs03	7.59	5.59	3.38	2.90	2.89	4.47
Gs04	7.64	5.64	3.43	2.95	2.95	4.52
Gs05	7.69	5.69	3.49	3.00	2.98	4.57
Gs06	0.00	5.70	3.99	2.99	3.19	3.17
Gs07	7.49	5.49	3.32	3.49	3.49	4.66
Gs08	7.19	5.29	3.70	2.99	2.69	4.37
Gs09	7.44	5.14	3.71	2.15	2.70	4.22
Gs10	7.69	4.99	3.70	3.49	2.71	4.51
Gs11	0.99	5.50	3.69	3.29	3.30	3.35
Gs12	7.30	5.79	3.30	2.99	2.99	4.47
Gs13	7.24	5.75	3.23	2.94	2.93	4.42
Gs14	7.19	5.70	3.19	2.90	2.90	4.38
Gs15	7.15	5.64	3.13	2.86	2.84	4.32
Gs16	7.09	5.59	3.07	2.80	2.80	4.27
Gs17	7.43	6.15	3.24	2.65	2.75	4.44
Gs18	7.50	6.19	3.30	3.00	2.79	4.55
Gs19	7.99	5.49	3.29	2.99	2.30	4.41
Gs20	7.30	5.50	3.69	3.29	2.29	4.40
Gs21	7.24	5.45	3.64	3.25	2.24	4.37
Gs22	7.19	5.39	3.59	3.20	2.19	4.31
Gs23	0.86	5.99	3.80	3.30	2.49	3.29
Gs24	3.84	5.34	3.74	3.39	2.59	3.78
Gs25	7.69	4.69	3.69	3.49	2.69	4.45
Gs26	7.39	5.09	3.44	3.59	2.59	4.42
Gs27	7.10	5.49	3.19	3.69	2.49	4.39
Gs28	7.24	5.29	3.31	3.65	2.54	4.41
Gs29	7.19	5.25	3.26	3.59	2.49	4.36
Gs30	7.49	4.69	3.49	3.29	3.29	4.45
Mean	6.61	5.48	3.45	3.12	2.76	
C.D. (P=0.05)	Isolates (A) = 0.35		Genotype (B) = 0.86			A*B = 0.19

Table 2 : Virulence (Disease reactions) of *Gloeocercospora sorghi* isolates on five sorghum lines

Isolates	Sorghum lines				
	SPH 1794	CSV 1955	CSH 13	SPH 1752	PC4
Gs01	R	MR	S	S	S
Gs02	R	MR	S	S	S
Gs03	R	MR	S	S	S
Gs04	R	MR	S	S	S
Gs05	R	MR	S	S	S
Gs06	R	MR	S	S	S
Gs07	R	MR	S	S	S
Gs08	R	MR	MR	S	S
Gs09	R	MR	MR	S	S
Gs10	R	MR	MR	S	S
Gs11	R	MR	S	S	S
Gs12	R	MR	S	S	S
Gs13	R	MR	S	S	S
Gs14	R	MR	S	S	S
Gs15	R	MR	S	S	S
Gs16	R	MR	S	S	S
Gs17	R	MR	S	S	S
Gs18	R	MR	S	S	S
Gs19	R	MR	S	S	S
Gs20	R	MR	S	S	S
Gs21	R	MR	S	S	S
Gs22	R	MR	S	S	S
Gs23	R	MR	MR	S	S
Gs24	R	MR	MR	S	S
Gs25	R	R	MR	MR	S
Gs26	R	MR	S	S	S
Gs27	R	MR	S	S	S
Gs28	R	MR	S	S	S
Gs29	R	MR	S	S	S
Gs30	R	MR	S	S	S

A. Resistant B. Moderately resistant C. Susceptible

Fig. 1: Virulence (Disease reactions) of *G. sorghi* isolates on sorghum varieties

Table 3 : Agressiveness of *Gloeocercospora sorghi* isolates on five sorghum lines

Isolates	Sorghum lines					
	SPH 1794	CSV 1955	CSH 13	SPH 1752	PC4	Mean
Gs01	1.29	6.09	7.06	4.09	2.79	4.27
Gs02	1.35	6.14	7.14	4.15	2.84	4.32
Gs03	1.40	6.19	7.19	4.20	2.90	4.38
Gs04	1.44	6.24	7.25	4.25	2.94	4.43
Gs05	1.49	6.29	7.29	4.29	2.99	4.47
Gs06	1.00	2.29	6.69	4.28	6.29	4.11
Gs07	1.29	7.29	5.69	3.70	6.70	4.93
Gs08	1.49	3.29	3.69	5.30	7.30	4.22
Gs09	1.74	3.79	3.34	5.64	7.15	4.33
Gs10	1.99	4.29	2.99	5.99	7.00	4.45
Gs11	0.99	6.29	6.99	2.30	3.29	3.97
Gs12	1.98	3.29	4.99	5.69	6.99	4.59
Gs13	1.95	3.24	4.94	5.64	6.95	4.55
Gs14	1.89	3.19	4.88	5.59	6.94	4.50
Gs15	1.84	3.14	4.83	5.54	6.84	4.44
Gs16	1.79	3.09	4.78	5.49	6.79	4.39
Gs17	1.65	2.24	3.25	7.24	5.64	4.00
Gs18	1.99	2.29	3.29	7.29	5.69	4.11
Gs19	2.00	5.29	6.29	4.99	6.98	5.11
Gs20	1.29	3.28	4.29	6.29	7.01	4.43
Gs21	1.24	3.25	4.23	6.25	6.93	4.38
Gs22	1.19	3.20	4.20	6.20	6.88	4.34
Gs23	0.99	4.30	5.69	4.29	6.29	4.31
Gs24	1.24	4.00	4.19	5.99	6.64	4.41
Gs25	1.49	3.69	2.69	7.69	6.99	4.51
Gs26	1.48	2.99	3.49	7.19	7.01	4.43
Gs27	1.50	2.29	4.29	6.70	7.00	4.36
Gs28	1.49	2.64	3.89	6.94	6.99	4.39
Gs29	1.45	2.59	3.85	6.89	6.94	4.35
Gs30	1.49	4.69	4.99	5.69	6.30	4.63
Mean	1.51	4.03	4.95	5.53	6.00	
C.D. (P=0.05)	Isolates (A) = 0.36		Genotype (B) = 0.90		A*B = 0.20	

Table 4: Virulence index of *Gloeocercospora sorghi* isolates on five sorghum lines

Isolates	Sorghum lines					
	SPH 1794	CSV 1955	CSH 13	SPH 1752	PC4	Mean
Gs01	0.99	1.88	7.09	5.09	3.80	3.77
Gs02	1.05	1.93	7.14	5.15	3.84	3.82
Gs03	1.09	1.99	7.20	5.20	3.90	3.88
Gs04	1.14	2.05	7.25	5.24	3.94	3.92
Gs05	1.20	2.09	7.29	5.30	4.06	3.99
Gs06	1.00	1.39	5.99	5.35	6.35	4.02
Gs07	1.19	2.30	6.20	4.20	6.69	4.12
Gs08	1.20	1.60	2.99	6.30	9.09	4.24
Gs09	1.24	1.74	2.80	6.20	8.94	4.18
Gs10	1.29	1.89	2.60	6.09	8.80	4.13
Gs11	0.98	2.09	6.69	3.10	4.00	3.37
Gs12	1.29	1.60	5.50	6.69	7.99	4.62
Gs13	1.24	1.66	5.44	6.64	7.95	4.59
Gs14	1.19	1.45	5.40	6.60	7.89	4.51
Gs15	1.15	1.51	5.34	6.54	7.84	4.48
Gs16	1.09	1.45	5.29	6.49	7.79	4.42
Gs17	1.24	1.35	3.95	8.23	7.04	4.36
Gs18	1.30	1.39	4.00	8.29	7.09	4.41
Gs19	1.29	2.05	6.70	5.99	10.15	5.24
Gs20	1.18	1.60	4.50	6.70	10.03	4.80
Gs21	1.14	1.55	4.45	6.60	10.04	4.76
Gs22	1.09	1.49	4.40	6.60	10.00	4.72
Gs23	1.00	1.69	3.99	4.90	8.59	4.03
Gs24	1.09	2.14	3.24	5.14	8.70	4.06
Gs25	1.20	2.60	2.49	5.39	8.79	4.10
Gs26	1.20	1.99	3.74	5.89	9.09	4.38
Gs27	1.21	1.39	4.99	6.39	9.39	4.67
Gs28	1.23	1.70	4.36	6.14	9.24	4.53
Gs29	1.15	1.64	4.31	6.09	9.20	4.48
Gs30	1.19	2.00	5.29	6.19	6.70	4.27
Mean	1.16	1.77	5.02	5.96	7.56	
C.D. (P=0.05)	Isolates (A) = 0.30		Genotype (B) = 0.75		A*B = 0.16	
			CV = 2.44			

across five sorghum lines was maximum in Gs 19 (5.11) and lowest in Gs 11 (3.97). Amongst the host lines, PC 4 and SPH 1752 had 6.0 and 5.53 mean severity scores, respectively, while SPH 1794 had lowest (1.51) across all the isolates.

Virulence index:

Variation was reported in virulence index of different isolates (Table 4). Mean virulence index was highest in Gs 19 (5.24) and lowest in Gs 11 (3.37). Means were also significantly different among the varieties with maximum value in PC4 (7.56) and minimum in SPH 1794 (1.16).

In the present investigation, on the basis of virulence sorghum lines clearly differentiated the isolates. Virulence and Latent period are the two important part of pathogenic fitness of the isolates, once the infection begins, the active host-pathogen interaction occurs and

aggressiveness or the severity of disease becomes an important key of the host resistance. Isolates were differentiated into three pathogenic races on the basis of disease reaction.

Conclusion:

The foregoing results in respect of pathogenicity of 30 isolates from sorghum indicated great variability among themselves. In view of presence of virulence in *G. sorghi* populations specific to five different sorghum lines, there is need to monitor the resistance stability of the existing available material through field survey and virulence analysis. This is important for strategic utilization and deployment of effective resistance to prevent or reduce high susceptibility of popular sorghum cultivars.

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