Prevalence of Pearlmillet Downy Mildew, *Sclerospora graminicola* in Gujarat and Pathogenic Characterization of its Isolates

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Abstract

Surveys were conducted in Gujarat state of India to assess the incidence of downy mildew (DM) caused by Sclerospora graminicola *in pearlmillet hybrids during the seasons of 2005 rainy and 2006 summer. Of the 23 private sector hybrids, three (Pioneer 7688, Proagro 9330 and -9440) were free from DM during the rainy season, 2005 and 11 (Bioseed 8510, HY 555, M-50, Nandi 5, Nandi 52, PAC 982, Pioneer 85M34, Pioneer 86M52, Proagro 9444, Raasi 2246 and Ritu) during the summer 2006. A public sector hybrid GHB 558 and a private sector hybrid Proagro 9444 were found resistant (<10% incidence) during both cropping seasons. Pathogenic diversity studied in a greenhouse experiment using nine isolates of* S. graminicola *collected from different locations and hybrids revealed that isolate Sg 441 from Brahmanwada in Mehasana district was the most virulent (mean incidence of 84% across 9 differentials) followed by Sg 445 and Sg 439 from Banaskantha district. However, based on virulence index (percent disease incidence × latent period^{-1}), Sg 445 was identified as a highly virulent isolate (virulence index of 16.5). This study provided two highly virulent isolates Sg 445 (Banaskantha) and Sg 437 (Jamnagar) to replace less virulent isolates Sg 348 (Anand) and Sg 200 (Jamnagar) that are being used for greenhouse screening of breeding lines targeted for Gujarat.*

Keywords: *Sclerospora graminicola*, downy mildew, pearl millet hybrids, resistance, virulence

Introduction

Downy mildew (DM), caused by *Sclerospora graminicola* (Sacc.) Schroet, is the most important biotic constraint to the sustained high productivity of pearl millet [*Pennisetum glaucum* (L.) R. Br.] hybrids in India. The hybrids are short-lived because of selection of virulent pathotypes from highly variable populations of the pathogen (Thakur *et al.*, 2003). The heterothallic nature of the fungus (Michelmore *et al.*, 1982; Thakur *et al.*, 2004; Pushpavathi *et al.*, 2006a) provides new variants in the progenies, which often matches the resistance genes in the hybrids and renders them susceptible. Pathogenic variability in *S. graminicola* populations has been studied by their reaction on a set of host differentials and also through a collaborative International pearlmillet downy mildew virulence nursery (IPMDMVN) at different locations in India and Africa (Thakur *et al.*, 2004; Pushpavati *et al.*, 2006b; Thakur *et al.*, 2006). The surveys for the prevalence of DM in the major pearlmillet growing states in India have revealed that several commercial F_1 hybrids being grown in different states become susceptible to the disease within 3-5 years (Thakur *et al.*, 2003; Rao *et al.*, 2005). However, there have been large variations in DM incidence of hybrids at different locations and seasons. Periodic monitoring of the crop is required to understand virulence shift in the pathogen and effective management of resistance in pearlmillet hybrids. This study reports the status of pearlmillet hybrids for DM resistance in farmers' fields in Gujarat and identification of new virulence of *S. graminicola*.

Materials and methods

DM survey and collection of isolates

The surveys were conducted during the rainy and summer crop seasons in 2005 and 2006, respectively, when the crop was at preboot to flowering stages. One pearlmillet field was sampled at approximately every 10 km along the roadside, depending upon crop intensity and hybrid diversity. In each field (0.4 ha), 5 random subplots (4 subplots at the corners

and one in the center) were selected and within each subplot a minimum of 50 plants were counted in 2-3 rows to record diseased and healthy plants and mean per cent DM incidence was calculated.

Nine DM-infected leaf samples as oosporic isolates were collected from 9 highly susceptible hybrids from six districts (Anand, Gandhinagar, Jamnagar, Kheda, Mehasana and Banaskantha) in Gujarat during the 2005 rainy season to study pathogenic diversity and evolution of new virulence(s) in *S. graminicola* (Table 1). Two isolates Sg 200 and Sg 348 collected during 1998 and 2001 surveys were included in the study to compare the virulence levels. These isolates were maintained through asexual generations in isolation chambers in greenhouse on seedlings of a hybrid Pioneer 7777, except Sg 200 and Sg 348 that were maintained on ICMP 451.

Host differentials

The host differential set included P 7-4, P 310-17, 700651, 7042R, IP 18292, IP 18293 and 852B from IPMDMVN (Thakur *et al.*, 2004), and two known DM susceptible lines - ICMP 451 and 7042S.

Inoculum preparation and inoculation

Sporangial inocula of the 11 isolates were raised on seedlings of a highly susceptible genotype 7042S in isolation chambers in a greenhouse at ICRISAT, Patancheru. Sporangia from sporulating leaves were harvested in ice-cold water and spore concentration was adjusted to 1×10^6 ml^{-1}. Pot-grown seedlings of the differential lines were spray-inoculated at coleoptile stage using an atomizer. The inoculated seedlings were incubated at 20°C with >90% RH for 20 h, and then transferred to greenhouse benches and incubated at 25±2°C and >95% RH for disease development for the next 2 weeks. The experiment was conducted in CRD with three replications, and 35-40 seedlings per replication.

Data recording and analysis

Latent period$_{50\%}$ (number of days after inoculation for typical symptom appearance on 50% of the infected seedlings) was recorded from 4th day onwards after inoculation. DM incidence was recorded 14 days after inoculation as percent infected plants. Quantitative differences in the virulence levels of the isolates were determined by calculating virulence index (VI = per cent disease incidence × latent period^{-1}) (Thakur and Rao, 1997).

Data sets were analyzed using ANOVA to determine significant differences among different isolates for latent period, DM incidence and VI. Based on the proximity matrix, calculated by using Euclidean distance measure, the cluster analysis was done using Average Linkage method (GENSTAT 9.1, Lawes Agricultural Trust, UK) to determine the similarity among the isolates and to classify isolates into virulence groups based on VI.

Results and discussion

On-farm DM surveys

A total of 72 fields in 18 taluks of eight districts - Ahmedabad, Anand, Banaskantha, Gandhinagar, Kheda,

Table 1. Downy mildew isolates collected from different cultivars and locations in six districts of Gujarat during 1998-2005

Isolate	Year	Cultivar	Location/District	Latitude (N)	Longitude (E)	DM%	Maintained on
Sg 200	1998	ICMH 451	Jamnagar/Jamnagar	22°28'	70°02'	13	ICMP 451
Sg 348	2001	ICMH 451	Gana/Anand	22°31'	72°54'	92	ICMP 451
Sg 432	2005	Hybrid?	Dabhan/Kheda	22°42'	72°48'	44	Pioneer 7777
Sg 435	2005	Hybrid?	Sunav/Anand	22°31'	72°47'	50	Pioneer 7777
Sg 437	2005	7042S+HB3	ARS/Jamnagar	22°28'	70°02'	90	Pioneer 7777
Sg 438	2005	GHB 577	Naniatalmalivas/ Banaskantha	24°22'	72°22'	23	Pioneer 7777
Sg 439	2005	GHB 558	Dangia/Banaskantha	24°15'	72°19'	5	Pioneer 7777
Sg 440	2005	Nandi 3	Teniwada/Banaskantha	23°59'	72°22'	14	Pioneer 7777
Sg 441	2005	Hybrid?	Brahmanwada/Mehasana	23°51'	72°22'	71	Pioneer 7777
Sg 442	2005	Hybrid?	Tarana/Gandhinagar	23°26'	72°34'	55	Pioneer 7777
Sg 445	2005	AHT-503	Danthiwada/Banaskantha	24°19'	72°19'	100	Pioneer 7777

Mehasana, Rajkot and Surendranagar in Gujarat were surveyed during rainy season, 2005. Of the 72 fields surveyed, there was no DM in Rajkot and Surendranagar (Table 2). In other districts, the mean DM incidence varied from 1% in Ahmedabad and Mehasana to 54% in Gandhinagar followed by 19% in Kheda and 13% in Anand. Two public sector hybrids, GHB 558 and GHB 577 had mean incidence of 2 and 23%, respectively in Banaskantha district. However, no DM was observed on GHB 577 in Surendranagar. Three private-sector hybrids (Pioneer 7688, Proagro 9330 and -9440) were DM-free, while Gowri, Nandi 3, -5 and PG showed 2 to 22% mean incidence (Table 2). Several unregistered hybrids recorded 2 to 54% DM incidence in Ahmedabad, Anand, Gandhinagar, Kheda and Mehsana districts.

In summer 2006, of the 71 fields surveyed in seven taluks of three districts - Mehasana, Banaskantha and Patan, 41 (58%) fields had DM incidence ranging from 1 to 93% in Mehasana and Banaskantha (Table 3). However, there was no DM in Patan district. Only three public sector hybrids were observed in the farmers' fields. Public sector hybrid, HHB 67 had 11% incidence, whereas, the other two hybrids GHB 557 and GHB 558 were DM-free. Of the 23 private sector hybrids, 11 (Bioseed 8510, HY 555, M-50, Nandi 3, Nandi 52, PAC 982, Pioneer 85M34, Pioneer 86M52, Proagro 9444, Raasi 2246 and Ritu) were DM-free and 6 (Chandini 511, Nandi 35, Nirmal 1651, NK 1616, Paras Sarpanch, and Proagro 9555) were resistant (1-10% mean incidence) while 6 (Nandi 32, Ajay VBBH 334, Paras Ganesh, Pioneer 7777, Rani and SeedTek 1104) showed 30-61% susceptibility. Several hybrids of unknown identity had a mean DM incidence of 34% with a range of 0-68%. However, it is important to note that several private sector hybrids were grown with metalaxyl-treated seed supplied to the farmers.

Public sector hybrid GHB 558 and private sector hybrid Proagro 9444 were resistant to DM in both the cropping

Table 2. Downy mildew incidence in pearlmillet hybrids in the rainy season crop, 2005

District	Hybrid	No of fields	DM inc. (%)a
Ahmedabad	Unknown	15	2 (0-5)[b]
Anand	Nandi 5	1	12
	Unknown	2	26 (2-50)
Banaskantha	Nandi 3	1	14
	GHB 558	5	2 (2-5)
	GHB 577	1	23
Gandhinagar	Unknown	3	54 (52-55)
Kheda	AHT 503	1	20
	Gowri	1	22
	Unknown	2	32 (20-45)
Mehasana	Gowri	1	0
	PG hybrid?	1	2
	Unknown	28	11 (0-70)
Rajkot	Unknown	1	0
Surendranagar	Pioneer 7688	1	0
	GHB 577	1	0
	Proagro 9330	1	0
	Proagro 9444	1	0
	Unknown	5	0

[a]Mean of fields
[b]Figures in the parentheses indicate range

Table 3. Downy mildew incidence in pearlmillet hybrids in the summer season crop, 2006

District	Hybrid	No of fields	DM inc. (%)a
Mehasana	Unknown	2	34 (0-68)[b]
	Bioseed 8510	1	0
	Chandini 511	1	10
	GHB 557	1	0
	HHB 67	1	11
	Nandi 35	1	5
	Nandi 3	1	0
	Nirmal 1651	1	2
	NK 1616	2	2 (2-2)
	PAC 982	1	0
	Pioneer 7777	3	0
	Pioneer 86M52	4	0
	Raasi 2246	1	0
	Ritu	1	0
Banaskantha	Ajay (VBBH 334)	2	30 (28-32)
	GHB 558	1	0
	HY 555	2	0
	M 50	1	0
	Nandi 32	2	42 (40-44)
	Nandi 52	1	0
	PAC 982	1	0
	Paras Ganesh	4	61 (54-68)
	Paras Sarpanch	2	<1
	Pioneer 7777	17	50 (2-93)
	Pioneer 85M34	1	0
	Pioneer 86M52	3	0
	Proagro 9444	5	0
	Proagro 9555	3	2
	Rani	1	40
	SeedTek 1104	2	47 (16-77)
Patan	Pioneer 7777	1	0

[a]Mean of fields.
[b]Figures in the parentheses indicate range.

seasons. Another important observation was the apparent effect of cropping sequences in reducing DM incidence. No DM was recorded in the pearlmillet fields where tobacco was planted in the previous season. It would be desirable to study the effect of root exudates of tobacco on survival and infectivity of oospores of *S. graminicola*. Crop rotation with cotton, coriander and onion has been reported to reduce the DM incidence in pearlmillet (Thakur *et al.*, 2003).

Pathogenic variation

There were significant ($P<0.001$) differences between isolates, genotypes and their interactions for DM incidence, latent period and virulence index. Per cent disease incidence varied from 1 to 100. Six of the 11 isolates infected all the 9 host differentials with high mean DM incidence (73-84%) across host differentials (Table 4). However, differential reaction was observed on IP 18292, IP 18293, P 7-4, P 310-17, 700651 and 852B for the remaining 5 isolates. The old isolates Sg 200 (Jamnagar) and Sg 348 (Anand) were least virulent with 32 and 26% mean DM incidence, respectively. Among the new isolates, Sg 441 from Brahmanwada in Mehasana district was the most virulent with 84% mean incidence followed by Sg 445 and Sg 439 from Banaskantha district, both showing 78% incidence. Large variation for latent period was also observed among the test isolates. While, Sg 445 on 852B had a latent period of 4 days, Sg 348 had the longest latent period of 14 days on the same genotype. Mean latent period across the differentials was also minimum (5 days) for Sg 445. Similarly, significant variation for VI was observed among the isolates. Isolate Sg 348 recorded the minimum VI (0.1) on P 310-17 and it was maximum (25.0) for Sg 445 on differential line 852B. The mean VI across host differentials ranged from 4.2 (Sg 348) to 16.5 (Sg 445).

A dendrogram of virulence index clustered the 11 isolates into 4 groups with more than 90% similarity (Fig. 1). Cluster I consisted of the least virulent isolates (Sg 200, Sg 348 and Sg 442) and cluster IV had the highly virulent isolates (Sg 441 and Sg 445). Isolates Sg 432, Sg 438, Sg 439 and Sg 440 formed moderately virulent cluster II, and the virulent isolates Sg 435 and Sg 437 were included in the cluster III.

The results confirm the emergence of new virulence in *S. graminicola* populations in Gujarat over time from 1998 to 2005. Temporal virulence shift as indicated by DM incidence was observed among isolates Sg 200 (1998) and Sg 437 (2005) at Jamnagar, and isolates Sg 348 (2001) and Sg 435 (2005) at Anand. Spatial variation in *S. graminicola* has also been reported (Pushpavathi *et al.*, 2006b) and the pathogen populations may have adaptation to the geographically diverse zones. Among six districts from where we collected the isolates, Banaskantha and Jamnagar are geographically diverse (Table 1). Therefore, we have selected the most virulent isolate Sg 445 from Banaskantha and the other virulent isolate Sg 437 from Jamnagar to replace the less virulent isolates Sg 348 (Anand) and Sg 200 (Jamnagar) for greenhouse screening of breeding lines targeted for Gujarat.

Table 4. Downy mildew incidence (%) of 11 isolates of *Sclerospora graminicola* on nine host differential lines

Isolate	IP 18292	IP 18293	P 7-4	P 310-17	700651	7042R	852B	ICMP 451	7042S	Mean
Sg 200	49	6	10	3	11	33	6	78	96	32
Sg 348	3	2	7	1	6	21	1	89	100	26
Sg 432	100	59	90	77	42	85	13	100	100	74
Sg 435	100	12	60	19	56	63	6	95	100	57
Sg 437	87	8	58	60	37	93	33	74	100	61
Sg 438	100	40	44	58	60	69	100	96	100	74
Sg 439	100	53	53	73	56	71	100	95	100	78
Sg 440	100	40	42	55	28	100	100	87	100	73
Sg 441	100	44	81	58	77	96	100	100	100	84
Sg 442	72	11	27	4	15	49	35	100	100	46
Sg 445	80	46	86	63	53	75	100	100	100	78
Mean	81	29	51	43	40	69	54	92	100	62

[a]Means of 3 replications

LSD ($P<0.05$) for isolate means =1.2; genotype means=1.1; isolate × genotype means =3.5

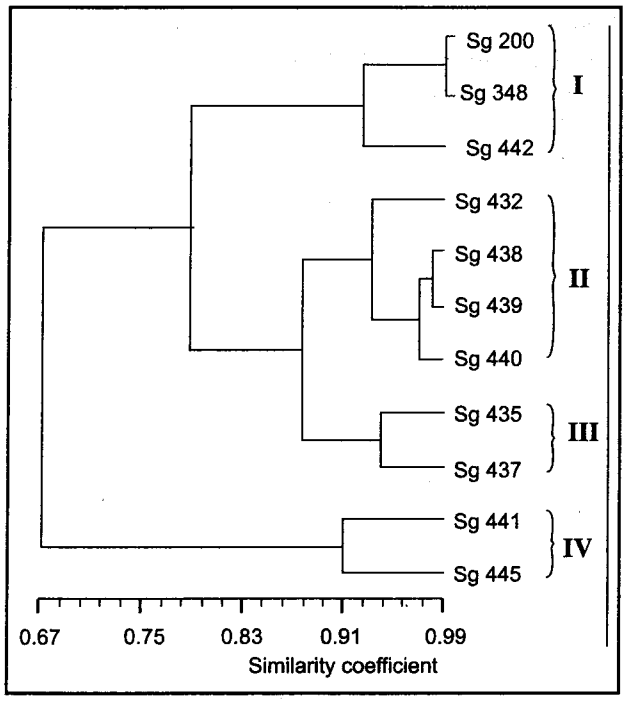

Figure 1. Grouping of *Sclerospora graminicola* isolates based on virulence index

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