Prevalence and Pathogenic Diversity in Pearl millet Downy Mildew Pathogen Populations in Maharashtra, India

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Abstract

Pearl millet fields were surveyed in Maharashtra, India during the rainy seasons of 2009 and 2010 to monitor on-farm downy mildew (DM) incidence and assess the pathogenic diversity among native populations of Sclerospora graminicola. *Of the 131 fields surveyed in seven districts in Maharashtra, DM was observed in 72 fields in the range of 1-90%. DM was quite severe in Ahmednagar, Jalgaon, Aurangabad and Jalna districts with mean disease incidence of 56, 40, 35 and 32, respectively. Severe DM was observed on Pioneer 86M32, B 2301, GK 419, Tulsi, 535, 452 and several hybrids of unknown identity; whereas hybrids MRB 204, Nirmal 40, Sathya, Super 515, Super Boss, Tulja, XL 51, Great 555, Nuzvid 2301, Paras 51 and Sandeep were free from DM. Twenty six* S. graminicola *isolates collected during 2009-10 from Maharashtra were evaluated for pathogenic diversity on seven pearl millet host differential lines along with three isolates of* S. graminicola *collected earlier from the same region. Mean disease incidence on host differentials varied from 5 to 80%.* S. graminicola *isolates Sg 542, Sg 543, Sg 544, Sg 545, Sg 547, Sg 549, Sg 550, Sg 552, Sg 553, Sg 554 and Sg 555 showed > 50% mean DM incidence across host differentials. Highly virulent isolate Sg 542 collected from Deogaon, Aurangabad has been selected for the greenhouse screening of pearl millet lines being developed for Maharashtra.*

Keywords: *Sclerospora graminicola,* downy mildew incidence, pathogenic diversity

Introduction

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is a staple cereal grown on about 29 million ha in the arid- and semi-arid tropical regions of Africa, Asia and Latin America with India having the largest area of 9.3 million ha (Manga and Kumar, 2011). In India, pearl millet is mostly cultivated in the states of Rajasthan, Gujarat, Uttar Pradesh, Maharashtra and Haryana. Currently, more than 90 single-cross F_1 hybrids are grown on about 60% of the total pearl millet area under cultivation in India (Thakur *et al*., 2006; Mula *et al*., 2007). Biotic agents, such as fungi and bacteria cause several diseases on pearl millet, thus lead to substantial yield losses (Thakur, 2008). Among the diseases, downy mildew (DM) caused by *Sclerospora graminicola*, is a widespread and most important disease of pearl millet. The economic loss due to a single epidemic of DM has been estimated to be £7.8 million (Hash *et al*., 2003).

Though host plant resistance has a major role in the control of DM, the genetic resistance in the single-cross F_1 hybrids is short-lived and the hybrids become susceptible within 4-5 years of cultivation (Singh *et al*., 1993; Thakur *et al.*, 2003). Recent reports of the surveys conducted by the All India Coordinated Pearl Millet Improvement Project (AICPMIP) have shown up to 80% DM incidence in the farmers' fields in Maharashtra, while in other states such as Rajasthan, Madhya Pradesh, Tamil Nadu and Karnataka less incidence (0"30%) was recorded (AICPMIP, 2011). Many pearl millet hybrids are introduced in Maharashtra by several seed companies and research organizations every year, which in turn provide a diverse gene pool for the selection of virulent pathogen populations (Thakur *et al*., 2003). This has contributed significantly to the evolution of new pathotypes of *S. graminicola* (Thakur *et al*., 1999; Sharma *et al*., 2010, 2011).

Field surveys for the prevalence of DM on pearl millet hybrid cultivated by farmers are required not only to estimate the pathogenic diversity in *S. graminicola* but also to monitor resistance stability of new hybrids introduced in that ecology. Therefore, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) in

collaboration with AICPMIP has been undertaking surveys to record on-farm disease incidence levels in the hybrid-intensive states such as Maharashtra, Rajasthan, Gujarat, Haryana and Uttar Pradesh (Thakur *et al*., 1999; 2003; Rao *et al*., 2005; Sharma *et al*., 2011). Further, isolation and characterization of *S. graminicola* isolates from different agro-climatic zones results in the selection of virulent pathotypes for the greenhouse screening of breeding material developed at ICRISAT for the target ecology (Thakur *et al*., 2001; Sharma *et al*., 2011).

In recent years, no reports were observed on pathogenic diversity in *S. graminicola* populations in Maharashtra. Therefore, the present study was undertaken to monitor on-farm pearl millet DM incidence in Maharashtra and characterize *S. graminicola* isolates for virulence diversity.

Materials and methods

Survey for the prevalence of downy mildew

Survey of farmers' fields was carried out in pearl millet cultivating districts of Maharashtra during the rainy seasons of 2009 and 2010. DM incidence in the pearl millet fields was calculated as per the standard procedures described earlier (Thakur *et al*., 1999; 2003).

Sample collection and isolation of *S. graminicola*

DM infected pearl millet leaf samples were collected from different locations in Maharashtra during the rainy seasons of 2009 and 2010. At least 10-20 km distance was maintained between each sampling site. The sporangia of each sample were harvested and the pathogen was established on susceptible pearl millet genotype (7042S) as per the standard procedure (Singh *et al*., 1993). All the sporangial isolates of *S. graminicola* were maintained in the isolation chambers under green house conditions at ICRISAT, India (Table 1).

Table 1. *Sclerospora graminicola* isolates collected from different pearl millet cultivars from farmers' fields in Maharashtra

Isolate	Year	Cultivar	Location
Sg 530	2009	Unknown hybrid	Karodi, Aurangabad
Sg 531	2009	Pioneer 86M32	Pimpalgaon, Nashik
Sg 532	2009	Pioneer 86M32	Shrirampur, Ahmednagar
Sg 533	2009	Pioneer 86M32	Newasa-1, Ahmednagar
Sg 534	2009	Pioneer 86M32	Newasa-2, Ahmednagar
Sg 535	2009	Pioneer 86M32	Gangapur, Aurangabad
Sg 536	2009	Unknown	NARP, Aurangabad
Sg 537	2009	MLBH-308	Ambad, Jalna
Sg 538	2009	452	Kannad, Aurangabad
Sg 539	2009	Unknown hybrid	ARS, Dhule
Sg 540	2010	Pioneer 86M32	Jambla, Aurangabad
Sg 541	2010	Krishidhan	Pimpalgaon, Aurangabad
Sg 542	2010	Pioneer 86M32	Deogaon, Rampari, Aurangabad
Sg 543	2010	Unknown hybrid	Pimpalgaon-1, Aurangabad
Sg 544	2010	Unknown hybrid	Pimpalgaon-2, Aurangabad
Sg 545	2010	Unknown hybrid	Pimpalgaon-3, Aurangabad
Sg 546	2010	GK 419	Thapti, Thanda, Aurangabad
Sg 547	2010	Pioneer 86M32	Shahagarh, Jalna
Sg 548	2010	Unknown hybrid	Dakkalgaon, Jalna
Sg 549	2010	Unknown hybrid	Hathnur, Aurangabad
Sg 550	2010	Unknown hybrid	Andaner, Aurangabad
Sg 551	2010	Unknown hybrid	Chalisgaon, Jalgaon
Sg 552	2010	Pioneer 86M32	Sindhkeda, Dhule
Sg 553	2010	Pioneer 86M32	Dondiacha, Dhule
Sg 554	2010	Nirmal 9	Indave, Dhule
Sg 555	2010	Selfed seed	NARP, Aurangabad

Pathogenic diversity among *S. graminicola* isolates

Twenty-six *S. graminicola* isolates collected from Maharashtra were evaluated for pathogenic diversity on seven pearl millet host differentials and two susceptible checks 7042S and ICMP451 along with three isolates (Sg 021, Sg 150 and Sg 332), collected earlier from the same region. Pathogenicity tests were conducted under greenhouse conditions. Sporangial inocula of the 29 isolates were raised on seedlings of a highly susceptible genotype 7042S in isolation chambers in a greenhouse. Sporangia from sporulating leaves were harvested in ice-cold water and spore concentration was adjusted to 5×10^5 ml^{-1}. Pot-grown seedlings of the differential lines and the two susceptible checks were spray-inoculated at coleoptile stage using an atomizer. The inoculated seedlings were incubated at 20ºC with >95% RH for 20 h, and then transferred to greenhouse benches maintained at 25±2°C and >90% RH for disease development for the next two weeks. The experiment was conducted in a completely randomized design with three replications, and 35 to 40 seedlings per replication. The diseased plants were recorded 14 days after inoculation and per cent incidence was calculated based on

the total and diseased seedlings. Data were subjected to analysis of variance using the GenStat statistical software package (Lawes Agricultural Trust, UK) to test the effect of isolates, host genotypes, and isolate × genotype interactions on DM incidence.

Results and discussion

On-farm survey

In all 131 fields were surveyed for DM incidence in seven districts on 26 and 52 ha area during the rainy seasons of 2009 and 2010, respectively. Of the 131 fields surveyed, DM was observed in 72 fields in the range of 1-90% (Table 2). DM was quite severe in Ahmednagar, Jalgaon, Aurangabad and Jalna districts with mean disease incidence of 56, 40, 35 and 32, respectively; however, no DM was observed in the three fields in Beed district. More than 20 pearl millet hybrids were observed in farmers' fields in the seven districts surveyed (Table 3). Low DM incidence (<10%) was observed on Pioneer 86M32 in Nasik and Beed districts; while the same hybrid recorded up to 80 and 90% DM incidence in Dhule and Aurangabad districts, respectively. DM was not observed in Jalgaon, Jalna and Dhule districts during 2009; whereas in 2010, 8-80% DM was observed in these districts. Thirty-two pearl millet hybrid cultivars (21 hybrids of unknown identity) were found infected with DM in the farmer's fields. Pioneer 86M32 was predominant hybrid in Maharashtra and was observed in 52 of the 131 fields surveyed covering an area of 28.9 ha. DM was quite high (up to 90%) on this hybrid and 35 of the 52 fields had 41% mean DM incidence (Table 3). In our previous surveys in Uttar Pradesh, India, severe DM was observed on the same hybrid during 2007-08 (Sharma *et al*., 2011). DM incidence was high (31% mean incidence) in several hybrids of unknown identity. Severe DM was observed on B 2301, GK 419, Tulsi, 535 and 452 with 34-60% mean incidence. However, hybrids MRB 204, Nirmal 40, Sathya, Super 515, Super Boss, Tulja, XL 51, Great 555, Nuzvid 2301, Paras 51 and Sandeep were free from DM. DM incidence in the susceptible hybrids keeps on increasing every year despite seed treatment with fungicides. This poses another threat of development of fungicide resistance in the pathogen populations (Thakur *et al*., 2003). Earlier surveys conducted by the ICRISAT and AICPMIP scientists have shown that several hybrids succumb to DM when grown on the same piece of land for 4-5 years (Thakur *et al*., 2003). Therefore, cultivation of susceptible hybrids should be avoided to prevent the buildup of pathogen inoculum in the farmers' fields and curtail the chances of evolution of new virulences.

Pathogenic variation in *S. graminicola* isolates

Mean disease incidence on host differentials varied from 5 to 80% (Table 4). All the test isolates exhibited > 90% DM incidence on susceptible check 7042S indicating sufficient inoculum load for a reliable screen. Isolate Sg 542 collected from Deogaon, Aurangabad was virulent on all the seven differential lines and recorded highest mean incidence of 80%; while Sg 021, an old isolate presently being used in the greenhouse screening at ICRISAT exhibited lowest (5%) mean incidence across differentials. Isolates Sg 542, Sg 543, Sg 544, Sg 545, Sg 547, Sg 549, Sg 550, Sg 552, Sg 553, Sg 554 and Sg 555 showed >50% mean DM incidence across host differentials. Among host differentials, P7-4 exhibited highest degree of resistance with lowest DM incidence (19%) across isolates; while 852B was highly susceptible with a mean incidence of 68% (Table 4).

In India, over the past 30 years, DM of pearl millet has been largely managed by the development and cultivation of resistant hybrid cultivars. However, in recent years DM

Table 2. Prevalence of pearl millet downy mildew (DM) in different districts of Maharashtra during 2009-10 rainy seasons

			Fields surveyed		DM incidence* (%)	
Districts	No. of villages	Total	DM infected	% infected fields	Mean	Range
Aurangabad	33	59	26	44	35	1-90
Ahmednagar	9	17	12	71	56	5-86
Jalna	9	22	18	82	32	2-83
Dhule	12	18	11	61	30	8-80
Jalgaon	5	8	2	25	40	9-70
Nashik	1	4	3	75	6	2-10
Beed	2	3	0	0	0	-
Total	71	131	72	-	-	-

*Based on the number of infected fields; mean of 5 random samples/field; 50 plants/sample

Table 3. Prevalence of downy mildew (DM) on different pearl millet cultivars in Maharashtra during 2009-10 rainy seasons

	Fields surveyed		DM incidence (%)*	
Cultivars	Total	DM infected	Mean	Range
535	1	1	38	-
B 2301	3	2	50	29-81
GK 419	2	2	40	35-45
Unknown Hybrids	34	21	31	1-87
Krishidhan	3	3	11	10-14
Selfed seed	2	2	19	10-28
MRB 204	7	0	0	-
Nirmal 40	1	0	0	-
Nirmal 9	2	1	12	-
Pioneer 86M32	52	35	41	5-90
Sathya	1	0	0	-
Super 40	1	1	10	-
Super 515	2	0	0	-
Super Boss	3	0	0	-
Tulja	6	0	0	-
Tulsi	2	2	34	18-50
XL 51	2	0	0	-
452	1	1	60	-
Great 555	1	0	0	-
MLBH 308	1	1	2	-
Nuzvid 2301	1	0	0	-
Paras-51	1	0	0	-
Sandeep	2	0	0	-
Total	131	72	-	-

*Based on the number of infected fields; mean of 5 random samples/field; 50 plants/sample

has been observed in different parts of India due to evolution of new virulent pathotypes (Thakur *et al*., 2008; Sharma *et al*., 2010; 2011). Disease reaction of the host differentials to the 26 new and three old isolates of *S. graminicola* from Maharashtra exhibited significant variations in the virulence levels of the pathogen populations. Previous studies have also indicated significant levels of pathogenic as well as molecular diversity among *S. graminicola* isolates collected from different agro-climatic zones of India (Pushpavathi *et al*., 2006; Sudisha *et al*., 2008; Sharma *et al*., 2011). The *S. graminicola* isolates collected from a single host (Pioneer 86M32) at different locations also exhibited variable disease levels on host differentials which indicate that different virulences could be selected on a same host genotype. Old pathotypes, Sg 021, Sg 150 and Sg 332, included in this study recorded comparatively low DM incidences than the new isolates. This shows that new virulent forms of *S. graminicola* have evolved over time in Maharashtra.

ICRISAT has a major research focus on development of pearl millet hybrid parent lines, which are disseminated to public organizations and private seed companies for use in developing F_1 hybrid cultivars. For the designation of parental lines, breeding lines are screened against different pathotypes in the greenhouse under high disease pressure and the lines found resistant (d"10% disease incidence) to at least two pathotypes are selected and disseminated as seed parents. One of the objectives of this study was to replace the old less virulent pathotypes presently being used at ICRISAT for the greenhouse screening of pearl millet lines targeted for Maharashtra with the new virulent pathotype. As a result of this study, highly virulent isolate Sg 542 collected from Deogaon, Aurangabad has been selected for the greenhouse screening of pearl millet lines being developed for Maharashtra.

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Table 4. Downy mildew incidence of *Sclerospora graminicola* isolates on pearl millet host differential lines

	Downy mildew incidence (%)*									
Isolate	P7-4	P310-17	700651	7042R	852B	IP18292	IP18293	Mean	ICMP451	7042S
Sg 021	4	2	2	23	0	0	2	5	89	97
Sg 150	10	4	10	21	33	0	14	13	69	99
Sg 332	17	0	35	43	0	0	0	13	98	100
Sg 530	12	0	0	21	3	5	3	6	95	100
Sg 531	15	43	24	100	100	5	19	44	100	100
Sg 532	42	46	45	100	22	11	27	42	96	100
Sg 533	4	37	44	49	65	6	13	31	64	100
Sg 534	35	14	14	82	17	63	20	35	98	100
Sg 535	40	51	45	74	81	6	10	44	100	100
Sg 536	13	13	22	9	94	52	15	31	97	98
Sg 537	10	10	13	26	10	7	7	12	100	100
Sg 538	17	9	17	18	78	54	17	30	86	100
Sg 539	15	11	7	76	86	21	14	33	100	96
Sg 540	11	27	16	70	96	81	18	46	59	99
Sg 541	4	33	55	57	92	0	26	38	60	99
Sg 542	28	100	67	84	100	100	83	80	100	100
Sg 543	30	92	78	62	100	0	29	56	31	100
Sg 544	24	74	58	93	100	23	87	66	56	100
Sg 545	45	100	29	100	100	100	74	78	92	100
Sg 546	17	24	58	85	88	4	23	43	21	100
Sg 547	12	30	54	77	100	98	52	60	98	100
Sg 548	11	28	30	33	0	2	31	19	28	99
Sg 549	5	61	51	100	100	52	98	67	100	100
Sg 550	23	32	56	92	99	99	63	66	100	100
Sg 551	24	37	13	17	1	0	5	14	100	100
Sg 552	14	56	81	92	100	18	20	54	100	100
Sg 553	23	94	93	100	100	5	35	64	100	100
Sg 554	16	35	44	81	100	100	24	57	100	100
Sg 555	19	11	75	63	100	74	90	62	100	100
Entry Mean	19	37	39	64	68	34	32		84	100

*Mean of three replications
LSD (P<0.05) for entry means = 0.97; for pathotype means = 1.75; for entry × pathotype means = 5.25

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