Evidence for temporal virulence change in pearl millet downy mildew pathogen populations in India

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Introduction

Downy mildew (DM) caused by *Sclerospora graminicola* is prevalent in most pearl millet (*Pennisetum glaucum*) growing parts of the semi-arid tropics. The pathogen is an obligate oomycetes and reproduces both by sexual and asexual processes. Heterothallism and sexual cross compatibility among the isolates of *S. graminicola* (Michelmore et al. 1982, Thakur et al. 2004, Pushpavathi et al. 2006a) lead to genetic and pathogenic variability in the pathogen population. Sporangia, the asexual spores, produced on the infected leaves help in secondary spread of the disease. In India, the disease is quite severe on single-cross F_1 hybrids and causes substantial yield losses (Singh 1995, Thakur et al. 2003).

Pathogenic variability in *S. graminicola* studied through a collaborative International Pearl Millet Downy Mildew Virulence Nursery (IPMDMVN) and on-farm DM survey has revealed differences in *S. graminicola* populations at different locations, including those within India (Thakur et al. 2004, Rao et al. 2005, Thakur et al. 2006). The on-farm DM surveys during 1994–2004 in hybrid-intensive states of Maharashtra, eastern Rajasthan, Gujarat and Haryana in India revealed increased DM susceptibility of a hybrid when grown in the same field for more than three consecutive crop seasons, indicating the emergence of new virulences at different locations/ environments over time (Thakur et al. 2003, Rao et al. 2005). In this article, we report the evidence for temporal virulence change in *S. graminicola* populations based on DM incidence data on a set of pearl millet lines from specific locations in India.

Materials and methods

Evaluation in DM nursery. Eighteen pearl millet lines (genetic stocks) that were identified as highly resistant in the downy mildew nursery at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India during 1990–93 (Singh et al. 1997) were re-evaluated in the same DM nursery during 2006. The DM nursery has been maintained in a particular field (RP 9C) at ICRISAT farm for over 20 years and has been operational since then mainly during the rainy season. The field soil is infested with oospores to provide primary inoculum and infector rows of highly susceptible lines are used to serve as inoculum donor through asexual spores (Singh et al. 1993). The experiment was conducted in a randomized complete block design with 2 rows of 4 m length and two replications. Disease incidence data were taken at 30 and 60 days after emergence by counting the total and infected plants in each plot.

We also compared the DM incidence data sets of selected pearl millet lines obtained from the DM virulence nursery conducted in the disease nursery at Agricultural Research Station, Durgapura, Rajasthan and at Agricultural Research Station, Anand, Gujarat during 2001 and 2006.

Evaluation in greenhouse. Seven pearl millet lines that were identified as highly resistant against the 1993 Patancheru pathotype of *S. graminicola* in a greenhouse screening were re-evaluated against the 2006 Patancheru pathotype (Sg 409) in the greenhouse by spray inoculating the seedlings with the sporangial suspension (Singh et al. 1993). Each time the *S. graminicola* isolate was the Patancheru field population maintained on a highly susceptible pearl millet line 7042S in isolation chambers in a greenhouse. Similarly, two isolates Sg 348 and Sg 435 collected in 2001 and 2005, respectively, from Anand were used to inoculate seven pearl millet lines in the greenhouse at ICRISAT, Patancheru to determine their virulence shift.

The major change in disease incidence (%) on a pearl millet line over time at a particular location was considered as a reflection of change in virulence of the pathogen population. This is based on basic assumptions that variables such as environmental factors and inoculum levels were optimal for disease development, and that the seed of each pearl millet line was genuine at both times of testing. The optimal conditions of disease development were ensured by using a universal susceptible check line 7042S each time. The field observations were tested by

greenhouse tests to overcome the influence of environmental variables.

Results and discussion

The 18 pearl millet genetic stocks that were identified highly resistant to DM (incidence of 0 to 7%) during the 1990–93 field screening recorded 55 to 100% incidence during 2006 in the DM nursery at ICRISAT, Patancheru (Table 1). The susceptible check line 7042S recorded 90% and 100% incidence during the two screenings.

At Durgapura, in the DM nursery 11 pearl millet lines including a susceptible check showed increased levels of incidence from 2001 to 2006 (Table 2) and some of the lines that were resistant (<10% incidence) in 2001 became susceptible (14–50% incidence) in 2006. A similar trend of increased DM incidence on nine pearl millet lines was observed at Anand when these were evaluated in the disease nursery in 2001 and 2006 (Table 2).

In a controlled greenhouse screening seven pearl millet lines that showed very low disease (0 to 6% incidence) against the 1993 Patancheru pathotype and were identified as good sources of resistance recorded 20 to 100% incidence in 2006 against a new Patancheru pathotype Sg 409 (Table 3). Similarly, in a greenhouse experiment, five of the six pearl millet lines that were resistant (2–7% incidence) to Anand pathotype (Sg 348) collected in 2001 became susceptible to a new Anand pathotype Sg 435 collected in 2005 (Table 3).

These results from three locations, Patancheru, Durgapura and Anand, clearly demonstrate the temporal change in virulence of *S. graminicola* populations at these locations. The field observations at Patancheru and Anand have been well supported by the greenhouse evaluation data. This is the first report of a clear temporal virulence change in *S. graminicola* populations in India, although indications of such temporal virulence change and clear spatial variability in virulence have been reported in several earlier studies (Thakur et al. 2004, Rao et al. 2005, Pushpavati et al. 2006b).

Such temporal virulence changes in *S. graminicola* populations signify the genetic potential of the pathogen to evolve virulent population to match the resistance of the new host cultivars and calls for a much closer monitoring of the virulence change in the pathogen populations. This is particularly important because of the large number of single-cross hybrids that are being cultivated by farmers in India and there have been rapid replacements of the hybrids, particularly by private seed companies that greatly influence the pathogen population through host-mediated selection pressure. This could be better done by developing genetic markers for the

Table 1. Downy mildew (DM) incidence of 18 pearl millet germplasm accessions evaluated during 1990–93 and 2006 in disease nursery at ICRISAT, Patancheru, India.

	Field DM incidence (%)	
Accession	1990–93[1]	2006
P 94/1/2-1	6	88
IP 8695-4	5	55
IP 8715-4	0	72
P 2914-3	0	80
P 2925-1	4	87
P 2947-2	5	58
P 2950	0	79
SDN 503	7	55
IP 8292	0	58
BLL-2	0	61
GL-37	0	92
YL-31	0	78
YL-7	0	100
YL-36	4	100
STWL-2	0	96
STYG-7	0	58
STYG-8	0	99
Ysrl-1	0	80
7042S (check)	90	100

1. Source: Singh et al. (1997).

Table 2. Downy mildew (DM) incidence on pearl millet lines screened in disease nursery in 2001 and 2006 at Durgapura, Rajasthan and Anand, Gujarat, India.

	DM incidence (%) at Durgapura		DM incidence (%) at Anand	
Line	2001	2006	2001	2006
P 7-4	5	21	1	11
P 310-17	9	14	10	24
700651	7	18	0	21
7042R	58	90	NA[1]	NA
852B	2	50	3	37
834B	3	19	0	32
843B	22	45	13	42
IP 18292	16	28	0	41
IP 18293	3	23	1	21
ICMP 451	20	53	1	30
7042S (check)	68	94	87	99

1. NA = Data not available.

Table 3. Downy mildew (DM) incidence on pearl millet lines screened against *Sclerospora graminicola* isolates from Patancheru and Anand in greenhouse.

	DM incidence (%)			
	Patancheru isolates		Anand isolates	
Line	1993[1]	2006 (Sg 409)	2001 (Sg 348)	2005 (Sg 435)
P 7-4	3	52	7	60
P 310-17	2	96	1	19
700651	6	20	6	56
IP 18292	NA[2]	NA	3	100
IP 18293	0	85	2	12
ICMP 451	0	91	89	95
81B	4	85	NA	NA
843B	6	100	NA	NA

1. Source: Singh et al. (1997).
2. NA = Data not available.

avirulence genes in the pathogen populations and resistance genes in the host cultivars in addition to the ongoing work of virulence survey through IPMDMVN and on-farm DM survey.

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