Field Surveys of Pearl Millet Downy Mildew – Effects of Hybrids, Fungicide and Cropping Sequence

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Abstract

Pathogenic and genetic diversity in *Sclerospora graminicola,* the pearl millet downy mildew pathogen, is a major cause of a reduced effective life of commercial F_1 hybrid cultivars in India. We conducted surveys during 1994–2000 of pearl millet crops in farmers' fields in Maharashtra, the state that has the largest area (*ca.*1 million ha) under hybrids, to assess the incidence of downy mildew in hybrids. Results indicated: i) a rapid increase in the number of hybrids grown in the region, from 3 in 1994 to 26 in 2000, with the majority of seeds produced and marketed by commercial seed companies; ii) a new resistant hybrid when grown consecutively in the same field for more than three crop seasons often became susceptible to downy mildew (with up to 100% incidence) indicating the emergence/selection of new virulence; iii) seed treatment with the systemic fungicide– metalaxyl was not always effective in significant reduction of downy mildew incidence and of oospore production in some hybrids; and iv) cropping sequences, where previous crops were cotton, coriander and onion, had significantly lower downy mildew incidence compared with other crops.

Key words: Pearl millet hybrids, field survey, downy mildew, *Sclerospora graminicola,* virulence, fungicide, crop rotation

Genetic resistance to downy mildew (*Sclerospora graminicola* (Sacc.) Schröet.) in pearl millet (*Pennisetum glaucum* (L.) R. Br.), particularly in single–cross F_1 hybrids has been short–lived (Hash, 1997; Singh *et al.*, 1993). Currently, there are more than 50 F_1 hybrids that are being grown by farmers in India (Talukdar *et al.*, 1999). During the past 20 years there has been an increase in cultivation of hybrids, and today over 50% of the 10 million ha of pearl millet in India is under hybrids. Pathogenic variability in *S. graminicola* is well known (Ball *et al.*, 1986; Sastry *et al.*, 1995; Singh and Singh, 1987; Singh *et al.*, 1993; Thakur, 1995; 1999; Thakur and Rao, 1997; Thakur *et al.*, 1998), and eight pathotypes have been identified in India (Anon., 1999). Emergence of new pathotypes has been attributed to the commercial cultivation of new hybrids (Thakur *et al.*, 1992; 1998; 1999) and high genetic potential of *S. graminicola* to produce new recombinants both through sexual and asexual reproduction (Ball *et al.*, 1986; Sastry *et al.*, 1995; Thakur *et al.*, 1992; 1998).

Monitoring downy mildew development in pearl millet hybrids and virulence in the pathogen is critical to effective utilization and deployment of resistance in India. In a partnership project on characterization of pathogenic variability in the pearl millet downy mildew pathogen between International Crops Research Institute for the Semi–Arid Tropics (ICRISAT) and the All India Coordinated Pearl Millet Improvement Project (AICPMIP) of the Indian Council of Agricultural Research, field surveys have been conducted in major pearl millet growing states of India during the past few years. ICRISAT has a strong focus on breeding downy mildew resistant inbreds that are subsequently utilized by AICPMIP and private seed companies, in partnership with ICRISAT, to develop resistant hybrids. Recently, ICRISAT developed eight agronomically elite A–lines with resistance to diverse Indian pathotypes of *S. graminicola* (Thakur *et al.*, 2001), and some of these lines are being utilized for production of commercial hybrids. In this article, we report the results of a systematic field survey conducted in Maharashtra from 1994 to 2000 to monitor the incidence of pearl millet downy mildew on various hybrids in farmers' fields and assess the variation in disease incidence as influenced by host cultivars, fungicide and cropping sequence.

Materials and Methods

The field surveys were conducted during the growing season (July–October) to coincide with the pre–boot to flowering stages (second to fourth week of August). During the survey, one pearl millet field was sampled approximately every 10 km along the roadside, depending upon cropping intensity and hybrid diversity. More fields were sampled in areas with a greater diversity of hybrids. Downy mildew incidence was recorded in each field (0.5 ha approx.) by assessing a minimum of 50 plants in each of five quadrates (2 m x 4 rows). One quadrate was situated in each of the four corners and one in the center of the field. The normal plant density of pearl millet in a farmer's field is about 150,000 ha^{-1} and average field size is 0.5 ha. Thus from each field the incidence data was based on a minimum of 250 plants, which represent about 0.33% of the plant population. Information on cultivar, field area, fertilization, seed treatment with fungicide, cultural practices and cropping systems, including previous crops were also collected on a questionnaire sheet by closely interacting with the concerned farmers.

During 1999 and 2000 we used a GPS (Global Positioning System, GPS 12XL, GARMIN Asia Corp., Hsin Tien, Taiwan, ROC) device to record longitude and latitude of each field location to better understand the cropping system and visit the same fields each year. Using the GIS computer software (Arcview GIS version 3.1, ESRI, California, USA), the field locations and downy mildew prevalence were mapped in relation to the seasonal rainfall pattern in seven districts of Maharashtra (Fig. 1).

From each field with >50% incidence, downy mildew–infected leaf samples (8 to 10) were collected and brought to the laboratory to examine oospore production. The leaf samples were cut into 10–cm pieces, dried under the sun in brown paper bags, and ground separately to make leaf powder. The grinder was washed with water and rinsed with alcohol and dried before grinding the next sample. A sub–sample of 5 g from each sample was used to examine oospore population by suspending 0.25 g of the leaf powder in 5 ml sterile distilled water amended with a drop of Tween–20. The suspension was vortexed and filtered through a double–layer muslin cloth. Drops (50 µl each) of the suspension were placed on microscope slides and examined using a compound microscope to record the presence of oospores. A total of 10 such drops were examined from each sample and the number of oospores counted.

Data were subjected to appropriate analysis using GENSTAT statistical package (Rothamsted Experiment Station, Herpenden, Herts AL52JQ, UK).

Results and Discussion

Effect of hybrids. There was a rapid increase in the number of hybrid cultivars grown by farmers in Maharshtra, from 3 in 1994 to 26 in 2000 in the areas that were surveyed (Table 1). The number of fields surveyed increased from 109 in 1994 to 513 in 1999, the percentage of fields with downy mildew varied from 8% in 1994 to 48% in 1996, and downy mildew incidence from 0 to 100% on some hybrids over the years. The cultivar diversity was greatest in Ahmadnagar, Aurangabad, Dhule, Jalgaon, Jalna, Nashik and Pune districts (Table 2) that are mostly endowed with light black soils

Table 1. Downy mildew (DM) survey of pearl millet in farmers' fields in Maharashtra during 1994–2000

Year	Number of fields		Number of hybrids	DM incidence range (%)
	Surveyed	With DM [a]		
1994	109	9 (8)	3	0–40
1995	210	21 (10)	6	2–90
1996	122	59 (48)	15	0–100
1997	190	76 (40)	17	1–90
1998	397	127 (32)	18	0–100
1999	513	77 (15)	23	0–90
2000	333	60 (18)	26	0–100

[a]Number of pearl millet fields with downy mildew, percentage in parenthesis.

Table 2. Number of pearl millet hybrids in farmers' fields in seven districts of Maharashtra during surveys 1994–2000

District	1994	1995	1996	1997	1998	1999	2000
Ahmadnagar	0	2	4	4	4	8	10
Aurangabad	1	2	9	8	5	11	14
Dhule	–[a]	–	1	3	11	14	11
Jalgaon	1	2	3	6	7	7	6
Jalna	–	2	3	2	2	3	5
Nashik	–	–	5	2	7	4	11
Pune	1	4	2	3	–	5	13

[a]Hybrids not found.

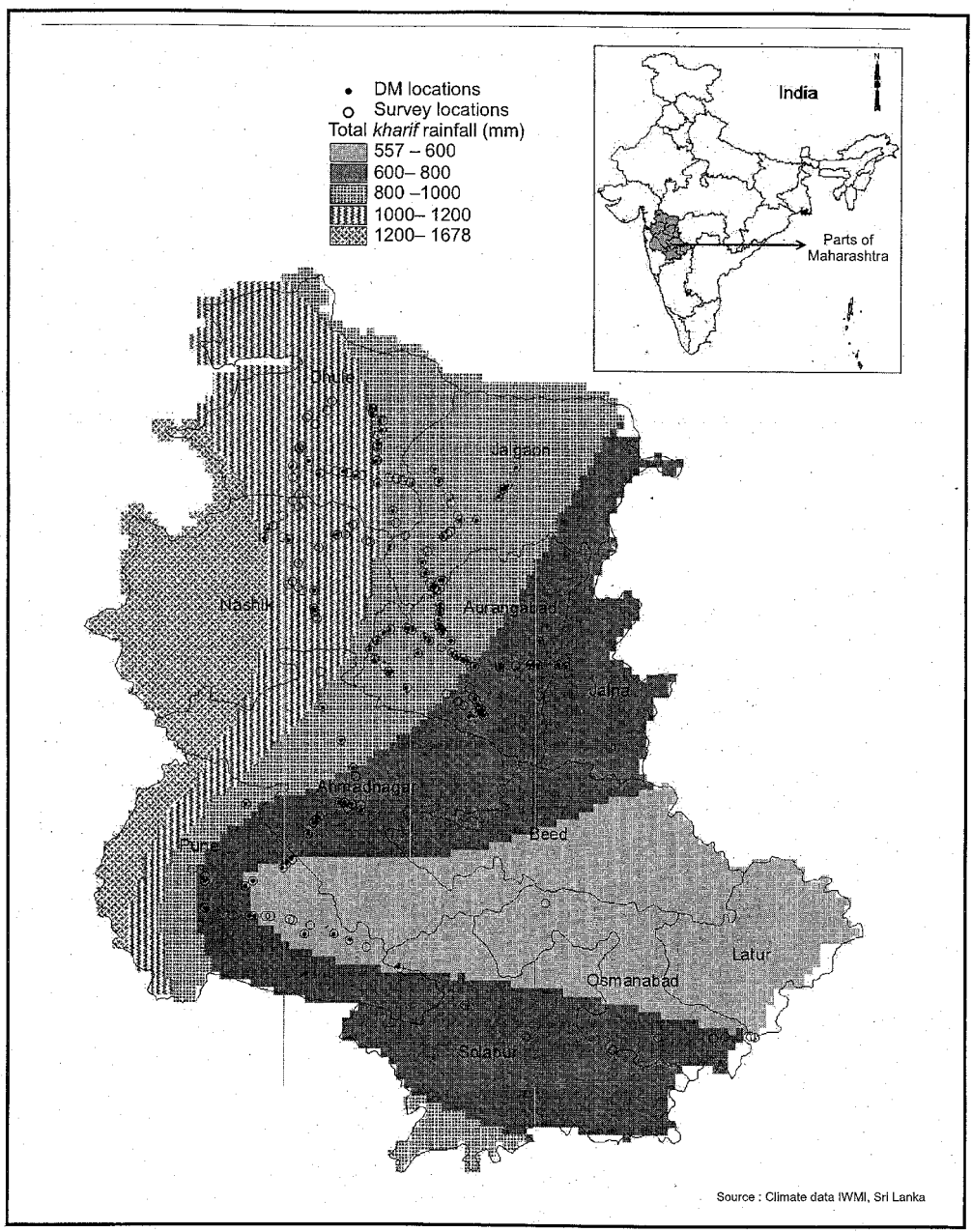

Figure 1. Seasonal (*kharif*) rainfall distribution, field locations (o) and downy mildew presence ⊙ during the pearl millet field survey in eight districts of Maharashtra, August 2000. The mapping of locations was done using latitude and longitude data collected using the GPS system. Note that: i) the parts of districts with high rainfall (6001200 mm) had greater numbers of hybrids and higher downy mildew incidence than those in drier areas; ii) the total numbers of locations shown are not represented in the data tables, because some of the hybrids encountered during 2000 were not common in previous years.

and an average rainfall of 600–1200 mm during June to October (Fig. 1). In the less fertile and drier parts, the predominant cultivars were an improved open–pollinated variety ICTP 8203 and local varieties (farmer's maintained mixture of cultivars).

Downy mildew incidence on 16 of the 26 hybrids that were cultivated at least for three seasons in various fields ranged from 0 to 100% (Table 3). Generally, when a hybrid cultivar was grown in the same fields consecutively for 3–4 years there was increased disease incidence, and by the third or fourth year the cultivar became highly susceptible (>50% incidence), unless the seed was treated with metalaxyl. For example, GK 1014 recorded 2% incidence in 1998, 40–60% in 1999 and 20–80% in 2000. Similarly, MBH 163 had low disease incidence for four seasons (1996–1999), but had up to 80% disease incidence in 2000. A similar trend was observed for MLBH 104, MLBH 267, Eknath 201, Proagro 7701, Proagro 9330, and Pioneer 7686 (Table 3), unless the seeds were treated with metalaxyl.

Effect of fungicide. During 1999 and 2000, many farmers grew pearl millet crops from metalaxyl (Apron SD 35)–treated seeds of susceptible hybrids. Downy mildew incidence in five popular hybrids (GK 1004, MLBH 104, MLBH 267, Proagro 7501 and Proagro 7701) ranged from 15–90% in fields sown with fungicide–treated seeds compared with 20–100% in fields sown with untreated seeds (Table 4). In MLBH 104 and Proagro 7701, fungicide treatment had no significant effect on disease incidence, while in GK 1004, MLBH 267 and Proagro 7501 fungicide treatment significantly reduced the disease over untreated seeds. In three of the five hybrids, oospore production was significantly reduced by the fungicide treatment of seeds, but in the other two hybrids (Proagro 7501 and MLBH 267), fungicide had no effect on oospore production (Table 4). In infected leaf samples of MLBH 104 there was abundant oospore production both from fungicide–treated and untreated plots, although the t–test was significant.

Effect of cropping sequence. In several cropping sequences, where the previous crop was cotton, fallow, coriander, or onion the downy mildew incidence in the pearl millet was less than those where it was wheat, or sorghum (Table 5). Significantly low disease incidence (1–7%) was recorded in three hybrids (MLBH 308, Proagro 7701, and Proagro 9330) in four fields where these were cultivated after onion. A similar effect was observed with MLBH 104 after coriander. A cotton–fallow–pearl millet sequence also recorded significantly low disease (25% mean incidence) compared with pearl millet–wheat–pearl millet or pearl millet–sorghum–pearl millet, sorghum–blackgram–pearl millet, or sugarcane–pearl millet sequence (58–74% mean disease incidence).

The number of hybrids cultivated by farmers in Maharashtra has increased rapidly during the past 10 years. This has contributed significantly to the evolution or selection of new virulence in *S. graminicola* (Anonymous, 1999; Sastry *et al.*, 1995; Thakur, 1995; 1998; 1999). When a susceptible hybrid was replaced by a new hybrid, it remained resistant for 3–4 years, depending on its genetic relatedness to the previous hybrid, fields of cultivation, and seed treatment with metalaxyl. This was demonstrated by the increased downy mildew incidence in MLBH 267 in 1996, Eknath 201 in 1996, GK 1004 in 1996, GK 1014 in 1999, Proagro 9330 in 1999, and MBH 163 in 2000 (Table 3).

Timely replacement of a popular hybrid that becomes susceptible to downy mildew is a real research and development challenge, both for public and private sectors. It takes 3–4 years for a new hybrid to become popular among the farmers, and it takes almost the same time to become susceptible after it has been cultivated over a large area. The results of our surveys have been useful, particularly for private seed companies (which provide 90% of the hybrids grown in Maharashtra), in their decision making process of whether to replace the susceptible hybrid with a new hybrid or treat the seed of the susceptible hybrid with fungicide.

The metalaxyl–treated seed, under normal field conditions (adequate soil moisture and temperature, and low oospore inoculum load) protects the crop from downy mildew up to 35 days after emergence (Singh and Shetty, 1990). The disease later appears with foliar symptoms on the fourth and fifth leaves and subsequently on the panicles as "green ear" and cause substantial grain yield loss (Gupta and Singh, 1996). The level of protection varies with cultivars and weather conditions (Singh, 1983; Singh and Shetty, 1990). The time lag between seed treatment and sowing (often more than 2 months of storage at 45° C or more) may reduce the effectiveness of fungicide (Chandrashekhar Rao *et al.*, 1988). Fungicide treatment of a susceptible hybrid seed costs farmers in two ways, first– the treated seed costs 20–30% more; and second – it does not provide adequate protection beyond 35 days, thus resulting in reduced yield. The infected plant tissues also contribute to increased soil inoculum for the next crop. In some cases, downy mildew infected plants from metalaxyl–treated seed did not produce oospores while in other cases there were abundant oospores. This variation in

Table 3. Downy mildew incidence on commercial pearl millet hybrids recorded during farmers' field surveys in Maharashtra, 1994–2000

Hybrid[a]	Downy mildew incidence range (%)							
	1994	1995	1996	1997	1998	1999	2000	Mean
MLBH 104	15–20 (2)[b]	5–90 (10)	0–80 (5)	10–80 (5)	10–80 (5)	5–85 (4)	40–100 (2)	41±2.2
MLBH 267	40–40 (5)	2–20 (2)	1–100 (17)	1–70 (4)	0–80 (8)	2–40 (9)	10–70 (6)	33±1.6
MLBH 287	–[d]	–	20–50 (2)	–	10–50 (5)	40–50 (1)	35–40 (1)	31±2.1
Eknath 201	–	5–15 (2)	20–90 (6)	40–60 (2)	–	2–90 (2)	–	43±3.7
GK 1004	–	20–20 (1)	80–80 (1)	40–90 (4)	2–100 (14)	2–70 (6)	20–75 (2)	52±2.1
GK 1014	–	–	–	–	2–2 (1)	40–60 (1)	20–80 (4)	44±4.4
Proagro 7501	–	–	–	2–80 (6)	1–80 (10)	40–80 (3)	30–60 (1)	36±2.3
Proagro 7701		40–40 (1)	1–1 (1)	60–85 (1)	2–60 (5)	1–90 (6)	2–60 (3)	31±2.7
Proagro 9330	–	–	–	–	0–0 (1)	5–60 (2)	1–60 (6)	15±3.1
JKBH 26	–	–	50–80 (1)	10–15 (1)	0–0 (1)	0–0 (1)	0–0 (1)	16±5.5
Pioneer 7686	–	–	–	20–40 (1)	0–70 (2)	15–45 (2)	2–20 (3)	21±3.1
MBH 163	–	–	2–5 (1)	0–10 (4)	2–5 (4)	2–10 (3)	10–80 (2)	9±2.3
MBH 204	–	–	–	–	0–0 (3)	0–0 (1)	2–10 (1)	1±0.5
PAC 931	–	–	0–0 (1)	–	–	15–30 (1)	0–0 (5)	3±1.3
Vijay 4	–	–	–	5–50 (4)	2–25 (4)	15–40 (2)	10–20 (1)	18±1.7
Shrada	–	–	0–0 (2)	1–2 (3)	0–0 (7)	0–0 (16)	0–25 (3)	1±0.3
ICTP 8203[c]	0–0 (5)	0–2 (1)	0–0 (7)	0–1 (1)	0–2 (8)	0–0 (14)	0–0 (8)	<1±0.2

[a]Source of hybrids: MLBH – from Mahendra Hybrid Seeds Co. Ltd. Jalna, Maharashtra (MS); Eknath– Nath Seeds Ltd. Aurangabad, MS; GK– Ganga Kaveri Seeds Pvt. Ltd. Secunderabad, Andhra Pradesh (AP); Proagro– Proagro Seeds Ltd. Hyderabad, AP; JKBH– JK Agri–Genetics, Secunderabad, AP; Pioneer– Pioneer Overseas Corp. Hyderabad, AP; MBH– Maharashtra Hybrid Seeds Co. (MAHYCO), Jalna, MS; PAC– Advanta India Ltd. Bangalore, Karnataka; Vijay– Vijay Seeds Co. Ltd., Jalna, MS; Shrada – Mahatma Phule Krishi Vidyapeeth, Rahuri, MS; ICTP– International Crops Research Institute for the Semi–Arid Tropics, Patancheru, AP.

[b]Number of fields surveyed and downy mildew recording made in 5 random plots with at least 50 plants per plot in each field.

[c]An open–pollinated variety.

[d]Hybrid not encountered.

Table 4. Influence of metalaxyl (Apron) seed treatment on downy mildew (DM) incidence and oospore production in different pearl millet hybrids grown in Maharashtra in 1999 and 2000

			DM incidence (%)[a]				Oospore production (no.)[b]		
Cultivar	Treatment	Number of fields	Range	Mean	t–value	p–value	Mean[c]	t–value	p–value
GK 1004	Apron	5	20–72	48			0		
	No Apron	11	20–100	63	2.92	0.005	284	18.96	0.001
MLBH 104	Apron	2	40–85	60			220		
	No Apron	6	40–100	67	1.15	0.258	327	4.83	0.001
MLBH 267	Apron	7	20–64	35			2		
	No Apron	10	20–80	43	2.39	0.019	2	0.62	0.542
Proagro 7501	Apron	3	20–49	28			75		
	No Apron	9	20–80	47	5.97	0.001	80	0.93	0.365
Proagro 7701	Apron	35	15–90	38			0		
	No Apron	20	10–60	38	0.17	0.865	253	20.06	0.001

[a]Based on downy mildew recording in 5 random plots with at least 50 plants per plot in each field.
[b]Number of oospore per milliliter of suspension.
[c]Mean of 10 observations.

Table 5. Influence of some cropping sequences on downy mildew (DM) incidence in pearl millet (PM) hybrids in Maharashtra during 1999 and 2000

	DM incidence (%)[a] on hybrids								
Cropping sequence	MLBH 104	MLBH 267	MLBH 308	Proagro 7701	Proagro 9330	PBH 47	GK 1004	GK 1014	Mean
PM–wheat–PM	45–86 (2)[b]	–[c]	–	80(1)	–	–	–	50(1)	65 (4)
PM–sorghum–PM	58–75 (2)	–	36(1)	–	–	–	66(1)	–	59 (4)
Sorghum–Blackgram–PM	58 (1)	–	–	–	–	–	–	–	58 (1)
Sugarcane–PM	–	–	–	–	–	–	74(1)	–	74 (1)
PM–fallow–PM	–	21–52(2)	–	26(1)	–	–	27(1)	–	31 (4)
Cotton–fallow–PM	–	10–52(2)	–	28–52(2)	–	2 (1)	5 (1)	–	25 (6)
PM–onion–PM	–	–	7 (1)	2 (1)	1(3)	–	–	–	3 (4)
Coriander–PM	9 (1)	–	–	–	–	–	–	–	9 (1)
SE									±5.6

[a]Mean of downy mildew recording in 5 random plots with at least 50 plants per plot in each field.
[b]Number of fields
[c]Information not available

oospore density could partly be due to the sample size and age of infected leaves at the time of sampling. Downy mildew infected, chlorotic leaves often do not contain oospores while necrotic tissues contain numerous oospores that contribute to soil inoculum.

A significant observation was the apparent effect of certain cropping sequences in reducing downy mildew incidence in pearl millet hybrids. Crops, such as wheat, sorghum, sugarcane, or blackgram appeared to increase disease incidence while cotton, fallow, coriander and onion drastically reduced the disease incidence (Table 5). It would be desirable to investigate the effects of root exudates of these crops on survival and infectivity of oospores of *S. graminicola*. In certain parts of Maharashtra where cotton, coriander and onion are common crops in pearl millet cropping systems, farmers should be advised of the advantages of using these

beneficial cropping sequences after their effectiveness is proved experimentally.

Our surveys represent a very small sample of the large pearl millet growing area in Maharashtra (1.8 million ha), and often it was not possible to find out if the farmers grew a particular hybrid for the first time. We have recorded very high disease incidence on some hybrids during our first or second year of survey, which would mean that the hybrids must have been cultivated for at least 2 years. Because there is no strict variety release regulation for the private seed companies in India, it is easy for them to market hybrids that have not been fully evaluated under the AICPMIP system.

The results also confirm the concept that genetically homozygous single–cross F_1 hybrid cultivars are more susceptible and resistance is more short–lived than heterogeneous open–pollinated varieties, which possess durable resistance to the highly variable populations of *S. graminicola*. An open–pollinated variety, ICTP 8203 occupies about 25% of the pearl millet area in Maharashtra and has consistently recorded a downy mildew incidence of 0–2% during the past seven years compared to several hybrids that have shown susceptibility within a short time (Table 3). Although ICTP 8203 gives relatively lower yield than the hybrids, it is preferred by farmers in the drier parts of the state (<600 mm rainfall) because of its better drought tolerance, higher resistance to downy mildew and lower seed cost. We believe that in India, both hybrids and open–pollinated varieties have specific niches for cultivation and this situation will continue in the future.

Despite the short effective life of hybrids, the genotypic diversity in pearl millet provided by different seed companies and public institutions, the small farm size, and the use of different cropping systems have diminished the chances for widespread epidemics as it often happens in large–scale monoculture systems of cultivation.

The surveys were useful for creating awareness among farmers about pearl millet downy mildew and its apparent adverse effect on yield, the measures that could help reduce the disease – the need to change the cultivar every 3–4 years, and follow suitable cropping sequences. We also noticed improvement in farmers' awareness since we began the survey in 1994 about hybrids and their companies, identification of diseases and fungicide. The future research efforts will be on developing an integrated disease management system involving host plant resistance, cropping system, and fungicide seed treatment.

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