Pathology

Pearl Millet Downy Mildew Research in India: Progress and Perspectives

RP Thakur[1]*, HS Shetty[2] and **IS Khairwal[3]**
[1. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, Andhra Pradesh, India;
2. Department of Applied Botany and Biotechnology, University of Mysore, Mysore 570 006, Karnataka, India;
3. All-India Coordinated Pearl Millet Improvement Project of the Indian Council of Agricultural Research, Agricultural Research Station, Mandor, Jodhpur 342 304, Rajasthan, India]
*Corresponding author: r.thakur@cgiar.org

Introduction

Downy mildew (DM), caused by *Sclerospora graminicola*, continues to be a major biotic constraint to pearl millet [*Pennisetum glaucum* (L.) R. Br.] production in India. The disease which had remained incipient on local landrace cultivars until the 1960s, became a serious threat to high-yielding, single-cross hybrids introduced into cultivation during the late 1960s. The first epiphytotics of DM occurred during the crop season of 1971 on the first popular hybrid HB 3 and caused substantial yield loss (Nene and Singh 1976). With the establishment of ICRISAT in 1972, pathological research on pearl millet began in 1974 to address the major diseases: downy mildew, ergot (*Claviceps fusiformis*), smut (*Moesziomyces penicillariae*) and rust (*Puccinia substriata* var *indica*). A Consultative Group meeting on downy mildew and ergot was held in 1975 at ICRISAT-Patancheru to review the status of research and identify priorities. The group, consisting of renowned plant pathologists and plant breeders from India and other countries, made several recommendations for downy mildew research relating to the internal seedborne nature of the pathogen, its likely transmission and methods to eliminate the pathogen from the seed; the relative roles of oospores, sporangia and seedborne inoculum in epidemiology; development of screening techniques; existence of physiologic races; multilocation evaluation of resistant lines to identify stable resistance; and development of chemical and cultural methods to supplement the principal host-plant resistance method of disease control (ICRISAT 1975). These recommendations formed the basis for downy mildew research encompassing basic, strategic and applied aspects. These are still being followed in our ongoing Indian Council of Agricultural Research (ICAR)-ICRISAT partnership research.

In this paper we discuss the advances made by the ICAR-ICRISAT partnership research on pearl millet downy mildew with the focus on several aspects such as seedborne nature of DM; field and greenhouse screening techniques; and host-plant resistance and pathogenic variability. Finally we suggest perspectives for future strategy for downy mildew management.

Seedborne Nature of DM

The first collaborative project between ICRISAT, the Downy Mildew Laboratory, University of Mysore (UoM) and the Indian Agricultural Research Institute (IARI), New Delhi began in 1976 to unravel the mystery of the internal seedborne nature and likely seed transmission of downy mildew (Shetty et al. 1980). After a series of discussions and experiments, important recommendations were made. They were subsequently ratified by ICAR for implementation by the plant quarantine authorities of India. These were: seed treatment for 10 min with 0.1% $HgCl_2$ (now Clorox 2.62% a.i.) with several rinses with sterile distilled water; hot water treatment at 55°C for 12 min followed by drying under shade; treatment with metalaxyl (2 g a.i. kg^{-1} seed); and growing-on test in the post-entry quarantine isolation area at ICRISAT-Patancheru until crop maturity. The plant quarantine authorities of India and ICRISAT have been strictly adhering to this protocol, and so far no downy mildew has been detected in any of the 22890 samples of pearl millet breeding lines and germplasm accessions imported from 96 countries since 1974.

Field and Greenhouse Screening Techniques

Some basic studies on biology and epidemiology at the UoM and ICRISAT clearly established the roles of oospores (sexual spores) and sporangia (asexual spores) in primary and secondary infection, and the influence of weather factors particularly temperature and humidity on DM disease development and spread (Safeeulla 1976; Singh and Williams 1980). These led to the development of a

field screening technique that used the basic 'sick-plot' concept combined with infector-rows to provide a uniform disease spread and indicator rows to provide a measure of the disease pressure in the disease nursery (Williams et al. 1981; Singh et al. 1993). The large-scale operation of the downy mildew screening facility at ICRISAT-Patancheru both during rainy and postrainy seasons (12 ha per year) laid the foundation for a systematic downy mildew resistance breeding program. The field screening technique was widely adopted by collaborating centers in India and Africa. Further studies on biology and epidemiology led to the development of a more precise greenhouse screening technique in which potted seedlings are inoculated with a known amount of inoculum and subjected to highly congenial conditions of humidity and temperature for infection and disease development (Singh and Gopinath 1985). This technique is precise, independent of the season, and both time-efficient and cost-effective. The technique has since been further refined to make it more effective, and is currently being used to screen breeding lines against the diverse Indian pathotypes of downy mildew.

Host-plant Resistance

The successful operation of field and greenhouse screening techniques resulted in the identification of a good number of germplasm and breeding lines with high levels of downy mildew resistance (Singh et al. 1997). A number of these lines were evaluated multilocationally through the establishment of a collaborative International Pearl Millet Downy Mildew Nursery (IPMDMN) to test the stability of resistance. The first IPMDMN, consisting of 25 promising pearl millet lines and controls, was established in 1976 and distributed to collaborators at 20 locations in Burkina Faso, India, Nigeria, Niger and Senegal. During its 20 years of operation, a number of lines with stable resistance to downy mildew were identified (Table 1); some of these have been utilized in breeding programs to develop hybrids and open-pollinated varieties (Hash et al. 1999). The results of IPMDMN also provided information on likely pathogenic variation in *S. graminicola* populations.

Table 1. Pearl millet lines identified as stable sources of downy mildew resistance across many environments in India and Africa (IPMDMN 1976–88).

Line	ICRISAT PMIC No.	Mean DM (%)	
P 7	ICML 12	2.0	(80 environments)
SDN 503	ICML 13	3.0	(80 env.)
700251	ICML 14	2.0	(80 env.)
700516	ICML 15	1.5	(80 env.)
700651	ICML 16	1.5	(80 env.)
P 1449	–[1]	2.0	(50 env.)
P 310	–	2.5	(50 env.)
Control (S)		60.0	(130 env.)

1. PMIC number not assigned.
Source: Singh et al. (1993).

Pathogenic Variability

With increasing area coming under pearl millet hybrid cultivation in India since the 1970s, downy mildew severity and spread have increased proportionately. The first downy mildew epidemic occurred on the most popular hybrid HB 3 in 1971, and the first report on pathogenic variability appeared in 1973 when NHB 3 was found susceptible at Gulbarga but resistant at Mysore (Bhat 1973; Shetty and Ahmad 1981). With increasing reports of pathogenic variability in *S. graminicola* and hybrids succumbing to downy mildew, a systematic study was initiated in the early 1990s following discussions between ICRISAT and ICAR scientists on characterization of the pathogen population. Accordingly, pathogenic variability in *S. graminicola* has been studied by establishing a collaborative International Pearl Millet Downy Mildew Virulence Nursery (IPMDMVN) and conducting an on-farm downy mildew survey and characterizing the isolates for pathogenic and genetic diversity.

The IPMDMVN, consisting of 11 pearl millet lines (as host differentials) was established at 17 locations in Burkina Faso, India, Mali, Niger and Nigeria during 1995–1999, and at 9–13 locations in India during 2000–2004. The results of the 1995–99 IPMDMVN revealed significant differences in *S. graminicola* populations at different locations (Thakur et al. 2004a; 2004b). The populations at Bagauda (Nigeria) and Durgapura (India) caused the most severe disease and those from Coimbatore and Aurangabad (India) the least. Based on disease incidence, the 17 *S. graminicola* populations were grouped into six major putative pathotypes (Fig. 1). The results indicated significant differential resistance, and that resistance in lines IP 18292, IP 18293, 700651 and P 310-17 was more stable than in others regardless of the location or season.

The on-farm downy mildew surveys conducted during 1994–2004 in the pearl millet hybrid-intensive Indian states of Gujarat, Haryana, Maharashtra and Rajasthan provided some useful results, including: (i) a rapid increase in the number of hybrids grown in these states, mostly hybrids from the private sector; (ii) a new hybrid when grown in the same field for more than three consecutive crop seasons often became susceptible, indicating the emergence/selection of a new virulence; (iii) seed treatment with the systemic fungicide metalaxyl was not always

effective in reducing downy mildew incidence, particularly in highly susceptible hybrids; and (iv) certain cropping sequences such as cotton, coriander and onion as previous crops had significant effect on reducing disease incidence (Thakur et al. 2003). The surveys have provided forewarnings on the performance of hybrids and their possible replacement in certain areas. For instance, the surveys indicated the likely resistance breakdown of HHB 67, the most popular hybrid in Haryana and parts of Rajasthan. This led to the development of HHB 67-2, a DM-resistant version of HHB 67, using marker-assisted backcross breeding. It was recommended for release in Haryana in 2005. This was the first successful demonstration of introgression of downy mildew resistance genes into hybrid parental lines using both conventional and DNA-marker technologies to produce downy mildew resistant hybrids in pearl millet through partnership research (Hash et al. 2003).

We have a collection of about 400 isolates (oospore inocula) of *S. graminicola* drawn from different pearl millet cultivars during the past 10 years of on-farm downy mildew surveys in the major pearl millet growing states of India. Some of the isolates that have caused high disease incidence (>80%) on popular hybrids have been characterized for their virulence and genetic diversity. In a recent study (Thakur et al. 2004b) 15 selected isolates, based on their pathogenic diversity on host differentials, were classified into five major virulence groups. Similarly, based on their genetic diversity using AFLP markers, these isolates were classified into five major clusters (Sivaramakrishnan et al. 2003). However, these two groups were not similar, as genetic diversity was not based on the virulence gene markers. In a recent study (RP Thakur, unpublished), 14 isolates of *S. graminicola* from Rajasthan were classified into nine distinct groups indicating both spatial and temporal variation in the

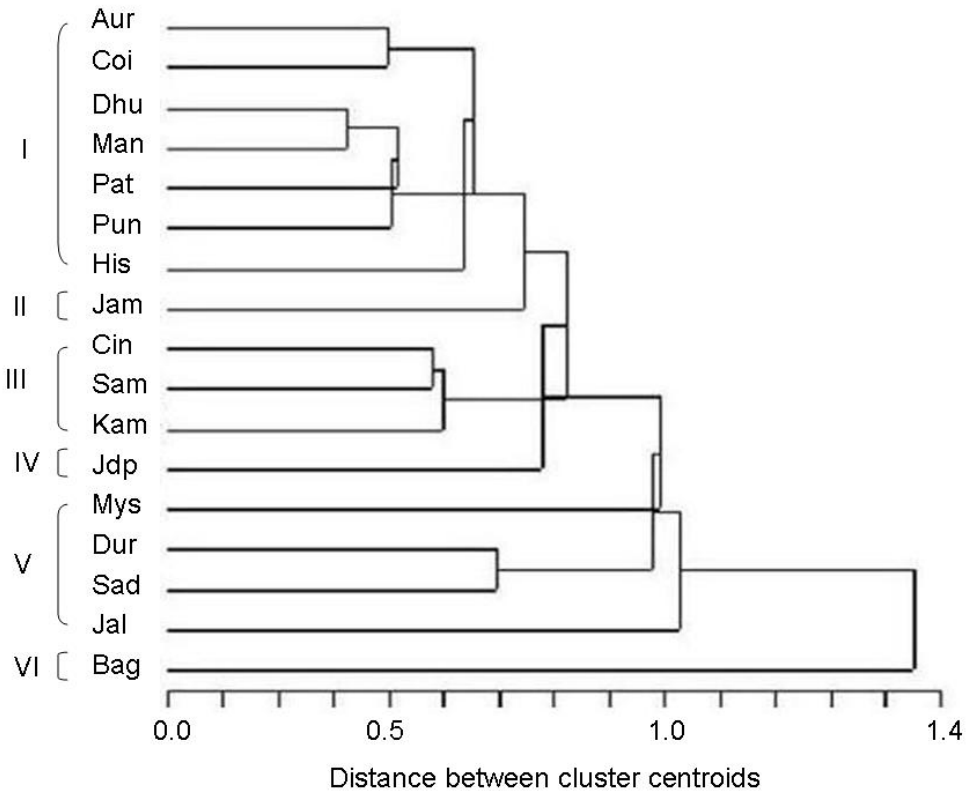

Figure 1. Grouping of *Sclerospora graminicola* populations from different locations into distinct clusters based on their virulence (downy mildew incidence on pearl millet differential lines): Aur = Aurangabad, Coi = Coimbatore, Dhu = Dhule, Man = Mandor, Pat = Patancheru, Pun = Pune, His = Hisar, Jam = Jamnagar, Cin = Cinzana, Sam = Samanko, Kam = Kamboinse, Jdp = Jodhpur, Mys = Mysore, Dur = Durgapura, Sad = Sadoré, Jal = Jalna and Bag = Bagauda.

Source: Thakur et al. 2004b.

virulence patterns of *S. graminicola* populations within Rajasthan (Fig. 2). Isolates from six locations (Jodhpur, Barmer, Churu, Bikaner, Mandor and Durgapura) were classified into diverse groups, and among the five isolates from Jodhpur, one collected in 2003 formed a virulence group distinct from the four collected in 1997. The results also supported host-directed virulence selection as a mechanism of virulence evolution (Thakur et al. 1992). Some of the highly virulent pathotype isolates have been established and are being used in greenhouse screening of breeding lines at ICRISAT-Patancheru.

Over the last three decades, there has been a progressive increase in the release of pearl millet hybrids and the spread of downy mildew. However, there have been no widespread epidemics of the disease in the past 10 years. This is because of the collaborative efforts of ICAR and ICRISAT which have helped us understand the disease better and effectively manage it. We consider this as a significant achievement and a demonstration of the strength of partnership research in managing this most important disease of pearl millet.

Perspectives

Despite the impressive progress on managing downy mildew in pearl millet, the battle is far from over. Given the dynamic nature of the pearl millet-downy mildew pathosystem and the high genetic potential for virulence evolution in the pathogen population in response to genetic changes in the host, an appropriate strategy has to be developed to manage this disease. In the light of the results of our past studies, we propose that future downy mildew management strategy in pearl millet should focus on: (i) virulence monitoring through on-farm downy mildew surveys and multilocation virulence nurseries; (ii) collection and characterization of isolates for virulence; (iii) identification of new virulence for use in screening for advanced breeding lines; (iv) breeding hybrid parental lines for resistance to single/multiple pathotypes; and (v) developing resistant hybrids for release and commercial cultivation. This paradigm of resistance breeding through monitoring virulence should be a continuous process to effectively manage downy

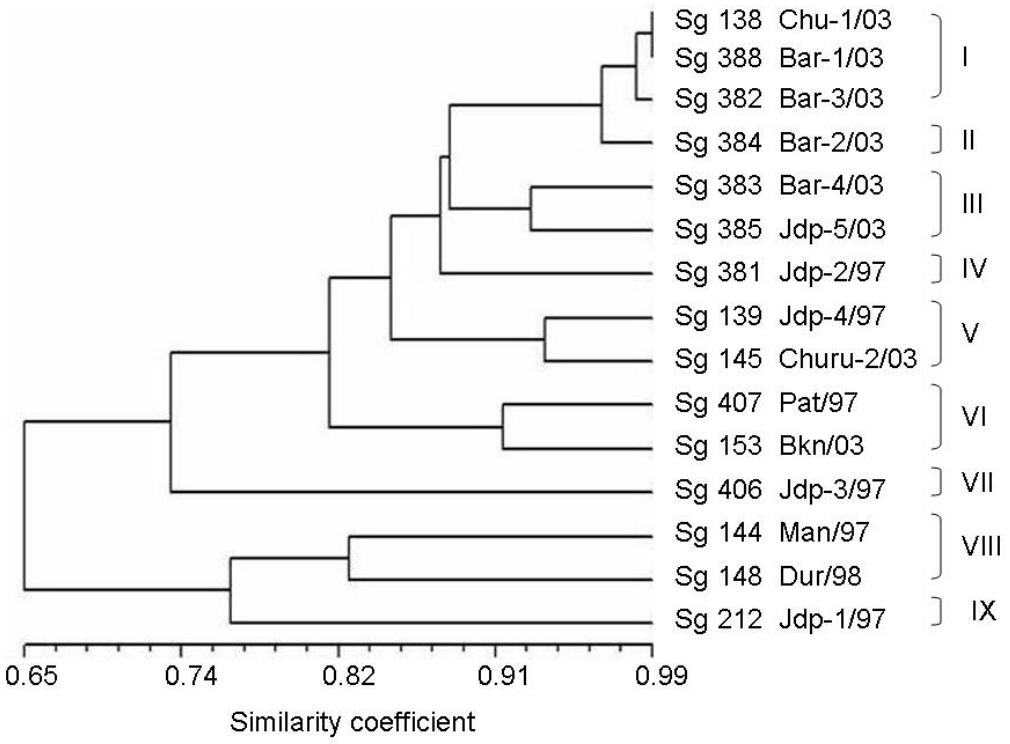

Figure 2. Dendrogram showing the classification of 14 isolates from Rajasthan and 1 from Patancheru into nine groups based on downy mildew incidence and latent period on 10 host differential lines: Chu = Churu, Bar = Barmer, Jdp = Jodhpur, Pat = Patancheru, Bkn = Bikaner, Man = Mandor and Dur = Durgapura.

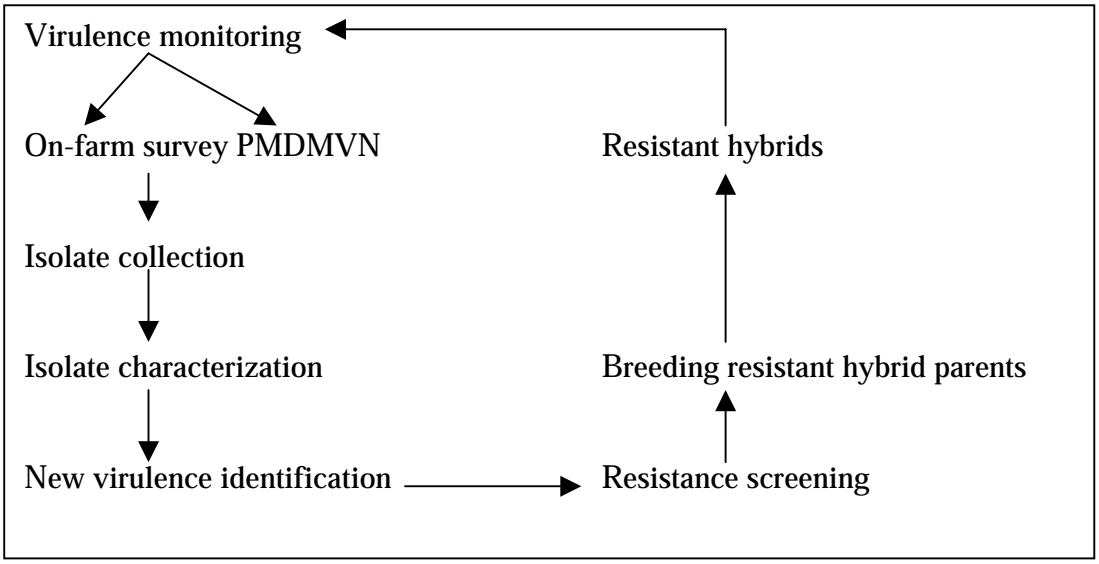

Figure 3. Strategy for managing pearl millet downy mildew through host-plant resistance. (PMDMVN = Pearl Millet Downy Mildew Virulence Nursery.)

mildew in pearl millet (Fig. 3). Use of molecular-marker techniques will be useful in identification of resistance genes, and transferring and pyramiding such genes in agronomically elite parental lines of pearl millet hybrids. Other approaches, such as judicious use of seed-treatment fungicides and suitable agronomic practices in combination with host-plant resistance would be desirable to prolong the commercial life of hybrid cultivars.

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