

Strategy for downy mildew resistance breeding in pearl millet in India

RP Thakur^{1*}, KN Rai¹, IS Khairwal² and RS Mahala³

1. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, Andhra Pradesh, India
2. All India Coordinated Pearl Millet Improvement Project, Agricultural Research Station, Mandor, Jodhpur 342 304, Rajasthan, India
3. Pioneer Overseas Corporation, Hyderabad 500 034, Andhra Pradesh, India

*Corresponding author: r.thakur@cgiar.org

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Abstract

Downy mildew (DM) caused by *Sclerospora graminicola* is a widespread and economically most important disease of pearl millet causing substantial annual yield losses, particularly in single-cross F₁ hybrids in India. Currently, in India about 50% of the 9 million ha under pearl millet cultivation is grown with more than 70 hybrids in which DM incidence has been highly variable, with some hybrids showing more than 90% incidence in farmers' fields. With increasing area under hybrid cultivation since the 1970s the disease has become more severe due to evolution of new virulent pathotypes in response to new hybrid genotypes. At ICRISAT, breeding for DM resistance using conventional pedigree breeding and more recently marker-assisted backcross breeding has been successful, and a large number of disease resistant hybrids have been developed and deployed. This has, to a large extent, helped in arresting the occurrence of widespread DM epidemics since the 1990s. In view of the increasing severity of the disease and evolution of new more virulent pathotypes, there is a need to develop a long-term DM resistance breeding strategy in India. In this paper, we discuss various aspects of the pearl millet-DM pathosystem, factors that influence disease resistance breeding and suggest short-, medium- and long-term strategies for DM resistance breeding.

Introduction

Downy mildew (DM) caused by an obligate parasite *Sclerospora graminicola* is quite widespread and economically most important disease of pearl millet (*Pennisetum glaucum*) in India and several countries in Africa. In India, the disease is prevalent in almost all pearl millet growing states and causes substantial annual losses. The disease is particularly more serious on single-cross F₁ hybrids than on open-pollinated varieties

(OPVs). This is due to narrow genetic base and uniformity of the hybrids than those of OPVs that are highly heterogeneous. Currently, about 50% of the 9 million ha under pearl millet cultivation is grown with single-cross hybrids in India (Rai et al. 2006). The DM incidence has been quite variable on different hybrids and more than 90% incidence has been recorded on some hybrids in farmers' fields (Thakur et al. 2003, Rao et al. 2007). The estimated annual grain yield loss due to DM is approximately 20–40% (Singh 1995, Hash et al. 1999, Hess et al. 2002) but this could be much higher under favorable conditions of disease development (Singh 1995, Thakur 1998, 2008). Most seed companies treat the seed with a systemic chemical fungicide metalaxyl to protect the crop from DM (Thakur et al. 2003, Rao et al. 2007). However, this treatment is effective only in case of moderately resistant hybrids in certain environments. The fungicide is ineffective in susceptible hybrids, in which the crop is protected only up to 40 days after emergence and the disease appears on nodal tillers and as 'green-ear' at the later stages of crop growth. The cost of treated seed is much higher and farmers have to pay additional price for such seed without any assurance from the seed companies of the protection from the disease. Also, as of now, no such regulation exists in India under which the concerned seed companies can compensate farmers for the crop loss. This chemical approach to DM management may also lead to the emergence of more virulent pathotypes.

With the increasing area under hybrid cultivation since the 1970s, the disease has become more severe and more widespread (Thakur et al. 2006). The most cost-effective management of the disease can be obtained by breeding DM resistant pearl millet hybrids. There has been considerable success in breeding for DM resistance using conventional pedigree breeding, and a large number of disease resistant hybrids have been developed and deployed (Khairwal et al. 2004). This has contributed

in arresting the occurrence of widespread DM epidemics since the 1990s (Thakur et al. 2006). Marker-assisted backcross breeding has further enhanced the ability and efficiency of DM resistance breeding (Hospital et al. 1992, Hash et al. 1999). However, in view of the increasing severity of the disease and evolution of new more virulent pathotypes (Thakur et al. 2004), there is a need to develop a long-term strategy for DM resistance breeding in India. In this paper, we limit our discussion to various aspects of the pearl millet-DM pathosystem and suggest a strategy for its genetic management.

In general, the long-term success of breeding for disease resistance is influenced by several factors that include: (i) the nature of the pathogen and diversity of virulence, (ii) availability, diversity and type of genetic resistance, (iii) screening method and selection environment, (iv) breeding methods, (v) utilization and deployment, and (vi) monitoring resistance/virulence. Each of these is discussed below.

Nature of the pathogen and diversity of virulence

Sclerospora graminicola reproduces both sexually by producing oospores and asexually by producing sporangia that liberate zoospores at maturity (Fig. 1). Oospores are thick-walled structures that can survive for several years on leaf debris and in soil and also contaminate the seed lots and thus could become externally seedborne. These are primary sources of inoculum in the field through contaminated seed and infested field soil. Once the seedlings are infected, sporangia are produced on the foliage which serve as a source of secondary inoculum for the spread of disease within and between fields (Fig. 2).

After landing on the young growing foliage, sporangia produce numerous zoospores that swim in the thin film of water on the leaf surface before producing infection hyphae. High humidity (>85% RH) with leaf wetness and moderate temperature range of 20–30°C are congenial for infection and disease development. The infection to pearl millet seedlings is systemic and the disease

symptom is expressed from the seedling stage as chlorotic strips to the flowering stage as green-ear in the panicle (Fig. 3).

The host-pathogen interaction in the pearl millet-DM system is expected to follow the general gene-for-gene concept (Flor 1971) as is well known in other obligate systems, such as wheat-rusts, wheat-powdery mildew and lettuce-DM. This concept is based on major R-genes for resistance in host and complementary virulence genes in the pathogen (Fig. 4). This is a simple concept and easy to explain how resistance genes are defeated and new virulence genes evolve over time and space. The hypothesis states that plant contains a single dominant resistance gene (*R* gene) that specifically recognizes the complementary avirulence gene (*Avr* gene) of the pathogen. Avirulence gene in the pathogen encodes a protein product that is recognized by the complementary *R* gene product of the plant, which results in induction of defense gene expression (hypersensitive reaction) and inhibition of pathogen growth (incompatible reaction). However, if the plants do not contain the *R* gene, the pathogen will be able to grow and infect them (compatible reaction), even though it contains *Avr* gene.

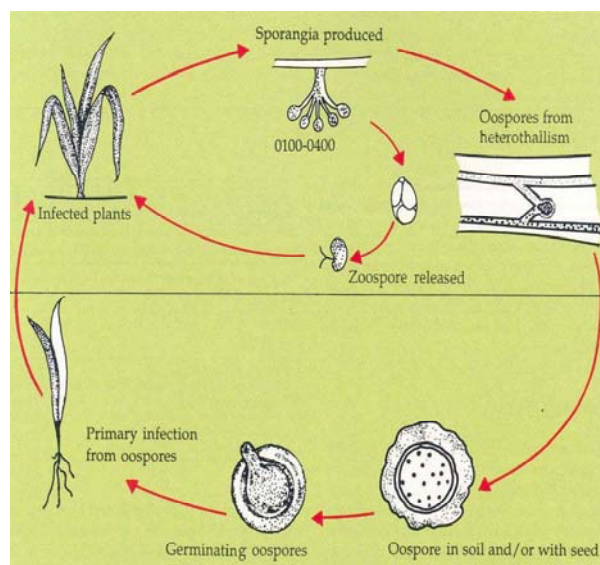


Figure 2. Disease cycle of pearl millet downy mildew.

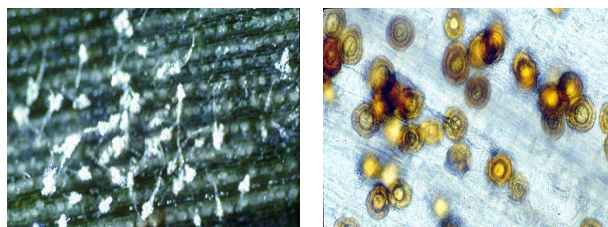


Figure 1. Sporangia (left) and oospores (right) of *Sclerospora graminicola*.



Figure 3. Symptoms of pearl millet downy mildew: infected seedlings (left) and green-ear panicle (right) .

Several *R* genes have been successfully employed through conventional breeding to confer near-complete resistance against specific races of pathogens in major crops (Hovmøller et al. 1997, McDonald and Linde 2003, Hovmøller 2007). The modern molecular work is based on this classical concept of gene-for-gene relationship. However, the major drawback of introgression of such *R* genes has been that they have been rendered non-functional when *Avr* genes mutate to virulent forms (McDonald and Linde 2003). The role of minor resistance genes and other trait genes, such as thick leaf cuticle genes, contributing to resistance cannot be ignored. Under natural ecosystems, the host-pathogen interaction phenomenon is not so simple and several other factors, such as weather variables and agronomic practices greatly influence the interaction and thus the resistance level of the cultivar.

Studies done at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and elsewhere have shown large pathogenic variability in *S. graminicola* populations from India and other countries (Ball 1983, Werder and Ball 1992, Thakur et al. 2002, 2004, Sivaramakrishnan et al. 2003). The pathogen is heterothallic, has rapid asexual generation cycles and can produce millions of spores in short time span. These characteristics enable the pathogen to produce new recombinants and rapid build up of mutants for adapting to the changing host resistance, chemical fungicide and other control methods.

Virulence diversity in *S. graminicola* is studied through a collaborative Pearl Millet Downy Mildew Virulence Nursery (PMDMVN) conducted under the Indian Council of Agricultural Research (ICAR)-ICRISAT partnership project. The nursery is grown annually at 10–12 locations of the All India Coordinated Pearl Millet Improvement (AICPMIP) centers in well-established DM nurseries. The PMDMVN consists of

40–50 entries, including putative differential lines and advanced breeding lines from ICRISAT and AICPMIP, and appropriate resistant and susceptible checks. Data are recorded twice on disease incidence, first at 30 days and second at 60 days after emergence, compiled, analyzed and the report is presented at the AICPMIP annual group meeting. The results of this multilocal testing provide useful information on virulence variability in the pathogen population and on the resistance stability of breeding lines under diverse environmental conditions. Lines showing stable resistance across environments (year × locations) can be useful for understanding the genetic basis of resistance (Thakur et al. 2004) as well as in resistance breeding. Such studies in the wheat-rust system have suggested that wheat genotypes that show stable resistance across many locations often contain multiple major or minor genes for resistance (Line and Chen 1995, Singh and Rajaram 2002). In addition to PMDMVN, information on new virulence is also obtained from the on-farm pearl millet surveys discussed below. Isolates (oospores) collected from PMDMVN locations, from a highly susceptible local land race and highly susceptible hybrids in farmers' fields are characterized and maintained at ICRISAT.

At present there are at least 11 diverse pathotypes (populations) of *S. graminicola* that have been identified (Table 1) from among 59 isolates from major pearl millet growing parts of India and were tested for pathogenicity and virulence on a set of putative host differentials in the greenhouse. These 11 pathotypes are being used selectively for screening breeding lines targeted for utilization in different pearl millet production zones of India.

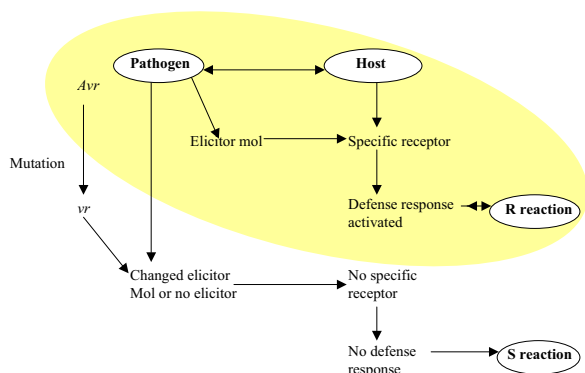


Figure 4. A conceptual gene-for-gene interaction between host and pathogen leading to resistant (R) and susceptible (S) reaction (Source: Flor 1971).

Table 1. Major virulent pathotypes of *Sclerospora graminicola* being used in greenhouse screening of pearl millet breeding lines at ICRISAT, Patancheru, India.

State	Location	Pathotype	Virulence index ¹
Rajasthan	Durgapura	Sg 212	7.99
	Jodhpur	Sg 139	6.20
	Barmer	Sg 384	14.38
Maharashtra	Jalna	Sg 150	4.31
	Ahmednagar	Sg 021	4.80
Gujarat	Jamnagar	Sg 200	4.92
	Banaskantha	Sg 445	16.46
Delhi	New Delhi	Sg 298	6.06
Haryana	Bhiwani	Sg 334	5.32
Andhra Pradesh	Patancheru	Sg 409	14.04
Karnataka	Mysore	Sg 048	11.25

1. Virulence index = Downy mildew incidence (%) × latent period⁻¹ (based on mean across 9 host differential lines).

Genetic diversity in isolates of *S. graminicola* has been reported using DNA fingerprinting (Sastry et al. 1995), and DNA markers, such as random amplified polymorphic DNAs (RAPDs) (Zahid 1997) and amplified fragment length polymorphism (AFLP) (Singru et al. 2003, Sivaramkrishnan et al. 2003, Pushpavathi et al. 2006). In a recent study (R Sharma, personal communication), using AFLP markers, 46 selected isolates from seven states in India were classified into seven distinct groups. Analysis of molecular variance indicated that variation in the *S. graminicola* populations was largely due to differences among the isolates within the states. In all these studies there were no correlations between virulence diversity and genetic diversity. Such genetic diversity studies will not be useful unless genes for avirulence and their markers are identified.

Availability, diversity and type of genetic resistance

Large numbers of germplasm accessions and breeding lines have been screened at ICRISAT and at the AICPMIP centers, and a number of resistant germplasm/lines have been identified (Singh 1995, Singh et al. 1997). Resistance stability of several of these lines, including P7 (ICML 12), SDN 503 (ICML 13), 700251 (ICML 14), 700516 (ICML 15), 700651 (ICML 16) and 7042R (ICML 22) has been confirmed through multilocal testing (Singh et al. 1994). Several other lines and germplasm accessions, including P 310-17, P1449-3, IP 18292, IP 18293, IP 18294, IP 18295 and IP 18298 with high levels of resistance have been identified (Singh et al. 1997, Thakur et al. 2004). Some of these sources have been strategically utilized to some extent in resistance breeding at ICRISAT and AICPMIP centers. Though these are useful sources of resistance, high levels of resistance have also been identified in many elite breeding lines and these have been used more extensively in breeding DM resistant hybrid parental lines in India. In general, there is enough geographical, morphological and genetic diversity in germplasm and breeding lines for DM resistance (Singh 1995, Singh et al. 1997).

Three types of resistance to DM – incomplete resistance (Singh et al. 1988), complete resistance (Singh 1995) and recovery resistance (Singh and King 1988) – have been reported in pearl millet. Incomplete resistance is usually polygenic, but could also be oligogenic. Genes governing this type of resistance confer incomplete resistance, exhibiting variable levels of dominance.

Most studies on genetics of DM resistance have shown resistance to be governed by major dominant genes with non-additive gene action (Deswal and Govila 1994, Singh and Talukdar 1998, Hash et al. 2003). Segregation

for host plant resistance has generally shown continuous variation (Singh et al. 1980, Basavaraju et al. 1981, Dass et al. 1984). However, there is clear evidence that the A₁ cytoplasm is not associated with susceptibility or resistance to pearl millet DM and that nuclear genes are involved in controlling the disease reaction (Anand Kumar et al. 1983, Yadav et al. 1993, Yadav 1996). Most of these studies used less defined resistant/susceptible lines and heterogeneous pathogen isolates. Both pearl millet and *S. graminicola* being allogamous and highly variable, and the disease measurement is taken on a continuous 0–100% scale, there is a greater possibility of identifying quantitative resistance with multiple genes involved. Several studies on molecular marker based genetic linkage maps for pearl millet have shown interesting results (Liu et al. 1994, Hash et al. 1995, Jones et al. 1995, 2002, Breese et al. 2002, Hash and Witcombe 2002) that will facilitate genetic manipulation of disease resistance. A number of quantitative trait loci (QTLs) for DM resistance have been identified on different linkage groups, and some of them are specific to different pathotypes (Hash et al. 1999, Hash and Witcombe 2001, Jones et al. 2002). DNA markers have been identified for about 60 different putative DM resistance QTLs in pearl millet (Breese et al. 2002, Hash and Witcombe 2002). Using RFLP-based marker-assisted backcross (MAB) method, several mapped QTLs have been transferred to backgrounds of elite inbred parental lines of a popular single-cross hybrid HHB 67 (843A × H 77/833-2).

It would be highly useful to strengthen research efforts on understanding the genetic nature of resistance and effectiveness of specific QTLs in different resistant lines for their effective utilization in resistance breeding.

Screening method and selection environment

Two types of screening methods are used: field screening and greenhouse screening. Both these screening methods are well established to identify and select resistance. Field screening is based on: (a) the disease sick-plot that provides primary inoculum as oospores in the soil on plant debris, (b) the use of infector rows of a highly susceptible cultivar that provides sporangia as secondary inoculum for the test lines, and (c) provision of perfo-irrigation system to create high humidity (>80% RH) and leaf wetness necessary for infection and disease development. This method has been refined over time and it is quite effective under proper management conditions (Fig. 5). Prevalence of high humidity (>80% RH), leaf wetness and moderate temperature (25–30°C) during the first 2–4 weeks of crop growth is critical for infection, disease development and disease spread. The nursery can be operated on a large scale both during the

rainy and postrainy seasons at locations in southern states of India because of the prevailing moderate temperature, if managed properly. However, in northern and western India, where average winter temperatures are low (<15°C), the disease development is adversely affected and the screening cannot be successful.

Greenhouse screening method, in contrast to field screening, can be operated throughout the year. However, at ICRISAT, Patancheru it is not used for 1–2 months during the hot summer (May–June) and severe winter (December–January) on account of heavy electricity cost to maintain the optimal temperature of 25°C. High humidity is maintained by a fogging system in the greenhouse that operates for 15 min at 30-min intervals by automated timer connected to a high-pressure pump (3hp). The entire process of pathotype maintenance, inoculum multiplication, inoculation and incubation has been well refined at ICRISAT (Fig. 6) and it has been quite effective in screening large number of breeding lines in a short time period (Singh et al. 1993, Thakur et al. 2006). About 7000 breeding lines are screened every year against diverse pathotypes. A large number of hybrid parental lines are also screened against specific single or multiple pathotypes (Tables 2 and 3). Major advantages of greenhouse screening include: independent of season, precise inoculation (with little chance of escape of seedling), rapid results (takes two weeks between inoculation and data recording), screening against multiple pathotypes at one place, highly reproducible and thus reliable, cost-effective and easy selection for resistance.

Downy mildew being a highly weather-sensitive disease, microenvironment in the field screening greatly influences the disease development. Despite adequate care and management of the field nursery, disease incidence is often quite variable across years/seasons and locations. However, the field screening provides important information on the general resistance levels of the lines against the highly heterogeneous pathogen population. In a field screening at a particular location, the known highly susceptible and highly resistant lines

generally show disease incidence true to their types, but other lines that are moderately resistant or susceptible provide more variable results and these could not be very well compared with the greenhouse incidence data. Field screening, in contrast to greenhouse screening, provide better opportunity for selection of greater number of resistant plants (to a natural population of the pathogen with moderate inoculum load) that can be selfed and seed obtained in the same season. Greenhouse screening, on the other hand, is more severe with high inoculum pressure, useful to screen large number of lines and discard susceptible lines/plants in a most economical and effective way. Resistant plants from greenhouse screen can also be transplanted in field/pots for generation advance.

A large number of hybrid parental lines and progenies are screened against single or multiple pathotypes in greenhouse and resistant selections are made. In some cases, resistant seedlings from the screening pots are transplanted in the field for advancing generation. During the past few years a number of parental lines with

Table 2. Evaluation of ICRISAT’s pearl millet breeding lines (A-/B-/R-lines) against diverse pathotypes of *Sclerospora graminicola* in greenhouse at ICRISAT, Patancheru, India, 2003–2007.

Pathotype	Total lines screened	No. of lines resistant (≤10% downy mildew incidence)
Jodhpur (Sg 139)	3413	1928 (56) ¹
Durgapura (Sg 212)	10212	4373 (43)
Jalna (Sg 150)	3595	1494 (42)
Jamnagar (Sg 200)	151	92 (61)
Patancheru (Sg 409)	320	149 (47)
Banaskantha (Sg 445)	702	216 (31)
Barmer (Sg 384)	53	20 (38)
New Delhi (Sg 298)	32	27 (84)
Total	18478	8299 (45)

1. Figures in parentheses are percentage values.



Figure 5. Downy mildew field screening system using infector rows with provision of perfo-irrigation at ICRISAT.



Figure 6. Downy mildew greenhouse screening system using fogging system at ICRISAT.

resistance to single and multiple pathotypes have been identified (Thakur et al. 2001, Rai et al. 2006). The lines showing resistance ($\leq 10\%$ disease incidence) to at least two pathotypes are designated and disseminated as seed parents. This breeding strategy has led to the development of more than 125 A-lines, most of which have been resistant to 2–5 pathotypes in the year they were designated and disseminated.

Breeding methods

Both conventional and molecular breeding methods have successfully been used in DM resistance breeding program (Hash et al. 1999, Hash and Witcombe 2002). The conventional breeding has mostly used pedigree selection for developing hybrid parental lines and recurrent selection for population improvement. In pedigree breeding, ecoregion-specific progenies selected for desirable agronomic traits, and grain and fodder yields are tested for resistance to the ecoregion-specific pathotypes. During inbreeding selection-generation advance, at least at two inbreeding stages, DM screening for resistance to specific pathotypes is done under greenhouse conditions at ICRISAT. In the molecular breeding program MAB method was used for transferring DM resistant QTLs into the hybrid parental lines (Hash and Witcombe 2001). Several DM resistant lines, such as IP 18292, 7042R and 700651 have been used in developing hybrid parental lines. A number of DM resistant QTLs effective against diverse Indian pathotypes of *S. graminicola* have been mapped on the pearl millet linkage groups and some of them have been transferred to the commercial B-lines (843B, 81B) and R-lines (H 77/833-2, ICMP 451). Development and commercial deployment of DM resistant version of HHB 67 is the first successful story of MAB in field crops in

public domain in India (Hash et al. 2006). We believe that both pedigree and MAB breeding methods have been quite effective in transferring DM resistance in advanced breeding lines and should be followed without much problem.

Marker-assisted breeding is currently not cost-effective, limiting its application selectively on commercial hybrid parents in few cases. But as more cost-effective tools are developed it could be increasingly used in the future. Instead of taking pedigree-derived progenies it may be much effective to begin DM screening from F_2 itself, if DM resistance breeding is the primary focus.

Use of doubled-haploid breeding technology (Thomas et al. 2003) could be useful to study inheritance of resistance and genetic diversity in hybrid parental lines and in development of pathotype-specific DM resistant inbreds in a short time and in gene pyramiding. It may serve as useful tool for marker-assisted selection (MAS) as well. Recently, CIMMYT (Centro Internacional de Mejoramiento del Maíz y del Trigo) has begun using this technology to develop drought tolerant inbred lines for tropical maize (*Zea mays*) for sub-Saharan Africa (<http://www.cimmyt.org/english/wps/news/2008/may/doubledHaploids.htm>) and several private seed companies, including Great Lakes Hybrids, a division of AgReliant Genetics LLC, are producing doubled-haploid corn hybrids with specific traits, such as disease resistance (<http://www.greatlakeshybrid.com/performance/research-information/doubled-haploid-breeding-technology/>). It promises to reduce costs and enhance effectiveness of producing better-adapted cultivars in a short time. The technology has also shown promise in other crops and thus can be tried in pearl millet as well. Since resistance breakdown is very fast in pearl millet and hybrid replacement rate is also rapid (Thakur et al. 2006), our goal should be to develop more resistant lines in shortest possible time. While MAS can help in improving existing

Table 3. ICRISAT's pearl millet breeding lines showing resistance to multiple pathotypes of *Sclerospora graminicola* in greenhouse screening at ICRISAT, Patancheru, India, 2003–2007.

Material	Total lines screened	No. of lines resistant ($\leq 10\%$ downy mildew incidence) to pathotypes ¹							
		JDP	JAL	PAT	JAM	DUR	NDL	BAN	BAR
2003 series of B-lines	17	10	13	10	9	11	NA ²	NA	NA
2004 series of B-lines	29	17	6	8	16	14	NA	NA	NA
2006 series of B-lines	31	18	16	12	15	11	NA	NA	NA
2007 series of B-lines	32	22	24	NA	21	21	27	NA	NA
Large-seeded B-lines progenies	14	13	0	14	14	14	NA	NA	NA
Proposed R-lines series-2007	27	14	17	14	13	11	NA	NA	NA
R-lines for making R-line Comp	107	NA	44	NA	40	40	NA	38	20

1. Pathotypes: JDP = Jodhpur; JAL = Jalna; PAT = Patancheru; JAM = Jamnagar; DUR = Durgapura; NDL = New Delhi; BAN = Banaskantha; BAR = Barmer.

2. NA = Data not available.

lines, doubled-haploid technology can consistently deliver new resistant lines in a short time.

Utilization and deployment of resistance

A common strategy of resistance breeding utilizing major genes involves: an effective screening method, availability of diverse germplasm, confirmed sources of resistance, knowledge of genetics of resistance, information on variability in virulence, effective utilization of resistance in breeding, and deployment and on-farm monitoring of performance of cultivar (Fig. 7). Genetic diversity and durability should be the main features of a resistance breeding program. These features are well represented in OPVs (WC-C-75, ICTP 8203, Raj 171) as they have shown resistance to DM on a large area for more than 20 years. However, the same is not true with hybrid cultivars. During the past 30 years, a number of DM resistant lines have been used in resistance breeding programs and some of the resultant hybrids have been commercially successful. There has been substantial progress in managing the risk of losses caused by DM epidemic during the past 15 years by diversifying the hybrid cultivars base, monitoring virulence, screening breeding lines to diverse pathotypes and breeding DM resistant hybrid parental lines at ICRISAT that are utilized by private and public organizations for developing hybrids. A study by Mahala et al. (2004) showed that more than 80 hybrids (by name) developed by public and private organizations were being grown by farmers in India.

There is no systematic and well-organized resistance breeding program in operation so far at most of the Indian national research centers. The major limitations include: (a) well-established DM sick plots; and (b) greenhouse screening facilities. In addition, there is lack of information on well-defined genetic resistance – R-genes/QTLs, utilization/introgression of specific R-

genes/QTLs and breakdown of specific resistance (R-gene). These aspects have to be addressed by creating research facilities and planning research to generate the information required for proper screening, monitoring virulence and developing a sound science-based DM resistance breeding program.

ICRISAT provides large number of diverse hybrid parental lines to private/public organizations in response to their selections during field days. These parental lines have information on resistance to as many as five diverse pathotypes, but also need to be evaluated against other pathotypes, if required. While utilizing these lines the organizations should keep record of specific DM resistance and monitor its heritability in the hybrids. Such hybrids could be screened for DM resistance to specific pathotypes in greenhouse and in the DM nursery at key locations before their release and commercialization. Proper monitoring of these hybrids in farmers' fields should be taken up for their resistance stability. There is also a need to strengthen the DM resistance breeding specific to pearl millet adaptation zones provided the existing zones serve the purpose for hybrid breeding programs.

Monitoring virulence/resistance

Virulence/resistance monitoring is done by a well planned on-farm survey in major pearl millet growing states of India (Thakur et al. 2003). During the past 10 years more than 3600 fields of at least 72 hybrids (by name) in 47 districts of 5 states [Maharashtra, Rajasthan, Gujarat (summer and rainy), Haryana and Uttar Pradesh] have been surveyed. Of these 44% of the fields were infected with DM incidence ranging from 1 to 100%. The results of the surveys have been regularly shared during the AICPMIP annual group meetings. These results have benefited the pearl millet researchers in monitoring resistance levels of their hybrids and planning their resistance breeding programs accordingly. This activity is very critical and needs strengthening through enhanced scientific and financial resources. The short growing period of the crop and appropriate time of DM observation (30- to 40-day-old crop) and a single survey team put limitation on the areas to be covered during the crop season.

An important finding from the surveys was that most of the seed supplied to farmers by private seed companies were treated with the fungicide metalaxyl (Ridomil/ Apron). The frequency of treated seed supply has increased over the past 5 years. This is a matter of great concern in relation to likely evolution of new pathotypes with higher virulence. The results have indicated that despite fungicide treatments, susceptible hybrids have recorded very high DM incidence (>80%) in some fields.

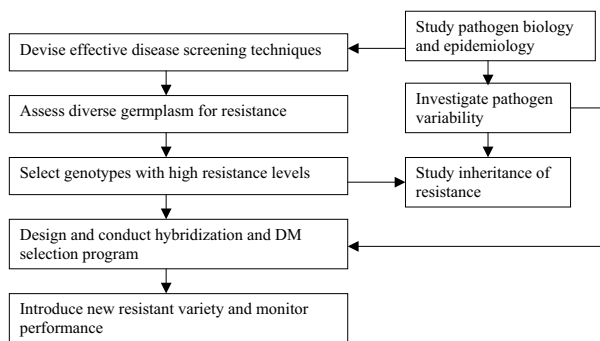


Figure 7. A generalized protocol for downy mildew (DM) disease resistance breeding.

This results in double losses both to seed companies and to farmers. There is a strong need for better understanding the DM pathosystem and develop a strategy to make the fungicide treatment more cost-effective. It is well known that fungicide seed treatment protects the crop only up to 35–40 days, and later the disease appears on the nodal tillers and on panicle on a susceptible hybrid. In case of moderately resistant (10–20% DM incidence) hybrids the fungicide treatment is more effective than in susceptible hybrids. The use of fungicide should be considered as a stop-gap arrangement for the replacement of moderately resistant hybrids, and the susceptible hybrids must not be

grown at all even with fungicide treatment. This strategy would greatly help in prolonging the commercial life of some popular hybrids and reduce the chances of evolution of new virulence in the pathogen population.

The survey results also indicated that seed supplied to farmers were from 1- to 2-year-old stocks and thus the treatment was too old to be effective in controlling DM in the field. Research has shown differential cultivar responses to metalaxyl treatment (Singh and Shetty 1990). In certain cultivars, metalaxyl treated seed when exposed at 40°C for 30 days loses its effectiveness and storage beyond 60 days prevented germination.

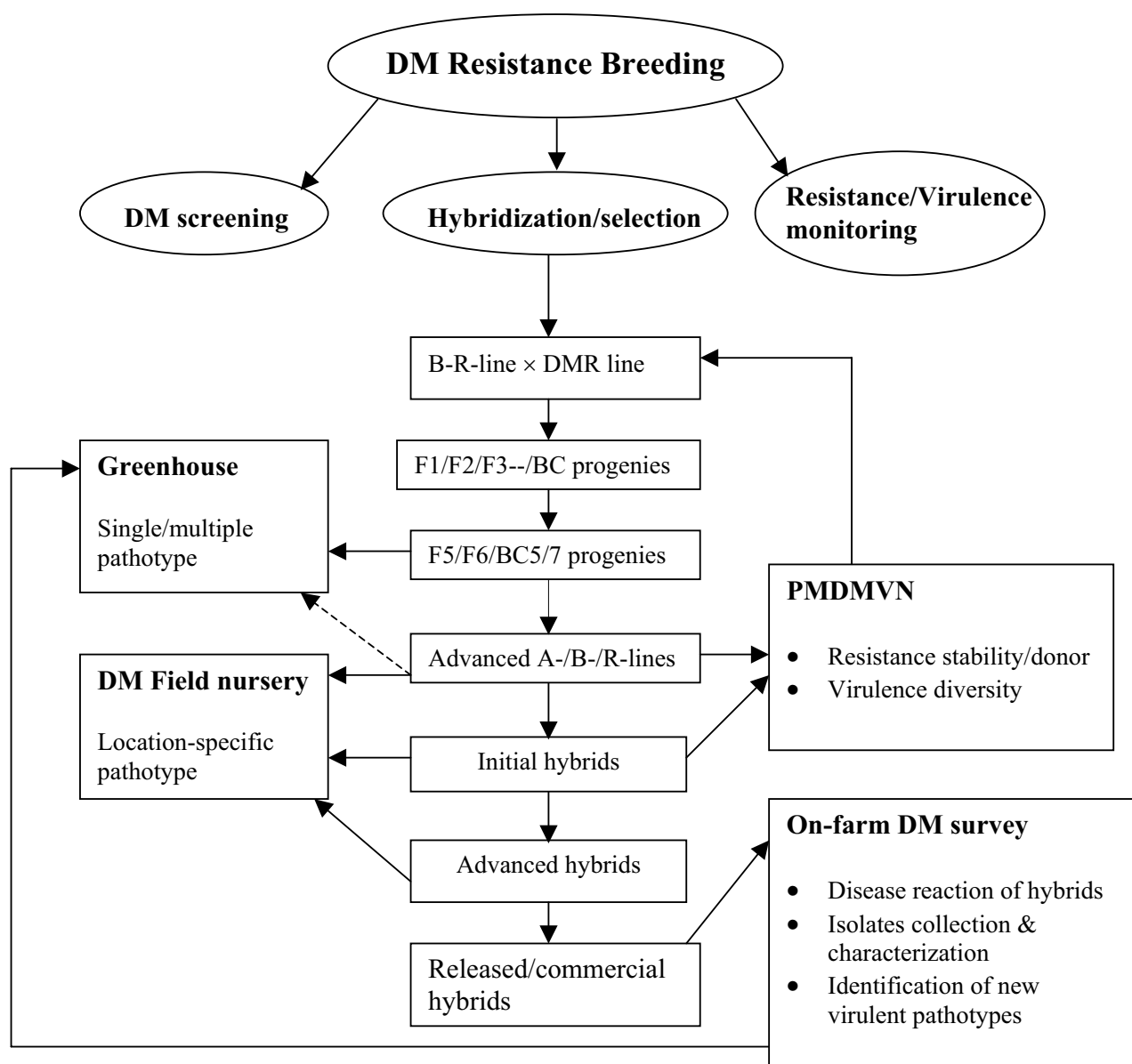


Figure 8. Proposed protocol for downy mildew (DM) resistance breeding in pearl millet.

Conclusions

Considerable progress has been made in understanding host-pathogen interaction, refining disease screening methods, identifying and utilizing resistant sources, and breeding DM resistant parental lines and hybrids. However, the disease is still a major challenge towards realizing the high yield potential of hybrids. Some of the research and development issues that need attention in different time frames can be divided into the following groups. In a short term of 1 to 3 years the focus should be to: (i) develop well-managed DM nurseries at key locations in each of the hybrid-intensive states under different adaptation zones (A1, A and B); (ii) develop greenhouse screening facilities at 2–3 locations; (iii) conduct well-organized on-farm survey, involving pathologists and breeders, one team in each zone during the rainy season crop, and a single team during the summer every year; and (iv) minimize the use of fungicide (metalaxyl) for seed treatment. In a medium term of 1–5 years it would be important to: (i) regularly replace the existing pathotypes with new more virulent pathotypes as they occur in different zones/states for greenhouse screening; (ii) screen breeding lines against representative pathotypes from each zone (2 pathotypes/zone); (iii) designate hybrid parental lines for resistance to specific DM pathotypes; (iv) evaluate hybrids and parental lines to specific pathotypes in greenhouse and DM nurseries prior to release and commercialization; and (v) provide timely information/feedback among the members on resistance performance of hybrids/parental lines. On a medium to long term of 3–8 years, attention must focus on: (i) identification of DM resistance genes/QTLs against specific pathotypes; (ii) development of genetically diverse and DM resistant parental lines; (iii) development of near-isogenic lines as host-differentials; and (iv) identification of genetic markers for avirulence.

Based on the above issues, we propose a protocol of DM resistance breeding in pearl millet (Fig. 8) for further discussion and refinement. We believe that with cooperation of the organizations involved in DM resistance breeding, we should be able to address the above issues towards developing an efficient and long-term DM resistance breeding strategy that would help realize and sustain the high yield productivity of pearl millet hybrids in India and contribute to global food security.

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