

Downy mildew of Pearl millet

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Abstract

Background: Downy mildew disease caused by *Sclerospora graminicola* is a very destructive disease of pearl millet. The disease is known to occur in Asia and Africa wherever pearl millet is grown as a food and fodder crop.

Challenges: Current status of knowledge about the symptoms of disease, pathogen variability, epidemiology and disease management along with disease resistance has been discussed in detail. Recent approaches of disease management through biological control of the disease have been discussed at length with particular emphasis on the use of BABA, cerebroside, enzymes, vitamins, botanicals and raw cow's milk with *Gliocladium virens*.

Conclusion: Advances made in the field of developing disease resistant varieties using various breeding strategies and molecular approaches have also been discussed in detail.

Introduction

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is an important cereal and forage crop of arid and subtropical regions of Indian subcontinent and several African regions. It also has great potential in the temperate zones as a warm season crop. It is believed that it was domesticated for not less than 3000 years ago as a forage or cereal crop in Africa. India is considered to be the secondary center of diversity². Pearl millet is predominantly a rainy season crop able to thrive well in the rainfall as low as 250 mm on relatively poor soils. Being most tolerant to drought and salinity the crop is largely grown in different countries of the world such as India, Nigeria, Chad, Tanzania, Mali, Niger, Ethiopia, China and Russia. Areas planted with pearl millet are estimated as 15 million hectares annually in Africa and 14 million hectares in Asia. India alone accounts for 10.3 million hectares with a total annual production of 7 million tones⁸⁰.

In India the major pearl millet cultivating states include Rajasthan, Gujarat, Maharashtra and Uttar Pradesh. Pearl millet is also known by several different common names including bajra in Hindi, bulrush, spiked millet and cattail in English, mil chandelles in Arabic and dukhn in French.

Downy mildew (DM) or 'green ear' disease caused by *Sclerospora graminicola* (Sacc.) Schroet. occurs destructively wherever pearl millet is grown⁵⁴⁶⁵⁶⁸⁰. Pearl millet downy mildew was first reported by Butler¹⁷ in India and described it the disease of ill-drained lands where it developed into epidemics of severity. In India, the pathogen is present in all the states where pearl millet is cultivated. Other published reports include detection in the temperate and tropical areas of the world, especially in Africa, Europe, China, Japan, Australia and various other countries of the world (Table 1). However, there is no report of its occurrence in the western hemisphere⁴³⁸⁶. Downy mildew is most widely distributed in all major pearl millet producing areas of Asia and Africa. The disease was considered with minor importance till 1970, as its incidence was sporadic on local cultivars. The first epidemic of downy mildew occurred in 1971 on the first popular hybrid HB 3 resulting in severe grain loss of about 4.6 million metric tons⁸⁰⁸⁶. Because of continued large-scale cultivation of the susceptible hybrids the disease caused serious epidemics during 1974, 1984, 1987 and 1988. Availability of data on grain loss is incomplete due to the greater variability from field to field, from farmer to farmer, and from season to season. On the basis of a few localized estimates in India the average annual yield losses can reach up to 40%, whereas 10-50% losses have been reported from Nigeria, however, worldwide annual grain yield losses may not exceed 20%⁵⁴¹¹³⁸⁷³². Information on extent of losses caused by Downy Mildew (DM) in pearl millet is rather scanty. Gupta and Singh²⁷ estimated the losses on pearl millet cv. HB 3. They observed that severe and systemic infection reduced fresh weight of the shoot along with the number of basal and nodal tillers. At 62% incidence 34% yield loss was recorded over the healthy crop.

Continent	Country	Reference
<i>Africa</i>	Chad, Egypt, Gambia, Malawi, Mozambique, Niger, Nigeria, Senegal, SouthAfrica, Zimbabwe, Burkina Faso, Ghana, Ivory Coast, Kenya, Mali, Sudan, Tanzania, Togo, Uganda, Zambia	Nane and Singh, 1976; Singh <i>et al.</i> , 1993, Singh, 1995, Wilson, 1999.
<i>Asia</i>	India, Pakistan, Israel	

Table1. Geographical distribution of *Sclerospora graminicola* on pearl millet.

In India the high yielding single cross F₁ hybrid cultivars based on an A₁ Cytoplasmic-nuclear Male-Sterility (CMS) having good tillering ability and a large number of compact, well filled ear heads were introduced in mid-sixties but the desired production potential could not be attained due to the attack of downy mildew pathogen⁶⁸⁰¹⁰³. In a few states of India yield losses caused by re-occurrence of DM in farmers' fields in last 40 years, led to the withdrawal of even promising cultivars that succumbed to the disease⁷⁹⁸⁹⁹⁰. Since all the earlier hybrids had A₁ cytoplasm as source of male sterility, it was a vacillation that probably this cytoplasm is the cause of downy mildew susceptibility. However, studies have confirmed that cytoplasm is not the cause, as different Male Sterile (MS) lines bred in same cytoplasm have been found to be resistant, hence MS lines with different genetic make up and resistance to downy mildew can be bred in the A₁ cytoplasm. Hybrids affected with more than 20% mean DM incidence have been proposed for their consideration toward withdrawal in a recently concluded on-farm DM surveys conducted during 1994-2004 in four states of India⁶⁴¹⁰⁶. They have further stated that despite a progressive increase in the release of pearl millet hybrids, no widespread epidemics of the disease have occurred in the past 10 years. Information on the disease in the form of published reviews is available⁶⁵⁴¹¹³⁸⁰⁸⁶⁸⁷. The present paper thus outlines the recent advances made on this disease during the past one decade.

Disease Symptoms

In this disease two types of symptoms are produced viz: 'downy mildew' and 'green ear'. Symptoms often vary according to the host, time of their expression and ambient conditions⁴³. The variation in symptom may occur due to systemic infection. Downy mildew infected plants develop severe disease syndrome from the very beginning and succumb even before reaching maturity. Reduction in the plant height, number of leaves and nodes are commonly observed in susceptible cultivars resulting in reduced grain and fodder yields. Changes in morphological characters in susceptible pearl millet due to downy mildew resulted in reduction of grain and fodder yields.

The disease normally appears in the form of chlorosis at the base of infected leaf followed by production of sporulation on the lower side of leaves known as the 'half-leaf' symptom. Abundant white asexual sporulation on the lower leaf surface of infected chlorotic leaf produce white downy growth under high relative humidity (>95%) and moderate temperature (20-22^o C). Subsequently the leaves turn reddish brown due to oospore production and dry ultimately. In acute cases shredding of leaves occur. Severely infected plants (up to 60%) are generally stunted because of root retardation and do not produce panicles.

At the time of panicle emergence green ear symptoms become visible. Symptoms appear on ear head with all possible degrees of proliferations and malformations. In malformation the florets are converted into leafy structures of diverse appearance (sometimes also referred as virescence). The leafy structures are chlorotic, and sometimes produce sporulation. Primary source of infection arrive from soil and systemically infect seeds. The invasion of the fungus in floral primordia plays a crucial role in deciding the extent of malformation. Generally four types of malformations have been observed: (a) the entire inflorescence is transformed into a green leafy tuft, which is symbolically referred as 'green ear'; (b) lower half of the ear-head is proliferated and the upper half contains normal flowers; (c) only bristles become long and no malformed leafy structures are formed, and (d) leafy tufts or frenching where the shoots remain stunted and produce leafy tufts at the top. In latent infections, green ear is the only manifestation of the disease. Kumar and Bhansali (unpublished) found that in all the diseased plants, as a rule, normal ears when outgrow the disease at primary shoots produce green ear followed by tufting and yellowing of leaves on the secondary shoots in successive downward order. This shows that leaf yellowing is an expression of the highest susceptibility, followed by tufting and green ear, and prevalence of some sort of disease escape mechanism in the plants.

Manifestation of changes in the infected flowers:

The bristles subtending the spikelets and all its parts: the glumes, palea, pistil and stamens are affected due to the disease and present varied pattern of suppression, proliferation and malformation. As a result of these changes the ears appear green and conspicuous. The stamens and pistil are generally transformed into leafy structures although sometimes all parts may be transformed. The pistil is most commonly affected and is hypertrophied into a long (5.0 to 7.5 cm), thick, curled (on drying) structures, while the stamens remain normal. The stamens are also transformed into varied proportions and turn leafy in structure. Occasionally the glumes are hypertrophied and grow larger than other floral parts.

Pathogen Description

There is no disagreement on the taxonomy of the downy mildew causing fungus. *Sclerospora* is now monotypic, the sole representative of Ito's *Eusclerospora* group. The very name *Sclerospora* is drawn from the thick-walled oospore with its dark-walled exosporium and adherent oogonial envelope. It appears that there are remote chances of a change in the name of *Sclerospora graminicola*⁵⁴⁹⁵. Phylogenetic relationship among sequences from Indian and African samples of *S. graminicola* was examined using a nested PCR technology by Viswanathan¹¹². She stated that all the samples from India, Mali, Nigeria and Niger, with the exception of Niger 4, formed a monophyletic group with the Oomycetes (*Peronospora*, *Phytophthora* and *Pythium*). Diagnosis of the fungus is based on two types of spores produced by *S. graminicola*. Asexual spores (sporangia, zoospores) are thin walled, hyaline, ellipsoid or elliptic and papillate measuring 15-22 x 12-21 μ m. Under natural conditions sporangia are produced in abundance during the night (03.00 h). Normal temperature (20 to 25^oC) and high relative humidity

(95-100% RH) favour sporangial production⁸⁶³⁰. *Sporangia* are actively ejected and germinate immediately, producing zoospores, or else die within a few hours. Under suitable conditions *sporangia* form in great profusion on the under surface of the diseased leaf (and, when conditions are favourable, also on the upper surface) forming a conspicuous and characteristic white 'downy' growth²³.

Oospores are the sexual spores. It has been observed that oospores can survive for 14 years under laboratory conditions but their viability is reduced after 4 years of storage. Different types of germination of *S. graminicola* oospores have been observed, which included germination by vesicle-like structures, by both vesicles and germ tubes, by typical irregular structures different from germ tubes and vesicles, by germ tubes and germination by extrusion of small round bodies/sporangia-like structures⁴⁶. Singh and Navi⁸² have reported that the optimum temperature for oospore germination was 28±2°C. Germination was highest when oospore treatment lasted for 24 h in sterile distilled water.

They found that dry oospores were more resistant to steam sterilization than wet oospores. To ensure the total destruction of oospores from soil, sterilization of wet soil for more than 2 h is needed. Oospore density in the soil was highly correlated with disease incidence at 90 days after sowing indicating the important role of oospores in disease epidemiology²⁵. The oospores play an important role as far as transmission of the disease is concerned. After sowing unsterilized seeds in sterilized soil 23.4% disease incidence was observed. It, therefore, suggests that seed borne spores are important in inciting downy mildew⁵¹⁷². However, no significant correlation was recorded between the mean incidence of downy mildew and oospore production⁶⁵.

Variability in pathogenesis

Sclerospora graminicola reproduces asexually by means of sporangia and sexually through oospores. The fungus is largely heterothallic with two mating types. These characteristics of the fungus make it highly variable and adaptable to diverse environmental conditions. Similarly, its host pearl millet is a highly outcrossing crop species. The information generated for the past few years on genetics of resistance, availability of host differentials, and development of molecular techniques, has made it easy to understand *S. graminicola*– pearl millet interactions.

Single cross F₁ hybrids have greatly contributed to increasing productivity of pearl millet. Early maturity, uniform crop stand and high harvest index of these hybrids have made them popular among the farmers. As a result hybrid cultivars cover about 55% of the total 10 million ha area under pearl millet in India with the cultivation of around 50 hybrids⁹⁸¹⁰³. By the time several races or pathotypes of *S. graminicola* have evolved in India. Stability of resistance in pearl millet lines developed at ICRISAT was studied through a collaborative International Pearl Millet Downy Mildew Virulence Nursery (IPMDMVN). The reactions to downy mildew of 11 pearl millet lines at 17 locations in India, Burkina Faso, Mali, Niger, and Nigeria from 1995 to 1999 were recorded^{88101103 104107}. Seven pearl millet lines (IP 18292, IP 18293, 700651, P310-17, P7-4, MBH 110 and 852B) provided differential reactions permitting classification of the 23 populations into 15 putative pathotypes at the global level (Table 2). Interestingly, the existence of a highly virulent pathotype of *S. graminicola* is reported from Jodhpur, Rajasthan, India¹⁰⁴.

Location/pathogen group	Host differential line								Putative pathotype
	IP 18292	IP 18293	700651	P310-17	P7-4	MBH 110	852B		
Madhav	R	R	R	R	R	R	R	R	I
Parasagar	R	R	R	R	R	R	R	R	
Cardinal	R	R	R	R	R	R	R	R	II
Shree	R	R	R	R	R	R	R	R	
Lakshmi	R	R	R	R	R	R	R	R	III
Kandhari	S	S	R	S	S	S	S	S	
Paragangra	S	S	R	S	S	S	S	S	III
Padma	S	S	R	S	S	S	S	S	
Kyree	R	R	R	R	R	R	R	R	IV
Pusa	R	R	R	R	R	R	R	R	
Shree	R	R	R	R	R	R	R	R	V
Shree	S	S	S	S	S	S	S	S	
Shree	S	S	S	S	S	S	S	S	V
Shree	S	S	S	S	S	S	S	S	
Parasachari	R	R	S	R	S	R	R	R	VII
Shree	R	R	R	S	R	R	R	S	VIII
Shree	R	R	R	R	R	R	R	R	VIII
Shree	R	R	R	R	R	R	R	R	IX
Shree	R	R	S	R	R	R	R	S	X
Shree	S	R	R	R	R	R	R	X	XI
Shree	R	S	R	S	R	S	S	S	XII
Shree	S	R	R	R	R	R	R	R	XIII
Shree	S	R	S	S	S	S	S	S	XIV
Shree	R	R	R	R	R	R	R	S	XV

Table 2. Populations of *S. graminicola* from 23 locations in seven countries identified into 15 putative pathotypes on seven host differential lines, based on field testing during 1992 to 1999.

Molecular aspects of pathogen variability

Various molecular methods have been used to study the mechanism of virulence and variability. Sivaramakrishnar⁹³ have reported pathogenic and genetic diversities among 15 isolates of *S. graminicola* on various differential lines of pearl millet cultivars in India. Based on mean disease incidence data they classified the 15 isolates into five major virulence groups. Using a specific set of primers designed for the conserved region they detected the presence of retrotransposable elements in the genome by amplification of part of the reverse transcriptase gene in a PCR reaction. Singru⁹¹ have studied a characteristic of retrotransposon activation responsible for mutation of the organism's genes. Accordingly, the DM fungus actively expresses the P5-like sequences in the host and the element is suppressed in a resistant host. Genomic library of the Path-6, a virulent isolate

with P5 retroelement, has been prepared, which belongs to the copia-like retrotransposon family. The library clones, designated as SgP5-1, SgP5-2, SgP5-3 and SgP5-4 were found to be distributed invariantly among genomes of five host-genotype-specific downy mildew pathotypes. A genomic library of this pathotype was constructed in the lambda gt11 vector. Repetitive DNA content was estimated as 8% based on colony hybridization of total fungal DNA with a genomic library. Digestion with isoschizomeric methylation-sensitive restriction enzymes revealed partial methylation of GATC and CCGG sequences in the genome. The presence of retrotransposable elements in the genome was detected by amplification of part of the reverse transcriptase gene in a PCR reaction, using a specific set of primers designed for the conserved region⁶⁸. By these methods genetic variability in host genotype-specific pathotypes of the pearl millet downy mildew pathogen have been studied at the molecular level. Micro-satellites (GAA) 61, (GACA) 4 and especially (GATA) 4 were quite informative and showed high levels of polymorphism among the pathotypes. The six pathotypes could be classified into five groups based on the cluster analysis of their genetic similarities, thereby confirming the existence of distinct host genotype-specific virulence in *S. graminicola* pathotypes. AFLP technique has been used for the detection of genetic variation in mildew isolates, to screen breeding material for the identification of resistant millet and monitoring changes in *S. graminicola* in relation to changes in host for effective disease management. AFLP technique was used to detect the extent of genomic variation among 19 fungal isolates from different cultivars of pearl millet grown in various regions of India. Fourteen AFLP primer combinations produced 184 polymorphic bands. An unweighted pair-group method of averages cluster analysis represented by dendrogram and principal coordinate analysis separated the mildew collections into four distinct groups⁹².

Environment and Agronomy

Temperature, humidity, rainfall, cloudiness and intensity of radiation are important environmental factors that influence the development and spread of disease. Apart from the host, edaphic, biotic and environmental factors influence the disease cycle of the pathogen. Studying the role of these factors in DM development Gupta and Singh²⁹ have stated that a pH value of 8.5 allowed disease to develop the most whereas the lowest disease incidence was recorded at pH value 7.5. Higher soil bulk density and moisture content did not favour the disease development. Addition of organic matter (farm yard manure) discouraged the development of DM. The addition of farmyard manure to soil or the addition of *Rhizobium*, *Azospirillum* or *Azotobacter inocula* as combined seed and soil treatment also reduced the disease with the best effects being from a cluster bean (*Cyamopsis tetragonoloba*) isolate of *Rhizobium* and from *Azotobacter chroococcum*. The direction of sowing also has a marked influence on the response of DM. Under arid environment around 40% less DM was observed in NW-SE direction sown crop as compared to N-S sown crop²⁸. Other important observations showed that the earliest symptom expression and the highest downy mildew were recorded when oospore powder was placed below and above the seeds in furrows. Incorporation of oospores over the seed in furrows gave better results than the present practice of pre-sowing furrow application of oospores below the seed for field screening of pearl millet cultivars for downy mildew resistance. The amount of oospore powder applied was correlated positively with disease incidence and negatively with the time taken to express disease symptoms. The correlation of interval between oospore application and sowing was negative with time taken to express disease symptoms and positive with disease incidence. A high negative correlation between the age of seedlings at the time of sporangial inoculation and disease incidence was observed. Barring sparse production of sporangia in the daytime, maximum production was observed at 03.00 h. The sporulation period in infected plants varied from 3 to 20 days³⁰.

Downy mildew and Tissue culture

There are many potential advantages in using tissue culture techniques to understand obligate nature of the parasite (*S. graminicola*). The inciting fungal organisms are cultured together with their host in controlled chemical and physical environment. Technique provides the attractive possibilities of a simplified experimental system for investigating the structure and physiology of host-parasite interaction. It also allows close scrutiny of pathogen, their products with an insight to the mechanism of resistance at cellular level.

Dual culture of *S. graminicola*

Downy mildew fungus has been successfully cultured on medium with no evident loss of fructifications. *S. graminicola* was first grown on host callus tissue and subsequently on a modification of White's basal medium contained casein hydrolysate, 2,4-dichlorophenoxyacetic acid (2,4-D) and kinetin¹⁰⁸¹⁰⁹. Dual cultures of host and fungal biotroph provide a controlled system to maintain isolates of pathogen and to study the interaction between host and pathogen. A few attempts have also been made to establish dual cultures using infected shoot tip and inflorescence axes or artificially inoculating healthy calli obtained from pearl millet seeds with *S. graminicola* sporangia^{714, 111}. Dual cultures can easily be established from malformed florets of pearl millet infected with *S. graminicola*. Malformed floral tissues usually produce more callus (86%) with fungus than shoot tips (25%). Fungal mycelium covers the entire surface of the callus within 30 days of placement of explants on the MS medium with 2 mg of 2,4-D. The infected calli also differentiate and produce plantlets when transferred to MS medium without 2,4-D¹¹¹.

Disease resistance through somaclonal variation

Pearl millet has considerable genetic variability for the improvement of germplasm. Tissue culture methods have been used for inducing desirable characters, genetic transformation and regeneration through embryogenesis⁹⁷⁹⁶⁵⁵. Many workers have demonstrated successful regeneration from protoplast. Therefore, somaclonal variation has shown great promise in enhancing the production and productivity of pearl millet. In tissue cultures, large number of population of callus cultures is required for the

induction and selection of disease resistant lines. In case of *S. graminicola* three different ways are used to induce disease resistance: 1) a tissue culture system for studying the host-pathogen interaction; 2) somaclonal variation as a source of disease resistance, and 3) selection, including the use of culture filtrate and toxic metabolites⁷⁵⁷⁷¹⁰.

Isolation of pathogen specific SG-toxin and development of toxin resistant pearl millet plants through callus cultures have been reported to be the most efficient method for inducing DM resistant cell lines. The adverse effect of SG-toxin on the callus initiation and its further new growth, regeneration of plantlets from callus and seedling growth was found in many susceptible pearl millet lines. Callus initiation decreases with increase in concentration of SG-toxin. In a recent study toxin resistant callus lines were regenerated via somatic embryogenesis in pearl millet cvs. ICTP 8203, ICMS 9555 and HB3 at 10% SG-toxin¹⁰¹¹. Such regenerated plants were tested for resistance to downy mildew under field conditions. Plants exhibited DM disease resistance throughout the growth period. Similarly, seed-derived callus of a PNMS 6B line of pearl millet also showed DM resistance under laboratory and field conditions⁷⁵⁷⁷.

Disease Management

Chemical control

Like other oomycetes, *S. graminicola* has a complex disease cycle where developmental forms differ in physiology and anatomy along with different impacts on host-parasite interactions, survival and distribution in space and time. The disease can efficiently be managed with the systemic fungicide metalaxyl. Seed dressing with Apron 35-SD (6 kg t⁻¹ seeds) and foliar application of Ridomil MZ72 (2 kg ha⁻¹) for seed crops have been recommended⁸³. However, looking to the acquired tolerance to metalaxyl in recent past a need to search for new systemic fungicides has been realized. Deepak¹⁹ tested the efficacy of 15 commercial and five experimental anti-mildew compounds both under greenhouse and field conditions and stated that fungicides of acylanilide series exhibited the highest efficacy of DM control. However, analysis of the cost: benefit ratio was uneconomic.

Biological control

With increasing environmental awareness the focus of managing plant diseases has been shifted towards viable and sustainable alternatives⁴. The biological control of downy mildew disease has been discussed by Shetty and Kuma⁷³. Basic and applied studies brought in the realization that host resistance is not absolute and chemicals become less effective due to the development of resistant pathogen population. In this regard, operability of induced systemic resistance is gaining importance and many studies have conferred the potential use of this procedure as a plant disease management strategy. The scientific basis of induced resistance has emerged with the identification and characterization of genes and biochemical pathways governing resistance in plants against invading pathogens. Based on this, agents of biological origin and certain chemicals have been gaining importance as resistance inducers in plants. have discussed about an interesting phenomenon of induced systemic resistance operating in pearl millet plants against downy mildew. They have experimentally demonstrated induction of resistance in pearl millet plants exposed to sub-optimal levels of *S. graminicola inoculum* against superinfection by the same strain of the pathogen. Innate defence mechanisms in plants can be triggered and enhanced by certain agents, which are referred to as inducers. Deepak²⁰ have reported the efficacy of cerebrosides (glycosphingolipids extracted from various plant pathogens), as resistance elicitors against DM of pearl millet. The resistance was of systemic nature and the time required for the resistance to build up was from 2 days onwards. Arun-Kuma⁵ have observed that a combination of raw cow's milk as seed treatment and *Gliocladium virens* as seed and soil treatments resulted in the lowest disease incidence (8%) and highest number of tillers of the crop. All the treatments increased plant height compared to the control. Arun-Kumar and Ma³ have investigated the biochemical constituents in treated and control plants to demonstrate the role of induced systemic resistance by raw cow's milk and *G. virens* in managing DM. Accordingly, the induction of resistance was established by the increased activities of defense related enzymes (peroxidase, polyphenol oxidase and catalase) and metabolites in healthy and DM infected leaves of treated pearl millet plants. They further emphasized the importance of integrating indigenous knowledge of using raw cow's milk with biocontrol agents as a logical strategy to induce resistance in low economic value crops largely grown by the resource-poor SAT farmers. Under field conditions Sharathchandra⁷⁰ have tested a commercial preparation Elexa (an aqueous chitosan formulation). Its seed treatment reduced DM severity to 42.5% and recorded 38% protection, whereas foliar spray to 7-day-old seedlings gave 67% protection and reduced severity to 25%. A combination of seed treatment and foliar spray to 7-day-old seedlings recorded 69% protection and reduced severity by 23%. The nature of disease control mechanisms is ascribed to induction of systemic resistance.

In an attempt to induce resistance in pearl millet for managing downy mildew certain biochemicals have also been tried. Seed treatment with amino acid proline (50 mM for 3 h) significantly enhanced seed germination and seedling vigour of pearl millet compared to the control besides protecting the pearl millet plants from downy mildew by offering 58% protection under greenhouse and 67% protection under field conditions. Studies revealed that 3 days were required for proline-treated plants to develop resistance, which was systemic and was sustained throughout the life of the plants⁶². Treating pearl millet seed with amino acids showed that under field conditions, Serine, DL-Tryptophan, DL-Leucine and DL-Isoleucine (25mM for 6h) reduced DM disease by 53, 51, 55 and 57%, respectively. The activation of phenyl propanoid pathway and accumulation of phenolic compounds showed evidence of amino acid-mediated induced systemic resistance⁷⁶.

Both seed and foliar treatments of *Pseudomonas fluorescens* controlled downy mildew, but efficacy was significantly higher when seed treatment was followed by a foliar application¹¹⁰. Under laboratory conditions, trichoshield (a talc formulation

consisting of spores of *Trichoderma harzianum*, *Trichoderma lignorum*, *Gliocladium virens* and *Bacillus subtilis*) seed treatment enhanced seed germination and seedling vigour of pearl millet significantly over the control; under greenhouse conditions vegetative growth parameters like height, fresh and dry weight, leaf area and number of tillers were significantly enhanced over the control. Trichoshield formulation provided better protection (52 to 71%) under field conditions against downy mildew in comparison with individual strains of *T. harzianum*, *T. lignorum*, *G. virens* and *B. subtilis*. Among the methods of application, foliar spray was found to be a more efficient delivery method than seed treatment or slurry treatment. Under field conditions, trichoshield treatment enhanced reproductive parameters and yield significantly over the control. Days required for 50% flowering was reduced by 4 days compared to the control. Yield enhancement of 28% over the control was highly significant⁶³.

Host-Plant Resistance

Host plant resistance is the only environment friendly and economical measure against the disease. Over the past thirty years considerable progress has been made in the field of host resistance. Research to improve screening systems to identify and use host-plant resistance has been successful allowing this serious threat to pearl millet cultivation in India to be largely controlled. Efforts were directed to search other sources of cytoplasm to avoid any situation as was faced in case of maize. Presently, several sources are available, but still A_1 cytoplasm is being used in most of the male sterile (ms) lines, it being agronomically better than other A_2 to A_5 cytoplasm. Efforts made at different national research centers in India led to the development of genetically different ms lines for use in the hybrid development programme.

Genetic diversification of the restorers was also made at different centers and private seed agencies, and now a large number of genetically diverse restorers are available. Combining inbred lines from ICRISAT, ARS Durgapura and Mandor, and CAZRI, Jodhpur made a Mandor Restorer Composite (MRC) and Mandor Early Restorer Composite (MERC). Seed of this composite was distributed by ICRISAT to NARS scientists and new restorer lines suitable for arid region having resistance to downy mildew have been developed. These efforts have now resulted in a broader genetic base of hybrids in India than it was a decade ago. Some of the new CMS systems (A_4 and A_5) in pearl millet, for which a large amount of the germplasm is in the maintainer class than in the A_1 system, offer further opportunity to accelerate diversification of the genetic base of pearl millet seed parents³¹⁵⁷⁶¹. These two new CMS systems also appear to have more stable male sterility than the A_1 CMS system⁶⁰.

Sources of resistance

The successful development and operation of field and greenhouse screening techniques resulted in the identification of a large number of germplasm and breeding lines with high levels of downy mildew resistance⁸⁹.

About 3500 germplasm accessions from almost all pearl millet growing countries have been evaluated at ICRISAT for their reaction to downy mildew. These downy mildew resistant sources [P7 (ICML-12), SDN 503 (ICML-13), 700251 (ICML 14), 700516 (ICML 15), 700651 (ICML 16)] have shown very high degree of stability across sites and years. Other sources of resistance are IP 16438, IP 16762, P310-17 and P 1449-3. Out of these P7, 700651, 700516 and P 310-17 have been utilized in breeding programmes³³.

Across the sites and years ICMP 423, ICMB 90111 and ICMA 90111 have been found resistant to DM. However, their resistance has been difficult to manipulate with pedigree selection, suggesting its complex inheritance. ICMP 85410 has been found downy mildew resistant as it derives its resistance from SC 14(M)-13499. Downy mildew resistance of this line has been found to be stable across the sites in western Africa. However, some of the pearl millet male sterile F_1 lines were highly resistant to 4 pathotypes (from Patancheru, Durgapura, Jalna and Jamnagar) of downy mildew in field at Andhra Pradesh, whereas seven ms lines of ICRISAT origin were found resistant to moderately resistant in western part of India⁸⁴⁷.

Reselected Sources of resistance

Selection of variability within susceptible parents led to the development of resistant types e.g. ICML 22 (7042 DMR) a downy mildew resistant version of susceptible 7042 (IP 2696) a landrace from Chaco⁸⁸. The line ICML 22 is being used at ICRISAT Asia Center for breeding of pollinators. Recent study on effectiveness within progeny selection for DM resistance in pearl millet revealed significant difference between the selected and unselected lines in 6 S3 progenies, with the DM incidence levels in the selected lines reduced from half to one-third in the unselected lines⁵⁸. However, the DM incidence in the six selected progenies varied from 14 to 46%, indicating that one-stage selection during the inbreeding process was not effective in improving the resistance of the crop to acceptable levels.

Recovery resistance

Recovery resistance is a phenomenon in which systemically infected plants outgrow the disease to produce healthy panicles. Here, pathogen and host co-exist, apparently without adversely affecting the yield. The phenomenon brings heritable changes in the recovered plants. Plants with this type of resistance quickly recover from the disease and subsequently behave like conventional resistant genotypes. Marker assisted selection would allow this type of resistance to be pyramided with more conventional resistances. Sources of recovery resistance are P1449, SDN 503 and ICMB 84184.

Sources of complete resistance

Accessions that show 100% resistance in greenhouse inoculation tests against major pathotypes in India and a large number of

field populations of *S. graminicola* in field disease nurseries in India and western Africa have been identified in the past. Five accessions viz. IP 18292, IP 18293, IP 18294, IP 18295 and IP 18298 were initially found to have zero disease incidences regardless of inoculum level⁸⁰³³.

Breeding methods

Conventional Breeding Methods

Conventional breeding procedures utilize greenhouse or field screening methods to incorporate adequate levels of downy mildew resistance (DMR) into breeding populations, parental lines and experimental open pollinated varieties that have superior agronomic performance and product quality. The following methods are being used in breeding for resistance to DM with varying degree of success:

Pure line selection: This is the type of selection within partially inbred lines. Pedigree selection and recurrent selection: These are the most widely and successfully used methods for improving downy mildew resistance.

Backcrossing: Conventional backcrossing procedures have seldom been used in breeding for resistance to pearl millet downy mildew. The only reported success using backcrossing is breeding of DMR seed parents MS 5054A and MS 5141A in the elite background of Tift 23 by IARI, New Delhi, India.

Induced mutations

Induced mutations have been used to produce MS 5071 B from Tift 23 B, and MS 5071 A was used in producing NHB series of hybrids with downy mildew resistance.

Selection for within line variability

Selection for DMR is done within a susceptible line using pedigree procedures. Large population of a susceptible line is grown in the disease nursery. Disease free plants are selfed and selfed progenies are screened panicle-to-row against the disease. A notable success is the development of ICMA 841A from susceptible 5141A and 5141B. This method provides an opportunity to revive once popular cultivars that have become susceptible to downy mildew.

Inheritance of Resistance

There have been conflicting reports about the nature of inheritance of downy mildew resistance mainly both the pathogen and the host is allogamous and highly variable¹⁰² and segregation for host plant resistance generally shows continuous variation. Regional variability in pathogen population used and difficulty in maintaining high and uniform disease pressure have led to conflicting conclusions³⁹.

It is clear that A₁ cytoplasm is not associated with susceptibility or resistance to downy mildew¹. However, there are ample evidences that genes in the nucleus control host plant resistance to this disease. Downy mildew resistance has been found to be dominant and variation in segregating population is continuous⁸⁷. The picture of mode of inheritance largely remains unclear and incomplete due to the variable pathogen population used in all these studies. Quantitative inheritance studies of downy mildew resistance in pearl millet have been more successful—identifying parental materials with the ability to transmit high levels of resistance. It has been concluded by a number of workers that non-additive gene action is responsible for much of the heritable variability⁴¹²¹. Such non-additive gene action can contribute substantially to general combining ability (GCA), since parents having dominant resistance can be expected to have high GCA for this trait when compared with more susceptible parents.

Regional variability of resistance causes additional hindrance to breeding¹²¹³²⁶⁷⁴⁷⁸¹⁰⁰. This regional variability has been found to be mainly due to genetic variability between pathogen populations rather than environmental differences between location⁹. Consequently, the testing of breeding material under multilocational trials has become expensive and time consuming. Selection for multilocational resistance could, therefore, theoretically be carried out by simultaneously screening breeding material in one location against a range of pathogen populations.

Marker-Assisted Selection (MAS)

MAS use highly heritable 'tags' as selection criteria that are genetically linked to the portion of genome controlling characters of interest. These markers can be morphological traits and proteins (including isozymes or DNA markers as RFLPs, RAPD and others). MAS are an excellent alternative to time consuming testcrosses, otherwise required to pyramid DMR genes. MAS and graphical maps based on markers can help breeders; more rapidly recover recurrent parent genotype in backcrossing programme, and more efficiently identifying desired recombinants in pedigree programmes²²⁴⁴³⁷. In order to use MAS in improvement of pearl millet downy mildew resistance it is necessary to have a:

- Donor parent with identified linked markers for some of its resistance genes.

- An elite parent with inadequate resistance, which has different marker alleles than the donor parents and lacks these resistance genes (at least the specific alleles linked to markers in the donor genome).

A system for efficiently determining genotype (or at least phenotype) at the marker loci in individual segregating plants derived from crosses between the resistant source and the elite parent.

MAS has considerable potential as a tool for pearl millet improvement. A saturated genetic linkage map based on molecular markers has been developed^{39,40}. This map was initially based on RFLP markers, since these can reveal an almost unlimited number of polymorphism and can be used directly to tag loci controlling traits of interest for MAS. Further, a skeleton map based on sequence tagged sites (STS – markers based on the polymerase chain reaction with less stringent requirements for large quantities of good quality DNA) has also been developed⁴⁹ that should reduce the cost of transferring this map to other breeding populations. Recent inheritance studies based on Molecular Marker Genetic Linkage Maps are yielding interesting results that will facilitate genetic manipulation of disease resistance^{39,40,36}. To date markers have been identified for at least sixteen different putative downy mildew resistant quantitative trait loci (QTLs).

In case of pearl millet, green house or laboratory screening for downy mildew resistance is efficient and relatively inexpensive against a single pathogen isolate or a few isolates from a single country. However, to pyramid resistances from different sources or select for resistance effective in other geographical areas would be time consuming and highly expensive. MAS method should be much faster and less expensive and should offer higher heritability for resistance (nearly 1.0) than routine multilocational screening against pathogen populations.

Development of bulk segregant analysis⁴⁸, similar in many ways to the concept of near isogenic populations¹⁶, has dramatically reduced the time and expense required to identify molecular markers for new resistance genes with large effect. However, it can miss genes of small effect. Molecular markers are also being used to characterize populations of pearl millet pathogen⁶⁷. Overall it looks like molecular markers and MAS will become important tools for pearl millet breeders in the decade ahead. Marker assisted selection offers breeders a new array of disease screening method with maximized heritabilities. Although not a universal solution to all breeding problems, MAS offers tremendous opportunities to breed for important characters that are otherwise difficult and expensive to assess.

Molecular markers linked to resistance genes would allow resistances to different pathogen populations to be selected for a single location in the absence of pathogen. Linkage drag and confounding effects of environmental variation associated with conventional breeding would also be eliminated. The ability to map genes contributing towards variation in complex traits with enough accuracy has only recently been made possible through the development of comprehensive molecular marker maps.

The first molecular-marker map for pearl millet has recently been constructed⁴⁵ so that QTL analysis is now possible. Following QTL analysis, Jones³⁹ found independent inheritance of resistance to pathogen populations from India, Senegal and populations from Niger and Nigeria. These results showed the existence of virulence differences in the pathogen populations from within Africa and between Africa and India. QTLs of large effect, contributing towards a large proportion of variation in resistance were consistently detected in repeated screens. No QTLs that were effective against all four pathogen populations was observed, demonstrating that pathotype-specific resistance is a major mechanism of downy mildew resistance in this cross. For all but one of the QTLs, resistance was inherited from the resistant parent and the inheritance of resistance tended to be the result of dominance or over-dominance.

Ultimately the goal of identified linked markers is their use as indirect selection tool in the breeding programme. In addition to the identified linked markers, it is necessary to have a low cost, high throughput marker service facility. At present SSR markers are considered appropriate for most species. It is predicted that SNP markers may become attractive in the coming years. A major limitation in MAS is the cost to select for the recurrent genome in the backcrossing programmes (normally, only markers linked to the trait/gene of interest are used).

Apart from this molecular mapping has also expanded knowledge of downy mildew resistance. It has shown that:

1. Many genes contribute to downy mildew resistance
2. These genes are scattered throughout the host genome
3. Pathogen strain specificity is the rule for each of these genes and
4. A large portion of resistance to a given pathogen population can be accounted for by relatively few genes.

Use of MAS for getting downy mildew resistant hybrid

Downy mildew surveys conducted in the pearl millet hybrid-intensive states of Gujarat, Haryana, Maharashtra and Rajasthan in India during 1994–2004 forewarned about the performance of hybrids and their possible replacement in certain areas⁴⁰⁵. The surveys indicated the likely resistance breakdown of HHB 67, the most popular hybrid in Haryana and parts of Rajasthan, cultivated on at least 400,000 hectares in these states. Being very early in maturity (within 65 days), it escapes terminal drought and also provides an opportunity for double cropping.

Since HHB 67 is highly preferred by the farmers, and no other hybrid in this maturity group being available, attempts were made to improve the parental lines of HHB 67 for DM resistance. The parental lines (843 A and H 77/833-2) of the original hybrid were improved for DM resistance through marker-assisted as well as conventional backcross breeding programmes at the ICRISAT campus at Patancheru. The gene for downy mildew resistance was added to the male parent, H 77/833-2, through marker-assisted breeding using ICRISAT elite parent ICMP 451 as the resistance gene donor. The gene for DM resistance was added to the female parent, 843A/B, from ICRISAT line ICML 22 through conventional backcross breeding. Field-testing of the new hybrid was done at various locations through All India Coordinated Pearl Millet Improvement Project (AICPMIP). The advantage of the marker-assisted selection was that male parent for HHB 67-2 could be developed in one-third of the time required for the developing the female parent by conventional selection methods. By identifying and marking the gene responsible for DM resistance in ICMP 451, it could be checked whether the gene had transferred to the next generation in the progeny of crosses between ICMP 451 and the male parent of HHB 67. By using molecular marker technology the presence of the gene can be tested even while the next generation is a seedling, saving precious breeding time. This is the first example of development of a DM-resistant version of hybrid HHB 67, using both conventional and DNA marker technologies to produce downy mildew resistant hybrid in pearl millet. It was recommended for release in the state of Haryana in the year 2003⁵.

Conventional breeding approaches will continue to make significant contributions to the genetic enhancement of productivity in pearl millet. However, the efficiency of such efforts can be increased considerably through the application of biotechnological tools to address areas as diverse as determination of heterotic patterns for more rational utilization of the germplasm in hybrid parent development and the genetic manipulation of traits that are too susceptible to the unpredictable environmental variations that are so characteristic of semi-arid tropical regions.

Physiology of host resistance

Recently some investigations have been made to find out the physiological attributes of a DM resistant pearl millet plant. Geetha²⁴ studied the Phenylalanine ammonia lyase (PAL) activity in pearl millet cultivars having different levels of DM resistance. PAL activity was elevated in resistant host cultivar and decreased in susceptible cultivars following downy mildew pathogen infection. The enzyme activation varied between cultivars and was correlated with the degree of resistance to downy mildew disease. An assay has been developed by Geetha²⁴ for identifying DM resistant and susceptible pearl millet genotypes at the seedling stage using arachidonic acid (AA). The acid induces a hypersensitive response (HR) comparable with HR induced by *S. graminicola* on coleoptile/root regions of 2-d-old pearl millet seedlings. A time gap in the appearance of cell necrosis among genotypes was related to the degree of resistance to downy mildew. The appearance of hypersensitive cell necrosis was rapid in genotypes having high resistance to downy mildew and was slow in genotypes with high susceptibility. This simple method of screening various genotypes in the absence of the pathogen aided the identification of DM resistant/susceptible host cultivars without the risk of introducing the virulent race of the pathogen. Similarly, a rapid method of detecting incipient infection of DM fungus in pearl millet has been reported by Bhansali¹¹. Dormant nodal buds isolated from systemically infected plants were having incipient infection of DM. Microscopic examination revealed that out of 45 meristems and bud scales 36 showed DM fungus when stained with 0.1% Trypan blue. They observed that the fungus colonized all embryonic leaves and continued to grow inside the developing leaves. Further symptoms produced from systemically sprouted nodal buds confirmed that the mycelium detected in meristems and bud scales of nodal, apical and floral buds were of *S. graminicola*. This method of detection may be useful in forecasting the presence of infection at an early stage of plant to avert the outbreak of DM in field grown pearl millet plants.

Differential induction of superoxide dismutase (SOD) in DM-resistant and -susceptible genotypes of pearl millet (*Pennisetum glaucum*) was observed. SOD activity was studied in resistant and susceptible pearl millet seedlings inoculated with *S. graminicola*. SOD activity increased by 2-3-fold in resistant seedlings upon inoculation. The activity was greatest in roots. SOD activity increased in all the resistant genotypes upon inoculation with *S. graminicola*. Native PAGE analysis showed four isozymes of SOD, three of which (SOD-1, -2 and -4) were Cu/Zn-SOD, whereas isozyme SOD-3 was Mn-SOD. This study also revealed increased intensity of all four isozymes of SOD in the resistant genotype upon inoculation⁸. The investigations conducted by Nagarathna⁵² with an enzyme lipoxygenase (LOX) indicated a good correlation between enzyme activity and their downy mildew reaction in field. Maximum activity was recorded in seeds of highly resistant genotypes and minimum activity of LOX was found in the highly susceptible genotypes. Seeds obtained from plants recovered from the downy mildew disease had more LOX activity than that of the original parent seeds. Thus, in seeds, the LOX activity can be used as a biochemical marker for screening different genotypes of pearl millet for downy mildew. Stomatal behaviour of DM infected plants showed lower leaf conductance and transpiration rate compared with healthy plants, indicating that infection elicited stomatal closure. Downy mildew infection inhibited stomatal opening at 12.00 h and pronounced stomatal closure was observed at 16.00 h, which resulted in decreased transpiration rate. Stomatal closure was more pronounced at the boot stage⁷¹.

Conclusion

Despite the advances in knowledge over the last 15 years, these diseases remain important constraints to sustainable crop production in the semi-arid regions of Africa.

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