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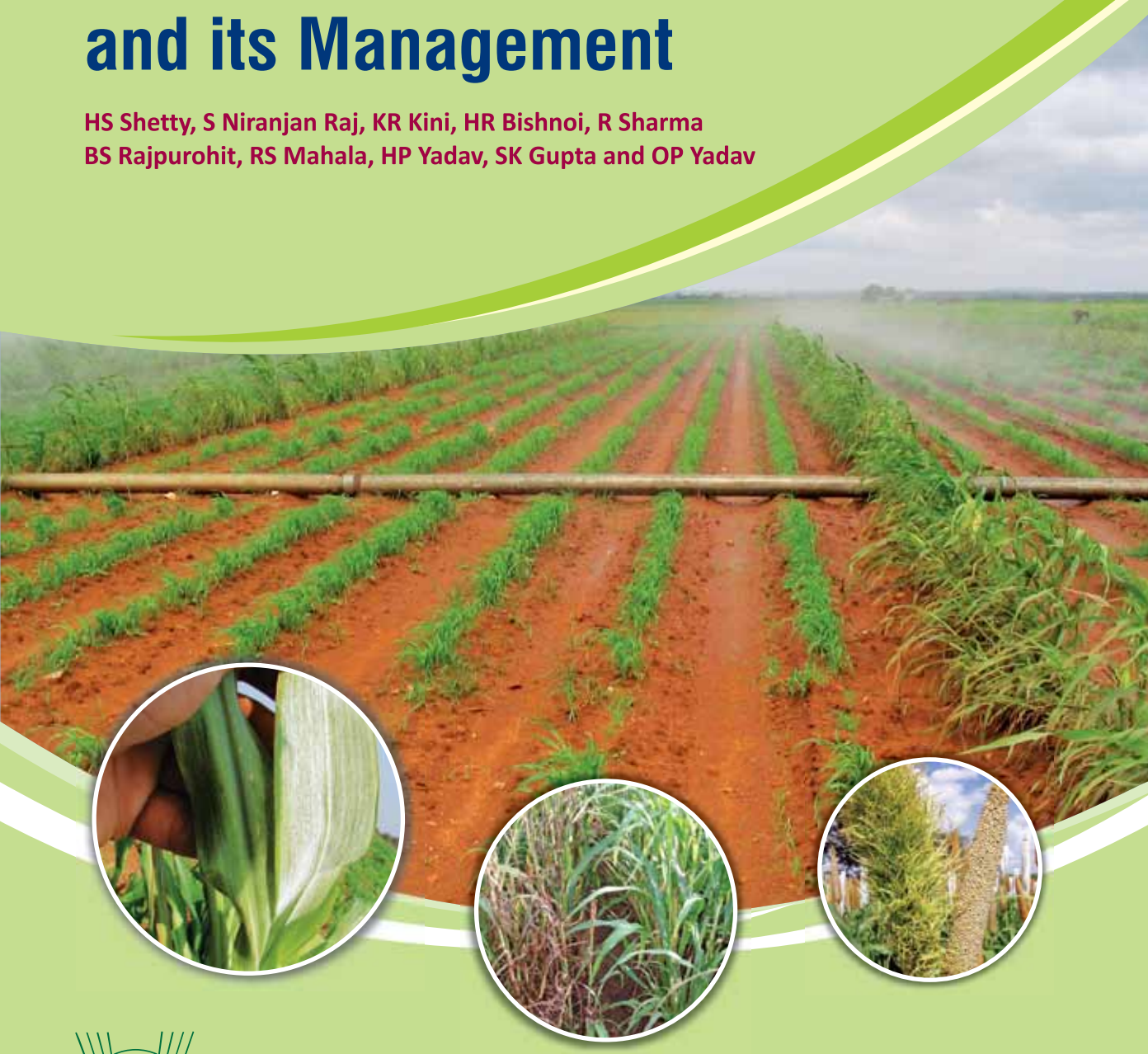

All India Coordinated Research Project on Pearl Millet
(Indian Council of Agricultural Research)
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Correct Citation: Shetty HS, Raj Niranjan S, Kini KR, Bishnoi HR, Sharma R, Rajpurohit BS, Mahala RS, Yadav HP, Gupta SK and Yadav OP. 2016. Downy Mildew of Pearl Millet and its Management. All India Coordinated Research Project on Pearl Millet (Indian Council of Agricultural Research), Mandor, Jodhpur – 342 304. 53 pp.

Lasertypeset & printed at
M/s Royal Offset Printers, A-89/1, Naraina Industrial Area, Phase-I, New Delhi-110028

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1. Introduction

Pearl millet (*Pennisetum glaucum* [L.] R. Br.) is the third most important rainfed cereal crop of India grown over 9 million hectares with an annual production of 9.5 million tonnes (Yadav and Rai, 2013). Globally, it is the sixth most important cereal grain cultivated on more than 30 million hectares which accounts for approximately 50% of world's millet production. Though the most of the crop area is in Asia (>10 m ha) and Africa (about 18 m ha), pearl millet cultivation is being expanded in some of the non-traditional areas, with Brazil having the largest area (about 2 m ha). It is also being experimented as a grain and forage crop in the USA, Canada, Mexico, the West Asia and North Africa (WANA), and Central Asia.

Pearl millet, being a C_4 plant, has a very high photosynthetic efficiency and dry matter production capacity. It is usually grown under the most adverse agro-climatic conditions where other crops like sorghum and maize fail to produce economic yields. Besides, pearl millet has a remarkable ability to respond to favourable environments because of its short developmental stages and capacity for high growth rate, thus making it an excellent crop for short growing seasons under improved crop management.

Pearl millet is primarily grown for food and dry fodder. Grains are mainly used for human consumption in the form of diverse food, mostly as leavened and unleavened flat breads and porridges. Pearl millet grains are highly nutritious with high levels of metabolizable energy and protein, have high densities of iron and zinc, and more balanced amino acid profile than maize or sorghum and therefore, the crop is gaining popularity as a health food. A significant portion of pearl millet grain is also used for non-food purposes such as poultry feed, cattle feed and alcohol extraction (Basavaraj *et al*., 2010).

2. Diseases of pearl millet

In comparison to wheat, rice, maize and sorghum, pearl millet is relatively less susceptible to pests and diseases (NRC, 1996). The number of diseases reported on rice are more than 350, 200 on wheat, 70 on maize, and 63 on sorghum whereas, about 50 diseases are reported on pearl millet. Pearl millet with its anatomy and the cell wall structure made up of tissues resistant to the pathogen penetration, is more biotic stress tolerant than rice and wheat.

Only five diseases are of major economic importance in India. These are downy mildew caused by *Sclerospora graminicola* (Sacc.) Schroet, blast caused by *Pyricularia grisea* (Cke.) Sacc [teleomorph - *Magnaporthe grisea* (Herbert) Barr], smut caused by

Moesziomyces penicillariae (Bref.) Vanky, ergot caused by *Claviceps fusiformis* (Loveless) and rust caused by *Puccinia substriata* Ellis & Barth var. *indica* Ramachar & Cummins. Downy mildew, smut and ergot directly affect grain yield. In addition, ergot can also reduce grain quality. Blast and rust affect stover yield and quality, but may reduce grain yield as well, if they appear early in the season.

Downy mildew is the most devastating disease of pearl millet particularly on susceptible and genetically uniform hybrids. Its biology, epidemiology and management aspects are complex as compared to the other major pathogens infecting pearl millet. During last few decades, concerted efforts have been made to address several issues in order to understand biology and epidemiology of downy mildew pathogen and host-pathogen interaction that have helped in development of resistant cultivar and management of disease.

3. Downy mildew

3.1 Host range

Sclerospora graminicola has the widest host range of the *Sclerospora* spp. with pearl millet and foxtail millet (*Setaria italica*) as its principal host originating in Africa (Harlan 1975, Brunken *et al*., 1977). It is believed that region of origin of the host is also the region of origin of the pathogen. Therefore, downy mildew pathogen has been associated with pearl millet since approximately 3500 BC. This is also supported by the fact that the most frequent sources of resistance to downy mildew are of African origin. It is also possible, but less likely, that the pathogen originated in indigenous Indian grasses and moved to pearl millet when this crop was introduced from Africa. The present widespread occurrence in Africa would then be explained by its introduction long ago on plant products from India, with many resistance sources among African pearl millet merely reflecting a long period of co-evolution in the regions with a wide range of host variability (Williams, 1984). On the contrary, Shaw (1981) suggested that *S. graminicola* has a temperate origin because it is circumpolar on species of *Setaria*, and that it has become adapted to plants in tropical habitats, particularly pearl millet. He opined *S. graminicola* to be not only primitive, but to have been circumpolar on the Paniceae since Pleistocene times because it might have coevolved in many locations with species of *Setaria, Panicum, Chaetochloa* and *Pennisetum*. Shaw (1981) also opined that *S. graminicola* has occurred in Africa since that continent was much more temperate, evolving and adapting to the changing climate along with its host. However, a few other graminaceous hosts have also been reported like *Zea mays, sorghum bicolor, Agrostis, Echinochloa crusgalli*, *Eleusine*,

Euchlaena maxicana, Panicum miliaceium, *Pennisetum leonis*, *Pennisetum spicatum*, *Saccharum* sp, *Setaria lutescens*, *Setaria verticillata*, *Setaria viridis*, and *Setaria magna* (Holliday, 1980). Singh and Williams (1979) tested 23 entries belonging to 11 genera including *Sorghum vulgare*, *Pennisetum americanum* (Syn = *P. glaucum*), *Zea mays*, *Paspalum* sp., *Setaria italica*, *Eleusine coracana*, *Heteropogon contortus*, *Echinochloa* sp., *Panicum miliaceum*, *Panicum miliare*, and *Euchlaena mexicana*. However, none of these except *P. glaucum* developed downy mildew under downy mildew nursery conditions at ICRISAT Patancheru, India in which a downy mildew susceptible control, 7042S, developed 100% downy mildew. Cultivars of all these genera, except *Echinochloa* sp., were also tested using a seedling dip inoculation technique (Singh *et al*., 1993), but none of them developed downy mildew except the cultivars belonging to *P. glaucum*. There is evidence of physiological specialization in *S. graminicola* as reported by Uppal and Desai (1931). They described two forms, one on many Setaria species like *Setaria italica*, *Euchlaena maxicana* and the other on pearl millet, but these two forms are not cross infecting pearl millet or *Setaria italica* as per experiments carried out at the downy mildew pathology laboratory, Mysore. Oospores of *S. graminicola* from *S. italica* also failed to infect 7042S. These examples clearly show a high degree of host specificity in *S. graminicola* (Singh and Luther, 1981). This suggests that the reported collateral hosts do not play a role in the disease epidemiology.

3.2 Symptoms

Pearl millet seedlings raised in the infested soil with downy mildew inoculum express the symptoms within 5-6 days of emergence. Symptoms are systemic and may appear from seedling up to flowering stage. Though pearl millet gets the disease throughout the crop growth period, it is most susceptible at coleoptile stage and the susceptibility decreases as the crop gets older. Typical downy mildew symptoms include sporulation on the abaxial leaf surface, chlorosis, stunted growth, and malformation of the earheads (Fig. 1). Initially the downy mildew symptoms appear on the second leaf and later on all subsequent leaves and panicles. Under favorable conditions, severe disease symptoms are seen on the first leaf itself. Symptoms on leaf

Fig. 1: *Typical downy mildew symptoms of pearl millet: A) stunted growth, B) chlorosis and C & D) malformed earheards*

initially appear as chlorosis (yellowing) at the base of the leaf lamina, and successively younger leaves show a progression of chlorosis. Half-leaf symptoms are characterized by a distinct margin between the diseased (basal portion) and non-diseased area towards the tip. During sporulation, massive asexual spores occur on infected chlorotic areas, generally on the abaxial surface of leaves, giving them a downy appearance (Fig. 2). Severely infected plants are generally stunted, and do not produce panicles. Green ear symptoms become visible at panicle emergence where the floral parts are transformed into leafy structures, which may vary in shape and size, and the transformation may be partial or total. This is sometimes referred to as virescence. These leafy structures can also be chlorotic, and sometimes support sporulation. In certain cases, green ear is the only manifestation of the disease. Oospores, sexual spores, are produced in infected leaves when compatible mating types of *S. graminicola* are present in the same tissue, or when homothallism is operative.

Fig. 2: *Abaxial side of* Sclerospora graminicola *infected pearl millet leaf showing profuse growth of the asexual sporangia*

3.3 Yield losses

Downy mildew or green ear disease is associated with pearl millet since long (Butler, 1907). The disease was restricted only to the landraces and local cultivars. Epidemics were not reported till the introduction of F_1 hybrids. In India, downy mildew epidemics caused substantial yield losses during 1970s and 1980s. Grain yield losses of 10% to 60% have been reported. The yield reducing potential of downy mildew is very high, and this was adequately demonstrated in HB 3, a popular hybrid, when pearl millet grain production in India was reduced from 8 million tons in 1970-71 to 5.3 million tons in 1971-72. This reduction was, to a large extent, due to a downy mildew epidemic, in which yields in some fields were reduced by 60 to 70%. The estimated annual grain yield loss due to downy mildew 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) and where a susceptible cultivar is repeatedly grown in the same field. Genetically uniform single-cross F_1 hybrids become susceptible more rapidly than heterogeneous open-pollinated varieties (Thakur *et al*., 2006) leading to heavy production losses.

3.4 Pathogen biology

S. graminicola, a type species of genus *Sclerospora*, belongs to group Chromista, phylum Oomycota, class Oomycetes, order Sclerosporales and family Sclerosporaceae. The pathogen is heterothallic, but homothallism also occurs (Michelmore *et al*., 1982). It produces both asexual (sporangia, zoospores) and sexual spores (oospores). Vegetative phase is in the form of mycelium colonizing the intercellular spaces in the host tissues like root, stem, leaf and panicle. The pathogen draws the nutrition from the host cell through a specialized structure called haustoria and haustorial mother cell. The hyphae are coenocytic, multinucleate and grow profusely in the tissues and at later stages produce plenty of asexual spores on the lower leaf surfaces. After the exhaust of sporulation, the pathogen switches over to sexual reproduction to produce oospores.

3.4.1 Asexual phase: Sporangia are produced on sterigmata located at the tips of sporangiophore branches. Fully developed sporangia are hyaline, thin-walled, ellipsoid or broadly elliptic and papillate, with dimensions of 15-22 × 12-21 µm. Sporangiophores are short, stout, determinate, and dichotomously branched structures that emerge from systemically infected leaves through stomata (Figs. 3, 4, 5). In nature, sporangia are

Fig. 3: *Confocal Laser Scanning Microscopic (CLSM) picture of* Sclerospora graminicola *sporangiophore on the pearl millet leaf surface (stained with Leucoaniline blue)*

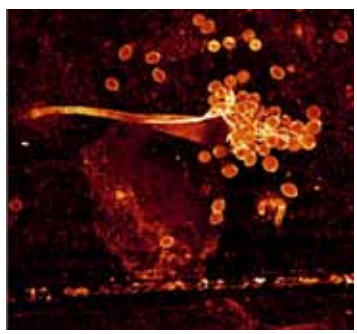

Fig. 4: *CLSM (UV laser) of* Sclerospora graminicola *young sporangiophore on pearl millet leaf surface (stained with Calcoflour White. Rotating 3D image.x200. xz direction)*

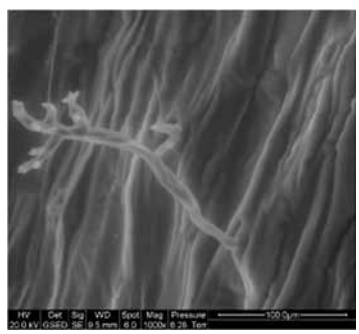

Fig. 5: *ESEM of pearl millet leaf surface showing* Sclerospora graminicola *sporangiophore emerging through stoma (enlarged view)*

produced during the night hours between 1 am to 4 am and under artificial condition, they can be harvested after 6 hours of incubation of infected leaves at 20 °C. Sporangial production can occur between 10 to 30 °C with an optimum production at 20 °C (Singh *et al*., 1987). Further, optimum sporangial production occurs between 95 and 100%

relative humidity (RH). No sporulation occurs below 70% RH. Under optimum conditions of temperature and RH, approximately 1.5×10^5 sporangia can be produced per cm^2 leaf area during one night (Singh *et al*., 1993). Sporangia germinate indirectly by producing zoospores. Mature sporangia after liberating from the sporangiophore produces wall less, biflagellate zoospores which swim freely in water (Fig. 6). The number of zoospores

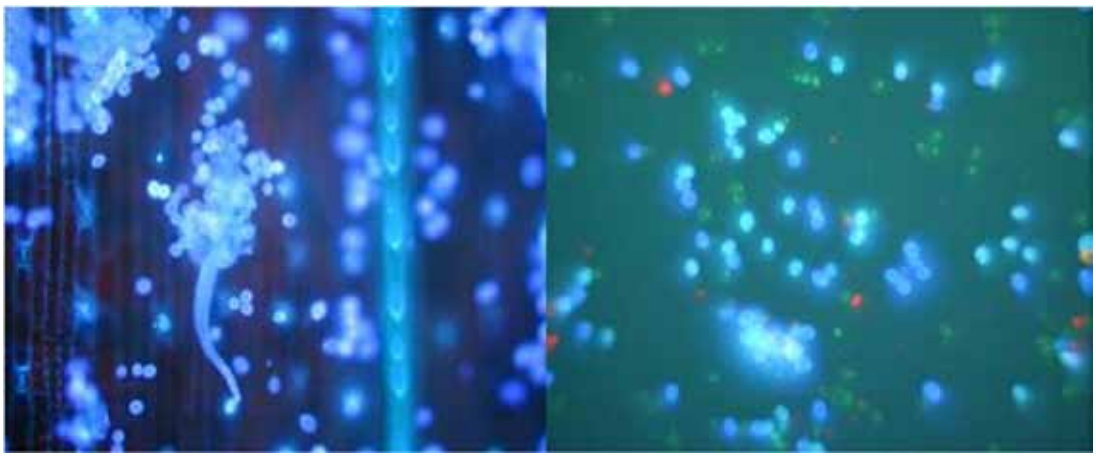

Fig. 6: *Sporangiophores, sporangium and zoospores of* Sclerospora graminicola

per sporangium may vary from 1 to 12 (Shetty, 1987; Ramakrishnan, 1963). Zoospores swim for 30-60 min, encyst, and then germinate by forming a germ tube. Sometimes, zoospores may germinate within the sporangium and the germ tube grows through the apical pore giving the appearance of direct germination (Shaw, 1981). Zoospores retain their infectivity for about 4 h at 30°C, and for a longer period at lower temperatures (Singh and Gopinath, 1990). Confocal microscope image of young sporangiophore of *S. graminicola* on pearl millet leaf surface showing a large number of nuclei before the formation of the sporangia stained with Syto 13 is shown in Fig. 7.

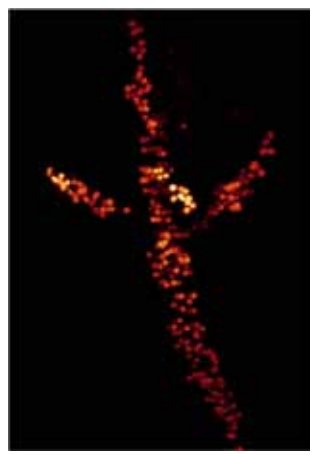

Fig.7: *CLSM of* Sclerospora graminicola *young sporangiophore on pearl millet leaf surface showing a large number of nuclei stained with Syto 13*

3.4.2 Sexual phase: Oospores are the sexual spores of *S. graminicola* and are brownish yellow, thick-walled, spherical resting spores (Fig. 8). Oospores are produced when the host completes its growth cycle and reaches senescence

and, simultaneously the pathogen completes asexual spore cycle and switches over to produce antheridia and oogonia which mate to produce oospores in plenty. Oospores are produced only when compatible mating types of *S. graminicola* are present in the same tissue, or when homothallism is operative. Mature oospores measure 32 µm (22-35 µm) in diameter. In *Sclerospora* spp. the oogonial wall is fused with the oospore wall, which is a major identifying feature of this genus. Reports suggest that oospores can survive from 8 months to 10 years under laboratory conditions (Nene and Singh, 1976). Oospores can germinate directly by germ tubes or indirectly by the liberation of zoospores, with great variation in the frequency of germination (Nene and Singh, 1976). The optimum temperature for oospore germination is 28±2°C. About one-year-old oospores give more infection (98%) than fresh or more than one-year-old oospores (Nene and Singh, 1976; Singh and Navi, 1996).

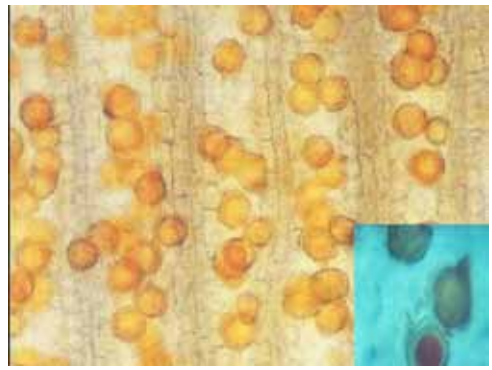

Fig. 8: *2,3,5-triphenyltetrazolium chloride (TTC) stained oospores of* Sclerospora graminicola*. The oospores which take up the red color are viable and unstained are non-viable*

3.5 Disease cycle

Oospores, present in soil, serve as primary source of inoculum, and infect the underground parts of plants, mostly at the seedling stage. The thick impermeable walls of the oospores protect them from desiccation. *S. graminicola* oospores mostly germinate directly by germ tubes. There are also reports of indirect oospore germination by the liberation of zoospores. However, so far there is no microscopic or photographic evidence of direct oospore germination or indirect mode by releasing the zoospores. The infection process begins with the formation of a germ tube which produces an appressorium (Figs. 9, 10, 11). This may be located at the junction of epidermal cells, directly over the epidermal cells or over stomata, depending upon the plant organ involved and the stage of development or maturation. Infection from oospores (whether direct by germ tubes or indirect by zoospores) may occur in the coleorhiza, radicle, lower portions of the coleoptile of seedlings, roots and underground portions of stem bases in older plants. Following the infection, pathogen grows intercellularly towards the meristem region. Systemic symptoms appear when the pathogen invades the developing leaves or inflorescence

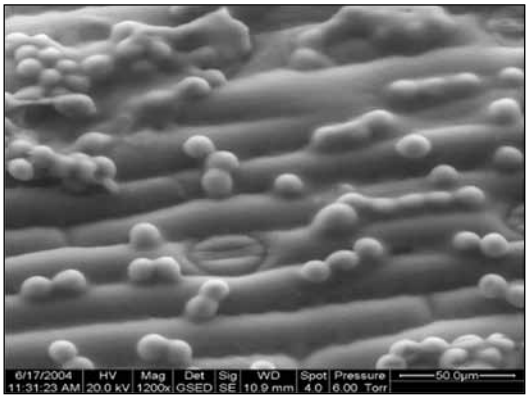

Fig. 9: *ESEM of pearl millet leaf showing* Sclerospora graminicola *sporangia deposited on the leaf surface. Note some sporangia deposited around the stomata*

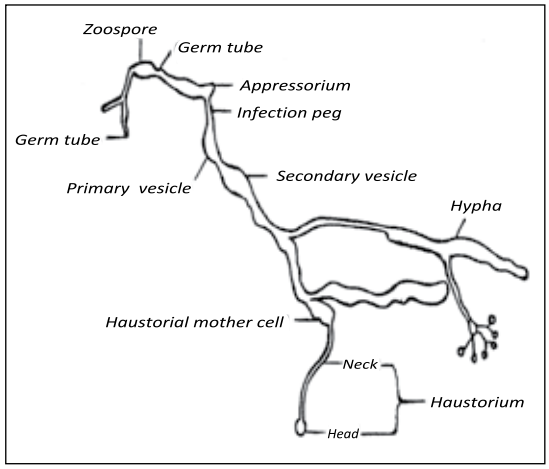

Fig. 10: *Different infection structures of* Sclerospora graminicola

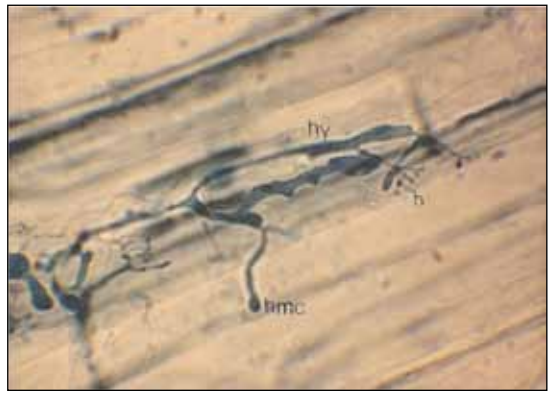

Fig. 11: Sclerospora graminicola *haustorial mother cells and haustoria in a highly susceptible pearl millet cultivar*

at the growing point. Without exception, younger the host plant, greater the susceptibility to systemic colonization by *S. graminicola*. Young seedlings are easily penetrated as they are succulent with their apical meristems protected only by a coleoptile, and are in close proximity to the soil. However, in older plants the apical meristem is encased by up to several leaf sheaths, making penetration to the meristematic region difficult. The systemic disease caused by *S. graminicola* is characterized by the invasion of the host apical meristem region by the pathogen and the appearance of symptoms when the organs that have been colonized during the process of tissue differentiation grow out and unfold. Thus symptoms appear sometime after the critical infection and colonization processes. Under humid conditions, systemically infected leaves produce abundant sporangia on the abaxial surface. Sporangia are important for the secondary spread of the disease within and among fields if environmental conditions are suitable (Singh and Williams, 1980). These sporangia release zoospores which germinate, penetrate the epidermis or stomata, and cause infection in successive manner. Plant inoculated at coleoptile stage produces systemic symptoms in young leaves in 4-7 days. If the

environment is suitable, infected leaves continue to produce sporangia until the tissues become necrotic or senesce. Oospores are not always found in systemically infected leaf tissue, presumably because only one mating type is present and homothallism is inoperative. *S. graminicola* survives as oospores in the soil along with infected leaf residue, and cause primary infection in the subsequent years. Oospores are transmitted on the seed surface, in soil, by wind, or by water. The complete disease cycle of *S. graminicola* is presented in Fig. 12.

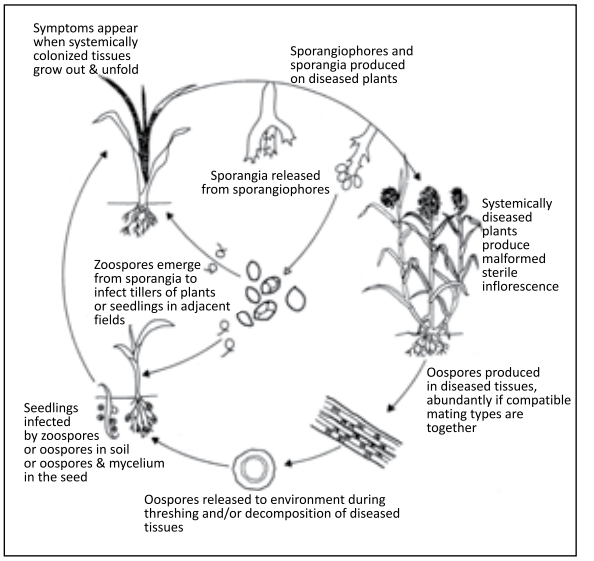

Fig. 12: *Disease cycle of pearl millet downy mildew pathogen* Sclerospora graminicola

3.6 Establishment of pathogen culture and maintenance of isolates

S. graminicola being an oomycete biotroph survives only on living host cell or tissue and has not been cultured on any defined synthetic medium. The isolates of pathogen collected during on-farm surveys are first established on the susceptible genotypes and then used in the virulence diversity studies as well as greenhouse screening of pearl millet lines for Downy mildew resistance. These isolates are maintained on the same host from which they were collected or on a highly susceptible line 7042S.

3.6.1 Establishment of culture from oospores: The downy mildew-infected old/mature leaf samples collected from fields are dried in shade, ground and strained to make a fine powder containing oospores (checked under microscope for the presence of oospores). Sterilized potting mixture (soil, sand, and farmyard manure in a ratio of 3:2:2 by volume) is infested with oospore inoculum and the pots (15 cm diameter) containing infested mixture are sown with original host genotype or a susceptible genotype 7042S @ 25 seed per pot. Pots are covered with a polythene bag and incubated at 30-35°C for 3-4 days for rapid seed germination. Pots are transferred to polyacrylic isolation chambers in a greenhouse at 25±2°C to avoid cross contamination from other isolates. Pots are watered adequately every day and observed regularly for downy mildew symptoms on the seedlings. As soon as infected seedlings are noticed, they are transplanted in

another pots containing sterilized soil and shifted to isolation chambers. The sporangia from infected seedlings are used for subsequent inoculation of seedlings for maintaining the isolate.

3.6.2 Establishment of culture from sporangia: Green infected leaves collected from the fields can also be used for establishing pathogen isolate in the greenhouse. Disease samples are collected and kept in polythene bags at low temperature. Small holes should be made in the polythene for aeration to keep the samples green for 2-3 days. The infected leaves are cleaned in the laboratory and incubated at 20°C in a humid chamber to induce sporulation. The sporangial inoculum harvested from the infected leaves is used to inoculate the pot-grown seedlings of the same host genotype from which the isolate was collected. The inoculated seedlings are incubated at 20°C for 16 h in the dark, and high humidity (>90%) is provided to facilitate infection process. The pots are then transferred to polyacrylic isolation chambers in the greenhouse at 25±2°C to avoid cross contamination from other isolates. Pots are watered adequately every day and observed regularly for downy mildew symptoms on the seedlings. Once infected seedlings are noticed, all healthy seedlings are removed from the pots. The sporangia produced on these infected seedlings are used for subsequent inoculation of seedlings to maintain the isolate.

3.6.3 Maintenance of isolates: Oospore/sporangia-derived isolates are maintained on pot-grown seedlings of its collection host or other susceptible host in isolation chambers through asexual generations (Fig. 13). Asexual inoculum from 30-40 days old seedlings grown in an isolation chamber is used to inoculate a new set of pot-grown seedlings of the same host genotype. The old set of infected seedlings are discarded, autoclaved and disposed off. This is done to maintain the fresh inoculum, prevent growth of saprophytes on ageing seedlings and avoid spread of the old isolates.

Fig. 13:. Sclerospora graminicola *isolates being maintained in isolation chambers in greenhouse*

3.6.4 Culturing pathogen on host tissue callus: A cell/tissue culture system referred to as 'dual culture' where the host and the pathogen are grown

together on a culture medium has been developed where pearl millet and *S. graminicola* can be grown together. Pearl millet shoot tip, hypocotyl region of germinating seeds, young healthy/malformed inflorescence are used as explants and cultured on the Murashige and Skoog tissue culture medium. After 30-40 days of culturing, pearl millet calli develop in the medium which are further sub-cultured and large numbers of culture flasks are maintained. Sporangia of the *S. graminicola* are aseptically inoculated to such host callus tissues where the pathogen infects and establishes on the host callus in 2-3 weeks time. Alternatively, downy mildew infected shoot tip or malformed inflorescence can be directly inoculated on to the culture medium to establish the dual culture (Upadhyaya *et al*., 1992; Geetha, 2002). The pathogen grows luxuriantly on the host callus, sporulates to produce sporangia/ zoospores. Dual cultures continue to grow up to 30-40 days, after which they are cut to 2-5 mm size explants and are again placed on the healthy calli which again grows as dual culture. Such dual cultures are being maintained continuously at Downy Mildew Research Laboratory, Mysore. Isolates of *S. graminicola* maintained as dual cultures on pearl millet host callus under laboratory conditions is shown in Fig. 14.

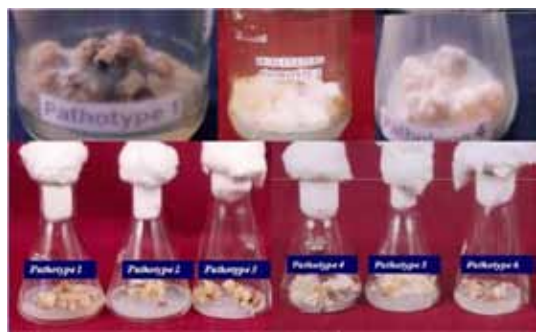

Fig. 14: *Pathotypes of* Sclerospora graminicola *maintained as dual cultures on pearl millet host callus under laboratory conditions*

3.7 Epidemiology

For disease development, inoculum source and survival, prevailing weather conditions and susceptibility/resistance of host are very important. Downy mildew has a compound interest type of disease cycle wherein primary inoculum source contributes to the secondary spread of the inoculum and several cycles of secondary inoculum spread are produced during the same crop season. Disease development is favored by high relative humidity (85-90% RH) and moderate temperature (20-30°C). Effective spread of inoculum from a focus depends upon prevailing weather conditions and wind velocity. Subsequent infection and disease development is guided by the susceptibility levels of the host on which inoculum gets deposited. On a susceptible host, under favorable weather and inoculum conditions, infection-to-disease development period (spore to spore) is about 7 days. Several crops of asexual spores are produced during a life cycle of the host that results in spread of the disease and increased yield losses.

Oospore-infested soil is the primary sources of inoculum which causes the first expression of the disease in a host population by infecting the seedlings. Severely infected young seedlings mostly die within 30 days without producing oospores. Oospores produced in the mature leaves, get mixed with the soil or seeds to initiate the disease in the next season. Secondary spread of the disease is through the airborne sporangia/zoospores. *S. graminicola* is known to infect about 14 species of graminaceous hosts belonging to eight genera (Safeeulla, 1976), however, the role of these hosts acting as primary source of inoculum and contributing to the downy mildew epidemiology of pearl millet under Indian conditions is not convincingly demonstrated.

3.7.1 Soil-borne inoculum: Oospore are primarily soil-borne, found in the soil or in the plant debris. Oospores when added to the soil or coated to the seed infect the germinating seedling and initiate the primary disease symptoms in 7-8 days. Large numbers of oospores are produced in downy mildew infected plants which can easily get incorporated in the soil during natural shredding of the crop or during harvest. Oospore survival in soil is reported to vary from 8 months to 10 years. It is generally difficult to show the germination of oospores. Therefore, vital stains such as 2,3,5-triphenyltetrazolium chloride (TTC) test is used to demonstrate viability of the oospores. In general 30-40% of the oospore produced are viable.

3.7.2 Seed-borne inoculum: Seed borne inoculums can be in the form of oospores sticking to the seed coat, or as a concomitant contamination with plant debris. Vegetative mycelium can also be seen in the various part of the seed tissues like seed coat, endosperm and embryonic tissues. However, only the mycelium in the embryonic tissue is infective (Shetty *et al.,* 1980). Shetty *et al*. (1977, 1978, 1980) demonstrated that inoculum may be present in pearl millet seeds either as external oospores, or in the form of internal dormant mycelium. Although these reports have indicated the possibility of the seed borne nature of *S. graminicola,* experimental evidence for the viability of the pathogen has not been demonstrated (Thakur *et al*., 2010). However, seed treatment is recommended to prevent introduction of *S. graminicola* through seed.

3.7.3 Air-borne inoculum: Chances of oospores acting as air-borne inoculum are less. However, only in tropical storm like conditions the oospores in the soil surface along with the plant debris can be carried with the high wind or storm to different fields or to uninfected areas. Mostly asexual spores contribute to the air-borne inoculum. Air-borne inoculum in the form of sporangia/zoospores can remain viable in the air for a few hours. After that the sporangia may desiccate and become non-infective. The air-borne sporangia

can infect the young seedlings or young crop by falling into the leaf whorl throughout the crop season. Seedlings infected by oospores as primary source of inoculum after 7-8 days produce asexual spores by sporulation. The inoculum from the asexual spores act as secondary source of inoculum and fresh plants continue to get infected including young inflorescence which produces green ear symptoms. Under favorable conditions, up to 35000 sporangia cm^{-2} are produced on the infected leaf and as many as 11 crops of sporangia are produced on successive nights. Sporangia are forcibly discharged up to 2.5 m distance from the sporangiophores. Sporangial liberation can continuously occur at 24°C, 100% RH and darkness. After liberation, some sporangia may fall to the ground and some may be carried by moving air. The air-borne sporangia under ideal conditions can travel up to 2-3 Km and deposit on the pearl millet crop. Air-borne state of *S. graminicola* is represented in Fig. 15. Sporangia can also germinate in the soil and produce zoospores which can survive, move against gravity in the soil, and remain infective up to 5 h showing that sporangia/zoospores deposited on the soil may act as secondary source of inoculum, causing infection in the seedling stage of the plant during the rainy season.

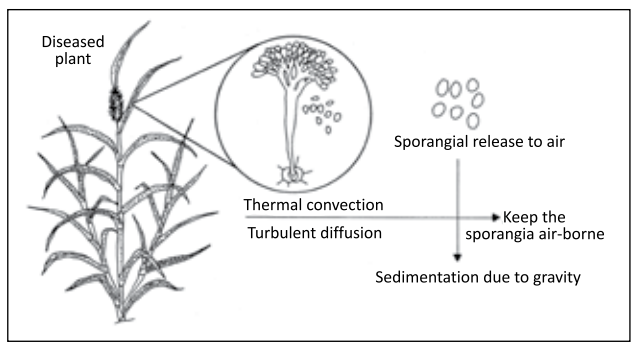

Fig. 15: *Air-borne state of* Sclerospora graminicola

The sporangium remains air-borne after taking off from the sporangiophores until it gets deposited on a substratum or the infection court. The viability of the sporangium is determined by temperature, humidity and wind speed. It is reported that nights are best suited for sporulation. The environmental conditions best suited for sporulation are also best suited for efficient dispersal. Safeeulla (1976) speculated that sporangia which fall onto the ground may liberate zoospores in the wet soil, which in turn may infect healthy plants through the roots.

3.8 Screening techniques

Reliable, repeatable and effective greenhouse and field screening methods have been developed for downy mildew of pearl millet (Safeeulla, 1976; Singh and Williams, 1980; Williams *et al*., 1981; Singh and Gopinath, 1985; Singh *et al*., 1993; Amruthesh, 2000; Chaluvaraju, 2002; Thakur *et al*., 2006). Screening techniques under greenhouse

conditions have been further refined (Singh *et al*., 1993; Weltzien and King, 1995; Jones *et al*., 2001) and are highly useful for testing breeding progenies between growing seasons. Field screening using a combination of infector rows and oospore-infested soil plots has been used effectively to screen for resistance to downy mildew at 11 locations under AICPMIP programme in India. Screening of a large number of germplasm materials is undertaken to identify resistance sources and use them in breeding programs. Resistance of identified sources is confirmed by repeated screening across locations.

3.8.1 Greenhouse screening: Though field screening technique has been widely adopted by pearl millet pathologists in India and Africa, further studies on biology and epidemiology of the pathogen has 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 season, and both time-efficient and cost-effective. This screening technique minimizes escape as every seedling is uniformly inoculated, can be operated throughout the year except during very hot (>42°C) and cold (<15°C) conditions. The technique is currently being used to screen breeding lines against the diverse pathotypes of *S. graminicola* of Indian origin. Different steps involved in the greenhouse screening are given (Fig. 16).

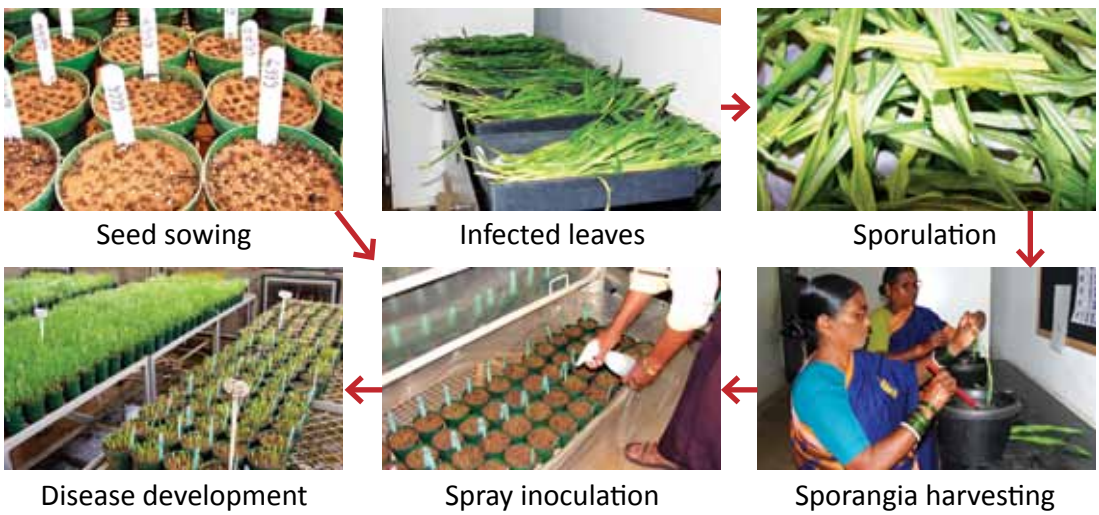

Fig. 16: *Steps involved in the greenhouse screening for downy mildew resistance*

3.8.1.1 Multiplication of inoculum: *S. graminicola* pathotype-isolates are multiplied on highly susceptible host genotypes like 7042S in the greenhouse for the production of sporangial inoculum for the inoculation of pearl millet lines to be screened. The pot-grown seedlings of the maintainer host are inoculated with the sporangial suspension of the pathotype-isolate, incubated at 20°C for 16 h in the dark, and then transferred to a greenhouse bay under misting for 4-5 days. The inoculated seedlings are grown for 25-30 days at 25±2°C under proper care of watering and fertilizers to produce good infected foliage, which sporulate profusely and provide a good amount of sporangial inoculum needed for mass inoculation. Several isolates can similarly be multiplied to generate large volumes of inoculum required for the inoculation of large number of breeding/ germplasm lines.

3.8.1.2 Growing seedlings of test lines in pots: The seed of test lines is sown in the plastic pots of various sizes (10 cm, 15 cm, and 17.5 cm diameter) filled with autoclaved potting mixture. The seed of test lines and susceptible checks is sown at uniform depth in holes made with the help of a dibbler stamp, with single seed per hole, to achieve uniform emergence of seedlings. The seeds in the pots are then covered with a 1 cm layer of potting mixture, irrigated properly and are maintained in the greenhouse at 35°C till seedling emergence.

3.8.1.3 Preparation of inoculum and inoculation: Infected leaves are collected from the pot-grown inoculated seedlings of different pathotype-isolates individually. The leaves are cut into pieces of 20-30 cm for proper handling and washed in the running tap water using cotton swab to remove old sporangial growth from the leaf surface and are wiped dry with tissue paper. The leaf pieces are placed in plastic tray lined with wet blotting paper (humidity chambers) keeping their abaxial surface facing upward. The trays containing infected leaves are then incubated in a BOD incubator at 20°C for 6 h in dark. The temperature in the incubator should be brought down to about 0°C after 6h of incubation of infected seedlings to prevent the release of zoospores from sporangia. After incubation, sporangia are harvested from sporulating leaves (using a soft camel hair brush) into ice cold sterilized distilled water (4°C) in a beaker suitably placed in an ice box. The sporangial suspension is filtered through a double-layered muslin cloth to remove the plant debris, and the sporangial concentration is adjusted to 1×10^5 mL^{-1} using a haemocytometer.

Dip inoculation, injecting the inoculum into the seedlings, and moist chamber inoculation have been described for the inoculation of pearl millet seedlings to screen against

S. graminicola under greenhouse conditions. However, spray-inoculation of the seedlings with the sporangial inoculum is the most common and reliable method of inoculation. Seedlings in each pot are counted at the coleoptile to first-leaf stage (2-3 days after sowing) and recorded on the plastic peg before inoculation to discount any seedlings emerging after inoculation. The pot-grown seedlings are spray-inoculated with the fresh sporangial suspension using pneumatic atomizer till run-off ensuring that every seedling has received uniform inoculum. The inoculated seedlings are covered with a polyethylene sheet immediately to provide high humidity required for infection, and incubated in the dark at 20°C for 16-20 h. These pots are then transferred to greenhouse benches at 25±2°C with misting to provide high humidity (>95% RH) and leaf wetness for disease development, for the next 14 days.

3.8.1.4 Data recording: The infected seedlings are counted in each pot 2 weeks after inoculation and the number is recorded on the same plastic label in the pot on which total seedling counts were recorded before inoculation. Thus, downy mildew incidence (%) in a particular line can be calculated from the number of total seedlings inoculated and number of infected seedlings. The test entries are categorized as highly resistant = 0-5% disease incidence; resistant >5-10% incidence; moderately resistant >10-20% incidence; susceptible >20-50% incidence and highly susceptible >50% disease incidence.

3.8.2 Field screening

3.8.2.1 The infector-row technique: A field-based downy mildew screening has been developed that is being effectively used to screen the breeding material for downy mildew resistance. The technique mainly utilizes sporangia from a susceptible pearl millet line as infection propagules. However, when the same field is used in subsequent years, oospore inoculum is built up in the soil resulting in development of "oospore-infested soil", and then both oospores and sporangia serve as inocula for screening. This technique has three basic components- infector rows as inoculum donor, indicator rows to provide the measure of uniform disease incidence/spread in the nursery, and test rows, the lines to be evaluated (Fig. 17). These are described below:

Establishing infector rows: Infector rows could be a mixture of two to three susceptible lines (local landraces and highly susceptible lines). For establishing infector rows, surface of the seed of infector lines is coated with infected leaf (collected in the previous season) powder containing oospores, by slurry treatment and drying the seed in the shade. The oospore-coated seed is sown on every fifth or ninth row throughout the entire length of

Fig. 17: *Field screening of pearl millet for downy mildew resistance*

the field. Infector rows are spray-inoculated at the coleoptile-to one-leaf stage (2-3 days after emergence) with sporangial suspension (1×10^5 sporangia mL^{-1}) during late evening hours after furrow irrigation. Perfo-irrigation is provided frequently to maintain high humidity (>90% RH) and leaf wetness to promote infection and disease development. downy mildew symptom development in the seedlings is monitored at weekly intervals by counting the total and infected seedlings in a stretch of 1 m length randomly at several places, to determine the disease incidence - percentage seedlings infected. About 70% downy mildew incidence in infector rows is considered adequate before planting the test rows.

Growing test rows: The infector rows provide sporangial inoculum for the screening of test rows, the lines to be evaluated for downy mildew resistance. The untreated seed of test lines is sown in the intervening 4 or 8 rows in between the infector rows about 3 weeks after sowing the infector rows (when >70% incidence occurs in infector rows). Each test line is sown in 2 rows of 4 m long and about 40 seedlings are maintained per row by thinning 2 weeks after seedling emergence. Perfo/furrow irrigation is provided for about 7 days after sowing the test rows to maintain high humidity (>90% RH) and leaf wetness to promote infection and disease development.

Growing indicator rows: Indicator rows are grown to measure uniform availability of sporangial inoculum in the disease nursery. Indicator row is a highly susceptible line, either a breeding line or a local landrace sown after every 10 or 20 rows of the test lines, at the same time as the test lines. High disease incidence levels in these lines indicate the uniform disease pressure in the nursery for a reliable disease screen.

3.8.3 Disease recording: Downy mildew incidence in the test lines is scored by counting the numbers of total and infected seedlings in each plot and is expressed as percentage of infected seedlings. Disease is scored twice, first at 30-days after emergence (pre-booting/flowering stage) and second at 60-days after emergence (soft-dough stage) of the crop. In certain lines, disease development is slow and thus number of infected plants increases from 30-day stage to 60-day stage of the crop, and sometimes the systemic latent infection is expressed as "green ear" in some plants at flowering. Thus, downy mildew incidence at 60-days stage is used to assess reaction of test line to downy mildew pathogen population at a particular location. Disease scoring can also be done at 10-day intervals up to crop maturity to determine disease progress, if required.

Based on disease incidence in the fields screen, the test entries are categorized as highly resistant = 0-5% disease incidence; resistant >5-10% incidence; moderately resistant >10-20% incidence; susceptible >20-50% incidence and highly susceptible >50% disease incidence. However, in order to relate the productivity loss to downy mildew severity, disease severity scores are taken on individual plant basis at crop maturity using a 1-5 scale (Thakur *et al.*, 2011), where

1 = no infection;
2 = 20% productive tillers infected;
3 = 50% productive tillers infected;
4 = 80% productive tillers infected;
5 = all tillers infected or total plant killed

Disease severity (%) = [{(1-1).n1+ (2-1).n2 + (3-1).n3 + (4-1).n4 + (5-1).n5}/ (5-1). N] × 100; where n1, n2, n3, n4, and n5 are total number of plants in each of 1 to 5 rating class, and N is the total number of plants in a plot. The productivity loss on individual plant basis could vary from zero when there is no infection (score 1) to 100% when all tillers get infected and the plant is killed (score 5).

3.9 Variability in pathogen

S. graminicola is a highly variable pathogen, and some isolates are generally more virulent than the others. The pathogen is largely heterothallic but homothalism has also been reported (Idris and Ball, 1984; Michelmore *et al.*, 1982). Existence of mating types

and their frequency greatly contribute towards the development of new recombinants in the pathogen populations (Pushpavathi *et al.*, 2006a). These characteristics of the fungus make it highly variable. In India, great heterogeneity is found in pathogenicity within populations, between seasons and among single-oospore isolates of *S. graminicola* (Thakur and Shetty, 1993). Two reference mating type isolates Sg 018 (*Mat*-1) and Sg 019 (*Mat*-2) have been identified in *S. graminicola* and are being maintained at ICRISAT, Patancheru, India. *S. graminicola* has the potential for rapid change in virulence pattern with host genotype-directed selection for specific virulence. As a result of evolution of host specific virulences, resistant genotypes lose their effective resistance within a short period and leads to the development of new pathotypes/races in the pathogen populations (Kolmer *et al.*, 2006). Therefore, pathogen populations in the major crop growing areas need to be periodically monitored and characterized to identify new pathotypes in the target area.

3.9.1 Pathogenic variation: The first evidence of pathogenic variation in *S. graminicola* was reported in 1973 when hybrid HB 3 was found to be resistant to one of the isolates of *S. graminicola* maintained at Mysore but susceptible at Gulbarga in India. These two isolates of downy mildew pathogen were considered as two different pathogenic races and referred to as race 1 and race 2. The race 1 isolated from HB 3 was more virulent and infected HB 3 hybrid whereas, the race 2 isolated from landrace cultivar Kalu Kombu was less virulent and did not infect HB 3 (Shetty and Ahmad, 1981). These isolates also varied in their morphological features like size and shape of asexual structures and number of nuclei in sporangia. However, these two distinct pathotypes are not clearly demonstrated as race 1 and 2 but they are regarded as different pathotypes.

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* is being studied by establishing a collaborative disease nursery, conducting on-farm downy mildew surveys and characterizing the isolates for pathogenic diversity on host differentials in the greenhouse screens.

3.9.2 Characterization of pathogen population through downy mildew virulence nursery: The International Pearl Millet Downy Mildew Virulence Nursery (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.,* 2004b). The results also indicated that resistance in the lines IP 18292, IP 18293, 700651 and P 310-17 was more stable than in others regardless of the location or season. The pathogen populations at Bagauda (Nigeria) and Durgapura (India) were found most virulent as they caused the most severe disease, and those from Coimbatore and Aurangabad (India) the least virulent. At present, the IPMDMVN consisting of 50-60 test entries from ICRISAT and AICPMIP, Mandor, is conducted at 11 locations– Durgapura and Mandor (Rajasthan); Hisar (Haryana); Anand and Jamnagar (Gujarat); Aurangabad and Dhule (Maharashtra); Gwalior (Madhya Pradesh); Patancheru (Telangana); Mysore (Karnataka); and Coimbatore (Tamil Nadu) in India. At each location the nursery is conducted in "oospores-infested plots" developed using oospore inoculum from the susceptible cultivars (7042S) using infector-row system. The results of this multilocational testing provide useful information on virulence variability in the pathogen population and on the resistance stability of breeding lines under diverse environmental conditions. Spatial variation in the virulence of pathogen populations has been revealed by the mean disease incidence levels in the test lines at different locations. During 2014, the mean downy mildew incidence across 65 test entries was highest at Jamnagar (34.1%) followed by Mysore (20.4%), and least at Aurangabad (3.6%). The variation in the pathogen populations was evident from the variable downy mildew incidence in the test lines across locations as well as by the mean disease incidence levels at different locations (Table 1). Considering the disease incidence at the soft-dough stage, the pathogen population at Mysore appeared most virulent with 50 test entries exhibiting >10% downy mildew incidence, followed by the pathogen population at Jamnagar (48 entries with >10% incidence). The populations at Aurangabad and Gwalior were least virulent with only 2-4 entries showing >10% downy mildew incidence. The results of disease nursery also provide useful information for the identification of pearl millet lines having stable resistance across locations/pathogen populations over the years for use in breeding programs. Pearl millet lines IP 18292, IP 18294, ICMR 01007, H 77/833-2 P5, ICMB 11333, 843-22B, JMSB-20091 have been found resistant across location in the disease nursery. Similarly, results of PMDMVN conducted during 2010-2014 indicate that resistance in a popular hybrid HHB 67-2 Improved through MAS is still effective.

Table 1: Downy mildew incidence (%) in PMDMVN-2014 at soft dough stage across 11 locations in India

Pedigree	Downy mildew incidence (at 60 days after emergence)												Resistance at locations (no.)
	Anand	Aurangabad	Coimbatore	Dhule	Durgapura	Gwalior	Hisar	Jamnagar	Mandor	Mysore	Patancheru	Mean	
P7-4	5.0	5.8	8.7	16.4	1.9	4.8	27.7	28.7	3.2	24.5	1.7	**11.7**	**7**
P310-17	9.7	3.7	2.7	1.7	1.8	1.8	17.0	29.2	1.1	22.7	10.5	**9.3**	**7**
700651	9.8	5.7	4.4	12.0	7.5	1.2	12.0	38.4	4.5	46.8	30.2	**15.7**	6
852 B	44.0	6.8	0.0	20.1	29.6	10.8	0.0	95.2	11.7	55.1	39.8	**28.5**	**3**
7042 R	43.2	3.0	2.3	7.2	36.9	62.9	97.0	96.4	23.9	49.1	59.8	**43.8**	**3**
IP 18292	0.0	0.0	3.0	3.8	3.3	0.0	0.0	5.6	4.1	10.3	8.7	**3.5**	**10**
IP 18293	3.6	4.9	0.0	3.0	1.7	1.1	0.0	26.3	1.1	21.3	8.2	**6.5**	**9**
IP 18294	0.9	1.4	0.0	7.8	4.3	1.2	3.3	10.0	4.1	24.8	8.0	**6.0**	**10**
ICMP 451	31.3	9.7	32.3	1.2	37.9	8.5	0.0	74.1	18.4	31.5	36.1	**25.6**	**4**
7042 S	86	82.5	96.1	83.9	78.4	93.8	94.7	100.0	96.2	94.5	100.0	**91.5**	**0**
ICMB 10444	0.0	5.3	2.4	42.1	0.9	0.0	13.6	0.0	0.5	10.1	4.8	**7.2**	**8**
ICMB 10555	0.0	0.0	6.0	2.4	3.8	1.1	27.9	23.9	2.8	8.9	1.8	**7.1**	**9**
ICMB 11555	0.0	0.0	3.8	0.0	0.0	1.0	1.5	12.2	0.0	15.7	2.7	**3.4**	**9**
ICMB 81	51.9	1.7	3.5	22.5	47.9	5.7	0.0	58.8	25.2	31.5	89.2	**30.7**	**4**
ICMB 14001	0.0	4.4	1.1	38.0	12.8	0.6	0.0	9.9	8.0	20.6	60.8	**14.2**	**7**
ICMB 14002	32.3	0.8	5.8	3.9	2.1	0.0	38.2	23.3	3.2	17.3	10.3	**12.5**	**6**
ICMB 14003	0.0	4.2	4.3	4.8	1.4	0.0	0.0	3.0	2.2	15.0	16.3	**4.6**	**9**
ICMB 14004	0.0	1.3	2.7	1.3	4.4	0.6	5.2	6.3	3.7	18.1	11.2	**5.0**	**9**
ICMR 08888	*	1.4	5.5	3.2	0.0	0.0	5.9	5.9	0.0	18.1	0.0	**4.1**	**9**
ICMR 08555	51.9	0.8	1.2	1.4	5.0	0.0	21.8	70.1	2.8	26.0	16.6	**18.0**	**6**
ICMR 14001	0.0	0.0	4.0	1.1	0.0	0.0	23.9	8.9	0.0	9.2	15.0	**5.6**	**9**
ICMR 01007	0.0	0.0	4.9	4.7	0.0	3.5	7.7	23.0	0.0	7.2	2.8	**4.9**	**10**
HHB 67-1 Improved	0.0	0.0	2.8	4.6	4.1	1.2	4.5	5.2	2.3	13.1	2.5	**3.7**	**10**
HHB 67-2 Improved	0.0	0.0	0.0	0.0	0.0	0.6	1.4	12.1	0.0	7.8	1.9	**2.2**	**10**
HHB 67 Original	0.9	2.0	10.4	2.0	5.0	2.0	0.0	24.7	11.9	43.2	12.3	**10.4**	**6**
H77/833-2-202	0.0	0.0	4.4	2.0	0.0	0.0	8.3	14.5	0.0	10.1	1.1	**3.7**	**9**
H77/833-2	0.0	0.0	4.4	0.0	0.0	2.6	7.8	25.9	0.0	7.3	19.6	**6.2**	**9**
H 77/833-2 P5	0.0	0.0	5.6	6.7	0.0	0.6	3.9	15.1	0.0	8.4	8.7	**4.5**	**10**
843-22B	0.8	0.0	6.5	2.0	12.3	7.7	15.8	41.9	8.3	14.4	0.0	**10.0**	**7**
843B	64.6	5.8	20.0	69.7	26.0	6.4	8.8	92.8	24.3	41.2	49.9	**37.2**	**3**
ICML 22	47.5	12.2	22.8	17.3	15.1	74.3	57	87.7	13.2	37.2	45.6	**39.1**	**0**
P 1449-P2	0.0	2.9	4.4	0.0	4.8	2.1	21.8	17.3	2.3	8.9	1.9	**6.0**	**9**
AIMP 92901-P3	0.0	4.2	2.8	1.3	0.0	0.0	4.7	18.4	0.0	8.0	4.0	**3.9**	**10**

Table 1 Continued ...

Pedigree	Downy mildew incidence (at 60 days after emergence)												Resistance at locations (no.)
	Anand	Aurangabad	Coimbatore	Dhule	Durgapura	Gwalior	Hisar	Jamnagar	Mandor	Mysore	Patancheru	Mean	
AIMP 92901-P8	6.7	6.9	4.4	3.9	0.0	0.0	2.3	2.9	0.0	7.5	2.3	**3.4**	**11**
ICMB 90111-P6	0.0	0.7	3.1	1.0	0.0	0.0	10.4	17.5	0.0	7.6	2.5	**3.9**	**9**
PIB 226	21.1	0.0	4.3	18.2	2.9	1.2	0.0	3.1	3.2	8.0	18.4	**7.3**	**8**
PIB 957	18.0	1.4	3.1	0.0	7.6	0.0	12.0	1.9	8.5	19.2	6.6	**7.1**	**8**
PIB 1234	0.0	0.0	4.6	1.4	6.5	2.5	0.0	33.5	4.4	25.6	7.3	**7.8**	**9**
PIB 654	0.0	1.3	3.0	0.0	0.0	2.8	0.0	6.3	0.0	13.9	21.6	**4.5**	**9**
PIB 626	0.0	0.0	2.8	4.3	0.0	0.6	11.5	30.4	0.0	18.5	3.5	**6.5**	**8**
PIB 686	0.0	2.2	2.9	0.0	0.0	0.0	0.0	3.6	0.0	16.0	4.2	**2.6**	**10**
214 B	0.0	0.0	3.1	0.0	0.0	1.3	0.0	47.5	0.0	16.3	6.0	**6.8**	**9**
543 B	29.3	0.0	10.1	0.0	1.3	2.9	0.0	70.6	1.6	15.6	11.0	**12.9**	**6**
BIB sum 458-462	51.0	3.0	3.5	38.3	32.5	3.0	13.0	55.0	18.9	8.4	21.5	**22.6**	**4**
BIB sum 503-510	21.7	3.4	21.0	14.8	28.2	8.4	14.9	83.5	13.1	23.4	17.9	**22.8**	**2**
BIB sum 472-482	56.6	1.6	31.3	13.2	13.2	1.3	23.1	86.6	7.3	34.2	43.0	**28.3**	**3**
Comp. sum 622-660	26.1	5.2	7.7	41.5	2.0	0.0	4.9	37.9	5.2	11.7	11.0	**13.9**	**6**
ARL-1	0.0	2.8	4.8	0.0	0.0	0.6	0.0	10.9	0.0	9.5	4.0	**3.0**	**10**
ARL-2	22.0	1.3	2.9	1.0	9.6	1.1	20.5	4.2	9.0	9.1	50.8	**12.0**	**8**
J-2480	0.0	0.0	3.3	0.0	6.0	2.1	22.7	43.1	2.8	22.9	52.5	**14.1**	**7**
J-2500	8.8	0.0	1.5	7.1	0.0	0.0	18.8	87.0	0.0	12.7	1.1	**12.5**	**8**
J-2495	25.6	0.0	4.6	0.0	2.9	0.5	0.0	4.5	2.4	11.0	0.0	**4.7**	**9**
J-2523	3.8	0.0	4.4	5.2	1.9	1.5	16.3	24.5	4.9	15.4	9.1	**7.9**	**8**
92-SB-13	0.0	4.1	2.8	0.0	17.5	1.1	0.0	24.0	15.3	17.0	0.0	**7.4**	**7**
JMSB-101	28.5	3.4	6.1	7.0	18.2	0.0	63.0	47.2	10.2	8.3	28.1	**20.0**	**5**
JMSB-9904	31.3	6.3	4.3	14.8	3.7	3.8	40.2	77.2	3.9	11.7	41.4	**21.7**	**5**
JMSB-20071	24.8	0.0	4.1	10.9	4.6	0.0	48.4	33.1	6.9	10.2	15.2	**14.4**	**5**
JMSB-20091	0.0	4.3	3.1	3.1	0.0	0.6	0.0	20.9	0.0	10.1	24.0	**6.0**	**8**
JMSB-20111	16.9	4.9	3.1	21.0	0.0	3.0	2.6	37.9	0.0	23.2	37.3	**13.6**	**6**
J-2578	10.9	1.5	0.0	9.9	3.8	0.0	40.1	21.9	5.6	19.9	13.4	**11.5**	**6**
J-2538	0.0	0.0	0.0	4.5	0.0	0.0	0.0	29.3	0.0	72.1	22.5	**11.7**	**8**
J-2510	0.0	0.7	4.7	26.9	0.0	9.8	20.0	95.3	0.0	17.8	49.9	**20.5**	**6**
JMSB 20101	31.8	3.9	0.0	0.0	1.4	0.0	0.0	4.3	1.2	20.0	1.8	**5.9**	9
JMSB 20082	0.0	5.5	3.0	2.9	0.0	0.0	36.6	20.7	0.0	13.7	1.3	**7.6**	**8**
JMSB 20042	0.0	1.4	1.5	0.0	3.7	0.0	9.9	42.2	5.4	18.3	2.9	**7.8**	**9**
Location mean	14.2	3.6	6.7	9.8	7.9	5.3	14.8	34.1	6.3	20.4	18.7		

** no data*

3.9.3 Monitoring virulence/resistance through on-farm surveys

Virulence/resistance monitoring is done by well-planned on-farm surveys in major pearl millet growing states of India (Thakur *et al.,* 2003). The field surveys are conducted in major pearl millet growing states i.e. Rajasthan, Haryana, Gujarat, Uttar Pradesh and Maharashtra. These surveys are conducted during July-October to coincide with the pre-boot to flowering stage. During the survey, pearl millet fields are sampled approximately every 10 km along the road side depending upon cropping intensity and hybrid diversity. More fields are sampled in areas with a greater diversity of hybrids. Downy mildew incidence is recorded in each field by assessing a minimum of 50 plants in each of five quadrates (2 m X 4 rows). Information on cultivar, field area, fertilization, seed treatments with fungicides, cultural practices and cropping systems is also recorded by interacting with farmers. Infected leaf samples are also collected during these surveys to study pathogenic variability among isolates based on their downy mildew reaction on set of host differentials. During 2000-2014 more than 2000 fields in five states [Maharashtra, Rajasthan, Gujarat (summer and rainy), Haryana and Uttar Pradesh] have been surveyed. Of these 51% of the fields were found infected with downy mildew. A systematic representation of number of field surveyed, number of downy mildew infected fields, number of hybrids grown and mean downy mildew incidence across

Table 2: On-farm surveys conducted in India to monitor the prevalence of downy mildew during 2000-2014

State	Year	No. of fields surveyed	No. of fields infected	Number of hybrids observed	DM incidence (%) range
Rajasthan	2002	164	37 (22)	16	1–25
	2003	224	210 (94)	7	1–60
	2004	109	59 (54)	9	1–20
	2006	121	101 (83)	24	1-100
	2008	35	14 (35)	5	2–62
	2009	24	4 (17)	7	2–30
	2012	32	4 (13)	8	2–16
	2013	62	9 (14)	7	2–20
	2014	9	1 (11)	1	Trace
	Total	780	439 (56)	84	1-100

Table 2 Continued ...

State	Year	No. of fields surveyed	No. of fields infected	Number of hybrids observed	DM incidence (%) range
Haryana	2003	209	197 (94)	4	1–30
	2004	102	88 (86)	4	1–45
	2009	29	4 (14)	9	1–25
	Total	340	289 (85)	17	1–45
Gujarat	2003	109	57 (52)	23	1–85
	2005	70	23 (33)	10	2–70
	2006	71	41 (58)	23	1–93
	2007	87	7 (8)	22	11–81
	2008	58	7 (12)	22	1–20
	2012	20	12 (60)	2	2–32
	Total	415	147 (35)	102	1–93
Uttar Pradesh	2007	56	48 (86)	12	2-100
	2008	41	37 (90)	5	10-100
	2012	33	7 (21)	6	1–74
	2014	35	12 (34)	6	1–28
	Total	165	104 (63)	29	1-100
Maharashtra	2000	333	60 (18)	26	1-100
	2009	48	12 (25)	10	10–70
	2010	83	60 (72)	15	8–90
	Total	464	132 (28)	51	1-100

Value in parenthesis shows percent downy mildew infected field

pearl millet growing states is given in Table 2**.** The results of the surveys are regularly shared during the AICPMIP annual group meetings. These results have benefited the pearl millet breeders in monitoring resistance levels of their hybrids and planning their resistance breeding programs accordingly. During on-farm surveys, it has been observed that most of the seed supplied to farmers by private seed companies is treated with the fungicide metalaxyl (Ridomil/Apron). This is a matter of great concern in relation to likely evolution of new pathotypes resistant to metalaxyl. Resistance to metalaxyl has already been reported in *Peronosclerospora sorghi*, the causal agent of sorghum downy mildew (Perumal *et al.*, 2008). It is well known that seed treatment with metalaxyl fungicide protects the crop only up to 35-40 days, and later the disease appears on the nodal tillers

and on panicle in a susceptible hybrid. Therefore, susceptible hybrids must not be grown at all even with fungicide treatment.

3.9.4 Characterization of *S. graminicola* isolates for virulence diversity based on reaction on host differentials

Isolates of *S. graminicola* collected from highly susceptible pearl millet hybrids and local cultivars during on-farm surveys in different states of India are characterized for pathogenic variation to monitor virulence change in the pathogen populations. Highly virulent isolates thus identified from different states are selected and used for screening breeding lines towards developing downy mildew resistant hybrid parental lines and hybrids.

The isolates collected from different fields/areas are established from oospores or sporangia on the pot-grown seedling in the greenhouse and maintained through asexual progenies. Isolates are maintained either on the same host from which they were collected or on a highly susceptible genotype 7042S. The isolates are characterized for pathogenic variation based on reaction on pot-grown seedlings of the 7 differential lines (P 7-4, P 310-17, 700651, 7042R, 852B, IP 18292, IP 18293) and two susceptible check lines ICMP 451 and 7042S. Sporangia from sporulating leaves of each isolate are harvested individually in sterilized distilled ice cold water using camel hair brush and filtered through double-layered muslin cloth. Spore concentration is adjusted to 5 x 10^5 ml^{-1}. Pot-grown seedlings of the differential lines and susceptible checks are spray-inoculated at coleoptile stage with the sporangia of each isolate using atomizer and covered immediately with a polyethylene sheet to provide >95% relative humidity. Seedlings are incubated at 20°C for 24 h and then pots are transferred to greenhouse benches at 25±2°C and >95% RH for disease development during the next 2 weeks. The line showing ≤10% downy mildew incidence is considered resistant to the isolate and the isolate is considered avirulent on that line; whereas line showing >10% downy mildew incidence is considered susceptible to the isolate and the isolate is considered virulent on that line. To assess pathogenic diversity among pathogen populations, 46 isolates of *S. graminicola* collected from seven states in India during 1992-2005 were selected for the virulence diversity study (Sharma *et al.*, 2010). Of the 46 isolates, 16 were from Andhra Pradesh, 13 from Rajasthan, 11 from Gujarat, 2 each from Maharashtra and Haryana and 1 each from Karnataka and Delhi. Differential reaction of the host genotypes to the test isolates was observed and on the basis of the reaction type, isolates were grouped into different pathotypes. The 46 isolates used in the study were grouped into 21 pathogenic groups (Table 3).

Table 3: Pathotyping of 46 isolates of downy mildew based on disease reaction on nine host differential lines

Pathotype	Downy mildew reaction on host differential lines									Isolate number
	P7-4	P310-17	700651	7042 R	852B	IP 18292	IP 18293	ICMP 451	7042 S	
1	S	R	S	S	R	R	R	S	S	A1, A9, M2, H3, R6
2	S	R	R	S	R	R	R	S	S	A6, H2, A13, A15
3	S	R	R	S	R	R	S	S	S	R7
4	S	R	R	R	R	R	R	S	S	A14
5	R	R	R	S	R	R	R	S	S	A2, A7, A8, A11, M1, G2
6	R	R	R	S	S	R	R	S	S	A3
7	S	R	S	S	S	R	R	S	S	A4
8	S	S	S	S	S	R	R	S	S	A12, R3
9	S	S	S	S	R	R	S	S	S	A10
10	S	S	S	S	R	S	S	S	S	G4
11	S	S	S	S	S	S	S	S	S	A16, R11, R12, R1, R9, R10, G9, G8, G7, G6, G11, G3
12	S	S	S	S	S	R	S	S	S	R8
13	S	R	S	S	S	S	S	S	S	G10
14	S	S	R	S	S	S	S	S	S	R4, R5
15	S	R	R	S	S	S	S	S	S	H1
16	R	R	R	S	S	S	S	S	S	K1
17	S	R	R	S	S	S	R	S	S	R2
18	R	R	S	S	R	S	R	S	S	G1
19	S	S	S	S	R	S	R	S	S	G5
20	S	R	S	S	R	S	R	S	S	R13
21	R	R	S	S	R	R	R	S	S	A5

R=downy mildew incidence ≤ 10%; S=downy mildew incidence > 10%

Characterization of isolates of *S. graminicola* collected from major pearl millet growing areas helps in selecting the pathotypes for use in greenhouse screening of pearl millet breeding material developed for different ecologies. A pathotype-isolate of *S. graminicola* is the most virulent isolate selected from a number of isolates collected from a particular pearl millet-growing area and characterized for virulence diversity. At present there are at least 17 diverse pathotypes (populations) of *S. graminicola* that have been identified from major pearl millet growing parts of India (Table 4). These 17 pathotypes are being used selectively for screening breeding lines targeted for utilization in different pearl millet

production zones of India. ICRISAT has a major research focus on development of parental lines, especially diversifying the genetic base of male-sterile lines, which are disseminated to public organizations and private seed companies for use in developing F_1 hybrid cultivars. As part of screening for downy mildew resistance, breeding lines are screened against different pathotypes of *S. graminicola* in greenhouse under high disease pressure (>85% disease incidence in the susceptible check) and those found resistant (≤10% disease incidence) to at least two pathotypes are designated and disseminated as seed parents.

Table 4: Downy mildew pathotypes being used at ICRISAT for greenhouse screening of breeding material

State	Pathotype (isolate) location	Collected from	Resistance source
Rajasthan	Sg 212 (old)	Jaipur	Several
	Sg 457 (new)	Jaipur	Several
	Sg 139 (old)	Jodhpur	Several
	Sg 526 (new)	Jodhpur	Several
	Sg 384	Barmer	Several
Maharashtra	Sg 150	Jalna	Several
	Sg 542	Deogaon	Several
Gujarat	Sg 200	Jamnagar	Several
	Sg 445	Banaskantha	Several
Delhi	Sg 561 (new)	New Delhi	Several
	Sg 298 (old)	New Delhi	Several
Haryana	Sg 519 (new)	Rewari	Several
	Sg 334 (old)	Bhiwani	Several
Andhra Pradesh	Sg 409	Patancheru	Several
Karnataka	Sg 048	Mysore	Several
Uttar Pradesh	Sg 492	Aligarh	Several
	Sg 510	Badaun	Several

3.9.5 Genetic diversity

It is important to study genetic variation in relation to virulence shift in the pathogen population to understand the changes in the genetic structure of the pathogen. In this context, molecular markers offer numerous genetic features to characterize diverse pathogen populations. DNA markers such as RFLP (Sastry *et al*., 1995), RAPD (Sastry *et al*., 2001) and AFLP (Singru *et al*., 2003; Sivaramakrishnan *et al*., 2003; Pushpavathi *et al*., 2006b) have successfully been used to study the genetic variation in the *S. graminicola*

populations. Apart from elucidation of genetic diversity, molecular markers can also be used to study evolution and monitoring movement/shift of pathogen populations over time and space.

AFLP analysis of *S. graminicola* revealed a high level of polymorphism among 46 isolates collected from the major pearl millet growing states of India (Sharma *et al*., 2010). A total 297 bands were scored following selective amplification with 3 primer combinations. Very high polymorphic information content (PIC) values of 0.985, 0.982 and 0.980 were observed for the primer combinations E-AT/M-CAG, E-TT/M-CAG and E-TG/M-CAT, respectively. However, no region/host specific amplicon was observed. The UPGMA-based dendrogram generated using Jaccard similarity coefficients clustered the test isolates into 7 groups (Fig. 18), however, with a low similarity value around 0.2. Analysis of molecular variance (AMOVA) revealed 93.2% of the total variance was due to the differences among the isolates within states and 6.8% was due to differences among the states. Despite the little contribution of variation among states towards the total variance, the value was statistically significant at (P<0.01). Genetic distance among the pathogen populations from different states was very low. Similarly, Sudisha *et al*. (2008) studied the genetic diversity among 27 isolates of *S. graminicola* using 20 RAPD and 19 ISSR primers (Fig. 19).

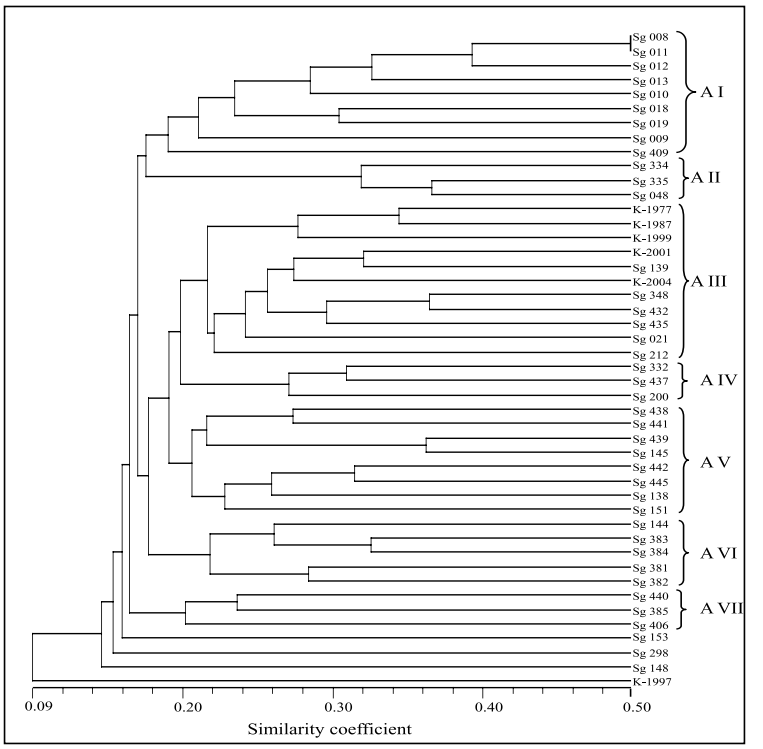

Fig. 18: *Dendrogram depicting genetic relatedness among 46 isolates of* Sclerospora graminicola *based on virulence index*

Simple sequence repeats (SSRs) or microsatellites are PCR-based molecular markers, which may be more desirable for population genetic analysis because this approach makes it simpler to obtain accurate polymorphic data due to co-dominance. Besides, these markers are highly reproducible, locus-specific, multi-allelic and abundant. In oomycetes like *Phytophthora* and *Plasmopara*, SSRs have been used for diagnosis and determination of mating type, studying genetic structure and population genetics. However, there is no report of development of PCR based SSRs markers from *S. graminicola* genomic DNA. Perumal *et al*. (2008) reported that of the 54 SSR primer pairs developed from *Peronosclerospora sorghi*, 37 could produce amplicons in *S. graminicola*, and can be used in the genetic diversity studies of pearl millet downy mildew. However, only 20 of the 37 reported SSRs could amplify reproducible fragments in the 23 Indian isolates of *S. graminicola*, and none of them was polymorphic (Sharma *et al*., 2009).

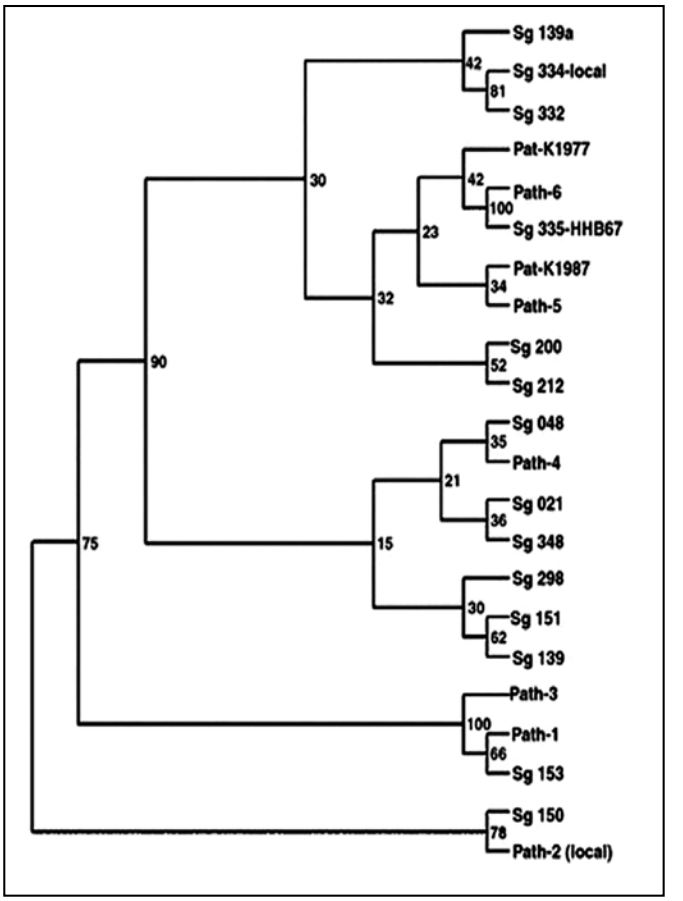

Fig. 19: *Dendrogram based on ISSR polymorphism of* Sclerospora graminicola *occurring on pearl millet by unweighted pair group method of averages (UPGMA) cluster analysis (numbers inside the branches are bootstrap values*

The high levels of genotypic diversity is because of frequent recombination events taking place in the natural populations of *S. graminicola*. However, no association between grouping of the isolates based on molecular data and pathogenicity data has been reported so far.

4. Downy mildew management

Management practices aim at reducing the disease impact on the crop and prevent crop loss. In this process, the main objective is to reduce the primary inoculum source and subsequently to prevent the secondary spread. This can be achieved by using disease resistant cultivars which is most economical and practical to farmers. In addition, cultural practices and chemical control can also be adopted by the farmers wherever it is feasible. Use of chemicals for foliar spray in pearl millet disease management is not desired by the farmers. Integrated approach for disease management includes using host resistance, chemicals and bioagents.

4.1 Host - plant resistance

Host plant resistance is a practical and economic method of disease management. The use of host plant resistance requires an effective and reliable screening technique to identify source material for breeding and incorporating resistance in the hybrids or improved cultivars. The resistant cultivar deployment and effective monitoring system for durability of resistance is also a good strategy in disease management. In pearl millet downy mildew, major concern is the breakdown of resistance to the disease. Therefore an effective monitoring system for pathogen virulence and resistance durability is required to manage the disease while using the resistant hybrids and improved cultivars.

4.1.1 Sources of resistance

Large numbers of germplasm accessions and breeding lines have been screened at ICRISAT and at the All India Coordinated Pearl Millet Improvement Project (AICPMIP) centers, and a number of resistant 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 multilocational testing (Thakur *et al*., 2011). Several other lines and germplasm accessions, including P 310-17, P 1449-3, IP 18292, IP 18293, IP 18294, IP 18295 and IP 18298 with high levels of downy mildew resistance have been identified (Singh *et al*., 1997, Thakur *et al*., 2004b). 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 downy mildew resistant hybrid parental lines in India. Recently, for the identification of

new and diverse sources of downy mildew resistance, pearl millet mini-core collection comprising 238 accessions was screened against eight pathotypes (Sg 384, Sg 409, Sg 445, Sg 457, Sg 510, Sg 519, Sg 526 and Sg 542) of *S. graminicola* collected from different geographical locations in India (Sharma *et al*., 2015). Multiple-pathotype (6-7) resistance was observed in some of the mini core accessions such as IP 9645, IP 11943, IP 14542, IP 14599, IP 21438 and IP 14537.

4.1.2 Genetics of resistance

To develop disease resistant cultivars, information on the number of genes and their pattern of inheritance is important. Most studies on genetics of downy mildew resistance have shown resistance to be governed by major dominant genes with both additive and non-additive gene action (Basavaraju *et al*., 1980; Basavaraju *et al*., 1981; Shinde *et al*., 1984). Variable expression of downy mildew has also been found in crosses of several populations of pearl millet (Pethani *et al*., 1980). Complete resistance to downy mildew in pearl millet genotype DMRP 292 was reported to be controlled by a single dominant gene (Singh and Talukdar, 1998). Incomplete resistance is usually polygenic, but could also be oligogenic. Genes governing this type of resistance confer incomplete resistance, exhibiting variable levels of dominance. Segregation for host plant resistance has generally shown continuous variation (Deswal and Govila, 1994; Hash *et al*., 2003).

Following development of molecular markers for pearl millet (Liu *et al*., 1994; Allouis *et al*., 2001; Qi *et al*., 2001; Yadav *et al.,* 2007, Yadav *et al.*, 2008), detailed marker-based genetic linkage maps have been produced (Liu *et al*., 1994 and 1996; Devos *et al*., 2000, Supriya *et al*., 2011). Using these linkage maps, genomic positions of quantitative trait loci (QTL) for pearl millet downy mildew resistance have been identified (Jones *et al*., 1995, 2002; Azhaguvel, 2001; Breese *et al*., 2002) and some of them are specific to different pathotypes (Hash *et al*., 1999, Hash and Witcombe, 2001, Jones *et al*., 2002).

In the molecular breeding programme, marker-assisted selection (MAS) was used for transferring QTL governing downy mildew resistance in parental line of a popular commercial hybrid HHB 67 (843A × H77/833-2) (Hash and Witcombe, 2001). Development and commercial deployment of downy mildew resistant version of HHB 67 is the first successful story of MAB in field crops in public domain in India with AICPMIP and ICRISAT collaboratative research work. Several additional downy mildew resistance QTL have been validated by integrated marker-guided backcross transfer to elite seed parent (ICMB 841 and ICMB 93333) and/or pollinator (J 2340 and ICMR 01004) backgrounds.

To date, while many pearl millet downy mildew resistance QTL have been mapped, essentially all confer pathogen-population-specific partial resistance of the sort expected from defeated major resistance genes. Only rarely has screening of a particular mapping population against a particular downy mildew isolate revealed the predominant role of an effective single major resistance gene. Therefore, the most promising approach for breeding downy mildew resistance that is both stable (across sites) and durable (across years), appears to be one based on pyramiding QTL for incomplete resistance from diverse sources in both sides of the hybrid parentage (preferably different effective resistances in seed parent and pollinator). Comparison of downy mildew susceptible pearl millet line 843B, to its conventional backcross derivative ICMB 99022, and the resistance donor ICML 22 has clearly demonstrated the effectiveness of pyramiding resistance alleles from ICML 22 (Hash *et al*., 2006). Parental line 843B had >90% downy mildew incidence against six isolates Sg 021, Sg 139, Sg 200, Sg 212, Sg 298 and Sg 384 of *S. graminicola*, whereas ICMB 99022 recorded 0-1% incidence, and the resistance donor ICML 22 had 4-16% incidence across these isolates. Thus, ICMB 99022 was recommended as a replacement for its susceptible but commercially successful recurrent parent 843B in hybrid breeding programs in India (Hash *et al*., 2006). Similar efforts are required to pyramid resistance alleles for these QTLs in the commercially successful parental lines of pearl millet hybrids that have succumbed to the new more virulent populations of the pathogen.

There had been some concern in past about the use of single source of cytoplasmic male sterility in hybrid breeding in India. There is clear experimental evidence that the A_1 cytoplasm is not associated with susceptibility to downy mildew and that nuclear genes are involved in controlling the disease reaction (Yadav *et al.,* 1993; Yadav, 1996).

4.1.3 Inducing disease resistance by priming

In crop plants like pearl millet, disease resistance can be achieved by using natural resistance of the crop which is offered by the innate immunity of the plants. It is also possible to induce resistance if the innate immunity of the plant is not enough to protect against the pathogen attack. The resistance can be induced by eliciting the defense system in plants by the bioagents like *Trichoderma harzianum*, *Pseudomonas fluorescens* and *Bacillus subtilis*. The same resistance can be achieved by using the cell wall components of these bioagents, glucan molecule of *Trichoderma harzianum* or the Lipopolysaccharides like components from bioagents like *Pseudomonas fluorescens* and *Bacillus* species. The resistance can also be elicited and induced by using the natural

compounds from the plants or some of the environmental safe chemicals like BABA, BTH and Chitosan. Therefore, many suitable disease resistance inducers have been identified under AICPMIP programme and field trials have given promising results. The advantage of inducing resistance is that it offers multiple resistance to major diseases in pearl millet.

More than 300 different biotic/abiotic agents have been evaluated for their efficiency to induce resistance against pearl millet downy mildew disease. Plant activators like BTH-Bion (Benzathiadiazole), Chitosan, Milsana (*Reynoutria sachalinensis*), Actigard (acibenzolar-S-methyl) and Phytogard (potassium phosphonate) have been identified as promising candidates for downy mildew disease management through seed priming (Table 5).

Table 5: Inducers of resistance against pearl millet downy mildew and their associated defense responses in the host

Inducer	Concentration	Disease protection (%)	Defense responses	Reference
β-Aminobutyric acid (BABA)	50 mM	72^{d}	Induced disease resistance, accumulation of lignin, callose, and HRGPs.	Shailashree *et al*., 2001
Benzothiadiazole (BTH)	0.75% aqueous solution	78^{e}	Induced resistance, reduced fungal biomass, increased HR.	Geetha and Shetty, 2002a
Cerebrosides, (glycosphingolipids)	100 mg ml^{-1}	67^{c}	Elicited resistance effectively against pearl millet downy mildew.	Deepak *et al*., 2003
PGPR – *Bacillus pumilus* INR 7	10g kg^{-1}	71^{cd}	Effectively induced systemic and durable resistance.	Niranjan Raj *et al*., 2003a, 2003b
Proline	50 mM	70^{cd}	Induced systemic and durable resistance	Niranjan Raj *et al*., 2004
L-methionine	15 mM	57^{a}	Increased transcripts of PR-1a, glucanases, chitinase, POX, and chalcone synthase.	Sarosh *et al*.,2005
Aspergillus niger (cell wall Carbohydrates)	0.5 mg ml $^{-1}$	67^{c}	Induced resistance, enhanced lignin and callose deposition, and increased POX activity.	Hindumathy *et al*., 2005

Table 5 Continued...

Inducer	Concentration	Disease protection (%)	Defense responses	Reference
Menadione sodium bisulphite (MSB)	20 mM	73[d]	Induced downy mildew disease resistance.	Pushpalatha *et al*., 2007
Ind-Ile-Me synthetic (1-oxo-indanoyl-l-isoleucine methyl ester)	75 µM	62[b]	Induced resistance, enhanced activities of PAL, POX, and HRGPs	Deepak *et al*., 2007c
NO donors - Sodium Nitro Prusside	1 mM	72[d]	Induced resistance , enhanced HR, lignin deposition and PAL activity	Manjunatha *et al*., 2008a
Silicon (silicon dioxide)	10 mM	78e	Induced resistance, increased silicon deposition, accumulation of HRGPs	Deepak *et al*., 2008
Chitosan	2.5 g kg^{-1}	80[e]	Induced resistance, increased chitosanase and POX activities.	Manjunatha *et al*., 2008
Datura metel extract	2% aqueous solution	79[e]	Increased POX, b-1,3-glucanase and chitinase activities	Shivakumar *et al*., 2009
Thiamine	20 mM	70[cd]	Increased LOX activitiy	Pushpalatha *et al*., 2011
Cellulysin	20 µg ml^{-1}	71[cd]	Increased LOX and glucanase activities	Pushpalatha *et al*., 2013
Lactuca sativa extract	100 µl aqueous solution	54[a]	Induced resistance, enhanced activities of PAL and POX	Mythrashree et al., 2013
Apron	6 g kg^{-1}	87[f]	-	-

x Percentage of downy mildew is the mean from two repeated experiments. Means followed by the same letter in a column do not differ significantly according to Duncan's multiple range test at $P = 0.05$.

4.2 Genetic diversification of hybrids

There have been downy mildew epidemics in pearl millet hybrids during 1970s and 1980s. This is due to inherent variability in the pathogen and shift in its virulence leading

to break down of the resistance. This phenomenon has been observed in hybrids like HB 1, HB 3, NHB 3, BJ 104, MBH 110 and MH 179 when these hybrids were grown on a large scale over years. Thus, in spite of overall accomplishment, pearl millet hybrid programmes have had setback due to downy mildew epidemics. A critical appraisal of the situation reveals that downy mildew epidemics in mid 1970s and 1980s were mainly due to lack of diversity in the parental lines of hybrids. Initially all hybrids were first based on Tift 23A, then on 5141A. Similarly the same pollinators were also repeatedly used in combinations with different male-sterile lines. Three restorers (J 104, K 560 and K 559) were male parents of nine commercial hybrids. Similarly two restorers (H90/4-5 and H77/833-2) were utilized in developing five hybrids. These facts indicate that outbreaks of downy mildew were due to a narrow genetic base among seed parents and restorers, rather than any undesirable effects of A_1 cytoplasm as has been clearly demonstrated by Yadav *et al.* (1993, 1996).

The recurring problem of downy mildew epidemics in pearl millet hybrids due to narrow genetic base of hybrids led to strengthening the research to diversify the genetic base of seed parents. Diversification of seed parents was further supplemented by the use of very diverse genetic material to derive pollinators. Both AICPMIP and ICRISAT are attempting to breed hybrid parents with a diverse combination of traits with a high level of downy mildew resistance. As a result, a large number of genetically diverse seed and pollen parents are being utilized in hybrid breeding (Yadav *et al.,* 2012). More than 100 hybrids have been released in the last 15 years by both public and private sectors utilizing such diverse breeding material and currently more than 125 hybrids are now in market. The exhaustive list of pearl millet hybrids released in India since 1985 has been recently provided by Yadav *et al.* (2012). The use of a large number of seed parents and pollinators clearly demonstrates the priority given to the genetic diversification of hybrid parental lines in pearl millet that has contributed to a great extent to contain downy mildew epidemics which had threatened hybrid technology *per se* in mid-1970s.

4.3 Chemical control

Use of chemicals in the form of fungicides is most favored disease management practice. In case of pearl millet, fungicide use is less popular because of difficulty to take up spray in vast areas by the farmers. Moreover, downy mildew pathogen being an oomycete organism is not controlled by the fungicides normally recommended for other fungal diseases. Seed treatment is more desirable since it can be applied before sowing very easily. General fungicides recommended for pearl millet seed treatment are Thiram and

Captan. These fungicides act as seed protectants. For oomycetes pathogen like downy mildew, a systemic fungicide metalaxyl is quite effective; but it is narrow spectrum oomyceticide.

Metalaxyl, $C_{15}H_{21}NO_4$, methyl N-(2, 6-dimethylphenyl)-*N*-(methoxyacetyl) DL alanine, is a oomyceticide with a unique combination of residual and systematic properties, is highly active both *in vitro* and *in vivo* against pearl millet downy mildew (Singh and Shetty, 1990). The mode of action of metalaxyl includes the inhibition of protein and ergosterol synthesis, by interference with the synthesis of ribosomal RNA. This action is systemic with protective and curative action, absorbed through the leaves, stems and roots. Metalaxyl is available in the form of Apron 35 SD, Ridomil MZ 72, Master 72% WP (Metalaxyl 8% + Mancozeb 64%). Seed treatment with Metalaxyl 35% WS at 6 g kg^{-1} seed controls the disease excellently for about the first 35 days after sowing. Foliar application of the Ridomil MZ 72 at 3 g l^{-1} arrests further development of the disease in systemically infected plants. Use of metalaxyl to control disease has been, however, found more economical in seed production plots than in commercial crop. Private seed companies are also using this chemical in treatment of commercial seed before selling to farmers.

Several new generation chemicals are also being tested for control of downy mildew. Safe and eco-friendly strobilurin group of fungicides/oomyceticides - Flint (Trifloxystrobin), Amistar (Azoxystrobin) and Stroby (Kresoxim-methyl) have been tested and evaluated which are effective in control of downy mildew. Similarly, Cyazofomid and Iprovalicarb were evaluated and found to be promising. Amistar is an effective strobilurin fungicide/ oomyceticide for control of downy mildew. Other strobilurin fungicides/ oomyceticides have been widely tested and are found to be very effective in controlling downy mildew disease (Deepak *et al*., 2004; Sudisha *et al*., 2004; Sudisha *et al*., 2007).

Presently, different biopolymers are mixed with the fungicide/oomyceticide and used as seed treatment to increase the efficacy of the compound in controlling the disease and also to increase the vigor of the seedlings. In this direction, various biopolymers derived from plants like *Acacia arabica*, neem, drum stick, papaya, atrocarpus and mimosops have been tested and recommended for pearl millet seed treatment at concentration of 1:2 w/v in combination with half dosage of Metalaxyl i.e., 3 g kg^{-1} which is highly effective in managing pearl millet downy mildew (Sudisha *et al*., 2009). Seed bio-priming of Arabica gum to pearl millet seeds along with half and full dose of Apron is shown in Fig. 20.

4.4 Cultural methods

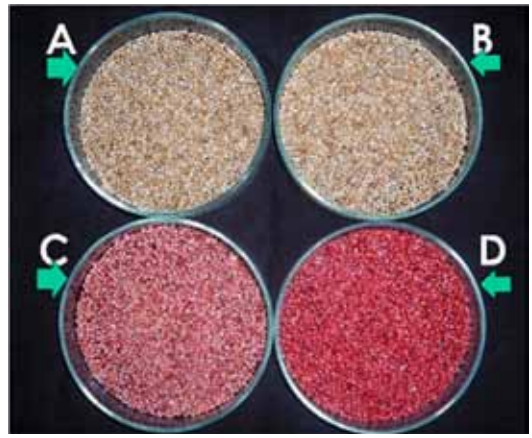

Fig. 20: *Seed bio-priming of Arabica gum to pearl millet seeds along with half and full strength of Apron A-Untreated control, B-Gum coated seeds, C-Gum + half dose apron, D-full dose of Apron (6g/kg)*

Cultural methods of disease management employ practices that make the environment less attractive to pathogens and less favorable for their survival, dispersal, growth and reproduction, and that promote natural control of pathogen or pest. The objective is to achieve reduction in disease pressure, either below economic injury levels, or sufficiently to allow natural or biological controls to be effective.

The major approach is by avoiding the pathogen coming in contact with the host. Removing the inoculum source by clearing the infected plant residue, burning the plant debris so that the inoculums present in the surface of the soil is inactivated, crop rotation, adjusting the sowing time, use of disease-free seed material for sowing, transplanting the planting material so that diseased material is eliminated, and cultivation of diverse hybrids are some of cultural interceptions that help in reducing the disease levels. A few cultural methods of downy mildew management in pearl millet are given below:

4.4.1 Sanitation: Use of disease-free seed and effective reduction of oospore-infested debris after harvest of the crop are prerequisite to reduce primary inoculum for the subsequent crops. Downy mildew-infected plant debris should be burnt, or the field should be ploughed deeply to bury the debris. This will help to reduce inoculum buildup in the upper root zone where infection is most likely to happen. Unfortunately, deep ploughing is not generally followed by farmers, due to more expanses and is difficult to perform.

4.4.2 Time of planting: Pearl millet crop sown very early in the season generally has a chance to escape the infection from *S. graminicola* than that sown late in the season due to less inoculum (sporangial) buildup and low infection by soil-borne oospores (Chahal *et al*., 1978; 1994). Early sowing of crop may receive more hot and dry conditions than later sown crop in the rainy season. The hot and dry conditions during the crop growth reduce the secondary spread of the disease by reducing sporangial production and their

infectivity. Though early sowing is likely to favor disease escape, but it depends upon rainfall pattern.

4.4.3 Direct sowing vs. transplanting: A transplanted crop of pearl millet suffers significantly less damage from downy mildew than a direct-sown crop, both in the rainy and post rainy seasons (Chandrasekhara Rao *et al*., 1987). This method can therefore be used to reduce downy mildew.

4.4.4 Crop rotation: Oospores of *S. graminicola* survive in soil and are considered to be the primary source of infection; therefore, crop rotation can be another disease management strategy. Thakur *et al*. (2003) observed the effect of certain cropping sequences in reducing downy mildew incidence in pearl millet. Crops such as wheat, sorghum, sugarcane and black gram appeared to increase downy mildew incidence while cotton, coriander and onion drastically reduced the disease incidence. Therefore, it would be desirable to investigate the effect of root exudates of these crops on the survival and infectivity of oospores of *S. graminicola*.

4.4.5 Roguing: Removal and complete destruction of infected plants from the field has been recommended for the control of downy mildew (Thakur, 1980). This can reduce the secondary spread of disease during the same season (Singh and Williams, 1980) and oospore buildup for the following season. Hence, farmers should be trained to identify the disease at an early stage and remove the diseased plants.

4.5 Biological control

In recent years, attention is diverted towards identifying an effective bioagent and their formulations to be used either as soil treatment or seed treatment. Under AICPMIP programme, a large number of bioagents have been identified and effective formulations have been developed to treat seed and soil for managing downy mildew in pearl millet. In addition, resistance can be induced in pearl millet by using bioagents or their components or natural plant and microbial components and also environmentally safe chemicals.

Downy mildew can be managed effectively by the use of bioagents like *Trichoderma harzianum, Pseudomonas fluorescens* and *Bacillus* species. Among several promising bioagents screened, *Pseudomonas fluorescens* UOM SAR 14, *Bacillus pumilus* INR 7, *Bacillus pumilus* SE 34 and *Trichoderma harzianum* Th UOM 1 have been found to be most promising. Their cultures are being maintained at Mysore and are ready for

commercial use. The bioagents of *Trichoderma harzianum* were used as seed treatment in the form of talc formulation at a concentration of 20 g kg^{-1} seeds and *Pseudomonas fluorescens* and *Bacillus* species were used as talc formulations at a concentration of 10 g kg^{-1} seeds (Table 6).

Table 6: Bioagents and their effect on pearl millet seed germination, emergence, seedling vigor, yield and downy mildew protection

Bioagent	Dose of bioagent	Seed germination	Seedling vigor	Seedling emergence	Yield (kg/ha)	Downy mildew protection (%)
Trichoderma harzianum UOM SAR1	20 g kg^{-1}	82^{a}	857^{c}	90^{b}	1306^{b}	67^{bc}
Trichoderma viridae UOM SAR 27	20 g kg^{-1}	$84a^{b}$	880^{d}	90^{b}	1396^{c}	63^{b}
Chaetomium globosum UOM SAR 39	20 g kg^{-1}	80^{a}	739^{b}	85^{a}	1312^{b}	52^{a}
Pseudomonas fluorescens	10 g kg^{-1}	90^{c}	$951f^{g}$	90^{b}	1476^{d}	80^{e}
Pseudomonas fluorescens UOM SAR 14	10 g kg^{-1}	90^{c}	964^{g}	92^{bc}	1497^{de}	77^{d}
Pseudomonas fluorescens UOM SAR 80	10 g kg^{-1}	88^{b}	$945f^{g}$	90^{b}	1430^{cd}	71^{c}
Bacillus subtilis UOM SAR 4	10 g kg^{-1}	92^{d}	1156^{i}	94^{c}	1571^{f}	70^{c}
Bacillus pumilus UOM SAR 16	10 g kg^{-1}	92^{d}	978^{h}	94^{c}	1538^{e}	68^{bc}
Apron	6 g kg^{-1}	82^{a}	922^{e}	90^{b}	1501^{de}	87^{e}
Untreated control	-	82^{a}	707^{a}	90^{b}	1124^{a}	-

x Percentage of downy mildew is the mean from two repeated experiments. Means followed by the same letter in a column do not differ significantly according to Duncan's multiple range test at P = 0.05.

4.5.1 Plant growth promoting rhizobacteria: Plant growth-promoting rhizobacteria (PGPR) are free-living, root-colonizing bacteria that exert beneficial effects on plants. PGPR are easy to deliver, improve plant growth, and activate resistance mechanism in pearl millet, and increase biomass production and yield. The application of PGPR as seed treatments has proved to be a beneficial component in disease management. These bacteria, apart from their action against pathogens, are good growth promoters, which is an added advantage. The PGPR act through release of growth promoting hormones, antibiosis, secretion of volatile toxic metabolites, mycolytic enzymes, parasitism and

through competition for space and nutrients. All these mechanisms may be found in single PGPR or in combination/consortium of PGPR. In addition, PGPR have broad spectrum of action, offer excellent and reliable control of pathogens, are safe to environment, and also beneficial in abiotic stress situations like drought.

A series of laboratory, greenhouse, and field experiments were conducted to evaluate strains of PGPR both as suspensions of fresh cultures (10^8 cfu ml^{-1} used 25 ml suspension for 5 g seeds) and as talc-based powder formulations (10^8 cfu/10 g talc for 1 kg seed) for growth promotion and management of downy mildew. All seed treatments with fresh suspensions and talc based powder formulations showed enhancement in germination and vigor index over the respective untreated control. Under experimental plot conditions, prominent enhancement in growth was also observed. Yield was enhanced by 40% and 37% over the untreated control by seed treatment with powdered formulations of strains *B. pumilus* INR 7 and *B. pumilus* SE34, respectively. Experiments showed varied degrees of protection by the PGPR both under greenhouse and field conditions (Table 7).

Table 7: Effect of seed treatment with fresh suspensions or talc formulations of plant growth-promoting rhizobacteria (PGPR) on downy mildew incidence under greenhouse and field conditions

Treatment	Downy mildew incidence (%)			
	Greenhouse		Field	
	Fresh culture spore suspension ($1x10^8$ cfu ml^{-1})	Talc based powder formulations ($1x10^8$ cfu ml^{-1})	Fresh culture spore suspension ($1x10^8$ cfu ml^{-1})	Talc based powder formulations ($1x10^8$ cfu ml^{-1})
Bacillus pumilus – INR7	40.6^{d}	44.6^{e}	30.2^{c}	36.6^{g}
Bacillus pumilus – SE34	46.7^{d}	51.9^{d}	38.6^{bc}	48.1^{ef}
Bacillus subtilis – GBO3	53.4^{c}	62.9^{c}	40.8^{bc}	53.1^{de}
Bacillus pumilus– 937b	55.0^{c}	59.4^{cd}	47.5^{b}	50.6^{e}
Bacillus subtilis – IPC11	60.5^{b}	64.9^{bc}	42.2^{bc}	56.7^{d}
Bacillus subtilis – T4	60.8^{b}	67.3^{b}	41.4^{bc}	62.5^{c}
Bacillus pumilus – 937a	65.3^{b}	65.4^{bc}	49.5^{b}	69.3^{b}
Apron	11.3^{e}	11.7^{f}	12.0^{d}	12.2^{h}
Untreated Control	93.9^{a}	93.2^{a}	93.5^{a}	94.4^{a}

x Percentage of downy mildew is the mean from two repeated experiments. Means followed by the same letter in a column do not differ significantly according to Duncan's multiple range test at P = 0.05.

With fresh suspensions, treatment with INR 7 resulted in the highest protection (57%), followed by *B. pumilus* strain SE34 and *B. subtilis* strain GBO3, which resulted in 50% and 43% protection, respectively, compared with the untreated control (Niranjan Raj *et al.,* 2003; 2004).

4.6 Integrated disease management

Integrated disease management (IDM) is gaining popularity in all crops. Integration of host resistance, fungicides/oomyceticides and bioagents, and other microorganisms to manage biotic and abiotic stress is also possible in pearl millet. It is therefore recommended that host plant resistance deployed in genetically uniform hybrids be backstopped with appropriate management practices (crop and cultivar rotation, and use of appropriate prophylactic seed dressings chemicals) to extend the economic life of the hybrid (Hash *et al.*, 1997; Witcombe and Hash, 2000; Hash *et al.*, 1999; Hash and Witcombe, 2002). This approach is also being practiced under AICPMIP programme. IDM module that includes host plant resistance (moderate level), half dose of metalaxyl (3 g kg^{-1} of seed), PGPR strain of *Bacillus pumilus* INR 7 (8 g kg^{-1} of seed) and Chitosan (2.5 g kg^{-1} of seed) is being tested at various AICPMIP locations for the management of pearl millet downy mildew (Table 8).

Table 8: Integrated disease management module developed for pearl millet downy mildew and evaluated at different locations of AICPMIP

Treatments	Emergence (%)	Downy mildew incidence (%)	Grain yield (kg ha^{-1})	Fodder yield (kg ha^{-1})
Chitosan (2.5 g kg^{-1} of seed)	90^{a}	45^{c}	1505^{c}	2666^{d}
B. pumilus (INR 7) (10 g kg^{-1} of seed)	92^{b}	33^{b}	1644^{e}	2878^{e}
B. pumilus (INR 7) + Chitosan	95^{c}	30^{b}	1685^{f}	2918^{f}
Pseudomonas fluorescens Pf1 (10 g kg^{-1} of seed)	90^{a}	48^{c}	1576^{d}	2514^{c}
Apron 35 SD 6 g kg^{-1} of seed	92^{b}	14^{a}	1497^{b}	2569^{b}
Control (untreated)	90^{a}	71^{d}	1430^{a}	2251^{a}

x Percentage of downy mildew is the mean from two repeated experiments. Means followed by the same letter in a column do not differ significantly according to Duncan's multiple range test at P = 0.05.

*Average from 2007 to 2010 across 8 locations.

5. Future thrust

Downy mildew management will continue to be major priority in pearl millet research. Therefore, additional sources of genetic resistance and effective disease management aspects need to be explored on regular basis. Effective, reliable and repeatable screening techniques for identifying the sources of host resistance have been developed which needs to be further supported by more sensitive and quick gene-based molecular markers. Such techniques can help predicting the downy mildew reaction under greenhouse and field conditions, and also cut short the time required for transfering the resistance into elite backgrounds.

Genetic and cytoplasm diversification of hybrid parental lines have been and going to be a key strategy to contain the downy mildew. A_1 system of cytoplasmic-genic male sterility (CMS) continues to be the main CMS source used in hybrid breeding in India and therefore it always involves the unforeseen risk of potential vulnerability of hybrids to diseases and insect-pests associated with the deployment of a single cytoplasmic background. Fortunately, other CMS sources are available in pearl millet. From among them, A_4 and A_5 are currently most potential sources of cytoplasmic diversification (Rai *et al.*, 1996, 2001, 2009). Male-sterile lines based on these sources are now available and their use in hybrid development would further diversify the cytoplasmic base of hybrids (Rai *et al*. 2012). Use of diverse base material in the development of both seed and pollen parents would further promote the genetic diversity in parental lines of hybrids.

Downy mildew pathogen produces numerous proteins and small molecules called 'effectors' that alter host cell structure and function. Many of these effectors are transported to the inside of the host cell where they suppress defenses responses and manipulate host cell machinery. Recognition of effectors by their cognate resistance (R) proteins in host cell results in a hypersensitive response that blocks further growth of the pathogen. Downy mildew R genes have been introgressed into hybrids but in the field the resistance is defeated within 5-7 years. The downy mildew pathogen can easily mutate its effector genes thereby avoiding recognition. Insight into the molecular interaction between effector and R protein can help in designing more rational breeding strategies. More R gene markers have to be characterized and functional analysis has to be carried out. Functionally characterized R genes can be further developed as gene based molecular markers and introgressed into hybrids through gene pyramiding within a short time so that durability of the hybrid increases.

Downy mildew pathogen is an outcrossing oomycete and has remarkable ability of developing new virulent pathotypes to overcome the resistance mechanism of genetically narrow hybrids. In order to stay ahead of new emerging virulent pathotypes, the more efficient and rapid development of inbreds would be very critical. The use of doubled haploid can be one such intervention to dramatically cut down the time period to develop new inbreds.

Continuously monitoring in the shift in virulence of pathogen and breakdown of resistance in the released hybrids in the farmers' fields is critically important to keep track of development of new virulent pathotypes of downy mildew pathogen in order to identify the sources of resistance and incorporate them in new parental lines of hybrids.

Identifying and formulating environmentally safe fungicides/oomyceticides, bioagents, plant growth promoting rhizobacteria, and disease resistance inducing components should continue. Analysis of the response of the host and pathogen interaction under changing climatic situations is important in the years to come.

A constant interface between pathologists and breeders would assist develop disease resistant and durable hybrids and varieties in pearl millet to prevent losses caused by downy mildew.

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