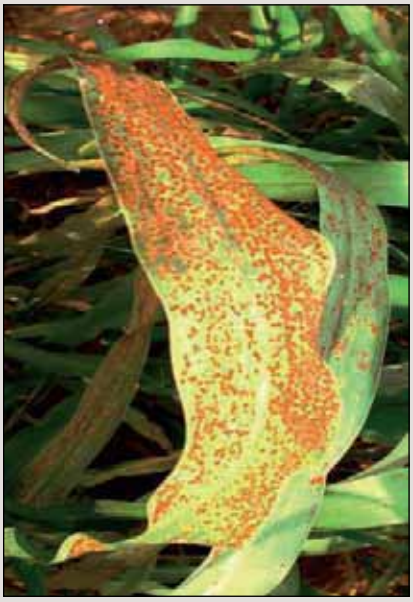
Screening Techniques for Pearl Millet Diseases

Information Bulletin no. 89







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Abstract

Pearl millet (*Pennisetum glaucum*), an important staple food crop in the semi-arid tropics of the world, is infected by a number of diseases. Important among these are downy mildew (*Sclerospora graminicola*), blast (*Pyricularia grisea*), rust (*Puccinia substriata* var. *indica*), ergot (*Claviceps fusiformis*) and smut (*Moesiziomyces penicillariae*) that individually or in combination cause substantial yield and quality losses. Effective and economic control of these diseases can be achieved by growing disease resistant varieties and hybrids. In order to develop disease resistant cultivars, it is important to have effective disease screening techniques that ensure identification of resistance. This information bulletin describes briefly about the economic importance, symptoms, pathogen biology and epidemiology for each of the above five diseases and provides the field and/or greenhouse screening techniques in detail in a step-by-step manner that is easy to follow. Photographs related to disease symptoms, pathogen morphology, disease cycle and rating scales have been included to facilitate easy identification and understanding. Information has also been provided on the available resistance sources and inheritance of resistance for each disease.

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About the authors

RP Thakur
Principal Scientist (Cereals Pathology) Retd.
ICRISAT, Patancheru 502 324
Andhra Pradesh, India

Rajan Sharma
Senior Scientist (Cereals Pathology)
ICRISAT, Patancheru 502 324
Andhra Pradesh, India

VP Rao
Lead Scientific Officer (Cereals Pathology) Retd.
ICRISAT, Patancheru 502 324
Andhra Pradesh, India

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Foreword

Pearl millet is infected by a large number of diseases caused by fungal, bacterial and viral pathogens, and nematodes. However, only a few are considered economically important, namely downy mildew (*Sclerospora graminicola*), blast (*Pyricularia grisea*), rust (*Puccinia substriata* var. *indica*), ergot (*Claviceps fusiformis*) and smut (*Moesiziomyces penicillariae*).

Downy mildew is the major constraint to attaining high grain yield potential of improved pearl millet cultivars in India and in western and central Africa. In the recent past, however, blast has emerged as a serious threat to pearl millet cultivation in India, while the severity of rust has also increased, most likely due to changing climate variables. As pearl millet is grown on the poorest soils under harsh climatic conditions, and generally by resource-poor farmers in the semi-arid tropics of western Africa and India, the use of host plant resistance is the most appropriate approach to managing these diseases.

In the last two decades, the status of downy mildew infestation has changed drastically in India with the commercialization of new hybrids giving rise to the development of new virulent strains of the pathogen. Meanwhile, severe outbreaks of blast disease have been recorded in some states during the past five years. Rust, which used to be limited to postrainy season crops, has become severe in the rainy season and summer crops as well. These new disease infestation trends point to the need to identify new sources of resistance for use in the pearl millet breeding program.

Progress in breeding for resistance to diseases depends on the effectiveness of screening techniques for the identification of resistance sources. Breeding for disease resistance has received top priority at ICRISAT, and significant progress has been made in developing effective greenhouse and field screening techniques. Using these techniques, numerous resistance sources have been identified and many resistant hybrid parental lines have been developed. As a result of the large-scale use of downy mildew resistant parental lines in hybrid development programs both by private and public sector, downy mildew is now under control in India and no large scale epidemics have occurred in the last decade.

This Information Bulletin on *Screening Techniques for Pearl Millet Diseases* edited by RP Thakur, Rajan Sharma and VP Rao provides valuable information on the importance of the diseases, their pathogen biology, and symptoms and epidemiology, and describes the screening technique for each disease. We are confident that this Bulletin will serve as a useful practical guide for students, researchers and policy makers, and all others who are interested in disease management through host plant resistance, in enhancing the productivity and production of pearl millet.

William D Dar
Director General
ICRISAT

1. Introduction

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is a staple food for millions of poor people living in the semi-arid tropical regions of Africa and Asia. The crop is predominantly grown in drier parts of West and Central Africa (WCA), Eastern and Southern Africa (ESA) and the Indian Subcontinent in South Asia (SA). Pearl millet is grown both for fodder and grain, and besides food for human consumption, the grain is also used for poultry feed and some industrial uses. More recently, pearl millet has become an important fodder crop in no-tillage crop farming systems in central Brazil.

Pearl millet is attacked by a large number of diseases caused by fungal, bacterial, viral and nematode pathogens. However, diseases that are considered economically important are only a few and include downy mildew, blast, rust, ergot and smut. Among these, downy mildew is the most destructive and widespread in India and countries in Africa. It is not yet reported from the Americas. Downy mildew infects the foliage and the panicles of the crop and causes severe losses. Blast and rust are mainly foliar diseases and they affect fodder quality and production of both fodder and grain. In recent years, blast has become more severe in many parts of India. Ergot and smut are highly tissue-specific and ovary-replacement diseases, they appear in low to moderate intensity and currently these are not of major consequence in India and elsewhere.

Research efforts at ICRISAT and some other research centers in India and the United States on various basic and applied aspects have led to improved scientific understanding of these diseases. Significant advances have been made in developing effective screening methods and breeding for resistance to these diseases. Of the several disease management options available, use of genetic resistance for breeding disease-resistant cultivars has received the highest priority because it is a highly cost-effective and feasible method for deployment at the level of resource-limited farmers in the semi-arid tropics. Disease control through seed treatment with fungicide has also been found effective for downy mildew, although the efficacy of fungicide lasts only for a short period and the disease appears in late tillers and at the flowering stage in the form of green ear. Rust and blast can be controlled by fungicide sprays in research and seed production plots, but this is not economical at the farm level.

Progress in breeding for resistance to diseases depends on the availability of effective resistance sources, mode of inheritance and effectiveness of screening techniques. Breeding for disease resistance has received top priority at ICRISAT, and effective screening techniques have been developed and information bulletins have been published for downy mildew (Singh et al. 1993), ergot (Thakur and King 1988a), smut (Thakur and King 1988b) and downy mildew and rust (Singh et al. 1997). Sources of stable resistance to these diseases have also been identified and some of these utilized in resistance breeding programs (Hash et al. 1999; Hash et al. 2006). During the past 10 years the scenario for downy mildew has changed drastically in India with

commercialization of new hybrids giving rise to development of new strains of the pathogen. At present, more than 20 virulent pathotypes of *S. graminicola* have been identified, and the new virulences evolve every few years. During the past five years there have been outbreaks of blast disease (*Pyricularia grisea*) in severe form in some states of India, for which very little information is available. Rust (*Puccinia substriata* var. *indica*), which used to be limited to post rainy season crops, has become severe in the rainy season and summer crops as well. Because of these developments, scientists at ICRISAT-Patancheru have refined the greenhouse technique for downy mildew and rust, and have developed greenhouse and field screening techniques for blast.

In this bulletin the refined and new screening techniques for downy mildew, rust and blast, including information on screening techniques of ergot and smut from the earlier publications have been described, thus bringing all relevant information into a single publication. Many of the earlier identified resistance sources for downy mildew and rust were re-evaluated to confirm their resistance and some new sources of resistance to downy mildew, rust and blast have been identified, all these form part of this bulletin.

The bulletin contains, for each disease, a brief introduction to highlight the geographical distribution and economic importance, symptoms, pathogen biology and epidemiology, and the screening techniques in detail, available sources of resistance and selected references.

We hope that this publication will be useful for students, researchers and policy makers, and all others who are involved with or interested in enhancing the productivity and production of pearl millet.

2. Downy mildew

2.1 Economic importance

Downy mildew is a highly destructive and widespread disease in most pearl millet growing areas of Asia and Africa (Williams 1984; Andrews et al. 1985a) and causes 20-40% grain yield losses annually worldwide (Singh 1995; Hash et al. 1999), and sometimes it could be much higher where a susceptible cultivar is repeatedly grown in the same field. Genetically uniform single-cross F_1 hybrid cultivars generally become susceptible more rapidly than heterogeneous open-pollinated varieties (Thakur et al. 2006) leading to heavy production losses. Downy mildew severity reached epidemic levels in India during the mid 1970s to 1980s when only a few single-cross hybrids were cultivated on a large scale (Fig. 1). However, since the 1990s no large-scale epidemics have occurred due to hybrid cultivar diversity for downy mildew resistance. Currently, more than 90 single-cross F_1 hybrids are grown on about 60% of the 9 million ha of pearl millet area in India (Thakur et al. 2006; Mula et al. 2007).

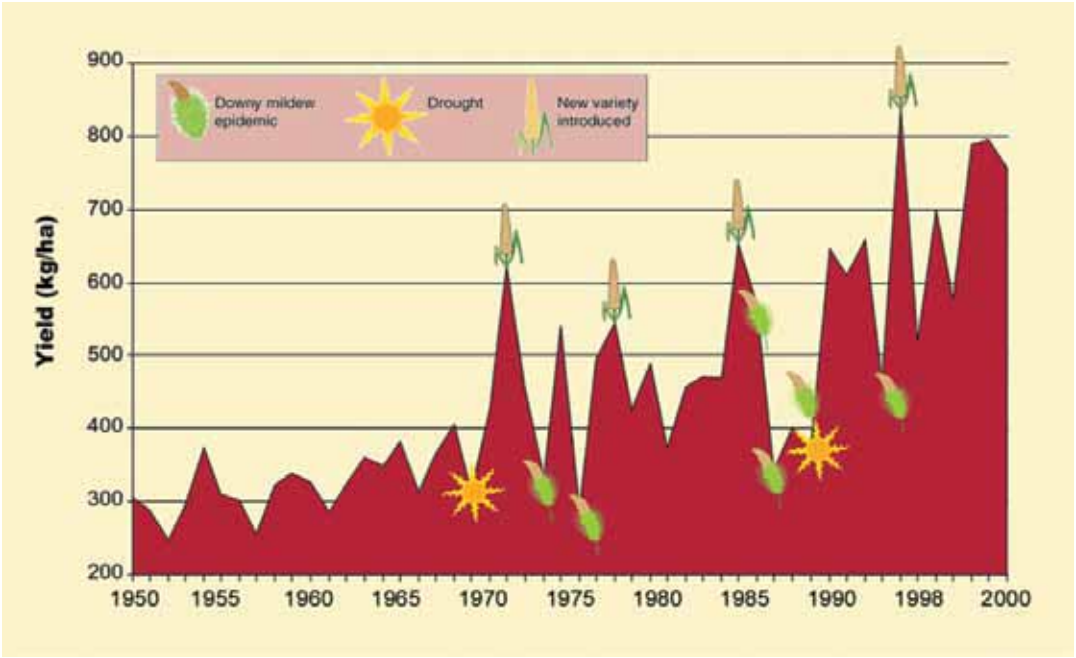

Fig 1. Boom and bust cycle due to downy mildew.

2.2 Symptoms

Primary infection occurs at the seedling stage from the infection caused by soil-borne oospores and systemic symptoms generally appear as chlorosis on the second leaf, and on all the subsequent leaves. Whitish growth of the pathogen in the form of spornagiophores and sporangia appear more on the abaxial leaf surface particularly during the morning hours. These spores get blown off by wind and rain splash and cause secondary infection in the field.

Fig 2. Field photo of a susceptible hybrid.

Young seedlings die under severe infection, and panicles produced on the infected plants develop green-ear symptoms (Figs. 2 and 3). Sporangia produced on the foliage of infected plants serve as the source of primary or secondary inoculum for nearby plants. Oospores are produced in the infected necrotic leaf tissues, which fall in the soil and serve as a source of primary inoculum for the next crop.

Fig 3. Downy mildew symptoms – foliar and green ear.

2.3 Pathogen

The pathogen causing downy mildew in pearl millet is *Sclerospora graminicola* (Sacc.) Schroet. This is an obligate oomycete parasite that reproduces both by sexual and asexual means. Sporangia, the asexual spores produced on the infected leaves, germinate to release motile zoospores. Zoospores are ephemeral and require a thin film of water on the leaf surface for swimming, encystment and germination to initiate infection. Oospores, the sexual spores, are produced by two compatible mating type thalli in the infected leaf tissue (Figs. 4 and 5). Sexual cross compatibility among isolates and heterothallism are well known (Michelmore et al. 1982; Pushpavathi et al. 2006). Pathogenic variability has been demonstrated in this pathogen from India (Thakur et al. 2006) and several countries in Africa (Werder and Ball 1992).

Variability in *S. graminicola* has been studied through a collaborative Pearl Millet Downy Mildew Virulence Nursery (PMDMVN), on-farm survey and bioassay of isolates on a set of putative host differentials (Thakur et al. 2004a, 2004b) and using DNA markers (Sastry et al. 1995; Sharma et al. 2010). More than 500 oospore isolates have been collected and about 100 representative isolates from major pearl millet growing states of India were analyzed to identify about 20 distinct pathotypes. Currently, 15 pathotypes are being used at ICRISAT-Patancheru to screen breeding lines in a greenhouse for the downy mildew resistance breeding program.

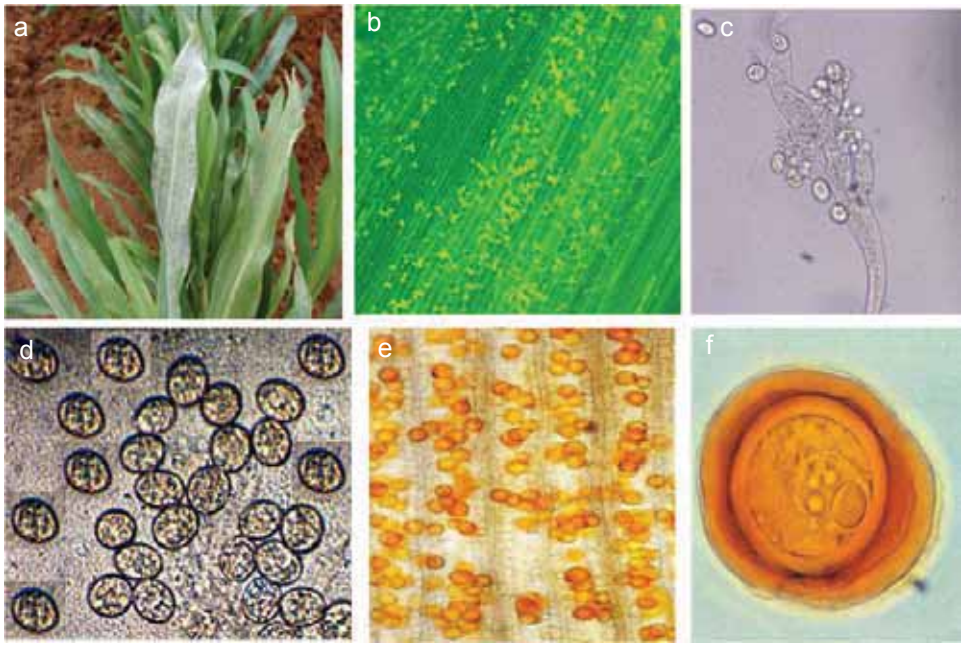

Fig 4. Sporangia and oospores of Sclerospora graminicola. (a) Downy mildew infected leaf, (b) an abaxial surface of pearl millet showing sporangiophores and sporangia; (c) sporangiophore bearing sporangia, (d) sporangia, (e) an infected leaf containing oospores, (f) mature oospore.

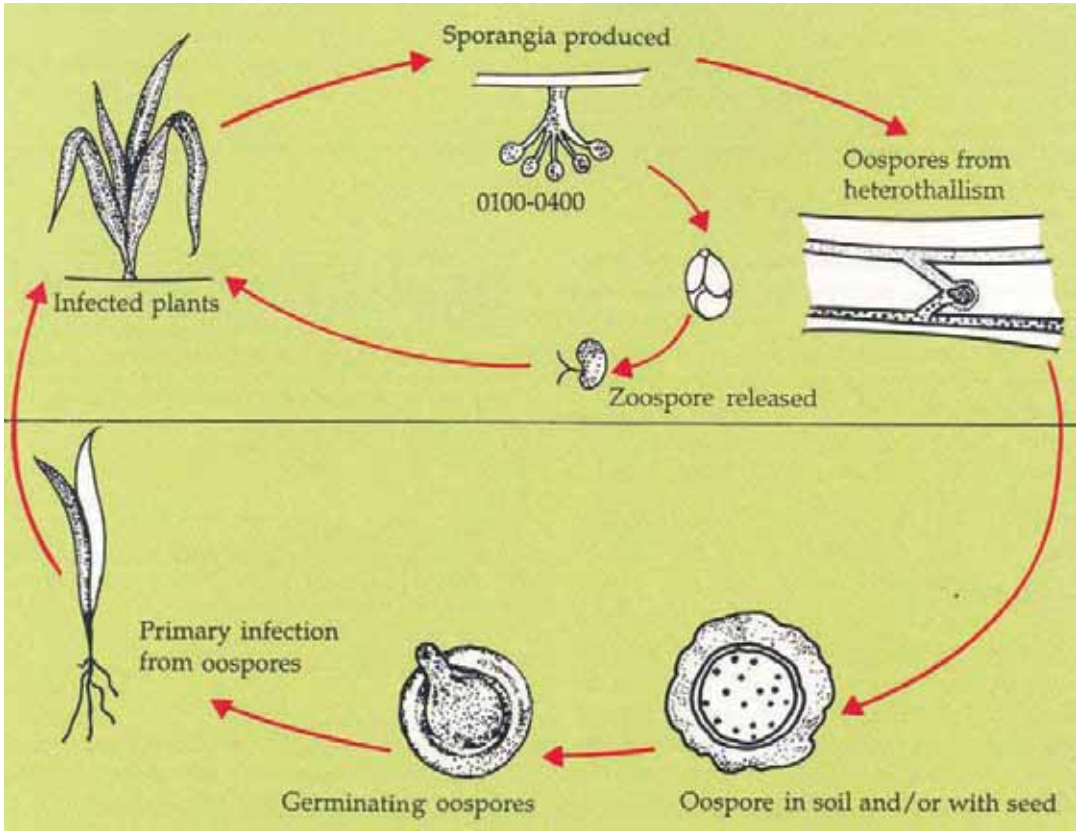

Fig 5. Downy mildew disease cycle (Singh et al. 1993).

2.4 Epidemiology

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 condition and wind velocity. Subsequent infection and disease development is guided by the susceptibility levels of the host on which inoculum get 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.

2.5 Screening Techniques

Both field and greenhouse screening techniques have been developed at ICRISAT (Williams et al. 1981; Singh et al. 1993; Singh et al. 1997) that are being extensively used worldwide. The greenhouse technique has been further refined to obtain more precise results.

2.5.1 Field screening

A field-based downy mildew screening has been developed that 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 a "sick-plot", 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 in the nursery, and test rows, lines to be evaluated. These are described below.

Establishing infector rows

These could be a mixture of two to three susceptible lines (local landraces and highly susceptible lines).

- Surface coat the seed of infector lines with infected leaf (collected in the previous season) powder containing oospores, by slurry treatment and dry the seed in the shade,
- Sow the oospore-coated seed on every fifth or ninth row throughout the entire length of the field,
- Spray-inoculate infector rows at the coleoptile to one-leaf stage (2-3 days after emergence) with a sporangial suspension (1×10^5 sporangia mL^{-1}) during late evening hours after furrow irrigation,
- Provide furrow or perfo irrigation frequently to maintain high relative humidity (>90% RH) and leaf wetness to promote infection and disease development (Fig. 6),

Fig 6. Field screening at ICRISAT.

- Monitor downy mildew symptom development in the seedlings, and count the total and infected seedlings in a stretch of 1 m length randomly at several places, to determine the disease incidence - percentage seedlings infected, at weekly intervals. About 70% incidence is considered adequate before planting the test rows.

Growing test rows

- Sow the test lines (untreated seed) in the intervening 4 or 8 rows in between the infector rows (when >70% incidence occurs in infector rows, about 3 weeks after sowing the infector rows).

Growing indicator rows

- Sow a highly susceptible line, either a breeding line or a local landrace at intervals, after every 10 or 20 rows of the test lines, at the same time as the test lines. Disease incidence level in these lines indicates the distribution of disease pressure in the nursery.

Disease scoring

- Count the numbers of total and infected seedlings in each plot and express as percentage of infected seedlings,
- Score the disease twice, first at 30-day after emergence (pre-booting/flowering stage) and second at 60-day 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.

Disease scoring can also be done at 10-day intervals up to crop maturity to determine disease progress, if required.

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, where

1 = no infection;

2 = 20% productive tillers infected;

3 = 50% productive tillers infected;

4 = 80% productive tillers infected; and

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).

2.5.2 Greenhouse screening

An effective greenhouse screening technique developed at ICRISAT (Singh et al. 1993) is useful for identifying resistance in large germplasm and breeding populations to different pathotypes of *S. graminicola* in a relatively small space and shorter time period under uniform conditions of disease development. This screening technique minimizes escape as every seedling is uniformly inoculated, can be operated throughout the year, [except during very hot (40+°C) and cold (<15°C) conditions], and it is rapid, reliable and cost-effective compared to field screening. Factors, such as depth of seeding in the pot, seed size and nature of breeding material (inbred and hybrid) greatly contribute to time of emergence of seedlings that interfere with inoculation time and uniform disease development. With our experience over the years, some of the methods have been refined to minimize the effects of these factors on seedling emergence. Steps involved in the greenhouse screening technique are described below.

Establishment of culture from oospores

- Collect downy mildew infected mature leaf samples from fields for establishment of isolate from the oospores,
- Dry the downy mildew-infected leaf samples in shade, grind and strain to make a fine powder containing oospores (check under microscope for the presence of oospores),
- Autoclave potting mixture (soil, sand, and farmyard manure in a 3:2:2 by volume) at 6.80 kg pressure for 2 h per day on two consecutive days,
- Infest the potted mixture with oospore inoculum, cover each pot with a polythene bag and incubate at 40°C for 3 weeks and sow with a susceptible genotype 7042S@ 25 seeds per pot (15-cm diameter) for rapid seed germination,
- Transfer the pots in polyacrylic isolation chambers in greenhouse at 25 ± 2°C to avoid cross contamination from other isolates,
- Water pots adequately everyday and observe regularly for downy mildew symptoms on the seedlings,
- After infected seedlings are noticed, remove all healthy seedlings from the pots,
- Use sporangia from infected seedlings for subsequent inoculation of seedlings for maintaining the isolate.

Establishment of culture from sporangia

- Collect green infected leaves from the fields and keep in polythene bags at low temperature. Small holes should be made in the polythene for aeration to keep the samples green,
- Clean the infected green leaves and incubate for sporulation at 20°C for 6 h,
- Use the sporangial inoculum harvested from the infected leaves to inoculate the pot grown seedlings of the same host genotype from which the isolate was collected,
- Incubate the seedlings at 20°C for 16 h in the dark; transfer the pots in polyacrylic isolation chambers to the greenhouse at 25 ± 2°C to avoid cross contamination from other isolates.
- Water pots adequately everyday and observe regularly for downy mildew symptoms on the seedlings,
- After infected seedlings are noticed, remove all healthy seedlings from the pots,
- Use sporangia from infected seedlings for subsequent inoculation of seedlings to maintain the isolate.

Maintenance of isolates

Isolates are maintained on pot-grown seedlings of its collection host or other susceptible host in isolation chambers through asexual generations (Fig. 7). 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 autoclaved before they are disposed. This is done to maintain the fresh inoculum, prevent growth of saprophytes on ageing seedlings and avoid spread of the old isolates.

Fig. 7. Downy mildew isolates being maintained in greenhouse at ICRISAT.

Multiplication of inoculum

- Inoculate the pot-grown seedlings from its maintainer host with the sporangial suspension of the isolate,
- Incubate the seedlings at 20°C for 16 h in the dark, and then transfer to a greenhouse bay under misting for 4-5 days,
- Grow the seedlings for 25-30 days at 25 ±2°C under proper care of watering and fertilization 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.

Growing seedlings in pots

- Fill the plastic pots with autoclaved potting mixture, up to the top 5 cm mark and water them. *(Plastic pots of various sizes (10-cm, 15-cm and 17.5-cm diameter) can be used for planting pearl millet seed depending on number of seedlings to be inoculated)*,
- Make uniform holes (1 cm) in saturated soil in the pots using a dibbler stamp (Fig. 8) (*This equipment, which facilitates equidistant sowing of seeds at equal depth in a pot thus reducing variability in emergence time due to sowing depth and distance between seedlings, is a new development*),
- Sow the seed of test lines and susceptible checks at uniform depth in holes made with the help of a dibbler stamp, single seed per hole, to achieve uniform emergence of seedlings (Fig. 9) *(variable seeding depth is a major factor influencing uniform seedling emergence)*,

Fig. 8. Dibbler stamp for making uniform sowing depth in pots.

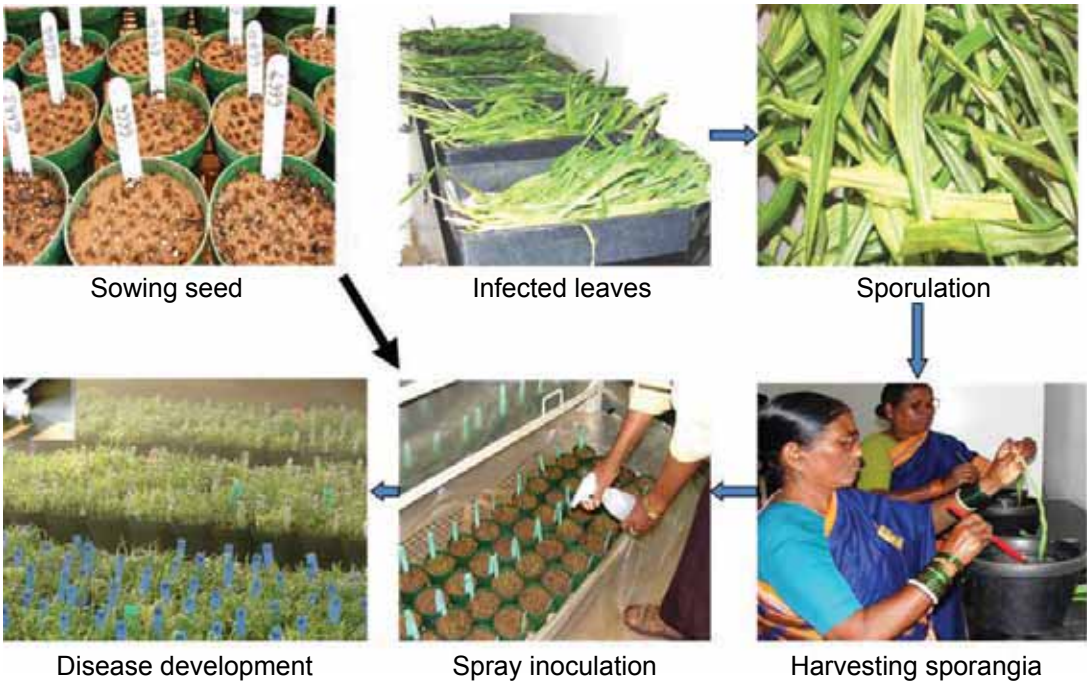

Fig. 9. Greenhouse screening for downy mildew resistance.

- Cover the seed with a 1-cm layer of potting mixture, irrigate properly and maintain these pots in the greenhouse at 35°C till seedling emergence.

Preparation of inoculum and inoculation

- Collect infected leaves from the pot-grown inoculated seedlings in the greenhouse as described above, and cut them into pieces of 20-30 cm for proper handling,
- Wash the leaf pieces in running tap water using cotton swab to remove old sporangial growth from the leaf surface and wipe dry with tissue paper,
- Place the leaf pieces in plastic tray in the humidity chambers (bottom tray and cover lined with wet blotting paper) keeping their abaxial surface facing upward,
- Incubate the trays containing infected leaves at 20°C for 6 h in dark and then bring to zero degree Celsius for the next 8 to 10 hours to prevent the release of zoospores from sporangia. (*Incubator with precise temperature control system, such as Percival make should be used to adjust temperatures for different time periods*),
- Next morning, harvest sporangia 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,

- Filter the sporangial suspension through a double-layered muslin cloth to remove the plant debris, and adjust sporangial concentration to 1×10^6 mL^{-1} using a haemocytometer, then transfer the inoculum to a pneumatic atomizer placed in the ice box,
- Count the seedlings at the coleoptile to first-leaf stage (2-3 days after sowing) in each pot and record the number on the plastic label. (*Seedlings in each pot are counted and recorded on the plastic label before inoculation to discount any seedlings emerging after inoculation)*,
- Transfer the above pots into the inoculation room on metallic shelves and organize them in rows,
- Spray-inoculate seedlings with the sporangial suspension using pneumatic atomizer till run-off ensuring that every seedling has received uniform inoculum,
- Cover the inoculated seedlings with a polyethylene sheet immediately to provide high humidity required for infection, and incubate in the dark at 20°C for 16-20 h,
- Shift the inoculated seedlings 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,
- Count the infected seedlings in each pot 2 weeks after inoculation and record the number on the same plastic label in the pot on which total seedling counts were recorded before inoculation. (*This method facilitates entering the data in computer from the plastic label and also to see the extent of infection in a pot*),
- Bring the plastic labels to the laboratory and enter the data on total and infected seedling for each entry in the computer,
- Validate the date and calculate the percentage disease incidence,
- Analyze data as required and prepare the report.

2.6 Resistance sources and utilization

A large number of germplasm accessions and breeding lines have been screened for resistance to downy mildew both in the field and greenhouse at ICRISAT, Patancheru, and promising lines from these have been evaluated in multilocation tests in South Asia – mainly in India, and in several locations of West and Central Africa over a number of years, and several lines with stable resistance have been identified (Table 1). Some of these lines have been used in a resistance breeding program at ICRISAT-Patancheru and in national programs in India and elsewhere (Thakur et al. 2006).

A wide range of agronomically improved breeding lines, particularly promising hybrid parental lines (A-/B- and R-lines) with high levels of resistance to single and multiple pathotypes have been identified. Availability of these resistance sources

has considerably enhanced the sustained development of hybrids with high levels of resistance to downy mildew. Several downy mildew resistant hybrid parental lines from ICRISAT have been utilized by both public sector and private seed companies in developing commercial hybrids that are currently being grown in India. Some of the resistant germplasm lines used in breeding are: P 7 (ICML 12), SDN 503 (ICML 13), 700251 (ICML 14), 700516 (ICML 15), and 700651 (ICML 16), P 310-17, P 7-3, P 1449-2-P1, IP 18298, P 1449-2, YL 18 and some advanced breeding lines (B-lines) with good levels of resistance – 863B-P2, ICMB 90111-P6, -01777, -02333, -03888, -03999, -05333, -06888, -95444, -96333, -96666, -98444 and -99022 (=843-22B).

2.7 Inheritance of resistance

Resistance to downy mildew has generally been reported to be inherited as a dominant, partially dominant (Deswal and Govila 1994; Singh and Talukdar 1998) or complex trait (Joshi and Ugale 2002). Quantitative Trait Loci (QTL) for downy mildew resistance have also been mapped (Jones et al. 1995) and results indicate both major and minor genes for resistance, but a single major gene is hardly enough to provide high levels of resistance in an inbred line of the hybrid (Hash et al. 2006). Downy mildew resistance QTLs specific to major pathotypes have also been identified in some of the advanced breeding lines (863B-P2, ICMB 90111-P6, P 7-3, P 310-17, P 1449-2-P1, PRLT 2/89-33 and IP 18293-P152) and a few of these have been introgressed in hybrid parental lines (Hash and Witcombe 2005) for developing commercial hybrids.

3. Pyricularia leaf spot or blast

3.1 Economic importance

Pyricularia leaf spot, also known as blast disease, is particularly important in pearl millet forage cultivars. It is an important disease in the southern United States and more recently it has emerged as a serious disease of dual purpose (grain and fodder) pearl millet hybrids in India (Lukose et al. 2007; Anonymous 2009). In India, the disease was first reported from Kanpur, Uttar Pradesh (Mehta et al. 1953) and remained as a minor disease for a long time. The disease causes chronic yield losses of grain (Timper et al. 2002) and forage (Wilson and Gates 1993).

3.2 Symptoms

The disease appears as grayish, water-soaked lesions on foliage that enlarge and become necrotic, resulting in extensive chlorosis and premature drying of young leaves. Depending on the resistance level of the host cultivar, the lesion size varies from small, roundish, elliptical, diamond shaped to elongated, measuring 1-2 mm to 20 mm (Fig. 10). Lesions are often surrounded by a chlorotic halo, which turns

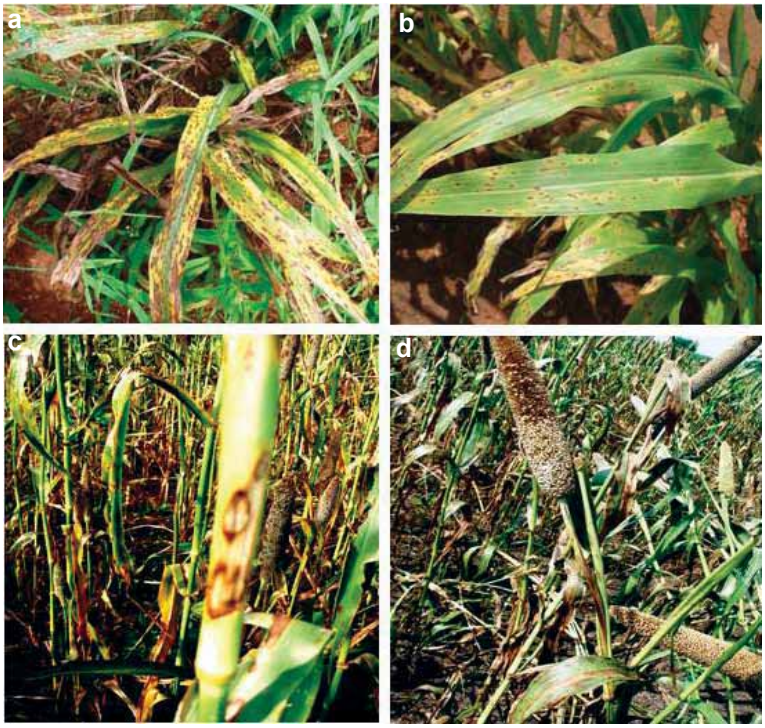

Fig. 10. Blast symptoms on: (a & b) foliage; (c) sheath; (d) peduncle and panicle.

necrotic, giving the appearance of concentric rings. The lesions are usually confined to interveinal spaces on the foliage. Lesions grow and coalesce to cover large surface areas and cause necrosis of tissues. In case of a susceptible cultivar the entire foliage gives a burnt appearance. Severely infected plants produce no grain or few shriveled grains in blasted florets.

Leaf blast on pearl millet has been found to be negatively correlated with green-plot yield, dry matter yield and digestive dry matter (Wilson and Gates 1993) thus affecting the productivity and quality of the crop.

3.3 Pathogen

Pyricularia grisea (Cooke) Sacc., [teleomorph - *Magnaporthe grisea* (Herbert) Barr], an ascomycete fungus, causes blight or blast in pearl millet. Asexual conidia are pyriform, hyaline, mostly three-celled with a small appendage on the basal cell (Fig. 11). Conidia measure approximately 17-31 x 6-9 μm and germinate by producing appresorium (Wilson 2000).

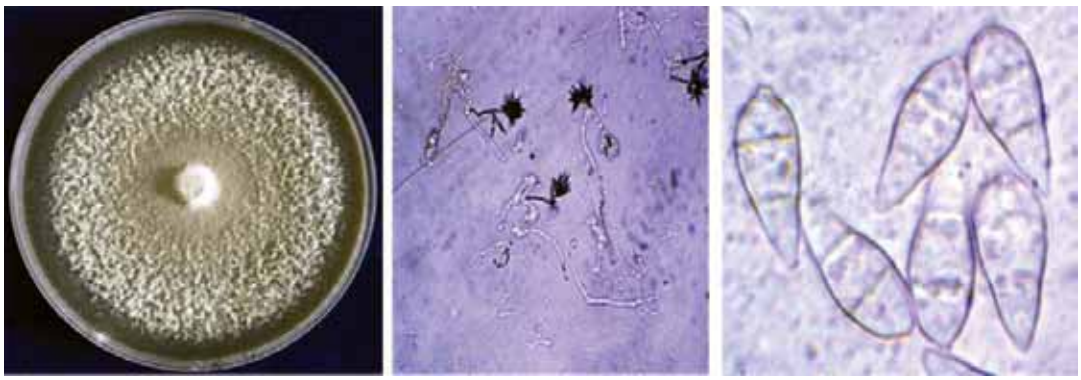

Fig. 11. Culture and conidia of Pyricularia grisea.

The pathogen can be easily isolated from infected leaf tissue and grown on synthetic media. The freshly harvested leaf portions with infection can be placed in glassine or paper envelopes in the refrigerator, where they will slowly dry and remain flat. The fungus can be isolated from these dried leaf samples for 3 years or longer.

3.4 Epidemiology

The pathogen (*P. grisea*) infects several cereal crops, including rice, wheat, pearl millet, finger millet, foxtail millet and grasses. The fungus is highly variable, but highly specialized in their host range. Thus *P. grisea* strains from rice do not infect pearl millet and vice versa. The rice blast pathogen is widely distributed and has many virulent

pathotypes, but there is no such evidence of the existence of pathotypes in pearl millet as of now. Prevalence of high humidity (>90% RH) and moderate temperature (25-30°C) favors infection and disease development. The disease becomes more severe during humid weather conditions especially with dense plant stands. The pathogen sporulates profusely in the lesions on foliage and the conidia can be easily dispersed by the wind and splashing rain. These spores can overwinter in stubble and can infect the next crop the following year. Conidia generated in the diseased plant can further spread the infection.

3.5 Screening Techniques

Screening for resistance to leaf spot or blast has generally been done under natural field epiphytotic conditions. Greenhouse screening has also been done at Tifton (Wilson et al. 1989). Both greenhouse and field screening methods have been standardized at ICRISAT and are described below.

Isolation and inoculum multiplication

- Isolate the pathogen on potato dextrose-agar (PDA) plates from infected leaves surface sterilized with sodium hypochlorite (2% solution), purify it through single-spore isolation and maintain the culture on PDA under refrigeration,
- Transfer mycelial plugs from PDA to oatmeal agar plates and incubate these at 28°C with 12 hours of darkness for 7-10 days,
- Harvest spores by flooding the plates with sterilized distilled water and scrapping off the growth with a spatula,
- Prepare the spore suspension of desired concentration (1×10^5 spore mL^{-1}) with the help of a haemocytometer,
- Add 2 drops of a surfactant (Tween-20) in a liter of spore suspension for uniform spore dispersal just before inoculation.

3.5.1 Field screening

- Grow test lines in the central four rows and a highly susceptible line on the first row and every fifth rows as infector/indicator rows,
- Spray-inoculate seedlings at pre-tillering and/or pre-flowering stages with aqueous spore suspension to run-off,
- Provide high humidity (>90% RH) by operating perfo- or sprinkler irrigation twice a day 30-60 min each in the morning (between 1000 and 1100) and in the afternoon (between 1700 and 1800) on rain-free days,

- Record disease severity at the hard-dough stage using a progressive 1-9 scale developed for rice blast (at International Rice Research Institute).

The disease severity rating scale has been modified to classify lines/plants into different disease reaction classes.

Foliar blast severity rating scale (1-9)

Rating scale	Symptoms and lesions	Disease reaction
1	no lesion to small brown specks of pinhead size	Highly resistant
2	large brown specks	Resistant
3	small, roundish to slightly elongated, necrotic gray spots, about 1-2 mm in diameter with a brown margin	
4	typical blast lesions, elliptical, 1-2 cm long, usually confined to the area between main veins, covering <2% of the leaf area;	Moderately resistant
5	typical blast lesions covering <10% of the leaf area	
6	typical blast lesions covering 10-25% of the leaf area	Susceptible
7	typical blast lesions covering 26-50% of the leaf area	
8	typical blast lesions covering 51-75% of the leaf area and many leaves dead	Highly susceptible
9	>75% leaf area covered with lesions and most leaves dead	

Disease development is growth-stage dependent (Wilson and Hanna 1992). Therefore, early-maturing varieties will express more severe leaf spot than late-maturing varieties at any given evaluation, so severities of pearl millet with widely differing maturities should be corrected for maturity or rated at a similar growth stage.

3.5.2 Greenhouse screening

Growing seedlings, inoculation and evaluation

- Prepare pots (15-cm diameter) filled with autoclaved soil-sand-FYM mix (2:1:1 by volume),
- Sow the seed of test lines and a susceptible check in pots (10 seeds/pot) in a greenhouse bay maintained at 35±1°C,
- Water the pots adequately and grow the seedlings for 10-12 days,
- Spray-inoculate 10-12-day-old seedlings (at the 3-leaf stage) with the aqueous conidial suspension (ca. 1×10^5 spores mL^{-1}) of *P. grisea*,

- Expose the inoculated seedlings to high humidity (>90% RH) and leaf wetness condition under misting for 5-7 days. *The exposure period to high humidity can vary depending on ambient conditions and susceptibility level of the test genotypes*,
- Transfer the seedlings to another bay with no misting, and water the pots regularly,
- Examine the seedlings visually daily for symptom development 3-4 days after inoculation. (*Highly susceptible genotypes can show symptoms within 4-5 days after inoculation*),
- Record disease severity scores 10 days after inoculation using the above 1-9 rating scale.

3.6 Resistance sources and utilization

Several lines of cultivated pearl millet and wild relatives of pearl millet have been identified with resistance to Pyricularia leaf spot (Wilson and Hanna 1992). Tift 186 and Tift 383 were also identified as resistant (Burton 1977, 1980), but more recent observations suggest that their resistance is no longer effective. More recently several ICRISAT-bred elite B-lines and R-lines were identified with high levels of resistance to blast through field and greenhouse screens, and these lines were also resistant to downy mildew (Thakur et al. 2009). Some of the breeding lines with high levels of resistance are: 863B, ICMB 01333, ICMB 01777, ICMB 02111, ICMB 03999, ICMB 93222, ICMB 97222, ICMR 06222, ICMR 06444 and ICMR 07555 (Table 1).

3.7 Inheritance of resistance

Expression of resistance in pearl millet to Pyricularia leaf spot tends to be dominant or partially dominant and is not affected by cytoplasm (Wilson and Hanna 1992). The wild accession of *P. glaucum* subsp *monodii* is reported to have three independent dominant resistance genes (Hanna and Wells 1989), and four landraces from Burkina Faso each had independent dominant resistance genes (Wilson et al. 1989). Resistance to the Indian isolate of *P. grisea* was found to be governed by a single dominant gene (Gupta et al. 2011).

4. Rust

4.1 Economic importance

Rust on pearl millet has been reported from many countries of Asia and Africa, and also from the US and Brazil. Substantial losses in grain yield and fodder quality may occur when infection occurs before flowering (Wilson et al. 1996). Rust is of greater importance on multicut forage hybrids where even low severity can result in substantial losses of digestible dry matter yield (Wilson et al. 1991). The disease has become widespread due to large-scale seed production in the summer season, and overlapping cropping in certain states of India. For hybrid seed production, the crop is grown in the post-rainy season during January-April that coincides with cool nights (15-20°C) and warm days (25-34°C). During this period the abundant dew formation occurring on the foliage in the mornings helps urediniospores to germinate and cause infection.

4.2 Symptoms

Infected leaves initially show pinhead chlorotic flecks, which later turn into reddish-orange, round to elliptical pustules on both surfaces (Fig. 12). Individual pustules (uredinia) are small and erumpent, and as the disease progresses they coalesce to occupy larger leaf surface. The infection generally begins from the distal end of the leaf and progresses towards the basal part. In severe cases, rust pustules appear on the entire leaf blade, leaf sheath, stem and culm. These pustules contain numerous urediniospores that become airborne as pustules burst. As the pustules age, teliospores also appear, although with low frequency.

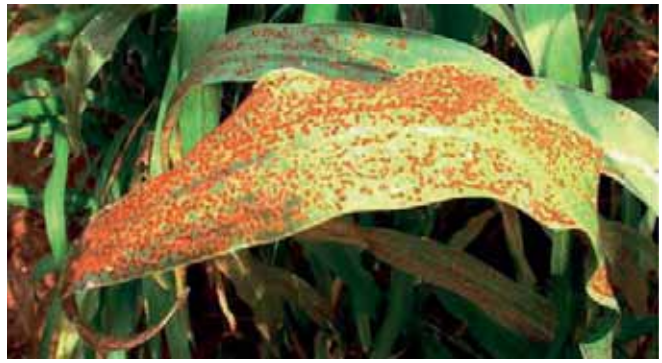

Fig. 12. Rust symptoms.

4.3 Pathogen

Puccinia substriata var. *indica* Ramachar & Cumm (syn: *Puccinia substriata* Ell. & Barth. var. *penicillariae* de Carvalho et al.; *Puccinia penniseti* Zimm), causes rust disease in pearl millet. A complete life cycle of the rust pathogen has recently been investigated in Brazil and the pathogen has been renamed as *P. substriata* var. *penicillariae* (syn. var. *indica*) (de Carvalho et al. 2006).

The rust pathogen is heteroecious and requires two unrelated hosts to complete its life cycle. Pearl millet, on which uredial and telial stages are produced, is called the 'primary host' (Fig. 13). Brinjal (egg plant – *Solanum melongena* L.) and *S. aethiopium*, on which spermatia and aeciospores are produced, are the 'alternate hosts'. Pearl millet leaves are first infected by aeciospores produced on the alternate host, which results in production of uredinia and urediniospores, and later telia and teliospores, which infect alternate hosts (Fig. 14).

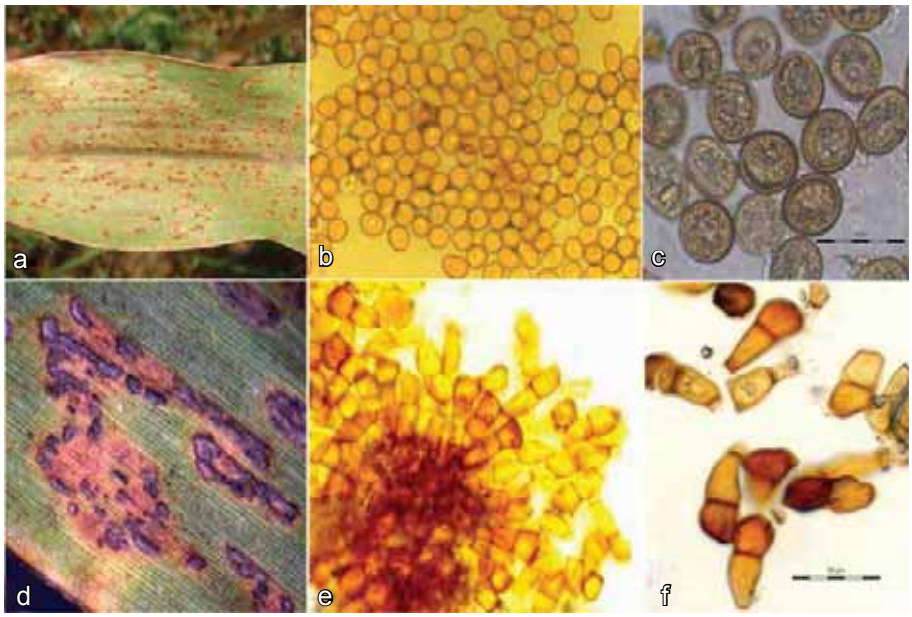

Fig. 13. Puccinia substriata var. indica – uredinial and telial stages on pearl millet: (a) uredinial pustules; (b) young urediniospores (20X); (c) mature ureddiniospores (40X); (d) Telial pustules; (e) a cross section of a telial pustule showing teliospores; and (f) individual teliospores (20X).

4.4 Epidemiology

The disease initiates from the infection by urediniospores available from the off season or volunteer pearl millet plants or by the aeciospores from *Solanum* species as described earlier. Once the infection is established in pearl millet, abundant production and release of urediniospores occurs in the field within 10 days after infection. Rapid production and release of urediniospores help secondary spread of the disease in a crop. The first visible symptoms appear as pinhead chlorotic flecks within 48 h after inoculation. These flecks later enlarge and contain numerous urediniospores. Occurrence of physiological races has also been reported from the USA (Tapsoba and Wilson 1996).

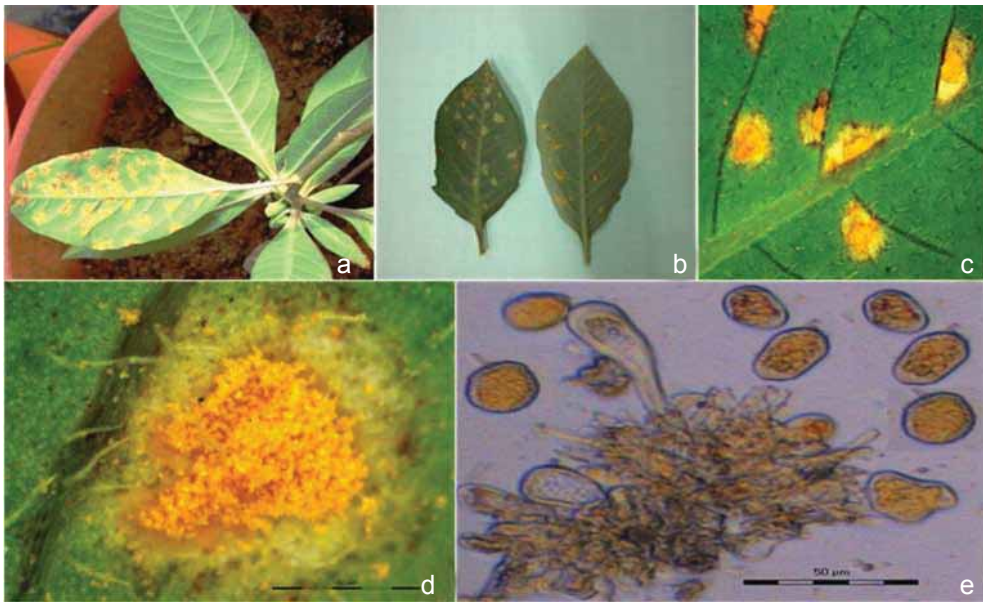

Fig. 14. Puccinia substriata var. indica on Euphorbia geniculata; (a) aecial stage on the lower surface of leaf; (b) spermagonia on upper surface and aecia on lower surface; (c) close-up view of aecia; (d) microscopic view of aecium (40x); (e) aeciospores (20x).

Clear, cool nights (<20°C) and dew formation favor infection and rust development. Rust is relatively more severe in the postrainy season crop than in the rainy or summer season crops in India.

4.5 Screening method

Screening under natural epiphytotic conditions can be done to identify resistance. There are known hot-spots where field screening can effectively be done, particularly during the late rainy season or postrainy season. However, there are chances that in certain years the natural disease pressure may not be adequate to discern resistant and susceptible genotypes. Therefore, it is desirable to screen lines under artificial epiphytotic conditions.

Screening for rust resistance has been done both in fields and greenhouses at the USDA-ARS, Tifton, Georgia (Wilson 1994) and at ICRISAT, Patancheru (Singh et al. 1997) using different methods. These are briefly described here.

Inoculum preparation

- Collect urediniospores from the infected foliage using a vacuum spore collector,
- Spread onto waxed paper or aluminum foil overnight in an air-conditioned room to allow evaporation of excess moisture,
- Transfer a small quantity (0.5cc) of urediniospores into a glass vial or self-sealing plastic bag. Date and store at -80°C,
- Prior to use, place the glass vials/plastic bags containing urediniospores in a water bath at 40°C for about 10 min to break spore dormancy,
- Prepare spore suspension in sterilized distilled water, add a drop of a surfactant (Twin 20) to ensure the uniform dispersal of spores and adjust the spore concentration (1×10^5 spores mL^{-1}) using a haemocytometer,
- Multiply urediniospores on a rust-susceptible genotype in the greenhouse, collect and store as described above.

4.5.1 Field screening

- Grow test lines in central four rows and a highly rust susceptible line as spreader/ infector rows on every first row and fifth row. (*This should be done during the postrainy season November-January to coincide with cool nights and morning dew*),
- Inoculate the spreader row by dispensing 2 mL of the urediniospore suspension (1×10^5 spores mL^{-1}) into the whorls of the plants 20-25 days after seedling emergence. *(Sporulation can be observed within 7 to 10 days after inoculation, and this will serve as inoculum donor for test rows*),
- Alternately, spray inoculate seedlings both in test lines and susceptible checks with urediniospore suspension twice at 25 and 40 days after emergence, and irrigate the crop by perfo-irrigation to provide high humidity for 2-3 days,
- Record rust severity on individual plants in a line (if segregating material) or entire line 25-40 days after inoculation (at the grain-filling stage) using a modified Cobb scale (Fig. 15),
- Record rust severity on lower leaves and top 4 leaves separately to indicate the disease progress. (*Under field screening, rust severity on top four leaves of a plant is critical as these leaves mainly contribute to photosynthesis and grain filling in the panicle. Plants showing high rust severity on the top four leaves often set poor seed, as seed remain shriveled and undersized)*.

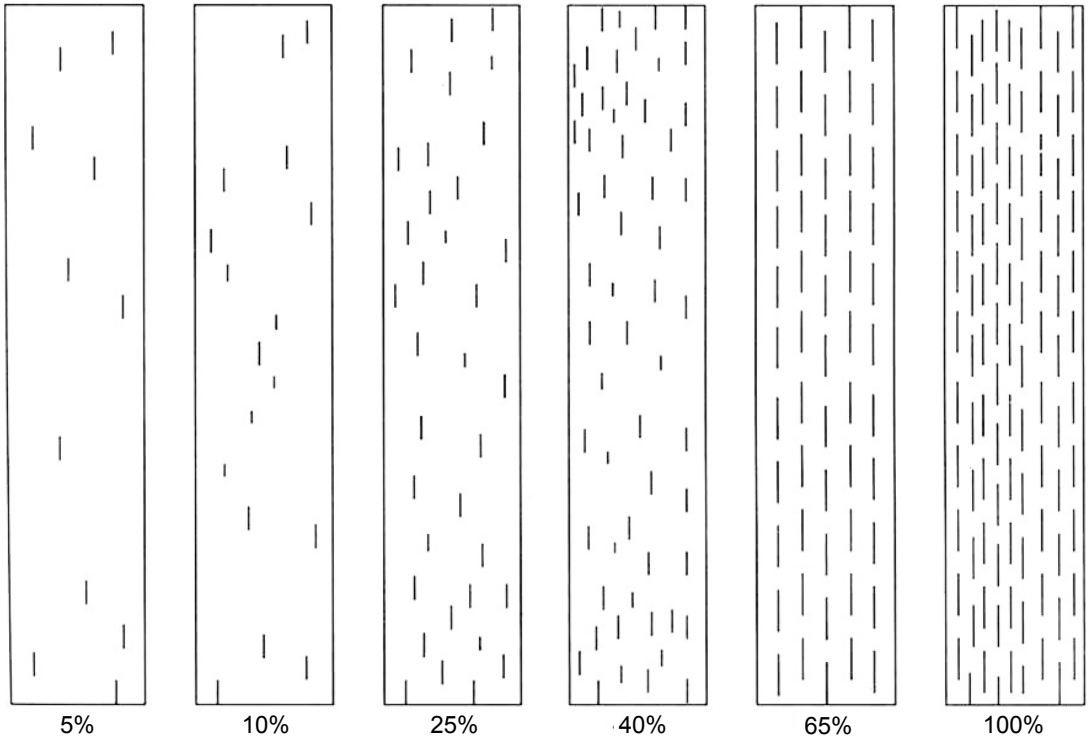

Figure 15. Rust severety rating scale (Singh et al. 1997).

4.5.2 Greenhouse screening

- Grow test line and a susceptible check in pots filled with potting mix of soil-sand-farm yard manure (2:1:1 by volume) @ 10 seedlings/pot (15-cm diameter),
- Spray-inoculate 12-day-old seedlings (at the 3- to 5-leaf stage) with an aqueous suspension of urediniospores (ca. 1×10^5 urediniospores mL^{-1}),
- Incubate the inoculated seedlings in a moist chamber (>95% RH, 25±2°C) for about 18 h and then shift them to greenhouse benches at 25±2°C under misting for 5-7 days,
- Transfer the seedlings to a normal bay at 25±2°C without misting,
- Record rust infection types 12 days after inoculation (*under optimum conditions, uredinia develop in 8-10 days after inoculation*).

Rust infection types are recorded on a 0-4 scale, where

0 = no visible flecks - Immune

1 = pinhead flecks with no sporulation - Hypersensitive reaction

2 = small scattered erumpent pustules with little sporulation – Resistant

3 = clear many erumpent pustules containing numerous spores – Susceptible

4 = many coalescing pustules covering most leaves – Highly Susceptible

Seedling screening in the greenhouse is useful to identify the hypersensitive reactions governed by strong resistance genes. Infection types 0, 1 and 2 indicate resistance, and infection types 3 and 4 indicate susceptibility. However, slow-rusting resistance may also be present (Wilson 1994). In addition, the modified Cobb scale can also be used for data recording, if required.

Greenhouse screening is useful for identifying resistance in large populations in a small space, and under relatively uniform conditions. However, multilocation field screening is necessary to identify resistance effective against the naturally occurring variable pathogen populations.

The screening technique is simple, effective and easily adaptable at any location with adequate greenhouse facilities.

4.6 Resistance sources and utilization

Following the screening of a large number of germplasm accessions at ICRISAT-Patancheru and Bhavanisagar, Tamil Nadu under natural disease pressure in the winter season planting, many accessions with resistance to rust have been identified (Singh 1990; Singh et al. 1997).

Pearl millet germplasm Tift # 3 and Tift # 4 have been shown to have resistance to rust in the USA (Wilson et al. 1989; Wilson and Burton 1991). In addition, many downy mildew resistant accessions also possess resistance to rust (Singh et al. 1997). Through multilocation testing of a number of these lines for 6-7 years, some stable sources of resistance have been identified (Singh et al. 1990b; Singh et al. 1987) (Table 1). Slow-rusting genotypes have also been reported (Wilson 1994; Pannu et al. 1996; Anand Kumar et al. 1995) and *P. glaucum* subsp. *monodii*, a wild relative of pearl millet had been found to be immune to rust (Hanna et al. 1982) but subsequently this resistance was overcome by new virulence (Tapsoba and Wilson 1996).

Thus there are several sources of resistance available, but these need to be characterized for their effectiveness against a wide range of pathotypes/races, including their stability, especially when transferred into elite genetic backgrounds. In a recent evaluation of hybrid parental lines at ICRISAT, one elite B-line (ICMB 96222) and three elite R-lines (ICMR 06999, ICMP 451-P8 and ICMP 451-P6) were found resistant both in field and greenhouse screens (Sharma et al. 2009) (Table 1). Some of the resistant lines used in breeding are: 700481-21-8 (ICML 17), IP 537 B (ICML 18), IP 11776 (ICML 19), IP 2084 (ICML 20), and P 24 (ICML 21) and IP 2696 (ICML 11).

4.7 Inheritance of resistance

Resistance to rust, in most cases, has been reported to be controlled by single dominant genes (Andrews et al. 1985b; Hanna et al. 1985; Ramamoorthi et al. 1995; Pannu et al. 1996). This makes utilization of resistance easy, but also indicates potential rapid breakdown of resistance by evolution of new virulence in the pathogen. Partial rust resistance tends to be inherited in a more complex manner, and additive and epistatic genetic variances can complicate the application of this type of resistance in hybrid development (Wilson 1997).

5. Ergot

5.1 Economic importance

Ergot is prevalent in most pearl millet growing areas of Asia and Africa. In India, the disease is more severe in genetically uniform single-cross F_1 hybrids than in open-pollinated varieties. Ergot susceptibility is related to the cytoplasmic male-sterility (CMS) system and lack of pollen at protogyny (Thakur and Williams 1980; Thakur and King 1988a; Thakur and Rai 2002). The disease is highly weather sensitive and causes substantial losses of both grain yield and quality under favorable weather conditions. Ergot sclerotia that replace grains in the infected panicle contain neurotoxic alkaloids. Sclerotium-contaminated pearl millet grains when used for human consumption or for cattle feed cause various types of toxin-induced symptoms (Mantle 1992).

5.2 Symptoms

The first symptom of ergot appears as cream to pink mucilaginous droplets called 'honeydew' oozing out from infected florets of the pearl millet panicle (Fig. 16).

Figure 16. Ergot symptoms – honeydew and sclerotia.

These droplets contain numerous asexual spores called conidia. Both macro- and microconidia are produced in the honeydew. These droplets dry out within 10-15 days and are replaced by hard, dark brown to black structures with a pointed apex called 'sclerotia', which protrude from the florets in place of grains. During harvesting and threshing these sclerotia fall to the ground and get mixed with the grain, and serve as a source of primary inoculum for the next crop.

5.3 Pathogen

Claviceps fusiformis Lov. (syn. *Claviceps microcephala* (Wallr.) Tul.), an ascomycete fungus, causes ergot disease in pearl millet. The fungus produces two types of conidia, macro- and microcondia both in honeydew and culture. Macroconidia are hyaline, fusiform, unicellular, and germinate by producing one to three germ tubes from the ends or sides. Microconidia are hyaline, globular, unicellular and germinate by producing only one germ tube. Both macro- and microconidia are produced on the tips of germ tubes, macroconidia are produced in chains. Sclerotia, the progenitor of sexual spores, vary in shape, size, color and compactness, and germinate by producing 1-16 fleshy purplish stipes. Each stipe bears at its apex a globular capitulum with numerous perithecial projections. Asci are interspersed with paraphyses in the perithecia, which contains thread-like ascospores- the sexual spores of the fungus.

5.4 Epidemiology

Under conditions of high relative humidity (80-85%) and moderate temperature (20-30°C) with cool nights (15-20°C), honeydew symptoms appear within 4-6 days and sclerotia become visible within 15-20 days after inoculation. Ergot sclerotia from the infected panicles fall to the ground at harvest, or get mixed with the seed during threshing and serve as a primary source of inoculum for the next crop (Fig. 17). Following rains, these sclerotia germinate and release numerous ascospores that are carried by air currents to flowering pearl millet panicles. These ascospores germinate and infect the florets through the stigma (Thakur et al. 1984). The subject is adequately covered by Thakur and King (1988a). Infection and disease spread are favored by overcast skies and drizzling rain that provides high humidity and moderate temperature at the flowering of pearl millet. The honeydew droplets contain numerous conidia of the pathogen. When honeydew droplets containing mycelial mass, conidia and sugary liquid dry out within 10-15 days, these are transformed into hard, dark brown to black structures, generally larger than the seed, called sclerotia, and these vary in shape and size (Chahal et al. 1985).

The role of pollination and length of protogyny in ergot epidemiology has been demonstrated (Thakur and Williams 1980; Willingale et al. 1986). Rapid pollination of stigma prevents infection and reduced protogyny period helps rapid pollen production that results in increased seed set and thus reduced ergot infection. Ergot becomes

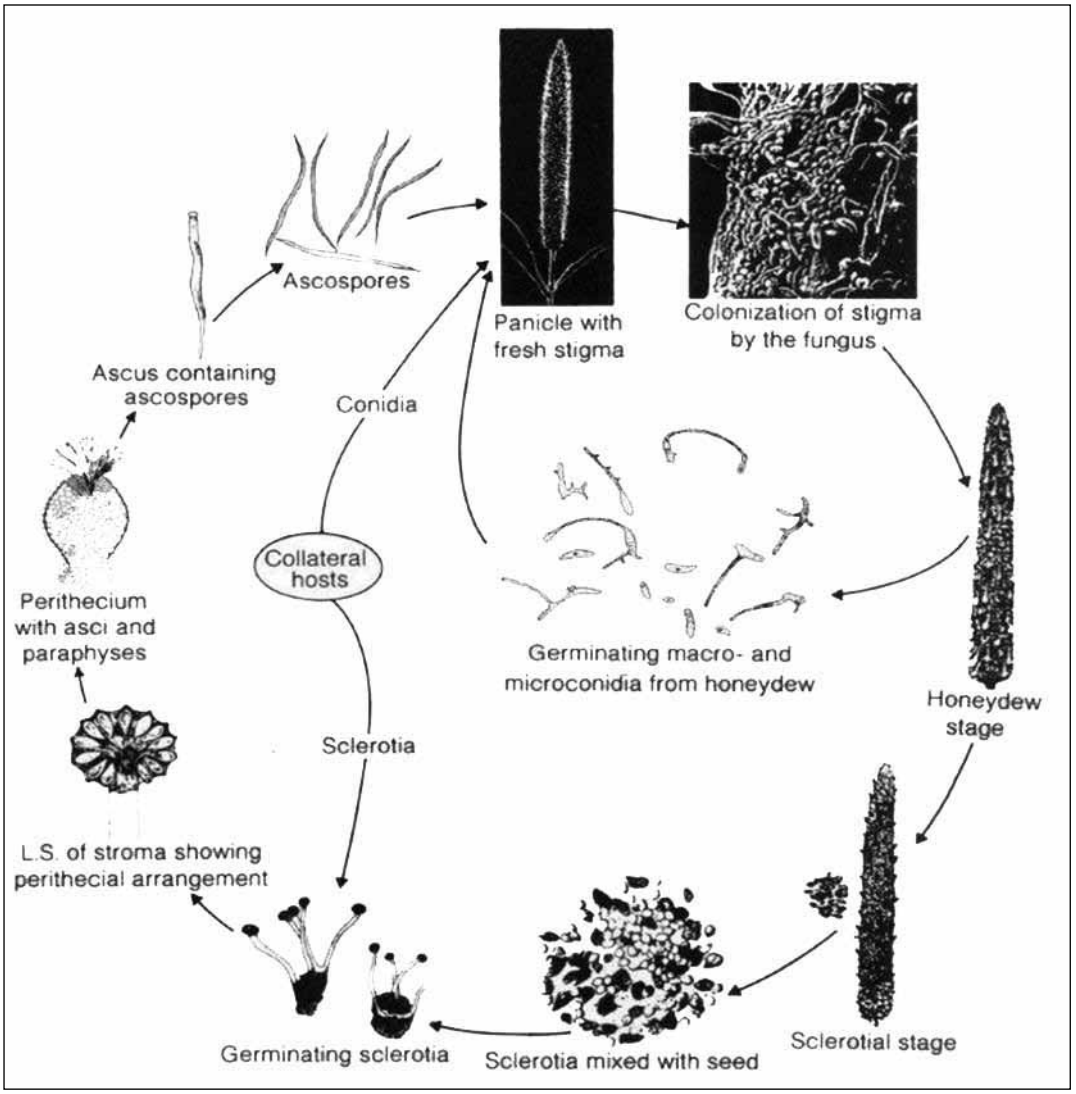

Figure 17. Ergot disease cycle (Thakur and King 1988a).

severe when pollination is inhibited by pollen wash caused by continuous rains during flowering (Thakur et al. 1992b) and cytoplasmic male-sterility (with no fertile pollen) favors infection by the ergot pathogen (Thakur et al. 1989).

5.5 Screening method

5.5.1 Field screening

An effective field screening technique developed at ICRISAT-Patancheru (Thakur and King 1988a) has been used in screening for ergot resistance. This is briefly described here.

- Collect the ergot-infected pearl millet panicles and store in a paper bag in a refrigerator,
- Prepare inoculum suspension by soaking and agitating the panicles in water or by suspending crushed sclerotia in water (5g sclerotia L^{-1} water) and filter the suspension through a double-layered muslin cloth,
- Spray the above conidial suspension (ca. 1×10^6 conidia mL^{-1}) onto fresh stigmas of an early-flowering susceptible genotype (using the technique described below),
- Use conidia formed in the honeydew of these infected panicles to make the inoculum suspension for subsequent inoculations,
- Bag panicles at the boot-leaf stage with parchment selfing bags to allow stigma emergence in a pollen-protected environment and avoid cross-pollination (at least 10 panicles/replication/entry),
- Remove the bags briefly (after 3-4 days) and spray-inoculate the panicles at the full-protogyny stage (>75% fresh stigma) (Fig. 18) with an aqueous conidial suspension (ca. 1×10^6 conidia mL^{-1}) prepared above,
- Replace the bags immediately after spray inoculation to avoid any pollen interference,
- Provide overhead sprinkling twice a day for 30 min each at 10 am and 5 pm on rain-free days to maintain high humidity (>80% RH) and panicle wetness,
- Remove the bags 10-15 days after inoculation when honeydew and sclerotial development become visible,
- Score each panicle 15-20 days after inoculation using the standard ergot severity scale (0 to 100% scale) to estimate the percentage of florets infected (Fig. 19),

Fig. 18. Fresh stigma.

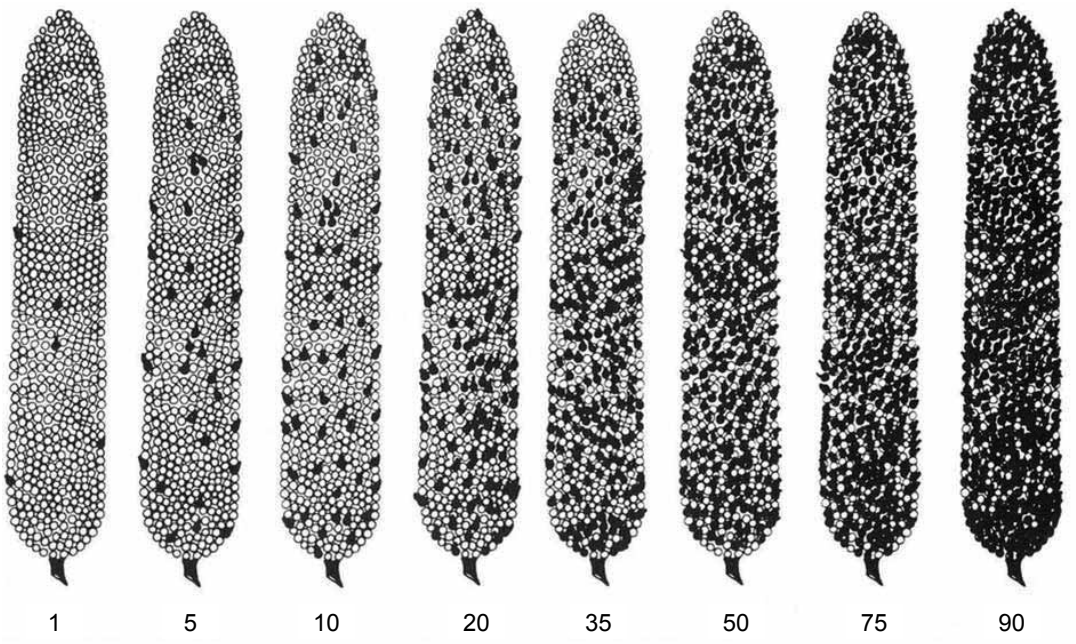

Fig. 19. Ergot severity (%) rating scale (Thakur and King 1988a).

- The percentage of florets infected is directly related to grain yield loss in a panicle. *Selection can be done of individual panicles that have adequate selfed-seed set and little or no ergot for further evaluation.*

This screening technique is effective, precise, and easily transferable. It is now being used at several locations in India and Africa. Sprinkler irrigation is essential to provide the high humidity necessary for good infection and disease development.

5.6 Resistance sources and utilization

It has been difficult to find a high level of resistance to ergot in germplasm accessions and breeding lines. Resistant lines were developed by crossing less-susceptible plants (<5% ergot severity) from a few lines and selecting resistant progenies using pedigree and recurrent selection under high ergot pressure (Chahal et al 1981; Thakur et al. 1982). Nearly 300 ergot-resistant inbred lines and populations of relatively narrow genetic base were evaluated for agronomic traits and reactions to smut, downy mildew and rust, and lines with multiple disease resistance were identified (Thakur et al. 1988, 1993). A total of 283 ergot-resistant lines have been deposited in the ICRISAT gene bank, and these were assigned accession numbers (IP 21209 to IP 21491) in 1993. Lines with stable resistance have also been identified through multilocation testing in India, WCA and ESA (Thakur et al. 1985; Thakur and King 1988c; Thakur et al. 1993). A few ergot resistant lines were found promising for high grain yield and

resistance to diseases in farmers' fields in ESA. Some of the ergot resistant lines used in breeding are: ICMPE 13-6-27 (ICML 1), ICMPE 13-6-30 (ICML 2), ICMPE 134-6-25 (ICML 3), ICMPE 134-6-34 (ICML 4) (Table 1).

5.7 Inheritance of resistance

The limited information available indicates that ergot resistance is a recessive polygenic trait with significant cytoplasmic × nuclear interaction, and for a hybrid to be resistant both parents must carry the same resistance alleles (Rai and Thakur 1995; Thakur and Rai 2002).

6. Smut

6.1 Economic importance

Smut is an important disease of pearl millet in India, western Africa and USA. Although, present in almost all countries where pearl millet is grown, no epidemics have been reported so far, and the extent of losses caused by the disease is quite variable (Thakur and King 1988b). Like ergot, it is a panicle disease that is more severe in CMS-based single-cross hybrids than in open-pollinated varieties (Thakur 1989). The estimated grain yield loss due to smut is 5-20%, although it can be higher under conditions favorable for disease development.

6.2 Symptoms

Smut symptoms appear on the panicle as green, shining smut sori in place of grains two weeks after inoculation; the sori mature within the next two weeks (Fig. 20). Matured sori turn brown and rupture to release dark-brown to black sporeballs of numerous teleutospores (Fig. 21).

Fig. 20. Smut symptoms – green and mature sori.

6.3 Pathogen

The basidiomycetes fungus causing smut of pearl millet is *Moesiziomyces penicillariae* Bref. Vanky (Syn. *Tolyposporium penicillariae* Bref.). The fungus produces teleutospores and sporidia. Teleutospores occur in compact, ball-like masses called spore balls in the infected florets. Spore balls vary in shape and size, and the number of teleutospores aggregated in a ball varies from 200 to 1400. Individual teleutospores do not separate readily, are mostly round, light brown and measure 7-12 um in diameter. Teleutospores germinate to produce four-celled promycelium on which sporidia are borne in chains (Fig. 21). These sporidia germinate to cause infection. Two sporidia of compatible mating types are needed to form a dikaryotic infection hypha, which penetrates through young emerging stigma of a pearl millet floret.

6.4 Epidemiology

Smut infection begins from soil- and seed-contaminated (not internally seed borne) inocula. Teleutospores from the previously infected florets are left in the soil and seed

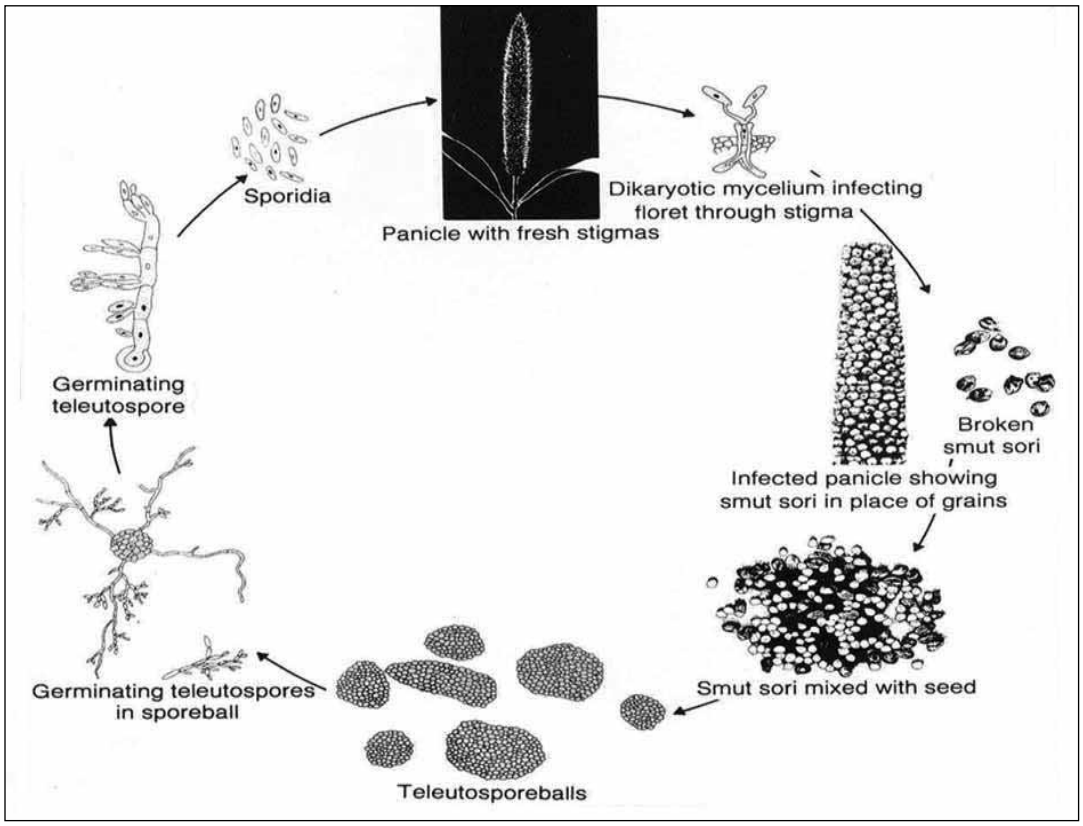

Fig. 21. Smut disease cycle (Thakur and King 1988b).

get contaminated at threshing (Fig. 21). Under favorable conditions of soil moisture and temperature, the teleutospores germinate in the soil and produce numerous airborne sporidia. These sporidia land on the flowering panicles and initiate infection through young emerging stigma. Two compatible mating type sporidia are needed to produce dikaryotic infection hyphae. Mature sori rupture to release masses of spore balls containing teleutospores, which germinate to produce the crop of sporidia. These sporidia play a major role in secondary spread of the disease. Because of the long latent period (about two weeks) secondary spread within a crop is limited, but the late tillers and late planted crops in the adjacent fields may get infected. Smut infection and spread is most favored by the prevalence of high relative humidity (80-95%) and optimal temperature (25-35°C) at the flowering stage of the crop.

As with ergot, rapid pollination is known to reduce or even prevent smut infection in pearl millet lines with shorter protogyny that facilitates self-pollination (Thakur et al. 1983a). The tightness of the flag leaf sheath around the emerging panicle is an additional mechanism of resistance through exclusion of inoculum. Tight flag leaf sheaths restrict infiltration of sporidia into the boot from dew or rain (Wilson 1995). Like in ergot, rapid pollination has been reported to reduce smut severity (Thakur et al. 1983a; Wells et al. 1987).

6.5 Screening method

6.5.1 Field screening

An effective field screening technique for smut resistance in pearl millet developed at ICRISAT-Patancheru (Thakur et al. 1983b; Thakur and King 1988b) is briefly described below.

Fig 22. Pure culture of the pathogen.

- Collect smut-infected pearl millet panicles and store under refrigeration for using as initial inoculum,
- Plate few teleutospores from smut sori onto potato agar or carrot agar and incubate for a week at 30-35°C to obtain the pure culture (Fig. 22) that contains numerous sporidia (Subbarao and Thakur 1983),
- Prepare an aqueous suspension of sporidia (ca 1×10^6 sporidia mL^{-1}) from a 7-day growth on the medium. *The sporidial suspension can also be obtained by soaking spore balls overnight in water,*
- Filter the suspension through a double-layered muslin cloth before using for inoculation,
- Inoculate a panicle by injecting 5-7 mL of sporidial suspension into the `boot' to fill the space between flag-leaf sheath and panicle. *Inoculate 10 or more plants in a row depending on experimental requirement,*
- Cover the inoculated boot with a parchment paper selfing bag,
- Provide sprinkler-irrigation 2-3 times daily, 30 min each, to maintain high humidity (>90% RH) during the period from inoculation to symptom expression,
- Open the bags 15-20 days after inoculation to allow the panicles to dry. *Infected florets show green smut sori in place of grains in the panicle,*
- Score individual inoculated panicles 25-30 days after inoculation using the standard smut severity scale to estimate the percentage of florets with smut sori (Fig. 23). *Individual panicles with little or no smut and good selfed-seed set can be selected for further evaluation.*

This screening technique is quite effective and is being used extensively in India, Africa and USA.

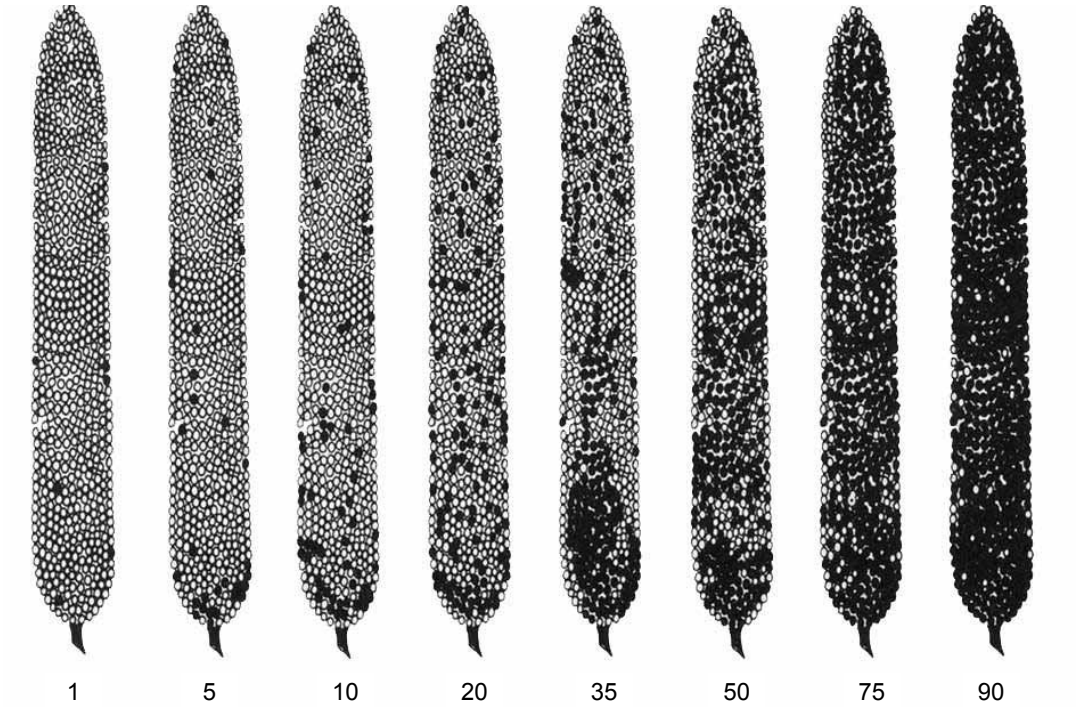

Fig. 23. Smut severety (%) rating scale (Thakur and King 1988b).

6.6 Resistance sources and utilization

Using the above screening technique a large number of smut-resistant lines have been identified from germplasm accessions originating from diverse agroecological zones of WCA (Thakur et al 1992a, b). Nearly 400 such lines of wide genetic base have been characterized for agronomic traits and reactions to other diseases (Thakur et al. 1992a) and deposited in the ICRISAT genebank, and these have been assigned accession numbers (IP 19685 to IP 20081). A number of these lines were evaluated at hot spots in India and countries in WCA and several lines with stable resistance to smut were identified (Thakur et al. 1986; Thakur and King 1988b, 1988d). Several hybrid parental lines, such as ICMA/B 98006, have been identified as having a good level of resistance, and these have been used in developing commercial hybrids in India (Rai et al. 1998).

Some of the resistant lines are: SSC FS 252-S-4 (ICML 5), ICI 7517-S-1 (ICML 6), EBS 46-1-2-S-2 (ICML 7), EB 112-1-S-1-1 (ICML 8), NEP 588-5690-S-8-4 (ICML-9) and P 489-S-3 (ICML 10) (Table 1).

6.7 Inheritance of resistance

Resistance to smut has been reported to be dominant (Thakur and Chahal 1987) and quantitative involving additive and non-additive effects (Phookan 1987; Chavan et al. 1988). The simply inherited *tr* allele conferring trichomlessness also confers some level of smut resistance (Wilson 1995).

7. Other diseases

There are other diseases, such as bacterial leaf spot and leaf streak, Bipolaris leaf spot, Cercospora leaf spot, Curvularia leaf spot, Exserohilum leaf spot, False mildew, maize streak virus, and several nematodes that occur infrequently in certain pearl millet growing areas (Wilson 2000). Currently, these have no major economic significance in India, but can turn into serious diseases in view of climate change and changing production environments. Limited efforts have been made in the pearl millet improvement program at Tifton, Georgia to develop an understanding of these diseases and undertake some breeding activities.

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Table 1. Sources of resistance to pearl millet diseases.			
Disease	**Resistance source**	**Resistance type**	**Reference**
Downy mildew	P 7 (ICML 12), SDN 503 (ICML 13), 700251 (ICML 14), 700516 (ICML 15), and 700651 (ICML 16)	Stable resistance	Singh et al. (1990a)
	863 B-P2, ICMP 423, ICMB 90111-P6, -01777, -02333, -03888, -03999, -05333, -06888, -95444, -96333, -96666, -98444, -99022 (=843-22B) etc.	Breeding lines with high level of resistance	
	IP 18295, P 1449-2, YL-18, IP 18298, IP 8289, IP 22396, YG-2, YG-8 and YM-16	Multiple pathotype resistance	Thakur et al. (2009)
Blast	ICMB 01333, - 01777, -02111, -02444, -02777, -03999, -93222, -97222, ICMR 06222 and -07555	Breeding lines with high level of resistance	Thakur et al. (2009)
	IP 5964 and IP 8913	Mini core accessions	
Rust	700481-21-8 (ICML 17), IP 537B (ICML 18), IP 11776 (ICML 19), IP 2084 (ICML 20) and P 24 (ICML 21)	Germplasm accessions with stable resistance	Singh et al. (1990b)
	ICML 11 (IP 2696)	Inbred with high level of resistance	Singh et al. (1987)
	P. americanum (L.) Leeke sub sp. *monodii*	A wild relative of pearl millet	Hanna et al. (1982)
	ICMB 96222, ICMR 01007, ICMR 06999, ICMP 451-P8 and ICMP 451-P6	Breeding lines with high levels of resistance	Sharma et al (2009)
Ergot	ICMPE 13-6-27 (ICML 1), ICMPE 13-6-30 (ICML 2), ICMPE 134-6-25 (ICML 3), and ICMPE 134-6-34 (ICML 4)	Stable resistance	Thakur and King (1988a, c)
	ICMPES 1, -2, -9, -15, -16, -22, -24, -26, -28, -32, -34 and -37	Sib-bulk lines with high level of resistance	Thakur et al. (1993)
Smut	SSC FS 252-S-4 (ICML 5), ICI 7517-S-1 (ICML 6), EBS 46-1-2-S-2 (ICML 7), EB 112-1-S-1-1 (ICML 8), NEP 588-5690-S-8-4 (ICML 9) and P 489-S-3 (ICML 10)	Stable resistance	Thakur and King (1988b, d)
	ICMSR Nos.	Sib-bulk lines	Thakur et al. (1992a, 93)
Ergot, smut and downy mildew	ICMP 1 (ICMPES-1), ICMP 2 (ICMPES-2), ICMP 3 (ICMPES-28) and ICMP 4 (ICMPES-32)	Sib-bulk populations with multiple disease resistance	Thakur et al. (1988)

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About ICRISAT

ICRISAT
Science with a human face

The International Crops Research Institute for the Semi-Arid-Tropics (ICRISAT) is a non-profit, non-political organization that conducts agricultural research for development in Asia and sub-Saharan Africa with a wide array of partners throughout the world. Covering 6.5 million square kilometers of land in 55 countries, the semi-arid tropics have over 2 billion people, and 644 million of these are the poorest of the poor. ICRISAT and its partners help empower these poor people to overcome poverty, hunger and a degraded environment through better agriculture.

ICRISAT is headquartered in Hyderabad, Andhra Pradesh, India, with two regional hubs and four country offices in sub-Saharan Africa. It belongs to the Consortium of Centers supported by the Consultative Group on International Agricultural Research (CGIAR).

Contact Information

ICRISAT-Patancheru (Headquarters)
Patancheru 502 324
Andhra Pradesh, India
Tel +91 40 30713071
Fax +91 40 30713074
icrisat@cgiar.org

ICRISAT-Liaison Office
CG Centers Block
NASC Complex
Dev Prakash Shastri Marg
New Delhi 110 012, India
Tel +91 11 32472306 to 08
Fax +91 11 25841294

ICRISAT-Nairobi (Regional hub ESA)
PO Box 39063, Nairobi, Kenya
Tel +254 20 7224550
Fax +254 20 7224001
icrisat-nairobi@cgiar.org

ICRISAT-Bamako (Regional hub WCA)
BP 320
Bamako, Mali
Tel +223 20 223375
Fax +223 20 228683
icrisat-w-mali@cgiar.org

ICRISAT-Niamey
BP 12404, Niamey, Niger
(Via Paris)
Tel +227 20722529, 20722725
Fax +227 20734329
icrisatsc@cgiar.org

ICRISAT-Bulawayo
Matopos Research Station
PO Box 776,
Bulawayo, Zimbabwe
Tel +263 383 311 to 15
Fax +263 383 307
icrisatzw@cgiar.org

ICRISAT-Lilongwe
Chitedze Agricultural Research Station
PO Box 1096
Lilongwe, Malawi
Tel +265 1 707297, 071, 067, 057
Fax +265 1 707298
icrisat-malawi@cgiar.org

ICRISAT-Maputo
c/o IIAM, Av. das FPLM No 2698
Caixa Postal 1906
Maputo, Mozambique
Tel +258 21 461657
Fax +258 21 461581
icrisatmoz@panintra.com

www.icrisat.org

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