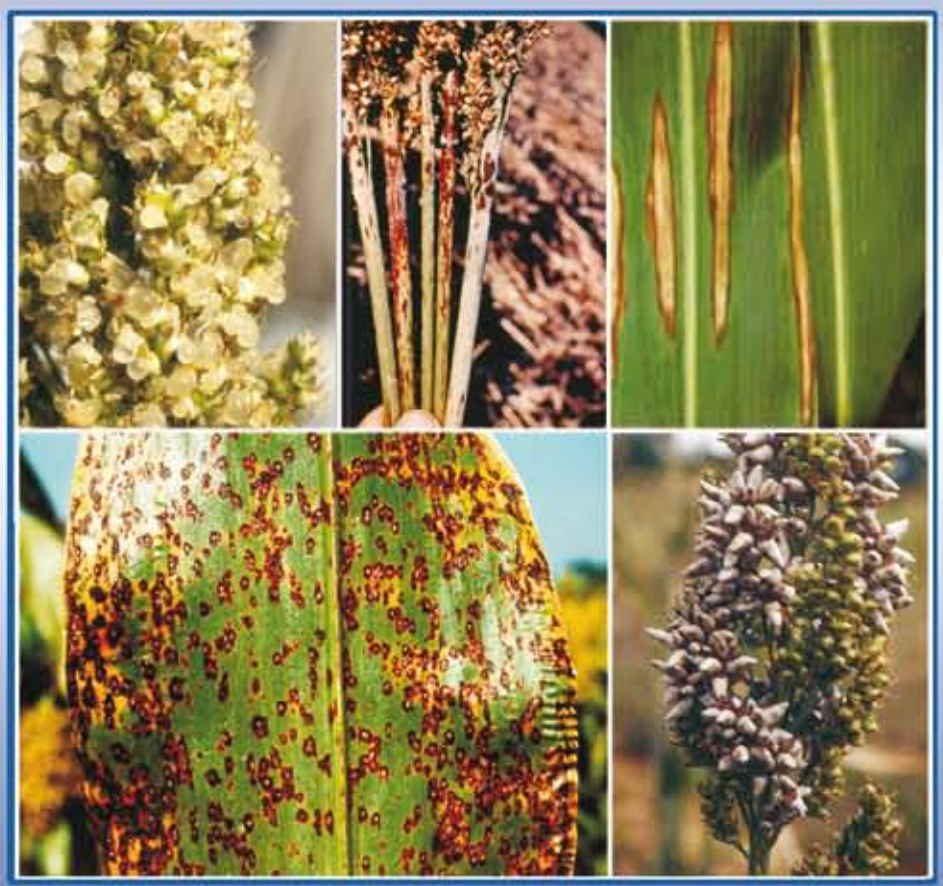
Screening Techniques for Sorghum Diseases

Information Bulletin no. 76



International Crops Research Institute for the Semi-Arid Tropics

Citation: Thakur RP, Reddy BVS and Mathur K. (eds.). 2007. Screening Techniques for Sorghum Diseases. Information Bulletin No. 76. Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics. 92 pp. ISBN 978-92-9066-504-5.

Screening Techniques for Sorghum Diseases

Edited by

RP Thakur, Belum VS Reddy and Kusum Mathur

Information Bulletin No. 76

ICRISAT

International Crops Research Institute for the Semi-Arid Tropics
Patancheru 502 324, Andhra Pradesh, India

2007

Authors

IK Das
Senior Scientist, Sorghum Pathology
National Research Centre for Sorghum
Rajendranagar, Hyderabad 500 030,
Andhra Pradesh, India
E-mail: das@nrcsorghum.res.in

RA Frederiksen
Professor (Retd.), Department of Plant Pathology and Microbiology,
Texas A&M University
College Station, Texas 77843-2182, USA
Present: 8248 Raintree Drive NE,
Albuquerque, NM 87122
E-mail: rfrederiksen@comcast.net

CW Magill
Professor, Department of Plant Pathology and Microbiology
Texas A&M University
College Station, Texas 77843-2182, USA
E-mail: c-magill@tamu.edu

Kusum Mathur
Associate Professor, Department of Plant Pathology
Rajasthan College of Agriculture
Maharana Pratap University of Agriculture and Technology,
Udaipur 303001, Rajasthan, India
E-mail: kusum.mathur@rediffmail.com

YD Narayana
Senior Scientist, Department of Plant Pathology
University of Agricultural Sciences,
Krishinagar
Dharwad 580005, Karnataka, India
E-mail: narayanayd@hotmail.com

LK Prom
Professor, Plant Pathology
USDA-ARS, Southern Plains Agricultural Research Center
College Station, Texas 77845, USA
E-mail: lprom@cgru.usda.gov

R Perumal
Associate Scientist, Department of Plant Pathology and Microbiology
Texas A&M University,
College Station, Texas 77843-2182, USA
E-mail: rperumal@ag.tamu.edu

SV Rao
Principal Scientist
(Genetics & Cytogenetics)
National Research Centre for Sorghum
Rajendranagar, Hyderabad 500 030,
Andhra Pradesh, India
E-mail: svrao@nrcsorghum.res.in

VP Rao
Lead Scientific Officer (Cereals Pathology)
International Crops Research Institute for the Semi-Arid Tropics
Patancheru 502 324, Andhra Pradesh, India
E-mail: vpr@cgiar.org

Belum VS Reddy
Principal Scientist (Sorghum Breeding)
International Crops Research Institute for the Semi-Arid Tropics
Patancheru 502 324, Andhra Pradesh, India
E-mail: b.reddy@cgiar.org

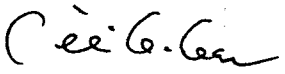
P Sanjana Reddy
Scientific Officer (Sorghum Breeding)
International Crops Research Institute for the Semi-Arid Tropics
Patancheru 502 324, Andhra Pradesh, India
E-mail: sanjana.reddy@cgiar.org

RP Thakur
Principal Scientist (Cereals Pathology) and Head, Plant Quarantine Laboratory
International Crops Research Institute for the Semi-Arid Tropics
Patancheru 502 324, Andhra Pradesh, India
E-mail: r.thakur@cgiar.org

Foreword

I am pleased to write a foreword for this important Information Bulletin on Screening Techniques for Sorghum Diseases edited by RP Thakur, Belum VS Reddy and Kusum Mathur. This contains several chapters contributed by experts from ICRISAT, Patancheru, Indian Sorghum Programs and Texas A&M University, USA. Sorghum crops are attacked by more than 50 diseases incited by fungi, bacteria, viruses and nematodes often resulting in substantial economical losses to growers. However, there are only few diseases that are more important than others in different sorghum growing regions of the world. In this bulletin, the authors have addressed globally important diseases and compiled the information on screening techniques so critical for identification of resistance and its utilization in breeding for developing disease resistant cultivars. It is well established that the most effective and economical method of controlling sorghum diseases is by growing resistant cultivars. Accordingly, developing disease resistant sorghum hybrids and varieties has been the major research focus at ICRISAT and in other programs in the world. It is in this context that a simple and reliable screening technique is required to easily discern resistant and susceptible lines. It is good to note that the authors have provided the background information on importance of the disease, pathogen, symptoms and epidemiology before describing the screening technique and resistance sources that have been identified and utilized in breeding at ICRISAT, in Indian Programs and elsewhere.

The authors have done a commendable job of compiling information pertaining to important diseases of sorghum in a simple and comprehensive manner. I am sure the bulletin will serve as a useful practical guide to sorghum researchers, teachers and students, and especially those who have interest in managing sorghum diseases through host plant resistance. I am pleased to note that small quantity seed of disease resistant lines reported in this bulletin can be made available to the requestor following the standard protocol of exchange of germplasm and breeding lines of ICRISAT.



William D Dar
Director General
ICRISAT

Acknowledgements

The editors would like to thank and appreciate the time and efforts of RS Rathore, former Head, Dept. of Plant Pathology, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur, Rajasthan, India; R Bandyopadhayay, Plant Pathologist, International Institute of Tropical Agriculture, Ibadan, Nigeria; Suresh Pande, Principal Scientist (Legumes Pathology), ICRISAT Patancheru and RA Frederiksen for their critical and positive review of the entire manuscript. The editors would like to express their appreciation to Clint Magill and Louis K Prom for reviewing chapters on Grain Mold, Anthracnose, Leaf Blight, Downy Mildew, Rust, Ergot and Smuts; to YD Narayana on Downy Mildew and Charcoal/Stalk Rot; to TG Nageswara Rao (Principal Scientist, Plant pathology) and G Shyam Prasad (Senior Scientist, Agril. Entomology), NRCS Hyderabad on Charcoal/Stalk Rot; to IK Das on Grain Mold; and to RDVJ Prasada Rao (Principal Scientist, Virology) National Bureau of Plant Genetic Resources, Rajendranagar, Hyderabad on Virus Diseases. The editors would also like to thank the authors of various chapters for their efforts in preparing and submitting the manuscript in a timely manner and responding to the queries promptly. Technical and secretarial help of SM Ahmed (ICRISAT, Patancheru) is duly acknowledged.

Contents

List of Figures

Introduction

RP Thakur, Belum VS Reddy and Kusum Mathur

Sorghum (*Sorghum bicolor* (L.) Moench) is the host of many diseases that are caused by fungi, bacteria, viruses, nematodes and parasitic plants. These diseases can be classified according to the plant parts on which they occur, such as seedling diseases, foliar diseases, root and stalk diseases, leaf sheath diseases, panicle and grain diseases, and storage diseases. Diseases are also classified according to the symptoms they produce on plants and are called seedling blight, root and stalk rots, leaf blight, leaf spot, rust, smut, ergot, wilt, downy mildew, grain mold, leaf stripe, leaf streak, leaf mosaic, etc. Of over 50 diseases reported and described on sorghum, only a few are economically important globally, several others are regionally and locally important in specific agroecosystems.

In most semi-arid tropical environments, economically important diseases of sorghum are grain mold, anthracnose, leaf blight, downy mildew, charcoal rot, rust, ergot, smuts and virus diseases - maize stripe and maize mosaic. These diseases, either alone or in combinations, cause substantial damage to crops resulting in heavy economic losses every year. Other diseases are of sporadic nature and do not account for substantial economical loss. Our strategy of disease management has been mainly through host plant resistance (HPR), which is economical, environment-friendly and technically feasible at farmers' level. Disease management through HPR involves development of a simple and effective screening technique to identify genetic resistance that could be appropriately utilized in breeding programs to develop disease resistant cultivars. At present, both conventional breeding and molecular breeding methods are being used to transfer resistance in breeding lines. In molecular breeding wherein genotyping has become easy and rapid, proper phenotyping is critical for the success of a resistance-breeding program.

In order to develop an effective disease screening technique, a sound knowledge of biology and epidemiology of the disease, availability of pure culture of a virulent strain of the pathogen, simple inoculation method, appropriate disease rating scales, availability of large variable germplasm, adequate laboratory, greenhouse and field facilities are required. In

addition, highly trained and committed scientific support staff is needed to carry out the day-to-day routine work in greenhouse and field screening trials. For example, watering pots with growing seedlings in the greenhouse seems to be a simple work, but our experience shows that it is a highly sensitive and very important day-to-day work that can be done by skilled workers only. For instance, under-watering or over-watering the pots can affect seedling growth and disease development, leading to erroneous results. Similarly, identification of disease symptoms on seedlings and proper data recording of disease reaction require expertise and experience.

Scoring plants for disease reaction has been a highly subjective exercise. Depending on the host-pathogen systems and objectives of the experiment, the disease scores are taken either on a visual qualitative scale of resistant, moderately resistant, moderately susceptible or susceptible, or on a quantitative rating scale of varying classes, such as percentage incidence, 1-9 scale, or 1-5 scale. The quantitative scores are then converted into qualitative form to describe disease reaction types of resistant or susceptible class. Both accuracy and precision of disease scores are required for properly classifying the lines into respective resistant and susceptible classes. In addition to several weather variables, pathogenic races/pathotypes, inoculum density, time of scoring, etc, that affect disease severity, a great deal of variability in disease scores, is also added by the technicians or pathologists who record the disease severity. This generally happens with nurseries that are evaluated multilocationally where at some locations pathologists or technicians are not well trained and experienced. Thus there is a strong need for training plant pathologists/technicians for data recording exercise to obtain reasonably precise and accurate results.

Over the years, screening techniques have been developed and refined for major sorghum diseases, such as grain mold, anthracnose, leaf blight, downy mildew, ergot, charcoal rot, rust, smut and virus diseases. For some of these diseases, both field and greenhouse screening techniques are available, while for others only field screening techniques are known. In some cases, pathologists have used the concept of "hot spot" to screen genotypes against a particular disease, but this has often failed due to highly erratic and unpredictable weather conditions. The basic underlying principle in developing a disease screening technique is to provide

adequate pathogen inoculum at the most susceptible growth stage of the crop under optimal environmental conditions for infection and disease development. This helps to clearly distinguish plants and lines as resistant or susceptible class. After the resistant plants/lines are identified, the resistance is confirmed by screening their next generation progenies under similar conditions, which provides information on heritability of resistance as well. Further, resistance stability is determined by testing the lines in different environments under high disease pressure through multilocation testing. The stable resistance sources thus identified are used in breeding programs to develop disease resistant hybrid parents and varieties. It is often desirable to study inheritance of resistance and number of genes involved before using such sources in breeding programs for effective transfer of resistance genes. A number of resistant lines for each of the major diseases have been identified and some of those have been effectively utilized in developing hybrid parental lines and varieties.

In this bulletin, the authors have provided a brief account of economic importance, geographical distribution, nature of pathogen, disease symptoms and epidemiology, and described in detail the screening techniques for major fungal and virus diseases, and listed some of the available resistance sources and their utilization. These screening techniques have been developed and refined over the years based on the new research information becoming available on biology and epidemiology, and are generally effective under the described situations. However, in order to make the screening more effective, these screening techniques could be modified to some extent to suit the particular climate and location. Among the four smut diseases, detailed screening technique is known only for head smut, which is more severe and prevalent than the other three smuts (long, covered kernel and loose kernel). Screening techniques for these have been proposed based on the available information on the nature, biology and epidemiology. These techniques would require validation to test their effectiveness under different conditions.

We believe that the authors have provided accurate and up-to-date information available on each disease described in this bulletin. We welcome comments from sorghum researchers to further improve these techniques that would greatly help enhance the efficiency and

effectiveness of disease resistance breeding, and thus increase the productivity and production of sorghum.

Selected References

Leslie JF (ed.). 2002. Sorghum and Millets Diseases. Ames, Iowa, USA: Iowa State Press. 504p.

Frederiksen RA and **Odvody GN (eds.).** 2000. Compendium of Sorghum Diseases. Second edition, The American Phytopathological Society, St. Paul, Minnesota, USA: APS Press. 78 pp.

Reddy BVS, Sharma HC, Thakur RP and **Ramesh S.** 2006. Characterization of ICRISAT-bred sorghum hybrid parents (Set 1). International Sorghum and Millets Newsletter (Special Issue), (Thakur RP, ed.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics. 47:1-5.

Thakur RP, Frederiksen RA, Murthy DS, Reddy BVS, Bandyopadhyay R, Giorda LM, Odvody GM and **Clafflin LE.** 1997. Breeding for disease resistance in sorghum. Pages 303-315 *in* Proceedings of the International Conference on Genetic Improvement of sorghum and pearl millet. 23 to 27 September 1996, International Sorghum/Millet Collaborative Research Support Program, Lubbock, Texas, USA.

Grain Mold

RP Thakur, VP Rao, Belum VS Reddy and P Sanjana Reddy

Introduction

Grain mold is the greatest constraint for optimum sorghum (*Sorghum bicolor* (L.) Moench) grain yield and quality. Early maturing, high yielding hybrids and improved varieties that are grown during the rainy season are particularly more vulnerable. Grain molding and grain weathering are two successive events occurring on sorghum caryopsis. Grain molding occurs during anthesis to physiological maturity while grain weathering succeeds the grain molding during which saprophytic fungi continue to grow on matured grains. Damage resulting from grain mold infection includes reduced kernel development, discoloration of grain, colonization and degradation of endosperm and germ, decreased grain density, decreased germination, decreased seedling vigor, and possible mycotoxin contamination. Such grains are not suitable for food and animal feed and thus fetch much reduced market price to the growers.

Pathogens

A large number of pathogenic and saprophytic fungi are associated with grain mold complex. The major pathogenic fungi are species of *Fusarium* (*F. thapsinum*, *F. proliferatum*, *F. andiyazi*, *F. nygamai* and *F. verticillioides*), *Curvularia lunata*, *Alternaria alternata*, and *Phoma sorghina*. Some of the strains of *Fusarium* species are known to produce mycotoxins, such as fumonisins, moniliformin and trichothecenes.

Symptoms

Initial symptoms of grain mold are discoloration of grains due to infection and colonization by mold fungi (Fig. 1). Grain discoloration varies from light whitish, pinkish, grayish, to shiny black depending on infection and colonization by individual fungal species. Often grains are colonized by multiple fungi (Fig. 2). In severe cases, grains turn completely black.

Fig.1. General symptoms of grain mold on sorghum panicles

Fusarium species

Phoma sorghina

Alternaria alternata

Curvularia lunata

Fig. 2. Sorghum grains colonized by specific mold fungi

In case of severe infection at anthesis, grain development is affected resulting instead in chaffy florets or small grains on the panicle.

Epidemiology

Under field conditions, infection occurs at flowering by the naturally available inocula of the mold fungi. However, under greenhouse conditions fungal inoculation of panicles is required. High humidity (>90% RH) and temperature range of 25-35°C are quite favorable for infection and mold development. Panicle wetness during flowering to grain development stages due to frequent rain showers or overhead sprinkler irrigation helps maintain high RH essential for mold development. Dry weather and cooler temperatures (<20°C) are not favorable for mold development. The rate of grain colonization is the fastest by C. *lunata* followed by *Fusarium* spp., *A. alternata* and others.

Field screening technique

- Classify the test entries/lines into different maturity groups (early, medium and late), and for each group plant the test entries and known susceptible and resistant checks in separate replicated field experiments.
- Tag 10 plants/entry/replicate at flowering for recording the disease severity.
- Provide over-head sprinkler irrigation for about 30 min each at noon and late evening (between 5 and 7 PM) on rain-free days from flowering until physiological maturity to provide high humidity (>90% RH) necessary for infection and mold development (Fig. 3). (Natural conditions may be more appropriate when screening for locally adapted varieties.)

Fig. 3. Field screening of sorghum lines for grain mold resistance

- Monitor physiological maturity stage (black layer formation at the hilum region of grains) of each line, and also monitor grain mold development in the susceptible check lines in each maturity group.

  Record the panicle grain mold rating (PGMR) on each panicle of the tagged plants at the right physiological maturity stage (when majority of grains in the middle of panicle have matured) using the visual progressive 1 to 5 grain mold severity rating scale. [*For a reliable screening, the disease scores in susceptible check line should be ≥ 4.0 on the 1 to 5 scale and ≥ 7.0 on the 1 to 9 scale.]*

- Calculate the average PGMR of the 10 tagged-panicles for determining mold severity of the line.

Grain mold severity rating scale: 1 to 5

Severity rating	Percentage grains molded on a panicle	Disease reaction class
1	0 to <1	Highly resistant (HR)
2	1-10	Resistant (R)
3	11-25	Moderately resistant (MR)
4	26-50	Susceptible (S)
5	>50	Highly susceptible (HS)

Grain mold severity rating scale: 1 to 9

In some cases, where smaller differences between test lines are to be identified, the following 1 to 9 rating scale can also be used.

Severity rating scale	Percentage grains molded on a panicle	Disease reaction class
1	0 to <1	HR
2	1-5	R
3	6-10	
4	11-20	MR
5	21-30	
6	31-40	S
7	41-50	
8	51-75	HS
9	76-100	

- Also record the percentage grains infected/molded by individual fungi on a panicle based on the typical symptoms produced by each fungus (Fig. 2).
- Harvest the panicles soon after PGMR recording, dry them and thresh individual panicles.
- Collect 20 g threshed grain sample from the bulk of the 10 panicles; spread them in a petridish and score for threshed grain mold rating (TGMR) using the above 1 to 5 or 1 to 9 severity rating scale with the help of a magnifying lens under proper lighting.
- Also record the percent grains infected/molded by the individual fungi based on the symptoms as described above.
- Compute the data both for PGMR and TGMR for each set of material and subject to statistical analyses to find significant differences between entries and classify lines into resistant and susceptible classes.
- Identify the lines showing low mold ratings and desirable agronomic traits for further testing and utilization in resistance breeding.

  [*In several studies we have found strong positive correlations (r = 0.95-98) between PGMR and TGMR, and therefore we suggest that recording PGMR data alone should be adequate to discern lines into resistant and susceptible classes. We consider that PGMR data are more realistic and proper representative of the field reaction than the TGMR data, which is recorded several days later and during this period mold severity can increase depending on the prevailing humidity conditions.*]

Greenhouse screening technique

Inoculum preparation

- Soak grains of any sorghum line, such as cv. Bulk-Y or SPV 104 overnight and drain the excess water in the morning.
- Add 80 g of soaked grain in a 250 ml conical flask and plug tightly, cover the plug with a paper secured with rubber band, or aluminum foil.

- Autoclave the grains in the conical flasks at 121°C and 15 lbs psi for 1 h and allow to cool.
- Inoculate the autoclaved grains with actively growing pure cultures of the specific mold pathogens (species of *Fusarium*, C*urvularia*, *Alternaria,* etc.) and incubate at 28±1°C for 10 days under 12-h photoperiod.
- Shake the flasks on every alternate day to promote uniform growth and development of the fungi on the grains.
- Suspend the infested grains in a measured volume of sterile distilled water and vortex it for 2-3 min to dislodge the spores into the suspension.
- Adjust the concentration of the resultant suspension to about 1×10^6 spores mL^{-1} using a haemocytometer, and add 1 ml Tween 20™ (polysorbate 20) in 1000 ml spore suspension.

Growing test lines in pots

- Sow surface-sterilized seed of test lines and appropriate check lines in autoclaved potting mix of soil, sand and FYM (2:1:1 by volume) in 30-cm diameter plastic pots outdoor.
- Thin the seedlings two weeks after emergence to maintain 5 plants per pot, and maintain at least 15 plants for each line at 5 plants/pot/replicate for a replicated experiment.
- Transfer the pots in the greenhouse (25±2°C) when the plants are at pre-flowering stage.

Inoculation of panicles with mold fungi and data recording

- Tag uniformly flowering plants at full anthesis (>80%) in each line.
- Spray-inoculate the tagged panicles at full anthesis with each of the fungal suspensions.
- Allow the inoculated panicles to dry for 2-3 h and then provide wetness (>95% humidity) by overhead foggers for 48 h (Fig. 4).

Fig. 4. Greenhouse screening for grain mold resistance

- Keep inoculated plants in separate greenhouse chambers to avoid cross contamination.
- Again provide panicle wetness for 72 h at physiological maturity to promote fungal growth and grain colonization.
- Record mold severity (PGMR) at physiological maturity on the tagged panicles using 1 to 5 or 1 to 9 rating scale as described earlier.
- Harvest the panicles, dry them and thresh, and record TGMR as mentioned earlier, if required.
- Plate 25 grains per plate per replication in a pre-sterilized humid chamber (blotter method), and incubate at 28±1°C for 5 days under 12-h photoperiod to promote grain colonization.
- Record percentage grain colonization by individual fungi, based on total and molded grain for each line.
- Compute and analyze the data for comparing the resistance level of test lines against each fungal isolate.

  [Under greenhouse conditions, resistance to individual or multiple mold pathogens can be confirmed. The major limitation, however, is for handling large number of lines unlike under field nursery conditions.]

Resistance sources and their utilization

At ICRISAT Patancheru, more than 7,100 sorghum germplasm accessions have been screened using field screening technique and about 150 of them from diverse geographical origins and race types having different plant traits have been identified to be resistant or moderately resistant to one or more fungi. Resistance of these accessions has recently been confirmed by field screening and these have been further characterized for morphological traits, such as plant height, glumes coverage of grain, glumes color and grain color, at ICRISAT.

Several resistant accessions (IS 2815, IS 21599, IS 10288, IS 3436, IS 10646, IS 10475 and IS 23585) have been used in breeding to develop restorer lines, varieties and hybrid parents. In a trait-specific breeding program, a number of grain mold resistant lines with maintainer reaction have been converted into male-sterile lines. Fifty-eight seed parents with A_1 cytoplasm with white grain, red grain and brown grain have been developed. Also, the grain mold resistant accession IS 9470 with A_1 (*milo*), A_2, A_3, and A_4 (maldandi), and IS 15119 with A_3 and A_4 (maldandi) cytoplasms have been converted into male-sterile lines and these have been characterized. More recently, some test hybrids developed using mold resistant advanced hybrid parents (A- and R-lines) have shown promising results for mold resistance and grain yield at ICRISAT.

Selected references

Bandyopadhyay R and **Mughogho LK.** 1988. Evaluation of field screening techniques for resistance to grain molds. Plant Disease 72:500–503.

Bandyopadhyay R, Mughogho LK and **Rao KEP.** 1988. Sources of resistance to sorghum grain molds. Plant Disease 72:504–508.

Bandyopadhyay R, Butler DR, Chandrashekar A, Reddy AK and **Navi SS**. 2000. Biology, epidemiology and management of sorghum grain mold. Pages 34–71 *in* Technical and institutional options for sorghum grain mold management: proceedings of an international consultation, 18-19 May 2000 (Chandrashekar A, Bandyopadhyay R and Hall AJ, eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics (ICRISAT).

Leslie JF, Zeller KA, Lamprecht SC, Rheeder JP and **Marasas WFO.** 2005. Toxicity, pathogenicity, and genetic differentiation of five species of Fusarium from sorghum and millet. Phytopathology 95:275–283.

Navi SS, Bandyopadhyay R, Reddy RK, Thakur RP and **Yang XB.** 2005. Effects of wetness duration and grain development stages on sorghum grain mold infection. Plant Disease 89:872-878.

Reddy BVS, Sharma HC, Thakur RP and **Ramesh S.** 2006. Characterization of ICRISAT-bred sorghum hybrid parents (Set 1). International Sorghum and Millets Newsletter (Special Issue), (Thakur RP, ed.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics. 47:1-5.

Thakur RP, Reddy BVS, Indira S, Rao VP, Navi SS, Yang XB and **Ramesh S.** 2006. Sorghum Grain Mold. Information Bulletin No. 72. Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics. 32 pp.

Williams RJ and **McDonald D.** 1983. Grain molds in the tropics: problems and importance. Annual Review of Phytopathology 21:153–178.

Anthracnose

RP Thakur and Kusum Mathur

Introduction

Anthracnose is an important disease in major sorghum growing areas of the world. The disease is more prevalent and severe in warm and humid environments where it causes substantial economic losses. Anthracnose appears on several plant parts. The pathogen causes seedling blight, leaf blight, stalk rot, head blight and grain molding, and thus limits both grain and forage production. Among these, foliar anthracnose is the most pronounced and devastating on the forage and grain sorghum cultivars. Yield losses of 50% or more can occur under severe conditions. Use of disease resistant cultivars, as a strategy to control this disease, has often met limited success because of the large genetic variation in the pathogen population that renders the resistance ineffective in a short period. However, with the continuing research efforts, a number of resistance sources have been identified and some of these have been used to produce anthracnose resistant commercial hybrids and varieties in several countries.

Pathogen

Colletotrichum graminicola (Ces.) Wilson (syn. C. *sublineolum* Henn. Kabat & Bub.) is the causal agent of anthracnose in sorghum. Conidia are produced terminally and singly on conidiophores among setae and occur as masses immersed in the mucilaginous substrate. They are hyaline, nonseptate, uninucleate and sickle- or spindle-shaped, measuring 4.9-5.2×26.1-38.8 μm. Acervuli produced on the infected host tissue are with or without setae, pink or dark brown and oval to cylindrical. Setae in an acervuli are long (up to 100 μm), dark and prominent and are intermixed with conidia and conidiophores.

More than 40 races/pathotypes have been reported from different geographical areas of the world, using different sets of putative host differentials. Since the first report of existence of races in C. *graminicola* in the United States in 1967, some 11 races/pathotypes have been

reported from the US and Puerto-Rico; 12 from Brazil; 9 from India; 15 from Nigeria and 2 from western Africa. However, the actual number of races/pathotypes and their genetic variability at the global level are uncertain, as no clear gene-for-gene relationship has been established for any host-isolate systems.

Symptoms

The foliar symptoms of anthracnose appear 30-40 days after emergence. Typical symptoms are small, circular, elliptical or elongated spots usually about 5mm in diameter (Fig. 5). These spots develop gray to straw-colored centers with wide margins that may be tan, red, or blackish purple depending on the cultivar and the pathogen population. Under conditions of high humidity, the spots increase in number and coalesce to cover larger leaf area. On the centers of the spots few to numerous small, circular, erumpent black dots develop; these are acervuli of the fungus. Under magnification, black hair-like structures called setae protruding from the acervuli are seen. In some cultivars that develop low levels of foliar infection, midrib infection occurs more prominently (Fig. 5). Elliptical to elongated lesions of straw color, with red purple or black embedded acervuli are seen on the midrib. The pathogen also infects leaf sheath and panicles including grains and rachis (Fig. 6). Variation in foliage and grain symptoms may be due to host reaction, host physiological status, the prevailing weather conditions and pathogenic variability.

Fig. 5. Anthracnose symptoms: chlorotic flecks, lesions with acervuli and lesions on midrib

Fig. 6. Anthracnose symptoms on leaf sheath and developing grains

Epidemiology

The fungus survives as mycelium and sporogenic sclerotia in the crop debris for up to 18 months on the soil surface. Thus, sclerotia in debris on the soil surface may act as a primary source of inoculum. However, not all isolates of C. *graminicola* produce sclerotia. Conidia germinate on the leaf surface in thin films of water, develop germ tubes and appresoria, and penetrate the epidermis directly or through stomata. Infection of seedling occurs from the inoculum in the crop residue and is enhanced by rain splash and contact of foliage with the soil. Severe infection and disease development occur during prolonged periods of cloudy, warm, humid and wet weather. Sorghum plants are more vulnerable to infection from flowering through the grain development phase.

Field screening technique

Inoculum preparation

- Soak grains of a sorghum line overnight and drain the excess water in the morning.

- Place 80 g of soaked grain in 250 ml conical flasks and plug tightly, cover the plug with a paper secured with rubber band, or aluminum foil.
- Autoclave the grains in the conical flasks at 121°C and 15 lbs psi for 1 h, and allow to cool.
- Inoculate the autoclaved grains with an actively growing pure culture of the local isolate of C. *graminicola* and incubate at 28±1°C for 10 days under 12-h photoperiod.
- Shake the flasks every alternate day to promote uniform growth and development of the fungus on the grains.

Field planting and inoculation

- Plant a known susceptible line, such as IS 3089 or IS 18442 in every 9th row in a field as infector rows or inoculum donor to test rows.
- Plant test entries/lines in between the infector rows 20 days later when foliar symptoms are prominently visible in the infector rows.
- Take out grain inoculum prepared above in trays and air-dry for 30 min.
- Place 2-3 infested grains per plant in whorls of 21-day old plants (Fig. 7).
- Inoculation should preferably be done in the evening hours, followed by light sprinkler irrigation to provide adequate moisture for infection.

Fig. 7. Field screening for anthracnose by whorl inoculation and symptoms on foliage

- Provide sprinkler irrigation twice a day 30 min each during morning and evening on dry days.
- Tag 10 plants/entry/replicate for recording the disease reactions.

Record disease reaction type (HR, R, MR, S or HS) and disease severity (based on 1 to 5, or 1 to 9 scale) as given below, at the soft-dough stage on the top four leaves that contribute most to grain development. [*For a reliable screening, the disease scores in susceptible check line should be ≥ 4.0 on the 1 to 5 scale and ≥ 7.0 on the 1 to 9 scale.]*

- Analyze the data for reaction type and disease severity for each line.

Anthracnose severity rating scale: 1 to 5

Severity rating	Symptom and lesion type (on top four leaves)	Reaction type
1	No visible symptoms, presence of chlorotic flecks	Highly resistant (HR)
2	1-10% leaf area covered with hypersensitive lesions without acervuli	Resistant (R)
3	11−25% leaf area covered with hypersensitive and restricted lesions without acervuli	Moderately resistant (MR)
4	26−50% leaf area covered with coalescing necrotic lesions with acervuli	Susceptible (S)
5	>50% leaf area covered with coalescing necrotic lesions with acervuli	Highly susceptible (HS)

Anthracnose severity rating scale: 1 to 9

Severity rating	Symptom and lesion type (on top four leaves)	Disease reaction class
1	0 to <1% leaf area covered with hypersensitive reaction with mild yellow flecks	HR
2	1-5% leaf area covered with hypersensitive lesions without acervuli	R
3	6-10% leaf area covered with hypersensitive lesions without acervuli	
4	11-20% leaf area covered with hypersensitive and restricted necrotic lesions with acervuli	MR
5	21-30% leaf area covered with hypersensitive and restricted necrotic lesions with acervuli	
6	31-40% leaf area covered with coalescing necrotic lesions with acervuli	S
7	41-50% leaf area covered with coalescing necrotic lesions with acervuli	
8	51-75% leaf area covered with coalescing necrotic lesions with acervuli	HS
9	76-100% leaf area covered with coalescing necrotic lesions with acervuli	

The 1 to 9 rating scale is useful for differentiating between lines with minor differences in resistance.

Greenhouse screening technique

Inoculum preparation

- Grow the fungal isolates in 0.1% oatmeal broth in a rotary shaker (25°C, 125 rpm, with cool fluorescent light) for 10 days.
- Separate conidia from mycelial lumps and medium by filtering the cultures through double-layered muslin cloth, and adjust

spore concentration (1×10^5 conidia mL^{-1}) with the help of haemocytometer.

- Add two drops of Tween-20™ to 100 mL inoculum just before inoculation.

Growing plants and inoculation

- Sow surface-sterilized seed of test lines and appropriate check lines in autoclaved potting mix of soil, sand and FYM (2:1:1 by volume) in 30-cm dia plastic pots outdoor.
- Thin the seedlings two weeks after emergence to maintain 5 plants per pot, and maintain at least 15 plants for each line at 5 plants/pot/replicate for a replicated experiment.
- Transfer the pots in the greenhouse (25±2°C) when the plants are 2-week old.
- Spray-inoculate 21-day-old plants with the inoculum using a hand-held atomizer. Allow the inoculated plants to dry for a while and then transfer these to a humidity chamber (25°C, RH >95%) for 24 h.
- Transfer the plants to greenhouse (25°C, RH >90%) benches and arrange the pots in a complete randomized design, and provide the mist to maintain high humidity.
- Record data on latent period (time in days for the appearance of first chlorotic/necrotic lesion) starting 4th day after inoculation on each test line.
- Record data for disease reaction types (HR, R, MR, S or HS) and disease severity (on a 1 to 5 or a 1 to 9 scale) as described above, 14 days after inoculation.

  [*For a reliable screening, the disease scores in susceptible check line should be ≥ 4.0 on the 1 to 5 scale and ≥ 7.0 on the 1 to 9 scale.]*

Resistance sources and their utilization

Several sorghum lines have been identified as highly to moderately resistant. Some of the lines with stable resistance are: IS 3547, IS 6958,

IS 6928, IS 8283, IS 9146, IS 9249, IS 18758, M 35610, A 2267-2, SPV 386 and ICSV 247. At ICRISAT Patancheru, in a trait-specific breeding program, some of these lines with white-grain have been used to develop resistant lines and hybrid parents. Some tolerant hybrid seed parents, such as ICSA/B 260 to ICSA/B 295 are available at ICRISAT. The breeding program of William Rooney and colleagues at Texas A&M University has identified several potential sources of genetic resistance to *Colletotrichum* isolates prevalent in Texas, USA.

Selected references

Casela CR and **Frederiksen RA.** 1993. Survival of *Colletotrichum graminicola* sclerotia in sorghum stalk residues. Plant Disease 77:825-827.

Mathur K, Thakur RP and **Rao VP.** 2000. Pathogenic variability and vegetative compatibility among isolates of *Colletotrichum graminicola* and C. *gloeosporiodes* causing foliar and grain anthracnose in sorghum. Indian Phytopathology 53: 407-414.

Mathur K, Thakur RP, Neya A, Marley PS and **Casela CR.** 2002. Sorghum anthracnose -problem and management strategy. Pages 211-220 *in* Sorghum and Millets Diseases (Leslie JF, ed.). Ames, Iowa, USA: Iowa State Press.

Marley PS, Thakur RP and **Ajayi O.** 2001. Variation among foliar isolates of *Colletotrichum graminicola* of sorghum in Nigeria. Field Crops Research 69:133-142.

Ngugi HK, Julian AM, King SB and **Peacocke BJ.** 2000. Epidemiology of sorghum anthracnose (*Colletotrichum sublineolum*) and leaf blight (*Exserohilum turcicum*) in Kenya. Plant Pathology 49:129-140.

Pande S, Mughogho LK, Bandyopadhyay RB and **Karunakar RI.** 1991. Variation in pathogenicity and cultural characteristics of sorghum isolates of *Colletotrichum graminicola* in India. Plant Disease 75:778-783.

Pande S, Thakur RP, Karunakar RI, Bandyopadhyay R and **Reddy BVS.** 1994. Development of screening methods and identification of stable resistance to anthracnose in sorghum. Field Crops Research 38:157-166.

Reddy BVS, Sharma HC, Thakur RP and **Ramesh S.** 2006. Characterization of ICRISAT-bred sorghum hybrid parents (Set 1). International Sorghum and Millets Newsletter (Special Issue), (Thakur RP, ed.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics. 47:1-5.

Rosewich UL, Pettway RE, McDonald BA, Duncan RR and **Frederiksen RA.** 1998. Genetic structures and temporal dynamics of a C*olletotrichum graminicola* population in a sorghum disease nursery. Phytopathology 88:1087-1093.

Thakur RP and **Mathur K.** 2000. Anthracnose. Pages 10-12 *in* Compendium of Sorghum Diseases, Second Edition (Frederiksen RA and Odvody GN, eds.)., American Phytopathological Society, St. Paul, Minnesota 55121-2097, USA: APS Press.

Thakur RP, Rao VP, Wu BM, Subbarao KV, Mathur K, Tailor HC, Kushwaha US, Dwivedi RR, Krishnaswamy R, Hiremath RV and **Indira S.** 2007. Genetic resistance to foliar anthracnose in sorghum and pathogenic variability in C*olletotrichum graminicola*. Indian Phytopathology 60:13-23.

Thomas MD, Sissoko I and **Sacko M.** 1996. Development of leaf anthracnose and its effect on yield and grain weight of sorghum in West Africa. Plant Disease 80:151-153.

Leaf Blight

Kusum Mathur, RP Thakur and Belum VS Reddy

Introduction

Leaf blight is an economically important and widespread disease of sorghum in highly humid areas of Asia, Africa and the Americas. When infection occurs at preflowering stage in susceptible cultivars, grain yield losses of up to 50% may occur. However, when infection occurs at flowering or later stages, disease development is slower and yield losses are minimal. Leaf blight infected plants may succumb to stalk rot pathogens as well. The disease is considered more important in dual purpose and fodder sorghums than in grain sorghum under Indian conditions.

Pathogen

Exserohilum turcicum (Pass.) KJ Leonard & EG Suggs (syns. *Helminthosporium turcicum* Pass; *Bipolaris turcica* (Pass.) Shoemaker; and *Dreschslera turcica* (Pass.) Subramanian & PC Jain) causes leaf blight in sorghum. Conidia are light gray, straight or spindle-shaped or curved with rounded ends. They are borne singly on the tips of conidiophores. They are three- to eight-septate and have a protruding basal hilum, and measure 10-20×28-153 μm. They germinate by polar germ tubes. The pathogen can also infect maize, Johnsongrass, teosinte and Sudangrass. However, there are some host-specific races/pathotypes known that do not infect other crop species. So far, there are no reports on existence of different races of *E. turcicum* from sorghum.

Symptoms

Symptoms are visible from the seedling stage to the crop maturity stage. Small, reddish or tan spots develop on seedlings, the spots later enlarge and coalesce resulting in wilting of young leaves. On mature plants, long, elliptical, reddish purple or yellowish lesions develop, first on lower leaves and later progress to the upper leaves and stem as well (Fig. 8). Lesion size varies depending on resistance levels of host genotypes, virulence

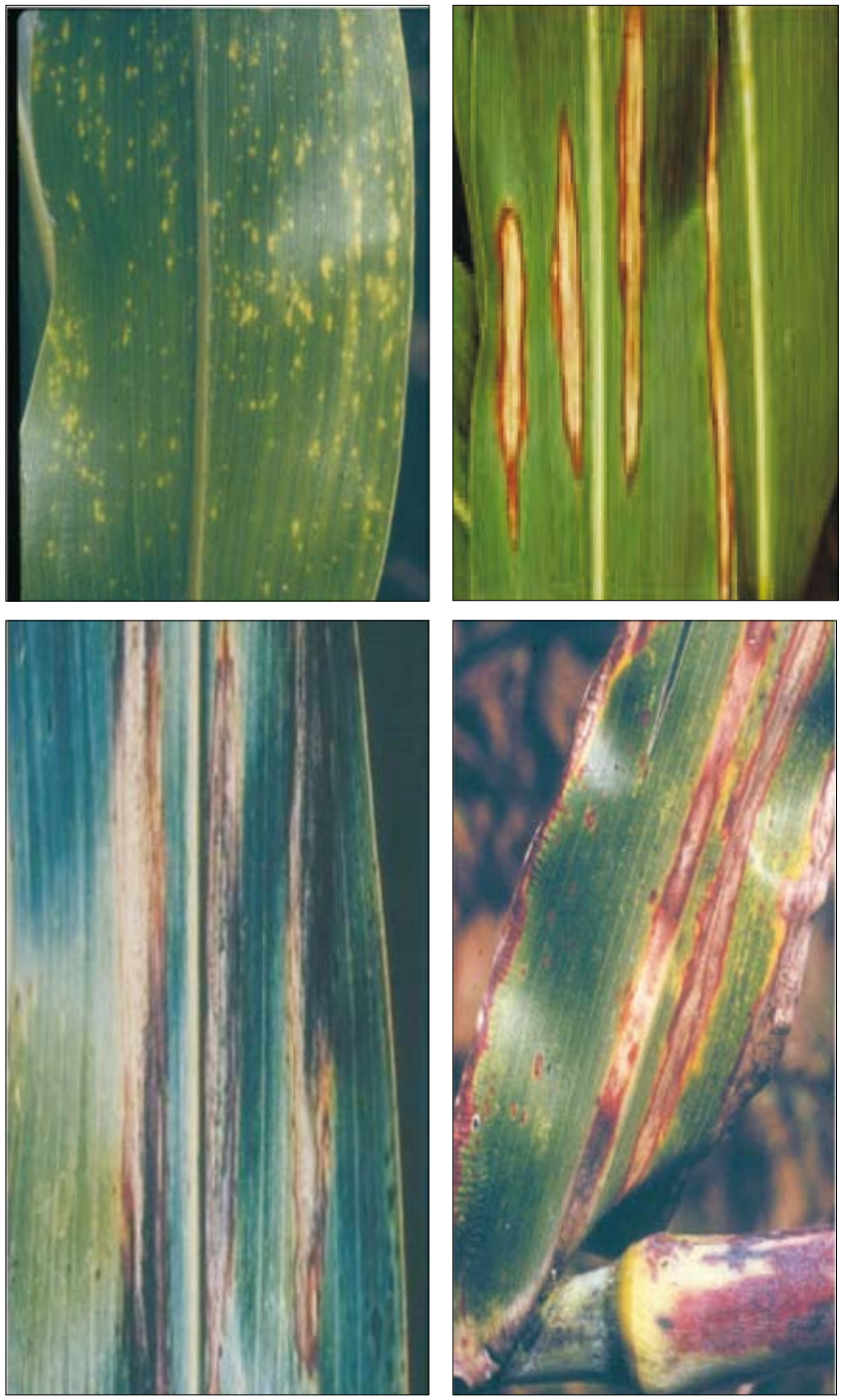


Fig. 8. Leaf blight symptoms: chlorotic flecks, enlarged lesions and sporulating lesions on foliage and on stem

levels of pathogen isolates and prevailing weather conditions. In humid weather, numerous grayish black spores are produced in the lesions in concentric zones. Unlike in anthracnose, grain is not infected, but the damage to green foliage results in grain yield loss.

Epidemiology

The pathogen survives in the field in crop debris as mycelia and conidia in the tropics and as chlamydospores in temperate climate. Both conidia and chlamydospores are airborne and can be transmitted to long distances. Infection and disease development is favored by moderate temperatures (18-25°C) and high humidity or heavy dew. Airborne conidia help in secondary spread of the disease. In addition to high humidity, temperature is the most critical weather variable affecting disease severity level after dough stage of the crop. Minimum temperatures between 14 and 16°C and mean temperatures of 20-22°C are most favorable for disease development.

Field screening technique

Inoculum preparation

- Soak grains of any sorghum line overnight and drain the excess water in the morning.
- Place 80 g of soaked grain in a 250-ml conical flask and plug tightly, cover the plug with a paper secured with rubber band, or aluminum foil.
- Autoclave the grains in the conical flask at 121°C and 15 lbs psi for 1 h and allow to cool.
- Inoculate the autoclaved grains with an actively growing pure culture of the local isolate of *E. turcicum* and incubate at 25±1°C for 10 days under 12-h photoperiod.

- Shake the flask every alternate day to promote uniform growth and development of the fungus on the grains.

Field planting and inoculation

- Plant known susceptible line, such as IS 18442 in every 9th row in a field as infector rows or inoculum donor to test rows.

  [*As the disease development is favored by moderate temperature, delayed planting during late August/early September would be more favorable for disease development than the normal June/July planting under Indian conditions.*]

- Inoculate the infector row plants by placing *E. turcicum* infested sorghum grains in the whorl (2-3 grains/plant) at 30 days after emergence and provide high humidity by sprinkler irrigation.
- Plant test entries 20 days later in between the infector rows.
- Inoculate the plants in test rows by infested sorghum grains 30 days after emergence as done for the infector rows.
- Inoculation should preferably be done in the evening hours, followed by light sprinkler irrigation to provide adequate moisture for infection.
- Tag 10 plants/entry/replicate for recording the disease reactions.
- Record disease reaction (based on R, MR and S) and disease severity (based on 1 to 5 or 1 to 9 scale) at soft-dough stage. Normally the top four leaves that contribute most to grain development should be scored.

  [*For a reliable screening, the disease scores in susceptible check line should be ≥ 4.0 on the 1 to 5 scale and ≥ 7.0 on the 1 to 9 scale.]*

Leaf blight severity rating scale: 1 to 5

Severity rating	Symptom and lesion types (on top four leaves)	Reaction type
1	No visible symptoms/chlorotic flecks	Highly resistant (HR)
2	Up to 10% leaf area covered with small restricted lesions	Resistant (R)
3	11–25% leaf area covered with small restricted lesions	Moderately resistant (MR)
4	26–50% leaf area covered with large coalescing lesions	Susceptible (S)
5	>50% leaf area covered with large coalescing lesions	Highly susceptible (HS)

Leaf blight severity rating scale: 1 to 9

Severity rating	Symptom and lesion types (on top four leaves)	Disease reaction class
1	0 to <1% leaf area with mild yellow flecks	HR
2	1-5% leaf area covered with hypersensitive small lesions	R
3	6-10% leaf area covered with hypersensitive small lesions	
4	11-20% leaf area covered with small necrotic lesions	MR
5	21-30% area covered with small necrotic coalescing lesions	
6	31-40% area covered with large coalescing necrotic lesions	S
7	41-50% leaf area covered with large coalescing necrotic lesions	
8	51-75% leaf area covered with large coalescing necrotic lesions	HS
9	76-100% leaf area covered with large coalescing necrotic lesions	

The 1 to 9 rating scale is useful for differentiating between lines with minor differences in resistance.

Greenhouse screening technique

Inoculum preparation

- Grow the fungal isolate in 0.1% oatmeal broth in a rotary shaker (25°C, 125 rpm, with cool fluorescent light) for 10 days.
- Separate conidia from mycelial lumps and medium by filtering the cultures through double-layered muslin cloth, and adjust spore concentration (1×10^5 conidia mL^{-1}) with the help of haemocytometer.
- Add two drops of Tween-20™ to 100 mL inoculum just before inoculation.

Inoculation

- Spray-inoculate 21-day old pot-grown plants with the above inoculum using a hand-held atomizer. Air-dry the inoculated plants and transfer these to a humidity chamber (25°C, RH>95%) for 24 h.
- Maintain 5 plants/pot/replicate with 3 replications for each line.
- Transfer the plants to greenhouse (25°C, RH>90%) benches and arrange the pots in a complete randomized design, and provide the mist to maintain high humidity.
- Record data on latent period (time in days for the appearance of first chlorotic/necrotic lesion) starting the 4^{th} day after inoculation on each test line.
- Record data for disease reaction types (HR, R, MR, S or HS) and disease severity (on 1 to 5 or 1 to 9 scale) as described above, 14 days after inoculation.
- Compute the data for severity and disease reaction types for each line.
- Identify resistant lines and confirm their resistance by further screening and utilization.

Resistance sources and their utilization

Several sorghum germplasm accessions with high to moderate levels of resistance have been identified. Some of these are IS 13868, IS 13869, IS 13870, IS 13872, IS 18729, IS 18758, IS 19669 and IS 19670. These were used in crosses with agronomically desirable breeding lines and several female parents were developed in a trait-specific breeding program at ICRISAT. Some leaf blight tolerant hybrid seed parents, such as ICSA/B 296 to ICSA/B 328 were developed during 1989 to 1998 and are available at ICRISAT, Patancheru.

Selected References

Bergquist RR. 2000. Leaf blight, pages 9-10 *in* Compendium of Sorghum Diseases, Second Edition (Frederiksen RA and Odvody GN, eds.). American Phytopathological Society, St. Paul, Minnesota 55121-2097, USA: APS Press.

Reddy BVS, Sharma HC, Thakur RP and **Ramesh S.** 2006. Characterization of ICRISAT-bred sorghum hybrid parents (Set 1). International Sorghum and Millets Newsletter (Special Issue), (Thakur RP, ed.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics. 47:1-5.

Downy Mildew

RP Thakur, VP Rao and P Sanjana Reddy

Introduction

Sorghum downy mildew is economically important and widespread in many tropical and subtropical regions of the world where sorghum and maize are grown. Several epidemics of sorghum downy mildew have been recorded in many countries both on sorghum and maize. The disease is highly destructive due to its systemic nature of infection resulting in death of plants or lack of panicle initiation. A linear relationship between disease incidence and yield loss has been reported in several cases.

Pathogen

Peronosclerospora sorghi [Weston and Uppal (Shaw)] infects both sorghum and maize. In sorghum, the fungus reproduces both by sexual and asexual means. Conidia, the asexual spores, are produced on the under surface of infected leaf and are borne on conidiophores protruding through stomata. They are hyaline, obovate, or sub-orbicular with thin wall and germinate directly by producing germ tube. Oospores, the sexual spores, are produced in the mesophyll between the fibrovascular bundles. They are spherical, hyaline to yellow and enclosed in irregular thick oogonial wall, and germinate by producing nonseptate, branched, and hyaline germ tube.

Symptoms

Both systemic and local infections occur. Systemic infection occurs when the young meristematic tissues of the growing seedlings are infected and the symptoms appear as chlorotic foliage and stunted, often resulting in death of seedlings. The first infected leaf shows chlorosis on the lower part of the lamina, which further grows to cover larger part of the leaf (Fig. 9). The other leaves on a plant that get infected subsequently show more chlorosis. Under cool and humid weather conditions, the abaxial surface of chlorotic leaves produce abundant spores (conidia) that appear

Fig. 9. Various stages of sorghum downy mildew symptoms

as white, downy growth. As the plant grows, new emerging leaves exhibit parallel stripes of green and white tissue; the white interveinal tissue dies and leaf shredding occurs. These shredded tissues usually contain numerous oospores that develop as a result of mating of compatible thalli.

The local lesions on foliage are the result of infection by conidia. These appear as stippled, necrotic lesions on leaf blades. The fungus produces white downy growth of conidiophores and conidia on these lesions. Conidia from these lesions get dispersed through wind currents to neighboring plants and cause disease. The local lesions become systemic when the conidia from these lesions infect meristematic tissues.

Epidemiology

Initial infection is caused by the oospores in the soil that germinate and invade the roots of sorghum seedlings. The pathogen moves upward systemically within the seedling, colonizes the meristematic tissues and induces leaf chlorosis. Conidia (15-27×15-29 μm) produced on such chlorotic leaves are air-dispersed to cause secondary infection to adjacent seedlings. Oospores (25-42 μm in diameter) formed in the diseased plants infest the soil and survive as resting spores during the fallow period. Other hosts, such as Sudangrass and perennial *Sorghum helpense* serve as source of conidial inoculum for the next cropping season. Low soil moisture and cool temperature (10°C) favor infection by oospores. High humidity and cool environment (15-20°C) favor conidial production, infection and disease development. The pathogen survives as oospores in the seed glumes and as plant debris mixed with the seed. Several physiological races of the pathogen have been reported.

Field screening

Establishment of a downy mildew nursery - sick plot and infector-row system

- Mix the downy mildew-infected leaf tissue (containing oospores) in the soil and plant the known susceptible entries to indicate the disease pressure. The process can be repeated for 2-3 seasons to get up to 50% or more infection in a highly susceptible line.
- Plant a known susceptible cultivar/line as infector/spreader rows on every 9th row.
- Inoculate the infector rows at the 1st leaf stage with conidial inoculum, maintained in the greenhouse, during late evening.

  [*Infector rows can be established by inoculating the germinating seed using the sandwich method described below and transplanting the infected seedlings on rows in the field.*]
- Maintain high humidity (>90% RH) through furrow- or perfo-irrigation for 3 weeks. This is essential for infection and disease development.

- Thin the plants in the infector rows by leaving the infected plants for good growth of the plants in the infector rows.
- Plant the test entries 3 weeks later in between the infector rows when there is >70% incidence in the infector rows (Fig. 10).

Fig. 10. Field screening for sorghum downy mildew using infector-row system

- Plant a known susceptible line as indicator rows after every 10 or 20 rows along with the test entries to compare the distribution of disease level in the nursery.
- Continue perfo-irrigation as required for maintaining high humidity essential for infection and disease development for the next 2-3 weeks.
- Record the total and diseased plants at 15 days after emergence, pre-tillering stage (30 days after emergence) and at soft-dough stage (60 days after emergence), depending on the objective of the experiment.

- Calculate the percentage disease incidence based on total and diseased plants at soft-dough stage for each plot/replication and finally for each entry.

  [*The disease incidence data at the soft-dough stage should be considered final as some lines show susceptibility at later growth stages*.]

Greenhouse screening

Inoculum maintenance and multiplication

- Prepare the potting medium (a mix of black soil, sand and FYM @ 2:1:1 by volume) and autoclave at 121.6 °C for 1-h cycle, for 2 cycles with an interval of 24 h.
- Infest the pot soil filled with the above potting medium with leaf powder containing oospores of *P. sorghi* and sow the seed of a highly susceptible sorghum line (DMS 652, IS 643) in a greenhouse. Seedlings show systemic infection 7-10 days after emergence and sporulation occurs on the infected leaves. Conidia from such sporulating leaves can be used to inoculate fresh seedlings of a susceptible line.
- Spray-inoculate the fresh batch of pot-grown seedlings of a susceptible line with the above conidial suspension as described below, and maintain the conidial inoculum through serial inoculation after every 30-40 days.
- Multiply the inoculum of the downy mildew isolate by spray-inoculating the pot-grown seedlings of a susceptible line at the 1-2 leaf stage with the above conidial suspension.

Inoculum preparation, inoculation and data recording

- Fill the pots of 15-cm dia and sow the seed of test entries, 35-40 seed/pot with 3 replications to maintain about 100 seedlings/entry.
- Similarly sow the seed of known susceptible and resistant lines to serve as controls.
- Harvest the systemically infected, sporulating leaves from the susceptible line, wash them in running tap water and use a cotton-

swab to remove the old spores from the leaf surface, and air dry them for some time to remove the surface water.

- Cut the above leaves into pieces of 20-30 cm and keep them in moist chambers (plastic tray with its lid lined with double-layered wet blotting paper) upside down to facilitate the sporulation on the lower leaf surface.
- Incubate the leaves in moist chambers at 18-20°C for 6-7 h with >90% RH in the dark.
- Harvest the conidia in ice-cold water using a camel hairbrush to prevent their germination.
- Filter the conidial suspension through double-layered muslin cloth and adjust the conidial concentration to 1×10^5 conidia mL^{-1} using a haemocytometer.
- Spray-inoculate the pot-grown seedlings at the 1st leaf stage and incubate the seedlings at 20°C for 16-20 h under high humidity (95-100% RH). Count the total number of seedlings in each pot just before inoculation and note it down on the plastic label of each pot (Fig. 11). *This ensures the exact number of seedlings that were inoculated and discounts those that emerged after inoculation.*
- Transfer the pots to a greenhouse at 25±1°C for 2 weeks and provide high humidity using fogger.
- Count the infected seedlings after 2 weeks and note the number on the plastic label of the pot (just below the total number of seedlings).
- Compute the data and calculate the percentage disease incidence for each entry/line from the total and infected seedlings per pot.

Sandwich inoculation technique

For greenhouse screening, sandwich inoculation technique has also been used in cases where seed amount is limited and for grasses, such as Sudangrass where seed size is small and germination quite erratic. The steps involved are as follows.

- Geminate the seed in moist petridish chambers (petridish lined with wet blotting paper) for 24-30 h at 35°C.

Fig. 11. Greenhouse screening for sorghum downy mildew by spray (top) and sandwich (bottom) inoculations

- Place the washed infected sorghum leaf pieces (4-5 cm) in moist petridish chamber with abaxial surface facing upward.
- Transfer the sprouted seed on the leaf surface and cover with another layer of the infected leaf pieces with abaxial surfaces downward touching the seed, and cover with the moist upper lid (Fig. 11).

  [*This method exposes both radicle and plumule to conidial inoculum under congenial conditions of infection.*]

- Incubate the seeded plates at 20 °C for 24 h in the dark for infection of the germinating seeds.
- Transplant the inoculated germlings in pots (50 germlings/pot of 15-cm diameter filled with the soil-sand-FYM mix) in the greenhouse at 25±1°C
- Record the infected and total seedlings 14 days after transplanting to determine the incidence level.

The rest of the procedures are the same as described above for the greenhouse screening.

[*Greenhouse screening has several advantages over field screening as it is rapid, requires less space, is not season dependant and a large number of lines can be screened every year. In addition, it is more accurate and precise. The greenhouse screening is particularly important when screening sorghum lines for different pathotypes/races under controlled conditions.*]

Resistance sources and their utilization

A number of sorghum lines and varieties have been identified having good levels of resistance in different parts of the world and these are being utilized in resistance breeding program. Some of the highly resistant lines/varieties are: QL 3, IS 3443, IS 8283, IS 1331, IS 2474, IS 3547, IS 5743, IS 7179, IS 8185, IS 8276, IS 8607, IS 8864, IS 8906, IS 8954, IS 22228, IS 22229, and IS 22230. In a multilocation test three accessions, IS 3547, IS 18757 and IS 8283 were completely free from downy mildew across several locations in India, Argentina, Brazil and Nigeria over several years of testing.

Some downy mildew tolerant hybrid seed parents, such as ICSA/B 201 to ICSA/B 259 were developed in a trait-specific breeding program at ICRISAT and these have recently been characterized.

Selected references

Bock CH and **Jeger MJ**. 1996. Downy mildew of sorghum. International Sorghum and Millets Newsletter 37:33-51.

Narayana YD, Mughogho LK and **Bandyopadhyay R.** 1995. Evaluation of greenhouse inoculation techniques to screen sorghum for resistance to downy mildew. Euphytica 86:49–53.

Pande S, Bock CH, Bandyopadhyay R, Narayana YD, Reddy BVS, Lenné JM and **Jeger MJ.** 1997. Downy mildew of sorghum. Information Bulletin no. 51. Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics. 32pp.

Reddy BVS, Sharma HC, Thakur RP and **Ramesh S.** 2006. Characterization of ICRISAT-bred sorghum hybrid parents (Set 1). International Sorghum and Millets Newsletter (Special Issue), (Thakur RP, ed.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics. 47:1-5.

Charcoal/Stalk Rot

IK Das, SV Rao and P Sanjana Reddy

Introduction

Charcoal/stalk rot of sorghum is a major disease in dry regions of Asia, Africa, Americas and Australia. The disease is relatively more severe and destructive on high yielding sorghum cultivars when grain filling coincides with low soil moisture in hot dry weather. In India, the postrainy (*Rabi*) sorghums that are generally grown on residual soil moisture often get exposed to soil moisture stress during the grain filling stage if there are no rains. Dry weather conditions during this time may further increase the moisture loss from the soil. Under such a situation, plants are severely stressed due to increased senescence in root and stem cells that adversely affects the production and translocation of carbohydrates in the plant parts. Depending upon the cultivars, weather conditions and disease severity, yield losses vary substantially. The disease reduces grain yield and stover quality. Loss in grain yield is mainly due to lodging of the crop and loss in stover quality (and yield) is due to rotting and decaying of the stalk.

Pathogen

Charcoal rot is caused by the soil-borne fungus *Macrophomina phaseolina* (Tassi) Goid. It belongs to Deuteromycotina and its perfect state is called *Sclerotium bataticola* Taub. The mycelium is aerial, superficial or immersed, hyaline to brown, septate, profusely branched or dendroid. The fungus is highly variable in the size of sclerotia and presence or absence of pycnidia. Sclerotia are loose type, brown to black, irregular in shape and size, and are highly variable within an isolate. Pycnidia stage is widespread in jute and garden beans but uncommon in soybean, maize and sorghum. *M. phaseolina* is present in most cultivated soils and can infect about 500 plant species worldwide, including a wide range of agriculturally important crops.

Symptoms

Infected roots and lower stem of the infected plant show water-soaked lesions that slowly turn brown or black. Affected stalks become soft at the base and often lodge even due to moderate wind or by bending the plants. Thus pre-mature lodging is the most apparent symptom of charcoal rot (Fig. 12). When infected stalk is split open, the pith is found disintegrated across several nodes. The cortical tissues are disintegrated and vascular bundles get separated from one another. Numerous minute, dark, charcoal-colored sclerotia of the pathogen are formed on these vascular tubes (Fig. 13). Normally the disease appears during post-flowering stage, but in some cases seedlings can be infected.

Fig. 12. Field symptoms of charcoal rot showing lodging of plants

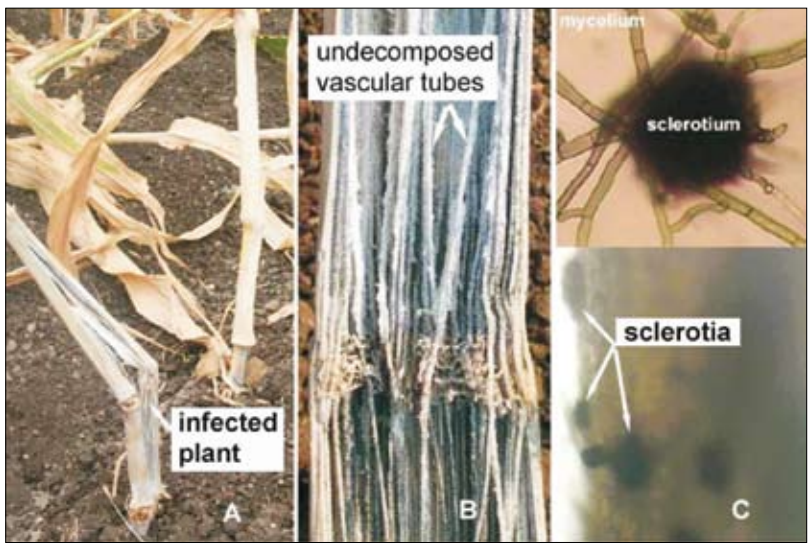


Fig. 13. Symptoms of charcoal/stalk rot showing tissue disintegration and presence of fungal propagules

Epidemiology

The pathogen is a soilborne fungus that survives as sclerotia on the infected plant debris. These sclerotia serve as primary source of inoculum, which can survive for 2-4 years in the soil. Dry weather, high temperature and low soil moisture are the important factors predisposing sorghum plants to infection by *M. phaseolina*. Infection occurs through damaged/injured roots and the fungal mycelium colonizes the xylem vessels blocking the translocation of water and carbohydrate to the upper plant parts. The prevalence of high soil temperature (35-38°C) and high soil moisture stress during the post flowering stage favor the disease development.

Under the condition of stress (moisture, temperature and photosynthesis) that often coincide with the onset of flowering, the host-defense-system is weakened and activity of *M. phaseolina* increases many folds leading to rapid and extensive rotting of roots and stalks that result in lodging of the crop. The pathogen produces at least six phytotoxins of which phaseolinone is the major product. Phytotoxins injure cell protoplast and often prime the infected plants towards more severe disease in later stages. High degree of variations in pathogenic properties in *M. phaseolina* and very strong relation of the disease with yield and environmental stresses, particularly moisture and temperature, makes the task of evaluating host resistance more challenging.

Field screening

Sick-plot method

- Grow test lines of similar maturity group in *Macrophomina* sick-plot (inoculum density of 100 – 150 microsclerotia g^{-1} soil) in a replicated trial. Include a susceptible check (repeated after every 20 lines) of similar maturity for comparing disease reaction.
- Maintain uniform plant spacing and induce uniform soil moisture stress after flowering by withholding irrigation and/or also by removing flag leaf.
- Record data on days to 50% flowering, plant stand and plant height at flowering.

- Record the number of charcoal rot infected and lodging plants, lesion length, number of nodes crossed by lesion, and grain yield at harvest (Fig. 14).
- Count the plants showing charcoal/stalk rot symptoms and calculate per cent incidence.
- Score individual plants, (at least 10/plot/replicate) for charcoal rot severity using a 1 to 5 scale, where:

  1 = one internode invaded, but rot does not pass through any nodal area;

  2 = two internodes;

  3 = three internodes;

  4 = more than three internodes; and

  5 = most internodes extensively invaded, shredding of stalk and death of plant.

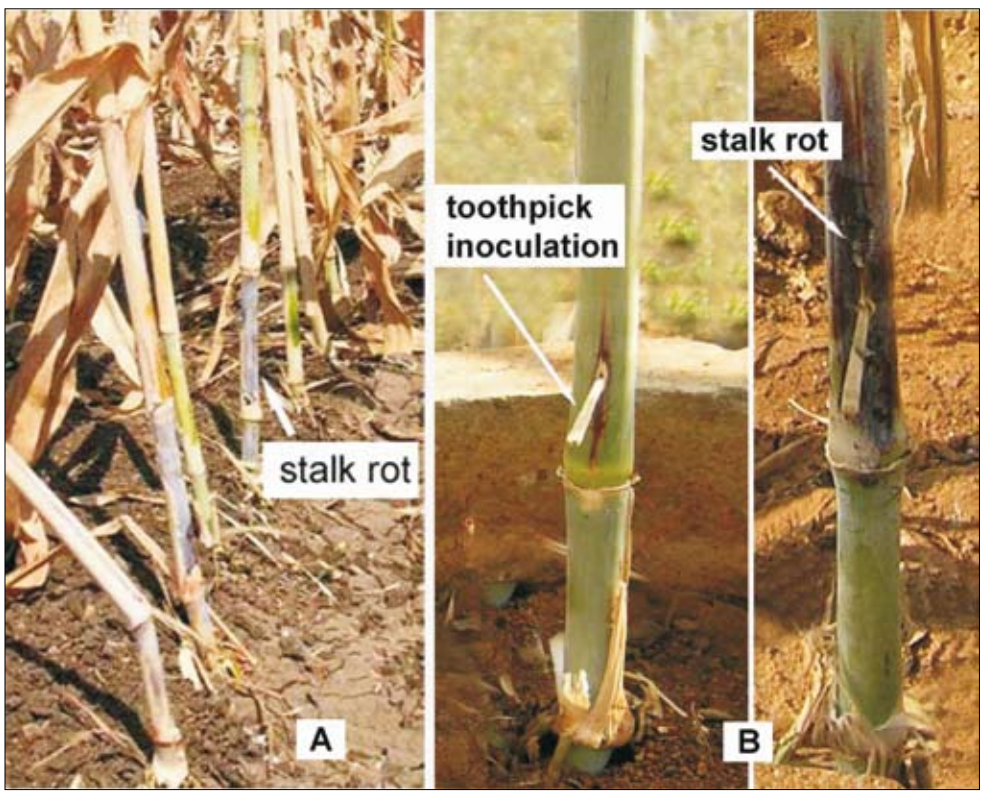


Fig. 14. Field screening for charcoal/stalk rot using sick plot and toothpick inoculation

- Compute the data for each line and compare charcoal rot incidence and severity of test lines with that of the known resistant and susceptible checks to identify resistant lines.

  [Note: *For reliable screening, the susceptible check should develop around 50% disease incidence. Disease escape due to non-uniform inoculum pressure in soil should not be confused with resistance.*]

Toothpick method

- Wash the toothpicks and air-dry. Tie these in bundles of 25-30 each, place these submerged in potato dextrose broth in screw capped glass bottles, and autoclave at 121°C and 15 lbs psi for 1 h and allow to cool.
- Inoculate the tooth picks with actively growing pure culture of the virulent local isolate of *M. phaseolina* and incubate at 25±1°C for 10 days.
- Insert toothpick infested with inoculum of *M. phaseolina* into the second internode of the stalk at 10-15 days after 50% flowering (Fig. 14).
- Inoculate 5-10 plants (uniform in growth, height, flowering and spacing) per row in each replication.
- Withhold irrigation at 50% flowering to ensure adequate soil moisture stress.
- After 25-35 days of inoculation, split open each stalk and observe for the presence of charcoal rot symptom.
- Count the plants showing symptom and score individual plants using 1-5 scale (as described above in the sick plot method).
- Compare the charcoal rot rating of test lines with that of the known resistant and susceptible checks to identify resistant lines.

  [*This method is very useful to study pathogenic variability among isolates of* M. phaseolina, *and can be used, with some modifications, to detect different levels of resistance in sorghum genotypes.*]

An *in vitro* screening technique to test sensitivity of seedling-tissues to phaseolinone, a predominant exotoxin produced by *M. phaseolina* in

cell-free culture filtrate (CFCF), can also be used, especially for preliminary screening of large number of breeding and germplasm lines in a short time. In this method, pre-determined concentrations of CFCF or the purified toxin, phaseolinone is used to screen 15-day-old seedlings of test lines along with resistant and susceptible checks. Susceptible seedlings show symptoms in 10 days and succumb. Resistance in test lines can be quantified in comparison with performance of check lines. The resistant lines can be tested in sick plot to validate the seedling performance.

Sources of resistance and their utilization

High level of genetic resistance is not available. However, sorghum genotypes that show stay-green trait (eg, E36-1 and B35) are generally tolerant to charcoal rot.

Some other lines, such as SLB 7, SLB 8, SLR 17, and SLR 35 are also reported to be tolerant to charcoal rot.

Drought tolerant, lodging resistant and nonsenescing sorghum genotypes are supposed to have good tolerance to charcoal rot. However, finding such genotypes with high grain yield under desirable agronomic background are often not easy.

Involving the stay-green trait sources in crosses with other high yielding lines, several improved hybrid parents were developed and some of these were characterized. Among the hybrid seed parents, ICSA/B 307, -351, -371, -373, -375, -376, -405, -589, -675, -678 and 702, and among male parents/varieties ICSV 21001 through 21025 are quite promising for stay-green trait.

Selected references

Karunakar RI, Pande S, Satyaprasad K and **Ramarao P.** 1993. Varietal reaction of nonsenescent sorghums to the pathogens causing root and stalk rot of sorghum in India. International Journal of Pest Management 39:343-346.

Mughogho LK and **Pande S**. 1984. Charcoal rot of sorghum. Pages 11-24 *in* Sorghum Root and Stalk rots – a Critical Review: Proceedings of the Consultative Group Discussion on Research Needs and Strategies for Control of Sorghum

Root and Stalk Rot Diseases, 27 November – 2 December 1983, Bellagio, Italy. Patancheru, AP 502 324, India: ICRISAT.

Rosenow DT. 1980. Stalk rot resistance breeding in Texas. Pages 306-314 *in* Sorghum Diseases, a world review: Proceedings of the International Workshop on Sorghum Diseases, 11-15 December 1978, Patancheru, Andhra Pradesh 502 324, India: ICRISAT.

Suchandra S, Mishra SK and **Siddiqui KAI.** 2000. Avirulent mutants of *Macrophomina phaseolina* and *Aspergillus fumigatus* initiate infection in *Phaseolus mungo* in the presence of phaseolinone; levamisole gives protection. Journal of Bioscience 25:73-80.

Pande S and **Karunakar RI.** 1992. Stalk Rots. Pages 219-234 *in* Sorghum and Millet Diseases: A Second World Review, (de Milliano WAJ, Frederiksen RA and Bengston GD, eds.). Patancheru, AP 502 324, India: International Crops Research Institute for the Semi-Arid Tropics.

Pande S, Mughogho LK and **Karunakar RI.** 1992. Incidence of charcoal rot in sorghum cultivars as affected by sowing date and plant density. Indian Journal of Plant Protection 20(2):162-170.

Pande S, Mughogho LK and **Karunakar RI.** 1994. Etiology of stalk rot and lodging in grain sorghum. International Journal of Tropical Plant Diseases and Review of Tropical Plant Pathology 12(2):117-137.

Pande S, Mughogho LK, Seetharama N and **Karunakar RI.** 1989. Effects of nitrogen, plant density, moisture stress and pathogen with *Macrophomina phaseolina* inoculation on charcoal rot development in grain sorghum. Phytopathologische Zeitschrift 126:343-352.

Reddy BVS, Sharma HC, Thakur RP and **Ramesh S.** 2006. Characterization of ICRISAT-bred sorghum hybrid parents (Set 1). International Sorghum and Millets Newsletter (Special Issue), (Thakur RP, ed.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics. 47:1-5.

Ergot

RP Thakur and VP Rao

Introduction

Ergot, also known as sugary disease, is widespread in most sorghum growing parts of the world. In Asia, sorghum ergot was first reported in 1915, in Kenya, Africa in 1925, in South America in 1995, in North America in 1996-97 and in Australia in 1996. The disease occurs due to infection of unfertilized ovaries in flowering sorghum panicles by the ergot pathogen, thus preventing fertilization and seed set. Rapid pollination and fertilization of ovaries prevents ergot infection in most sorghum lines. However, male-sterile lines (A-lines), which are used as female parents in hybrid seed production, lack fertile pollen and thus are more vulnerable to ergot infection than other lines, such as male-fertile lines. Also, the single-cross hybrids where pollination is delayed due to lack of pollen or pollen wash due to rains also get infected. Ergot is thus a major problem in hybrid seed production fields where A-lines are used as female parents. The disease can be highly damaging to sorghum hybrids when favorable weather conditions prevail at flowering.

Pathogen

The sorghum ergot pathogens are known by different names according to the symptoms they cause and the geographical regions where they were first detected. *Sphacelia sorghi* McRae is the causal agent of honeydew or sphacelial/conidial stage. After the sclerotial stage was discovered, the pathogens were identified as C*laviceps sorghi* P Kulkarni et al. in Asia, C. *africana* Frederickson, Mantle & de Milliano in Africa, and C. *sorghicola* Tsukiboshi, Shimanuki and Uematsu in Japan. Today, C. *africana* is the most widespread of the three C*laviceps* spp. and is found throughout Asia, Africa, the Americas, and Australia. These pathogens produce macroconidia and/or micro and/or secondary conidia. C. *africana* forms macroconidia, microcondia and secondary conidia. Macroconidia are oval to oblong (5-8×9-17 μm), slightly constricted at the center, and have two polar vacuoles; microconidia are spherical (2-3 μm dia); and

secondary conidia are borne on sterigmata like structures with a distinct protruding hilum. Sclerotia germinate to form stromata that appear as pale with globose proliferations with fully extended pigmented stipes; capitula are subglobose and internally purple and contain perithecia. Perithecia contain asci and each mature ascus produces eight filiform and hyaline ascospores of 1.2×45 μm size.

Claviceps sorghi, like C. *africana* also produces all three kinds of conidia. Macroconidia are hyaline, unicellular, elliptical to oblong (7.3×13.3 μm) with round ends; microconidia hyaline and round to obovate (3×4 μm); and secondary conidia pyriform (7.2×12.1 μm) and hyaline. Sclerotia germinate to produce 2-3 stromata with stipes, bronze to deep terracotta color, capitula buff colored and papillate, perithecia with ostioles; asci cylindrical with tapering ends, each producing eight filiform and hyaline ascospores.

Claviceps sorghicola produces only one type of conidia. These are small and oblong to elongate (3.7×8.2 μm). Sclerotia are long, straight to curved, purplish to black with longitudinal grooves. One to four stromata arise from each germinating sclerotium; stipes brown to bronze, capitula globose, dark brown and papillate; perithecia long and wide (200-300 ×105-140 μm), ovate to pyriform; asci long, cylindrical and hyaline with thick apical cap; each ascus produces eight filiform and hyaline ascospores.

Symptoms

The first symptom is exudation of honeydew droplets from the infected florets on sorghum panicles (Fig. 15). Depending on the weather conditions and host genotype, the honeydew color could be dirty white to brown and viscous. Under favorable conditions, honeydew exudation is profuse and the droplets drip down on foliage and on the ground appearing as whitish patches. These honeydew droplets contain numerous conidia, mostly macro- and microconidia that germinate to produce secondary conidia. The honeydew exudates impart a typical fermented smell that can be realized from a few meters away from the infected panicles.

The infected florets do not set seed and develop sclerotia in some florets (Fig. 15). These sclerotia are compact masses of whitish mycelium,

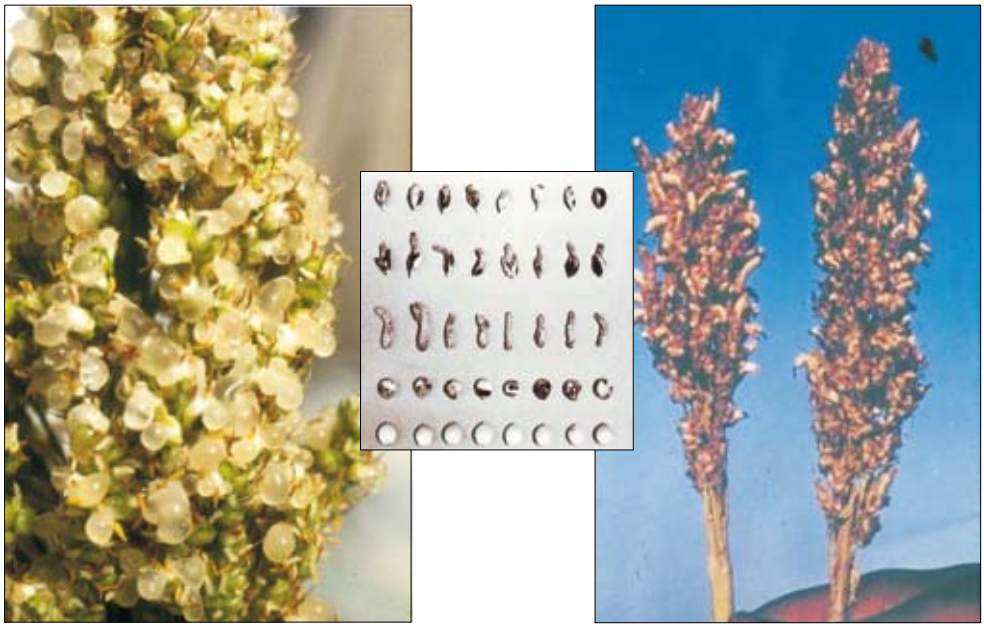


Fig.15. Symptoms of sorghum ergot: honeydew and sclerotia on panicles, and sorghum grains and sclerotia (inset)

which later turn brown and become hardened. These are elongated structures, larger than grain, protruding from the glumes. Sclerotial shape and size vary (large protruding from glumes to small concealed in glumes) depending on the host genotype, *Claviceps* species and weather conditions.

Epidemiology

Weather conditions that reduce pollen production and ovary fertilization increase the chance of ergot infection. Sorghum florets are vulnerable to infection from stigma exertion to ovary fertilization. Cool conditions (<15°C) and frequent rains favor ergot infection, disease development and spread within and between fields. Mean temperatures of 19-21°C and high relative humidity (95-100%) favor ergot development.

Sclerotia mixed with seed or present in soil serve as primary inoculum by releasing airborne ascospores or conidia contained on sclerotial grooves that land on stigmatic surface to initiate infection under favorable conditions. Honeydew produced on the infected florets contains numerous macroconidia and secondary conidia that help in secondary spread of the disease. Secondary conidia produced on collateral hosts or

from continually grown sorghum could also help in the establishment of the disease.

There is a strong interaction between pollen grains and ergot conidia on stigmatic surface of sorghum florets. Rapid pollination and fertilization prevents ergot infection and only unfertilized ovaries get infected by ergot conidia. Thus factors that promote pollination and fertilization prevent infection and the factors that delay anthesis, pollination and fertilization promote infection. Low night temperature (<10 °C) during flower initiation (2-3 weeks after emergence) to flowering reduces pollen viability and thus increases ergot infection. Male-sterile lines (lack of fertile pollen) are most susceptible while other lines that have reduced pollen production also get infected to varying degrees.

Field screening technique

Since ergot development is favored by cool weather during flowering, planting of sorghum lines for screening should be adjusted accordingly.

Inoculum preparation

Initial inoculum for the season is obtained from the infected panicles collected in the previous season and stored in a refrigerator. A conidial suspension is prepared by soaking and agitating the panicles in water or by suspending crushed sclerotia from the previous season in water (5 g sclerotia L^{-1} water) and filtering the suspension through a double-layered muslin cloth. This inoculum (ca 1×10^6 conidia mL^{-1}) is sprayed onto fresh stigmas of an early-flowering known susceptible line (using the technique described below), and the conidia formed in the honeydew are suspended in water for subsequent inoculations.

Inoculation and evaluation

An effective field screening technique developed for pearl millet ergot (C*laviceps fusiformis* Loveless) at ICRISAT, Patancheru has been widely adopted for sorghum ergot as well with some refinement and this is being used in resistance breeding programs. The steps involved are described below.

- Bag panicles as they emerge from the boot-leaf with selfing bags to allow proper stigma emergence and avoid cross-pollination.
- Remove bag (generally after 3-4 days) briefly and spray-inoculate the panicle at the anthesis initiation stage (visible fresh stigma stage) with an aqueous conidial suspension (1×10^6 conidia mL^{-1}) produced from honeydew of infected panicles, and replace the bag immediately.
- Inoculate a minimum of 10 panicles/entry/replicate.
- Provide high humidity (>90% RH) with over-head sprinklers twice a day for 30 min each at noon and evening on rain-free days until bags are removed 10-15 days after inoculation.
- Score each panicle 15-20 days after inoculation for ergot severity as percentage of florets infected (0 to 100% scale). Most infected florets produce honeydew and not sclerotia as in pearl millet ergot.
- Select individual panicles that have adequate selfed-seed set and little or no ergot for further evaluation.
- Calculate the mean severity percentage over 10 panicles for each entry and compare their resistance level for selection and utilization.

Sources of resistance and their utilization

Germplasm accessions IS 8525, IS 14131 and IS 14257 have been reported to have high level of resistance to ergot and these have been used in resistance breeding program. Some tolerant advanced breeding lines, such as ICSB 70, ICSB 84 and ICSB 102; ICSR 22, ICSR 54, ICSR 117 and ICSR 160 have been developed at ICRISAT.

Ergot being more a localized problem particularly in seed production plots, not much breeding efforts have been made to develop resistant hybrid parental lines and varieties.

Selected References

Bandyopadhyay R, Frederickson DE, McLaren NW and **Odvody GN.** 1996. Ergot – a global threat to sorghum. International Sorghum and Millets Newsletter 37:1-32.

Bandyopadhyay R, Frederickson DE, McLaren NW, Odvody GN and **Ryley MJ.** 1998. Ergot: A new disease threat to sorghum in the Americas and Australia. Plant Disease 82:356-357.

Tegegne G, Bandyopadhyay R, Mulatu T and **Kebede Y.** 1994. Screening for ergot resistance in sorghum. Plant Disease 78:873–876.

Thakur RP and **King SB.** 1988. Ergot disease of pearl millet. Information Bulletin no. 24. Patancheru, Andhra Pradesh 502 324, India: International Crops Research Institute for the Semi-Arid Tropics. 24pp.

Reddy BVS, Sharma HC, Thakur RP and **Ramesh S.** 2006. Characterization of ICRISAT-bred sorghum hybrid parents (Set 1). International Sorghum and Millets Newsletter (Special Issue), (Thakur RP, ed.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics. 47:1-5.

Rust

RP Thakur, VP Rao and P Sanjana Reddy

Introduction

Rust is a widespread disease in most sorghum growing parts of the world. Epiphytotics of rust have been reported to occur in cool and humid regions where sorghum is grown for forage. Rust infection substantially reduces forage quality and grain yield. Under favorable environmental conditions, rust development is fast and it affects panicle exsertion and grain development resulting in poor grain yield.

Pathogen

The fungus causing rust in sorghum is *Puccinia purpurea* Cook. It produces urediniospores and teliospores on sorghum. In the rust pustules, urediniospores and teliospores are interspersed with numerous paraphyses, which are usually short, clavate, hyaline and bent inward. Urediniospores are unicellular, pedicellate, oval to elliptical, walls fulvous or rust color, finely echinulated and 30-42×22-30 μm in size. Teliospores are bicelled, oblong to ellipsoidal, 25-32×40-60 μm in size, hyaline to yellowish, short and dark brown, and smooth walled. The fungus also attacks Johnson grass.

Symptoms

The typical symptoms of rust appear as scattered purple or red flecks on both surfaces of leaves (Fig. 16). The color of flecks varies depending upon plant pigmentation. In resistant plants, these remain restricted while in susceptible, these enlarge and appear like blisters that are brown to dark red pustules called uredinia. These uredinia are about 2.0 mm long and lie parallel to and between veins. As the leaf matures, the dried epidermis over the pustules ruptures, releasing the aggregates of reddish brown urediniospores. Gradually, most uredinia are converted into telia and the color of pustules changes from reddish brown to dark or blackish brown. New teliosori are also produced independent of uredinia on leaf surfaces, especially on the lower surface. On peduncles, reddish brown

Fig. 16. Symptoms of sorghum rust on leaf, peduncle and grains

to blackish brown uredinia and telia are seen in streaks as elongated lesions (Fig. 16).

Epidemiology

Urediniospores thrive on ratooned and successively planted sorghum, volunteer sorghum plants and on several perennial and collateral hosts. Secondary spread of the disease occurs by means of airborne urediniospores. Infection and disease development is favored by light intermittent rains and morning dew. Generally cool and humid weather favors epidemic development. *Oxalis corniculata* is an alternate host on which the aecidial stage develops. However, the role of aeciospores in disease development and spread is not important. Normally, sowing late during the rainy season or postrainy season leads to ideal conditions for rust development.

Screening techniques

Inoculum preparation

Urediniospores collected from the infected foliage in the previous season are used as inoculum. Urediniospores are collected from susceptible plants using a vacuum spore collector. These urediniospores are spread

onto waxed paper or aluminum foil overnight in an air-conditioned room to allow evaporation of excess moisture. Approximately 0.5 cc urediniospores are transferred into individual self-sealing plastic bags that are dated and stored at –80°C. Prior to use, plastic bags containing urediniospores are placed in a water bath at 40°C for about 10 min. The urediniospores are then suspended in water and a drop of a surfactant is added to ensure the uniform dispersal of spores. The spore concentration can be adjusted by using a haemocytometer. Urediniospores can be multiplied on a rust-susceptible genotype in the greenhouse, and stored as described above.

Field screening

- Plant the sorghum lines late in the rainy season or postrainy season to expose plants to cool weather favorable for rust development.
- Spray-inoculate sorghum seedlings with urediniospore suspension (1×10^5 spores mL^{-1}) twice, at 25 and 40 days after sowing.
- Irrigate the crop by perfo-irrigation to provide high humidity for 2-3 days after inoculation.
- Record rust severity 30 days after inoculation (at the grain-filling stage) using a modified Cobb's scale (Fig. 17) on the top four leaves of individual plants in a row or the entire row as required.
- Calculate the mean rust severity for the line across replications.

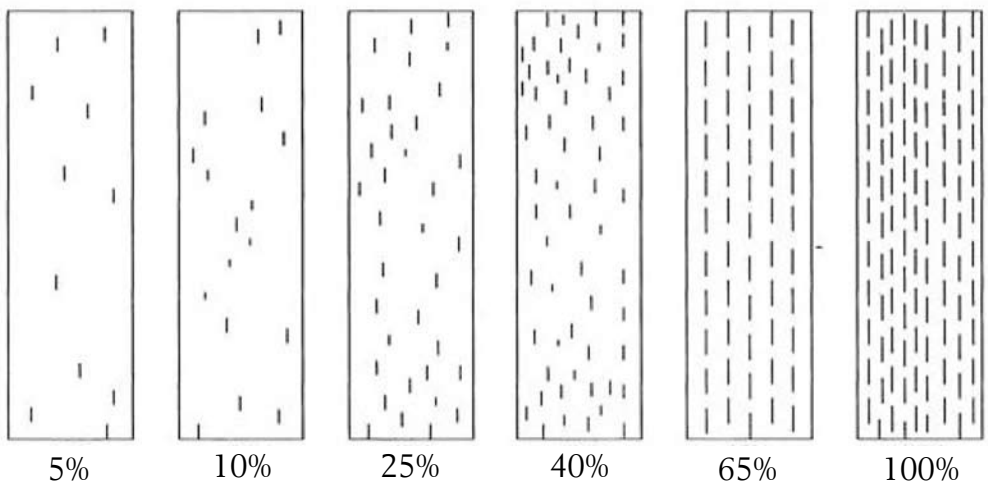


Fig. 17. Modified Cobb's scale for rust severity

For a large-scale field screening, rust susceptible genotypes can be used as inoculum donor by planting these as border rows and inoculating the plants with urediniospore suspension (approximately 5×10^5 urediniospores mL^{-1} water) about 30 days after sowing. Infection occurs within 7 to 10 days after inoculation. These infected plants serve the source of inoculum donor for the test rows planted about 2 weeks later than the infector rows.

Greenhouse screening

- Spray-inoculate pot-grown seedlings at the 3- to 5-leaf stage, with an aqueous suspension of urediniospores (1×10^5 urediniospores mL^{-1}).
- Maintain the inoculated seedlings in a moist chamber (>95% RH, 25±2°C) for about 18 h.
- Shift the seedlings to greenhouse benches at 25±2°C.
- Record rust severity data 15 days after inoculation using the modified Cobb's scale. (Under optimum conditions, pinhead chlorotic flecks appear within 3 days after inoculation and uredinia develop in about 8 days after inoculation).

[*Greenhouse screening is useful for identifying resistance in large number of lines in a small space, and under relatively uniform conditions. Screening against different pathotypes/races can effectively be done in the greenhouse. However, field screening is necessary to identify resistance effective against the variable pathogen populations prevalent in different sorghum growing areas under changing weather conditions.*]

Sources of resistance and their utilization

Several sorghum germplasm accessions and advanced breeding lines are known to possess good level of rust resistance under natural infection conditions. There have been no systematic efforts to screen sorghum lines by artificial inoculation. However, several hybrid seed parents, such as ICSA/B 329 to ICSA/B 350 have been developed in a trait-specific breeding program at ICRISAT and these have been characterized for agronomic traits.

We believe that with increasing importance of fodder and sweet sorghum, resistance to rust will become an important trait for an optimal productivity of a cultivar.

Selected References

Bandyopadhyay R. 2000. Rust, pages 23-24 *in* Compendium of Sorghum Diseases, Second Edition (Frederiksen RA and Odvody GN, eds.). American Phytopathological Society, St. Paul, Minnesota 55121-2097, USA: APS Press.

Reddy BVS, Sharma HC, Thakur RP and **Ramesh S.** 2006. Characterization of ICRISAT-bred sorghum hybrid parents (Set 1). International Sorghum and Millets Newsletter (Special Issue), (Thakur RP, ed.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics. 47: 1-5.

Singh SD, Wilson JP, Navi SS, Talukdar BS, Hess DE and **Reddy KN.** 1997. Screening techniques and sources of resistance to downy mildew and rust in pearl millet. Information Bulletin No. 48. ICRISAT. 36 pp.

Smuts

There are four different kinds of smuts affecting sorghum. These are head smut, long smut, loose kernel smut and covered kernel smut. Of these, head smut is more widespread and damaging while the other three smuts occur in relatively low frequency but are potentially important in several sorghum growing regions of the world. Because of their potential economic importance, these are being described here.

Head smut

P Ramasamy, RA Frederiksen, LK Prom and CW Magill

Introduction

Head smut is common in many parts of sorghum growing regions of the world. Africa has been suggested as the origin of the pathogen, although different races infect sorghum, corn and Sudangrass over a wide geographical area, including Europe, North and South America, Mexico, Africa, Asia, Australia, New Zealand, and the West Indies. In recent years head smut severity has increased due to cultivation of some susceptible sorghum cultivars or the appearance of more virulent races. It is one of the few smuts not controlled by seed treatment or management other than host resistance. Consequently, head smut is considered as a potentially important disease because of pathogen variability.

Pathogen

Head smut is caused by *Sporisorium reilianum* (Kühn) Clinton (Langdon and Fullerton) (syns. *Sporisorium holci-sorghi* and *Sphacelotheca reiliana*). It is an important soil borne fungal pathogen classified under division Basidiomycetes, order Ustilaginales. The smut spores are 9 to 14 μm in diameter and germinate to form a 4-celled or branched promycelium that bears sporidia terminally and near the septa. The sporidia may sprout to form yeast-like secondary sporidia or may germinate to form a

germ tube that penetrates meristematic tissue in the sorghum seedling. The pathogen is soilborne and the plants get infected at the seedling stage. The infection is systemic and progresses with the plant growth and finally expresses in the inflorescence at the boot leaf stage. In the United States, four physiological races have been identified among sorghum isolates on the basis of their reactions on a series of host differentials.

Symptoms

Head smut typically affects the inflorescence and rarely the foliage. A white peridium initially covers the sorus; large ruptured sori reveal distinct vascular strands. Symptomatic plants show no elongation of the peduncle with sterile panicles bearing sori of various sizes. The pathogen infects plants at the seedling stage and results in complete inability of infected plants to produce grain both in primary and secondary tillers. Other symptoms are reduction in plant height, phyllodial phenotype of the panicle, premature tillering and occasional chlorophyll degeneracy in localized areas of the leaves (Fig. 18). Some cultivars are dwarfed; others are stunted due to a lack of elongation of the peduncle. Smutted plants also have weakened root systems and commonly exhibit more severe stalk and root rots than smut-free plants. Parts of inflorescence

Sorghum head replaced by black spore mass (sorus)

Phyllodial phenotype

Infection in a nodal tiller

Fig. 18. Symptoms of sorghum head smut

that do not form gall usually show a blasting or proliferation of individual florets. Smut galls may also develop occasionally on the leaves and stems in some cultivars of sweet sorghum and Sudangrass.

Epidemiology

The pathogen is soilborne and survives in the form of teliospores in smut sori. Wind and rain scatter the smut spores on to the soil surface and in plant debris, where they lie dormant and overwinter. When sorghum seed is planted the following season, the smut spores germinate along with the seed. The germinated spore forms a germ tube that penetrates the exterior of the plant either in roots or mesoderm to reach the meristematic tissue in the sorghum seedling. Spore germination is high in moist soil with the temperature range of 27 to 31°C. The fungus develops only in actively growing meristematic tissue. However, the spores may also adhere to the seed surface and spread to new fields through seed. However, there is no report of internal seedborne nature of this disease. A dry soil with a temperature of approximately 24°C until the plants reach the 3-4 leaf stage is considered the most ideal for infection. Soil temperatures below 21°C and above 31°C seriously reduce the percentage of infection. Once this fungus infests soil, the spores can survive for a decade and hence planting of disease free or chemically treated seed does not prevent further infection.

Screening technique

Field screening

Field screening using conventional method of conducting trials at hot spots and relying on natural infection has not been effective because of variable environmental factors and erratic distribution of inoculum in the soil. Hence, in addition to natural infection, an inoculation technique in which seedlings are injected with sporidial suspension of the pathogen using a modified and simplified hypodermic syringe technique based on the method developed by Edmunds (1963) can be followed. However, the greenhouse screening method described below has been found more reliable and effective than the field screening technique.

Greenhouse screening

Inoculum collection

- Collect smut sori from head smut infected sorghum plants by bagging the individual panicles and allow these to dry at room temperature for a week before the teliospores are passed through a fine metal sieve to separate them from plant debris.
- Store clean teliospores in sterile plastic culture tubes at 4°C for use.

  [*Because field isolates of* S. reilianum *normally carry some bacterial and fungal contaminants with sori, the isolate of the fungus can be increased on the greenhouse grown susceptible plants*.]
- Grow susceptible sorghum plants in pots filled with soil + teliospores mixture in a greenhouse to obtain sori from smutted plants with abundant teliospores.

Inoculum preparation

- Wash dry teliospores of a defined isolate in 70% ethanol for 5 min, suspend in sterile water, plate on Potato Dextrose Agar (PDA). Incubate the PDA plates in the dark at 28°C for 72-96 h.
- Transfer the isolated sporidial colonies into 250 ml flasks containing 100 ml Potato Dextrose Broth (PDB) and incubate on a rotary shaker for 4 days.
- Filter the suspension using cheesecloth and make the sporidial inoculum with a concentration of 1×10^5 spores mL^{-1}. This suspension is used to inoculate seedlings with the help of a 30-ml hypodermic syringe.

Inoculation of seedlings

- Suspend irrigation to the pot-grown seedlings one day before inoculation and water them the day after injection.
- Inject inoculate 18-20 days old seedlings (approx. 10 cm height) below the apical meristem (Fig. 19) between 4 and 7 PM.

Seedlings at 3-4 leaf stage, 15-20 days old, and 10 cm tall ready for inoculation

Freshly prepared ice-cold inoculum

Injecting inoculum below the apical meristem

Fig. 19. Greenhouse screening for sorghum head smut

- Prepare fresh inoculum (milky white color) daily for each experiment and use 0.5-1.0 mL of sporidial suspension to inject each seedling continuously until drops of the inoculum are seen at the top of the leaf. [At the maximum of 100-150 individual plants/day/person should be enough for effective injection.]
- At the flowering stage, record the plants showing sorus development and determine the percentage infection. Plants showing no sorus development are considered resistant and there are no intermediate reaction types.

  [*The greenhouse screening using syringe inoculation is more effective, reliable and time saving and economical than field screening.*]

Resistance sources and utilization

Head smut resistant sorghum lines Tx2962 through Tx2978 were developed and released by the Texas Agricultural Experiment Station, the Texas A&M University System, Lubbock, TX, in 2006. These lines are generally earlier in maturity and shorter in plant height than the standard check Tx2783. The head smut resistant line BTx635 is one of the most popular lines in the production of hybrid sorghum. This

line is believed to possess resistance against infection by all four races of *S. reilianum*. Some of the highly susceptible lines are BTx7078 and SC170-6-17.

Selected References

Edmunds LK. 1963. Use of sporidial hypodermic injection to test sorghum for head smut resistance. Plant Disease Reporter 47:909-913.

Frederiksen RA. 2000. Diseases and disease management in sorghum. Pages 497-533 *in* Sorghum - Origin, History, Technology and Production (Smith CW and Frederiksen RA, eds.). New York: John Wiley & Sons, Inc.

Long Smut

RP Thakur

Introduction

Long smut, first reported from Egypt in 1887, is now known to occur in many countries in Africa and Asia, but has not been reported from Americas, Europe and Australia. It is known to be more prevalent in semi-arid tropical parts of Asia and Africa. The disease is reported both in cultivated and wild sorghum species. In certain cultivars, under favorable conditions grain damage could be 5% or more, thus causing substantial economic loss to farmers.

Pathogen

Long smut is caused by *Sporisorium ehrenbergii* Vánky (syns. *Tolyposporium ehrenbrgii* (Kühn) Patouillard). The teliospores are packed in spore balls that are dark brown and vary in size ranging from 30-230 μm diameter. The teliospores are of 9-15 μm diameter, lighter in color with smooth surface. Teliospores easily germinate in water droplets, potato agar or nutrient agar and produce a thick-walled three- to six-celled promycelium. The promycelium bears sporidia at the septa and terminal ends. Sporidia are 8-24 μm in diameter, hyaline, spindle-shaped and mostly single celled. The fungus grows like yeast cells on potato agar or nutrient agar and produces numerous sporidia within 7 days at 30°C.

Symptoms

Long smut appears as elongated, cylindrical, slightly curved sori, longer than normal grain (Fig. 20). The sori have a whitish thin membrane that ruptures to release black powdery mass of spore balls that can be easily blown by the wind. The long smut sori are much longer (2-4 cm) than those of covered kernel smut, and these are unevenly distributed on the panicle unlike the covered kernel smut sori. Each sorus contains 8-10 longitudinal filaments (remnants of the vascular elements of ovary) and teliospores are held between the filaments and the membrane wall.

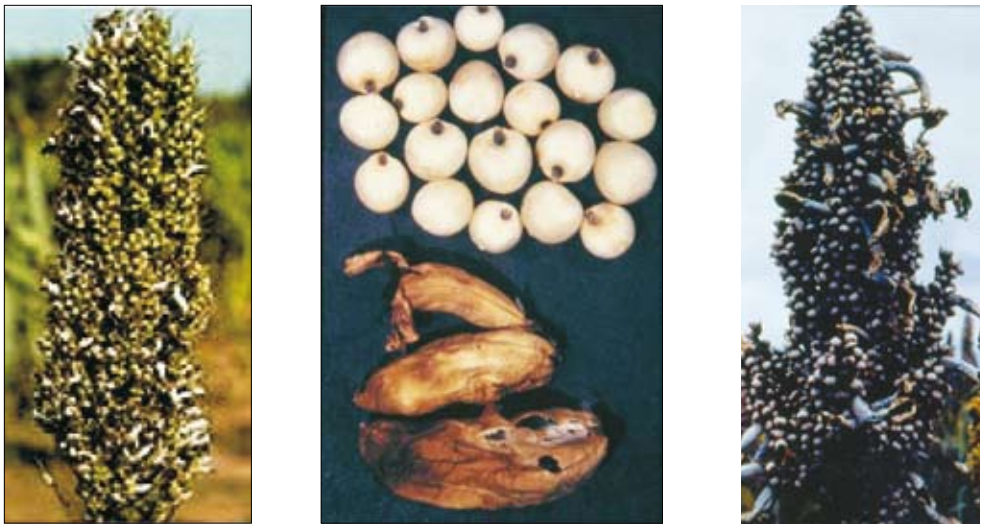


Fig. 20. Symptoms of sorghum long smut (on sides), smut sori and sorghum grains (center)

Epidemiology

The fungus *S. ehrenbergii* survives as teliospores on seed surfaces and in soil. The pathogen can move from one location to another through surface contamination of seed. Under favorable condition of soil moisture, the teliospores germinate and release numerous sporidia that may land in the boot leaf of sorghum plants and cause infection to emerging florets. Airborne teliospores may also settle on the flag leaf and germinate in water droplets accumulated at the flag leaf sheath to produce sporidia, which can infect the opening florets in the panicle. It is well known that the most susceptible stage for infection is the boot-leaf stage as also reported for smut of pearl millet. Relatively high temperature (30-35°C) and high humidity (>80% RH) favor disease development. Artificially inoculated florets take about two weeks to produce smut sori, and therefore, secondary spread of disease within a crop field is minimal except on late emerging panicles. However, late-planted sorghum in nearby fields may become infected through airborne spores. There are no reports on existence of races or pathotypes in *S. ehrenbergii*.

Screening technique

Inoculum

Prepare aqueous suspension of sporidia (1×10^6 sporidia mL^{-1}) obtained from a 7-day-old growth of *S. ehrenbergii* incubated at 30°C on potato agar (or PDA). The sporidial suspension can also be obtained by soaking the spore balls overnight in water in a conical flask and filtering the suspension through a double-layered muslin cloth before using for inoculation. The disease is similar to that of smut of pearl millet caused by *Moesziomyces penicillariae* (Bref.) Vanky (syn. *Tolyposporium penicillariae* (Bref.) for which an effective screening technique has been developed at ICRISAT.

Inoculation and disease scoring

- Inoculate the panicle by injecting about 10 mL sporidial suspension into the "boot" to fill the space between the flag-leaf sheath and panicle, and cover each inoculated panicle with a paper selfing bag. In each sorghum line, 5-10 panicles can be inoculated depending on the need and objective of the experiment.
- Sprinkler-irrigate twice, 30 min each before noon and in the evening on rain-free days to maintain high humidity for about a week after inoculation.
- Open the bags 15-20 days after inoculation and allow the panicles to dry for 2-3 days.
- Score the inoculated panicles for the percentage florets with smut sori.
- Select individual panicles that have no or very few smut sori and good seed set for further evaluation.
- Compute the mean and range of infection for each line, analyze the data and classify lines into resistant or susceptible classes.

Resistance sources and utilization

As the disease is not severe in many sorghum cultivars, there appears to be a reasonable level of resistance available in modern hybrids and varieties. However, there have been no efforts to systematically screen the world germplasm collections to identify resistance and use them in breeding programs.

Selected References

Kollo AI. 2000. Long smut, Pages 22-23 *in* Compendium of Sorghum Diseases, Second Edition (Frederiksen RA and Odvody GN, eds.). American Phytopathological Society, St. Paul, Minnesota 55121-2097, USA: APS Press.

Thakur RP and **King SB.** 1988. Smut disease of pearl millet. Information Bulletin no. 25. Patancheru, Andhra Pradesh 502 324, India: International Crops Research Institute for the Semi-Arid Tropics. 17 pp.

Covered Kernel Smut

RP Thakur

Introduction

Covered kernel smut is highly widespread and considered to be a major disease in all sorghum-growing regions. Covered kernel smut is the most common disease of sorghum where untreated seed is sown.

Pathogen

The fungus causing covered kernel smut is *Sporisorium sorghi* (syn. *Sphacelotheca sorghi*). It infects all groups of sorghum, including Johnsongrass. The pathogen is seedborne and infection is systemic, which begins at the seedling stage and progresses to the inflorescence. Smut sori are generally smooth, oval, conical or cylindrical, and vary in size from those small enough to be concealed by the glumes to those over one cm long. The disease is generally kept under control by seed treatment with chemical fungicide. Several distinct physiologic races of the covered smut fungus are known.

Symptoms

Normally in an infected panicle individual ovules are replaced by conical to oval smut sori (teliospores or chlamydospores) that are covered by persistent peridia that are larger than normal grain (Fig. 21). Initially each sorus is covered with a light pink or silver-white membrane, which later on ruptures to reveal the brownish-black smut spores. The infected kernels (smut sori) break open, and the microscopic spores adhere to the surface of healthy seeds where they overwinter.

Epidemiology

When a smut-infested kernel is planted, the teliospores (mostly 4 to 7 μm in dia) germinate along with the seed, forming a 4-celled promycelium (epibasidium) bearing lateral sporidia. The sporidia germinate and

infect the developing sorghum seedling. Once inside the seedling, the fungus grows systemically, apparently without damaging the plant until heading. The infected plants tend to boot earlier. At that time, the teliospores replace kernels and are surrounded by a membrane. At maturity, the membrane ruptures to release the teliospores that contaminate seed or soil. Soilborne teliospores are not considered important in infecting seedlings. The incidence of smut decreases when seed is planted in progressively warmer (15.5 to 32°C) wet soils.

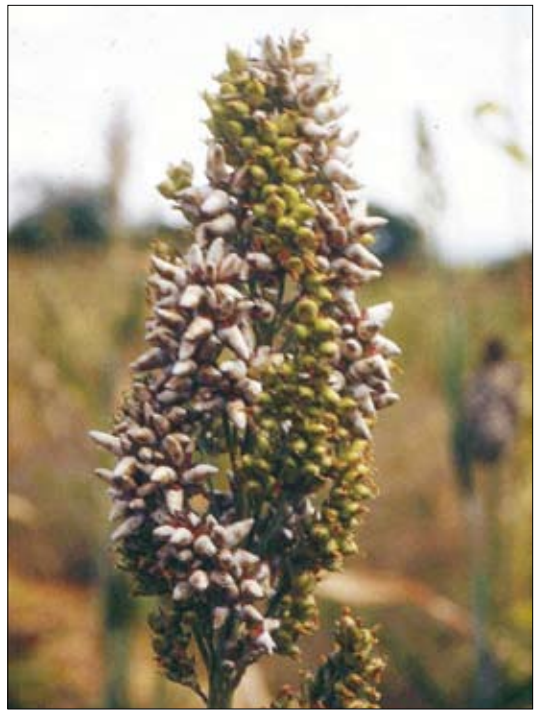


Fig. 21. Symptoms of sorghum covered kernel smut

Screening technique

As the disease is of relatively low economic significance, no systematic research efforts have been made to develop screening techniques, although variation in susceptibility to covered kernel smut in sorghum lines is well known. Since the pathogen is seedborne and it can be effectively controlled by seed treatment with a protectant fungicide, the disease has been kept under control so far. However, in order to develop an eco-friendly control measure, use of disease resistant hybrids and varieties is important.

The proposed screening technique both for covered kernel and loose smuts is described below.

- Surface coat the seed with smut spores (teliospores) before sowing
- Grow plants under normal conditions following the recommended agronomic practices.

- Bag the panicles at the boot leaf stage with paper selfing bags to promote infection.
- Alternately, inject-inoculate the panicles with sporidial suspension as in case of long smut (*S. ehrenbergii*), and bag the inoculated panicles.
- Provide sprinkler irrigation as for long smut on dry days to enhance infection.
- Open the bags 15 days after inoculation and allow the panicles to dry for 2-3 days.
- Score each panicle for the percentage of florets bearing smut sori.
- Select panicles with no infection, obtain mature seed from these and reevaluate to confirm the resistance.

Resistance sources and their utilization

As the disease is controlled by seed treatment with fungicide, not much information is available on resistance sources.

Selected References

Frederiksen RA. 2000. Covered kernel smut. Page 21 *in* Compendium of Sorghum Diseases, Second Edition (Frederiksen RA and Odvody GN, eds.). American Phytopathological Society, St. Paul, Minnesota 55121-2097, USA: APS Press.

Loose Kernel Smut

RP Thakur

Introduction

Loose kernel smut is common in most sorghum growing regions except Australia and some parts of Asia, including Malaysia and Indonesia. This smut is less widespread than covered kernel smut. Loose kernel smut attacks all groups of sorghums, including Johnsongrass, although certain varieties in some groups are immune or highly resistant. Sudangrass is usually not infected.

Pathogen

Loose kernel smut is caused by the fungus *Sporisorium cruentum* (Kühn) Vánky (syn: *Sphacelotheca cruenta* (Kühn) Potter). Teliospores are light yellowish brown or dark brown, minutely echinulated, globose to subglobose and measure 6 to 10 μm in diameter. The teliospores germinate by forming a thick, usually 4-celled promycelium bearing lateral sporidia, like in other smut fungi. Spore germination occurs at optimal temperatures of 28-32°C, and the fungus can easily be cultured on agar medium. It produces yeast-like colonies and numerous sporidia on nutrient agar or potato agar. The fungus is heterothallic and is able to hybridize with both the *S. reilianum* (head smut) and *S. sorghi* (covered kernel smut). Several races of *S. cruentum* infecting sorghum cultivars have been reported.

Symptoms

Normally, all kernels in an infected panicle are smutted (Fig. 22). Partial infection is uncommon. Individual infected kernels are replaced by small smut sori that are 2.5 cm or longer, pointed and surrounded by a thin gray membrane. Some kernels may be transformed into leafy structures or escape infection completely. The smut sori are surrounded by a thin gray membrane. This membrane usually ruptures soon after the panicle emerges from the boot to release powdery, dark brown to black teliospores. These teliospores can be blown away by wind leaving a long,

black, pointed, conical, often curved structure (columella) in the center of the gall.

Infected plants are stunted, panicles on them appear earlier than the healthy plants and most spikes with smutted glumes show hypertrophy. Abundant side branches (tillers) may also develop. Occasionally, the tillers are smutted, while the primary panicles remain healthy.

Fig. 22. Symptoms of sorghum loose kernel smut

Epidemiology

When the seed contaminated with teliospores are sown in the field, the spores germinate to produce sporidia. These sporidia germinate and infect the developing sorghum seedling. Most infections, however, result from the teliospores producing hyphae, which penetrate young seedlings before emergence. Seedling infection occurs over a wide range of soil moisture and pH at a temperature of 20 to 25°C. The fungus continues to grow systemically within the plant unobserved until heading, when the long, black, pointed smut galls develop in place of normal kernels.

Secondary infection may occur when spores from a smutted head infect late-developing heads of healthy sorghum plants. Infection is limited and no further systemic spread of spores occurs from the infected plants. Teliospores in the soil are not important in terms of infecting seedlings.

Screening technique

Since the disease is easily and effectively controlled by treating the seed with a protectant fungicide, which prevents introducing the pathogens into uninfested fields, no systematic research has been done to develop screening technique, although variation in susceptibility level of sorghum lines and cultivars is well known. Although seed treatment provides protection against seedling blight fungi in the soil and promotes plant growth, there is a strong need to develop an effective field screening

technique to identify genetic resistance in sorghum germplasm accessions and utilize these to breed resistant cultivars. The screening technique should be similar to that reported earlier for the covered kernel smut.

Selected References

Mughogho LK. 2000. Loose Kernel Smut. Pages 20-22 *in* Compendium of Sorghum Diseases, Second Edition (Frederiksen RA and Odvody GN, eds.). American Phytopathological Society, St. Paul, Minnesota 55121-2097, USA: APS Press.

General comments on smuts

Because there are reports of existence of physiologic races of the three sorghum smut fungi (*S. sorghi*, *S. cruentum* and *S. reilianum*), which can also hybridize with one another, it seems extremely difficult to develop highly resistant cultivars. However, it would be interesting and scientifically challenging to see if resistance to one pathogen is also effective against another. There are reports that sorghum lines resistant to races of covered kernel smut are usually also resistant to races of loose kernel smut.

Virus Diseases

YD Narayana

Introduction

Viruses and virus-like diseases infecting sorghum are distributed worldwide and cause varying levels of economic losses in grain yield and fodder quality. Of 23 viruses that are known pathogens on sorghum, five are economically important: sugarcane mosaic virus (SCMV), maize dwarf mosaic virus (MDMV), maize stripe virus (MStV), maize mosaic virus (MMV), and sorghum mosaic virus (SrMV). In general, the extent of losses depends on the stage of the crop at infection, with earlier infections resulting in greater losses.

In recent years, the incidence of MStV-S and MMV-S, both isolates of maize viruses, have been increasing in the postrainy season sorghums in peninsular India. Both these viruses together cause considerable losses in vegetative growth and grain yield of sorghum. The reduction in stover yield is particularly important and can cause significant losses in income from crop-livestock production systems in rural and peri-urban India, since sorghum crop residue is an important feed for cattle populations. The two most important virus diseases are dealt with below.

Pathogen

Two economically important sorghum viruses prevailing in India and other semi-arid tropic parts of the world belong to isolates of maize stripe virus (MStV) and maize mosaic virus (MMV). The MStV-S infecting sorghum is serologically related to MStV and belongs to *Tenuivirus* group. The virus particles are flexuous branched filaments having <10 nm width and contain eight species of nucleic acids- four ssRNAs and 4 dsRNAs. The MMV-S infecting sorghum is serologically related to MMV and belongs to subgroup plant *Rhabdovirus* with four major proteins.

Insect Vector: In nature, both MStV-S and MMV-S viruses are transmitted by a delphacid planthopper *Peregrinus maidis* (Ashmead) (Fig. 23), which is one of the major pests on sorghum in India. The important

characteristics of virus-vector relations are that the acquisition access period for the insect is 4 h, minimum incubation period of virus in the vector is 8 days, inoculation access period of 1 h and the insect transmits virus in a persistent manner. However, once the insect acquires the virus, it continues transmitting it for the remainder of its life, even passing the virus through eggs to the progenies in case of MStV-S, but not MMV-S.

*Fig. 23. Various stages of planthopper (*Peregrinus maidis*) on a sorghum leaf*

Symptoms

The earliest symptoms of viral diseases under field conditions often resemble those associated with herbicide, insecticide, or fungicide damage, or with genetic abnormalities. The disease symptoms may include chlorosis, yellowing, chlorotic streaks, stripes or rings, reddening of leaves, necrotic spots, dwarfing/stunting, rosetting, excessive tillering, sterility and delayed flowering.

Maize Stripe Virus (MStV-S). The characteristic symptoms include the appearance of chlorotic stripes/bands between the veins on infected leaves, stunted plants with small internodes, partial exsertion of the panicle (Fig. 24) with fewer seed setting or no panicle emergence, depending on the crop stage at infection.

Fig. 24. Symptoms of maize stripe virus on sorghum: susceptible to resistant leaf, infected seedling and partial exsertion of panicle

Maize Mosaic Virus (MMV-S). The symptoms include the appearance of fine continuous chlorotic or broken streaks between the veins on leaves (Fig. 25) that may become necrotic as the disease progresses, severe stunting of plants with shortened internodes and fewer seed setting on panicles.

Fig. 25. Symptoms of maize mosaic virus on sorghum

Epidemiology

In nature, both MStV-S and MMV-S are transmitted by a delphacid planthopper *Peregrinus maidis* in a persistent manner. The vector *P. maidis* is also a common pest on sorghum in Asia and Africa that could become a potential threat to sorghum production. Johnsongrass, one of the wild graminaceous hosts, serves as a perennial reservoir for both MStV and MMV and also for the vector. The persistent and transovarial nature of virus transmission across insect generations contributes greatly to the perpetuation of the disease. After the harvest of rainy season sorghum crop, the virus infected ratooned sorghum serves as source of primary inoculum and also harbors the insect vector, which helps in spread of the diseases for the postrainy sorghum crop.

Screening Techniques

For both MStV and MMV diseases, a similar screening technique is used. The group seedlings inoculation has been found to be simple and efficient (98% virus infection) for large-scale screening.

Field Screening

- Grow a sorghum line (CSH-9 or SPV 351) highly susceptible to MStV-S/MMV-S in every fifth row as infector rows leaving four blank rows.
- Infest the seedlings at 3-4 leaf stages by placing 2 viruliferous planthopper (mass multiplied and maintained in greenhouse) in to the leaf whorl of each plant.
- Plant the test entries next day after infestation in the four blank rows in between the two infector rows. Also plant resistant and susceptible check entries with the test entries.

  [The viruliferous insects placed in whorls inoculate the virus into infector row plants and also multiply, and the subsequent generations of insects move to young seedlings in the test rows and infect them.]
- Maintain 30-40 plants/row (4m)/replication for a 2-3 replicated experiment.
- Record data on the total number of plants and the number of plants showing the symptoms of the diseases.
- Compute the data from each plot and calculate the percentage incidence for each entry. Compare and contrast the data of test entries in relation to resistant and susceptible checks for selection and reporting.

Greenhouse screening

Collection and maintenance of inoculum

- Collect the cultures of MStV-S and MMV-S from naturally infected sorghum plants in the field.
- Feed young nymphs of planthopper onto the infected leaves for 4 days and incubate them on healthy plants for one week to get viruliferous insects.
- Expose group of seedlings of each test entry at the 3-4 leaf stage to the viruliferous insect for 2 days under caged condition, and then

spray the seedlings with an insecticide (0.2% monocrotophos) to kill the insects.

- Maintain the seedlings showing the characteristic virus symptoms in an insect-proof greenhouse for further use.
- Confirm the virus culture from seedlings by testing against antiserum of MStV-S or MMV-S using ELISA.

Collection and mass rearing of viruliferous planthopper

- Collect the planthopper (*P. maidi*s) colonies from apparently disease-free sorghum plants in the field using an aspirator.
- Allow the insects, after identification, to feed on healthy plants at the 4-5 leaf stage for 10 days in an insect-proof cage.
- Collect the adult insects from disease-free seedlings and maintain them on 4-week-old healthy sorghum plants for mass multiplication in insect-proof cages.
- Collect the second- and the third-instar nymphs of *P. maidi*s from healthy colonies and allow for virus acquisition by confining them in leaf whorls of virus infected plants for 4 days.
- After acquisition, incubate the colonies for 10 days on healthy sorghum plants to get viruliferous adults for inoculating sorghum plants in field or/and greenhouse.

Seedling inoculation

- Raise a group of 15-20 seedlings (2-leaf stage) in a polyethylene bag filled with a mix of autoclaved soil, sand and FYM (2:1:2 by vol.) and cover them with a cylindrical plastic bottle (20 cm long×7.5 cm in diameter) with provision for aeration by covering the top with nylon mesh and a small hole on one side to release viruliferous insects into the bottle.
- Inoculate 10-day old seedlings (2-leaf stage) grown in polyethylene bags with viruliferous insects (2 insects per seedling) in cylindrical cages and allow 48 h for inoculation access. Disturb the insects after 24 h and then at the end of 48 h either remove the insects for

reusing them for fresh inoculation of seedlings or spray with 0.2% monocrotophos to kill them, if not required.

- Maintain the seedlings in the insect-free greenhouse for expression of disease symptoms for 3 weeks.
- Record the data by counting the total and infected plants with the characteristic symptoms of the virus 3-4 weeks after inoculation.
- Compute the data and calculate the incidence percent.

[*The method is simple and efficient to get 98% virus infection and used for large-scale greenhouse screening of sorghum genotypes to identify resistant sources*.]

Resistance sources and their utilization

Sorghum lines IS 9600, Q 104, SPV 932 and ICSB 15 have been found resistant/tolerant (<10% infection) to MStV-S in a recent screening. No such information is available on resistance to MMV-S. Resistance breeding for virus resistance is yet to be initiated.

Selected References

Naidu RA, Harikrishnan R, Manohar SK, Reddy DVR, Ratna AS, King SB and **Bandyopadhyay R.** 1989. Maize mosaic virus on sorghum. Annals of Applied Biology. 114:301-310.

Narayana YD and **Muniyappa V.** 1995a. Survey, symptomatology and detection of an isolate of maize stripe virus on sorghum in Karnataka. Indian Phytopathology 48:171-176.

Narayana YD and **Muniyappa V**. 1995b. Effects of sorghum stripe virus on plant growth and grain yield of sorghum. Indian Journal of Virology 11:53-58.

Narayana YD and **Muniyappa V.** 1996a. Virus-vector relationships of planthopper *(Peregrinus maidis)* - borne sorghum stripe tenuivirus. International Journal of Pest Management 42:165-170.

Narayana YD and **Muniyappa V.** 1996b. Evaluation techniques for the efficient transmission of sorghum stripe virus by vector *(Peregrinus maidis)* and screening for disease resistance. Tropical Agriculture 73:119-123.

Navi SS, Bandyopadhyay R, Blümmel M, Reddy RK and **Thomas D.** 2003. Maize stripe virus: a disease of sorghum emerging in South India. Intl. Sorghum and Millets Newsletter 44:126-128.

Peterschmitt M, Ratna AS, Sacks WR, Reddy DVR and **Mughogho LK.** 1991. Occurrence of an isolate of maize stripe on sorghum in India. Annals of Applied Biology 118:57-70.

Notes

Notes

Notes

Notes

Notes

Notes

About ICRISAT®

The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) is a non-profit, non-political organization that does innovative agricultural research and capacity building for sustainable development with a wide array of partners across the globe. ICRISAT's mission is to help empower 600 million poor people to overcome hunger, poverty and a degraded environment in the dry tropics through better agriculture. ICRISAT belongs to the Alliance of Centers of 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-Niamey (Regional hub WCA)
BP 12404
Niamey, Niger (Via Paris)
Tel +227 20722529, 20722725
Fax +227 20734329
icrisatsc@cgiar.org

ICRISAT-Bamako
BP 320
Bamako, Mali
Tel +223 2223375
Fax +223 2228683
icrisat-w-mali@cgiar.org

ICRISAT-Bulawayo
Matopos Research Station
PO Box 776,
Bulawayo, Zimbabwe
Tel +263 83 8311 to 15
Fax +263 83 8253/8307
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

Visit us at www.icrisat.org

ISBN 978-92-9066-504-5 Order code IBE 076 402-2007