

Sorghum Grain Mold

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Abstract

Grain mold, the most important and widespread disease of sorghum worldwide, is a major constraint to sorghum productivity. Grain mold development is particularly severe in the short-duration hybrid cultivars and varieties that are grown during the rainy season under warm and humid conditions. It is caused by a number of unspecialized fungal pathogens that severely affect grain mass, seed viability, grain quality and market price. A large volume of literature is available on studies related to various aspects of grain mold, including biology, epidemiology, association with mycotoxins and management methods. In this bulletin, attempts have been made to briefly describe the important findings of research done at ICRISAT and elsewhere, and to emphasize some of the recent developments on management of grain mold, including refined screening techniques, sources of resistance, genetics and mechanisms of resistance, resistance breeding and other management options, including an integrated management approach.

Cover: Moldy sorghum grains on different types of panicles, and a mold-free panicle on the extreme right.

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Foreword

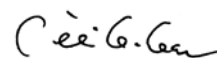
I am very pleased to write a foreword for this important publication on sorghum grain mold, jointly compiled by plant pathologists and breeders from different institutions. Grain mold is a problem in most sorghum-growing environments where warm and humid conditions prevail. Given grain mold's damage-causing potential that has a bearing on production, quality, and nutritional value involving mycotoxins, it has received top priority at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) during the last three decades.

ICRISAT's research focus has mainly been on managing grain mold through host plant resistance that involves developing effective screening techniques, identifying sources of resistance, and breeding for resistance through collaborative efforts of pathologists and breeders. Grain mold is a complex problem involving many fungal pathogens and several plant traits, and biochemical parameters in imparting resistance. Given this scenario, ICRISAT in partnership with the Indian Council of Agricultural Research (ICAR) and other organizations such as Iowa State University, has studied several aspects of grain mold and developed field, laboratory and greenhouse screening techniques. These techniques have been used to screen a large number of germplasm accessions and breeding lines to identify resistance and enhance resistance breeding. Screening techniques too have been refined and made simpler.

Currently, a number of resistant sorghum lines are available with varying grain color, plant height, and panicle architecture, and some of these have been utilized in generating advanced breeding lines, including hybrid parents (A-, B- and R-lines) with moderate to high levels of resistance. Several of the resistant and advanced breeding lines have been shared globally with sorghum researchers in both public and private institutions. I am happy to note that there is good potential to develop grain mold-resistant white-grain hybrids that are most desirable as food in India.

Apart from describing the findings of vital research done at ICRISAT and elsewhere, this bulletin elucidates on recent developments in grain mold management, laying emphasis on an integrated management strategy to manage the disease.

The authors have done a commendable job of compiling information pertaining to the disease in a simple and comprehensive manner. I am sure the bulletin will serve as a useful guide to sorghum researchers, students, and farmers, especially those who have interest in managing grain mold.



William D Dar
Director General, ICRISAT

1. Introduction

Grain mold is a major disease of sorghum (*Sorghum bicolor* (L.) Moench) that affects grain production and quality. The disease is particularly important on improved, short- and medium-duration sorghum cultivars that mature during the rainy season in humid, tropical and subtropical climates. Photoperiod-sensitive cultivars that mature after the rains often escape mold infection. Sorghum cultivars with white grain pericarp are particularly more vulnerable to grain mold than those with brown and red grain pericarp. Grain mold can be broadly defined as preharvest grain deterioration caused by several fungal species interacting parasitically and/or saprophytically with developing grain. Grain weathering, on the other hand, is a post physiological maturity problem when grain turn discolored and tissues are damaged by fungal colonization due to wet weather. Damage due to grain mold has been associated with losses in seed mass, grain density, seed germination, storage quality, food and feed processing quality, and market value. Some of the mold fungi are producers of potent mycotoxin that are harmful to human and animal health and productivity. Management of grain mold in the rainy season sorghum cultivars, particularly hybrids, is a difficult proposition. Of the several approaches that have been tried, host-plant resistance appears to be the most viable and effective method of managing grain mold. ICRISAT has invested considerable efforts towards understanding biology and epidemiology, developing screening methods, identifying resistance sources and utilizing these in breeding grain mold resistant sorghum hybrid parental lines (Bandyopadhyay et al. 2000; Reddy et al. 2000; Stenhouse et al. 1998). In this bulletin we describe briefly some of the recent advances in sorghum grain

mold research especially in relation to host-plant resistance and some other aspects of disease management, and also provide some information on alternative uses of molded sorghum grains by industries.

2. Economic significance

Production losses due to sorghum grain mold range from 30% to 100% depending on cultivar, time of flowering and prevailing weather conditions during flowering to harvesting (Singh and Bandyopadhyay 2000). It is difficult to estimate accurate losses caused by the disease since it involves the assessment of losses from production to marketing and finally utilization of the grain or seed. On a conservative scale, the annual economic loss due to sorghum grain mold in Asia and Africa has been estimated to be US\$ 130 million (ICRISAT 1992). Certain grain mold pathogens have consistently been associated with losses in seed mass (Castor and Frederiksen 1980; Indira et al. 1991; Somani and Indira 1999), grain density (Indira and Rana 1997; Castor 1981; Ibrahim et al. 1985), and germination (Castor 1981; Maiti et al. 1985). Other types of damage that arise from grain mold relate to storage quality (Hodges et al. 1999), food and feed processing quality, and market value.

3. Grain mold symptoms

One of the first visible symptoms is pigmentation of the lemma, palea, glumes, and lodicules. Depending on the fungus involved, the grain maturity stage and severity of infection, the symptoms could be highly variable (Fig. 1). Severely infected grain is fully covered with mold; partially infected grain may look normal and discolored; while



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

some apparently normal grain may not show external symptoms but produces mold fungi on blotter after surface sterilization. Fungal growth occurs at the hilar end of the grain, and subsequently extends on the pericarp surface. Severe infection in the field results in multicolored grains due to various colored fungal mycelium and sporulating structures depending on the fungal pathogen involved in colonizing sorghum grains. Discoloration of the grains due to fungal infection is more prominent on white-grain than in brown/red-grain sorghums (Fig. 2).

Early infection at anthesis results in blasted florets, poor seed set and small shriveled grains on the panicle (Fig. 3). Severely infected grain disintegrates even under slight pressure.

Infection by *Fusarium* spp. at early stages of flowering damage large areas of panicle including peduncle and rachis branches and spikelets, resulting in blighted panicle, and it is called as head blight disease.

Grain colonization by mold fungi vary in color and texture depending on the fungus involved. *Curvularia lunata*, the fastest colonizing fungus appears as shiny, velvety black, fluffy growth on grain surface (Fig. 4). *Fusarium* spp. generally produce pinkish white mycelium, powdery in appearance during early stages which later becomes pinkish fluffy (*F. pallidoroseum* – Fig. 4), and fluffy white (*F. verticillioides*, formerly a part of *F. moniliforme*- Fig. 4). *Phoma sorghina* produces pin-like small, round, black pycnidia embedded in grain and produces a



Fig. 2. Moldy grains on white- brown- and red-grain sorghum panicles.



Fig. 3. Blasted florets on sorghum panicles due to early infection by grain mold pathogens.

thick dirty black crust with rough surface on the pericarp (Fig. 4). *Alternaria alternata* appears as dull with grayish black mycelium, often sparse and in stripes (Fig. 4). *Bipolaris australiensis* appears as dark black mycelium on the grain surface (Fig. 4). *Cladosporium oxysporum* grows as grayish mycelium with powdery appearance on the grain (Fig. 4), while *Colletotrichum graminicola* produces small, black, enlarged acervuli studded with clusters of setae forming concentric rings on the grain (Fig. 4).

Post maturity colonization produces the “moldy appearance” of grain in humid environments. Although grain integrity is not affected by such superficial molding, market value of such grains is reduced due to moldy appearance. If there is rain during crop maturity, there is every possibility that grains will exhibit the mold symptoms and also sprout while in the panicle (Fig. 5). Sometimes it is difficult to

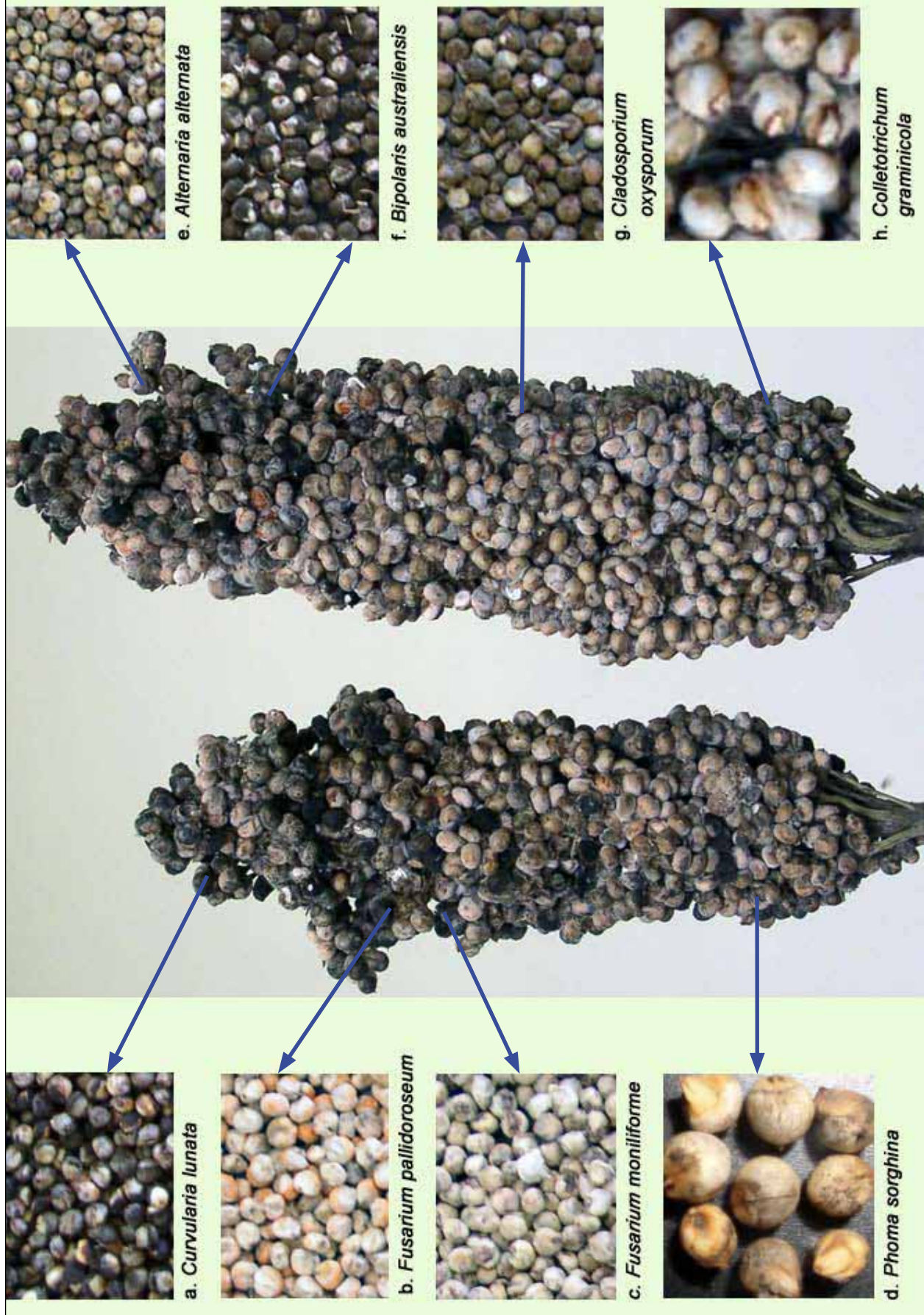


Fig. 4. Grains colonized by different grain mold pathogens on sorghum panicles.



Fig. 5. Sprouted grains on a moldy sorghum panicle.

differentiate between early infections and post maturity colonization in the field unless monitored properly. Both conditions occur together, and late-season colonization can mask symptoms of infection occurring during grain development.

4. Biology and epidemiology

A number of pathogenic and saprophytic fungi are involved in the sorghum grain mold complex. Some of these fungi have both

pathogenic and saprophytic phases at different stages of grain development. The growth and sporulation patterns of these fungi are highly variable and sensitive to changing weather conditions. Studies related to major grain mold fungi are briefly described here.

4.1. Fungi involved in mold complex

Fungi belonging to more than 40 genera are reported to be associated with sorghum grain mold (Navi et al. 1999). Several fungal species of the genera, *Fusarium*, *Curvularia*, *Alternaria*, *Phoma*, *Bipolaris*, *Exserohilum*, *Aspergillus*, and *Penicillium* have been found associated with grain mold disease. Of these, *Fusarium andiyazi*, *F. proliferatum*, *F. sacchari*, *F. verticillioides*, *F. thapsinum*, *F. nygamai*, *F. pseudonygamai* (formerly all inclusive in *F. moniliforme*), *Curvularia lunata*, *Alternaria alternata*, *Phoma sorghina*, *Bipolaris australiensis* and *Exserohilum turcicum* have been identified as major mold pathogens in various studies (Fig. 6). In India, major fungi involved in grain mold complex are *F. verticillioides*, *F. thapsinum*, *Curvularia lunata*, *Phoma sorghina*, *Colletotrichum graminicola*, and *Alternaria alternata*. Among these, *F. verticillioides*, *C. lunata* and *A. alternata* being more pathogenic than others (Thakur et al. 2003b). Frequency of occurrence of these fungi varies with location and environmental conditions during the cropping season. A recent study (Thakur et al. 2003b; Thakur et al. 2005) on variability among grain mold fungi through multilocation evaluation of selected sorghum genotypes at five Indian locations for three rainy seasons revealed predominance of *F. verticillioides* at Parbhani (Maharashtra state), of *C. lunata* and *P. sorghina* at Patancheru (Andhra Pradesh state), and of *A. alternata* both at Parbhani and Patancheru (Thakur et al. 2005). Majority

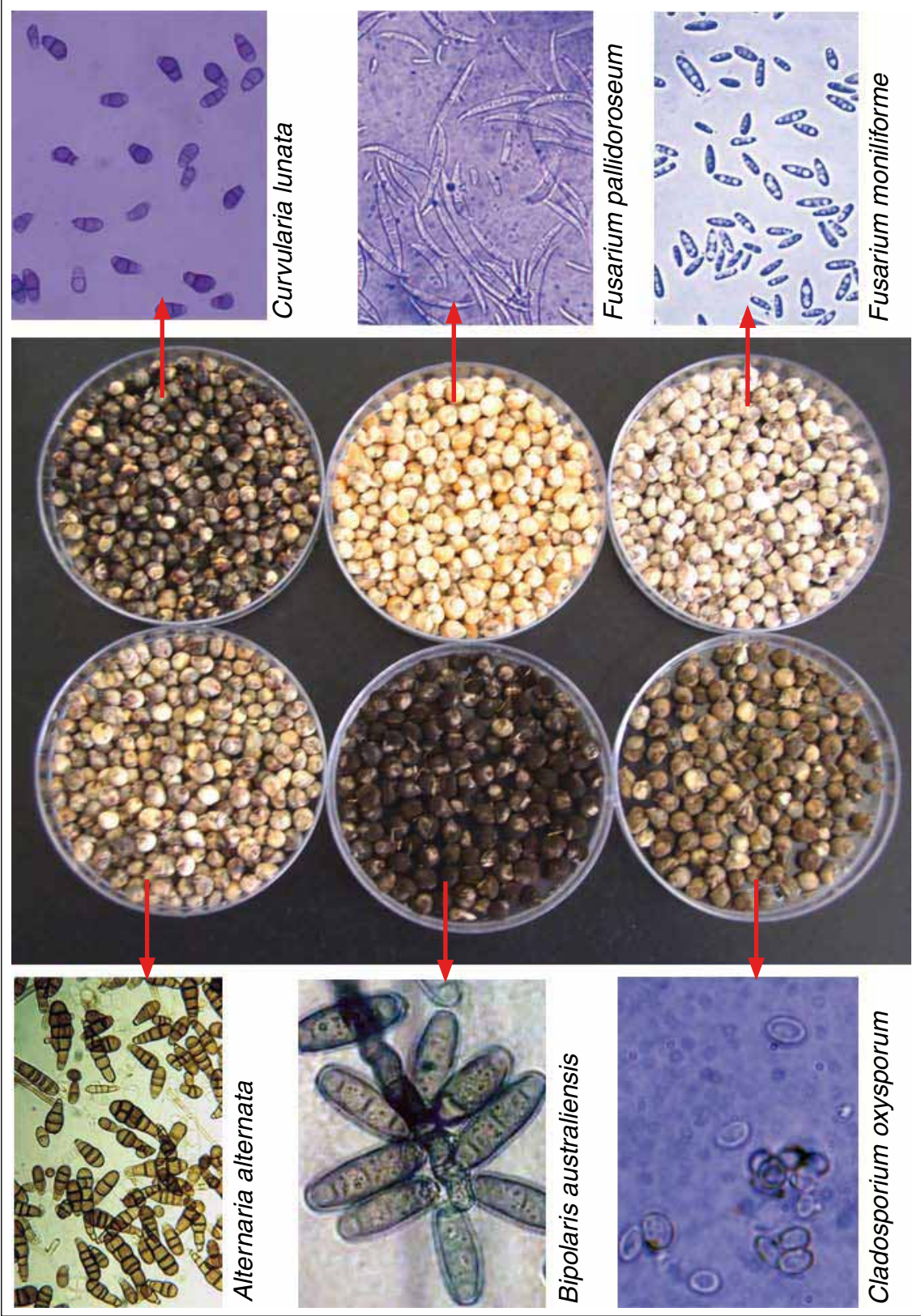


Fig. 6. Sorghum grains colonized by different mold fungi and their conidial forms.

of other fungi, including *Aspergillus niger*, *A. fumigatus*, *Cladosporium* spp., *Bipolaris* spp., *Exserohilum* spp., *Epicoccum* spp., *Fusarium semitectum*, and *Olpitrichum* spp. were generally saprophytes and contribute to post maturity grain weathering.

4.2. Mold fungi and mycotoxins

Several mold fungi involved in grain mold complex produce toxins as secondary metabolites that are harmful to human and animal health and productivity. These mycotoxins are fumonisins, moniliformin, fusaproliferin, fusaric acid, fusarins, beauvericin, and gibberellic acids. Fumonisins, a group of mycotoxin produced by *F. verticillioides* and related species, have received worldwide attention. *Fusarium* spp. is emerging as a major complex within the complexity of grain mold. Several species of *Fusarium*, including *Fusarium andiyazi* (Fig. 7a), *F. proliferatum* (Fig. 7b), *F. sacchari* (Fig. 7c), *F. verticillioides* (Fig. 7d), *F. thapsinum* (Fig. 7e), have been identified to be associated with moldy sorghum grains. Isolates/strains of some of these species have been shown to produce varying levels of fumonisins and moniliformin (Leslie et al. 2005). The first report of fumonisins toxicity to human and poultry came from the Deccan plateau of India (Vasanthi and Bhat 1998). An outbreak of poisoning, characterized by abdominal pain and diarrhea, attributed to the ingestion of fumonisins-contaminated maize and sorghum, has been reported from several villages in India (Bhat et al. 1997).

4.3. Weather variables and mold development

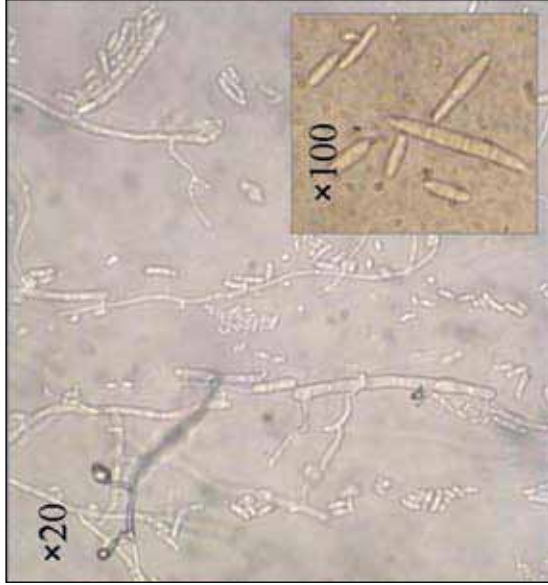
Infection by grain mold fungi and mold development are highly influenced by relative humidity and temperature. Humid and

warm conditions during flowering and grain development stages favor infection and mold development while dry conditions prevent it. In a recent study, Navi et al (2005) showed that frequency of infection by a fungus increased with the increase of wetness duration up to 72h. For different growth stages at which panicles were inoculated, the infection frequency varied among the fungi, indicating that individual fungi might have different windows for maximum infection during the grain development stages. Effects of panicle wetness periods were highly significant on infection by *C. lunata*, *F. verticillioides*, and *P. sorghina*. The influence of post inoculation incubation temperatures regime was highly positive and significant for infection by *Cladosporium oxysporum* compared with other fungi, such as *C. lunata*, *F. verticillioides* and *P. sorghina* (Navi et al. 2003).

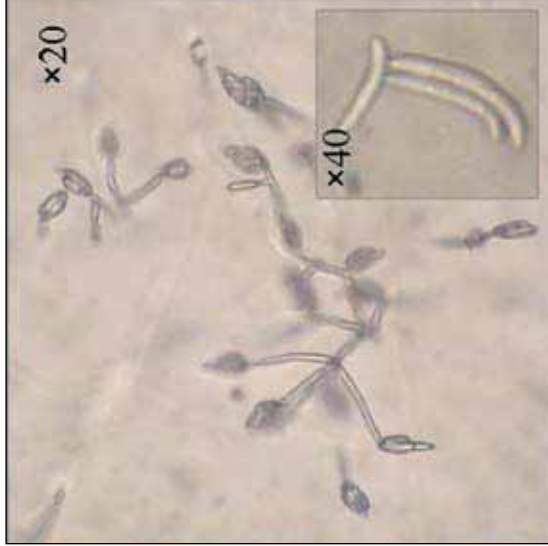
In a spore sampling study (Das et al. 2004) of mold fungi during the rainy season crop there was an increase in the inoculum load (number of spores m⁻³ air sampled) along the crop maturity, and the inoculum load reached its peak during the normal grain maturity period. This was explained by a strong correlation between the weather parameters and crop age to spore production in major mold pathogens. Spore production was more in warm temperature (25-28°C) and high relative humidity (100%) and it decreased with drop in temperature below 15°C and rise in temperature above 30°C. When there was a sudden rise in the relative humidity following rainfall, the inoculum load also jumped several times (Indira and Muthusubramanian 2004).

4.4. Infection process

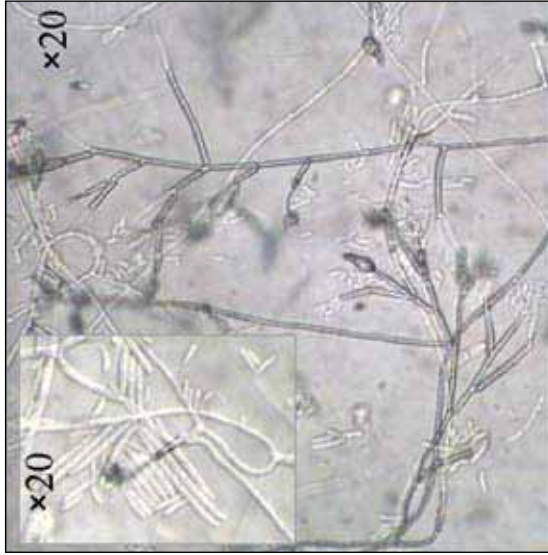
Infection and colonization of flower tissues occur prior to grain maturity (Forbes 1986).



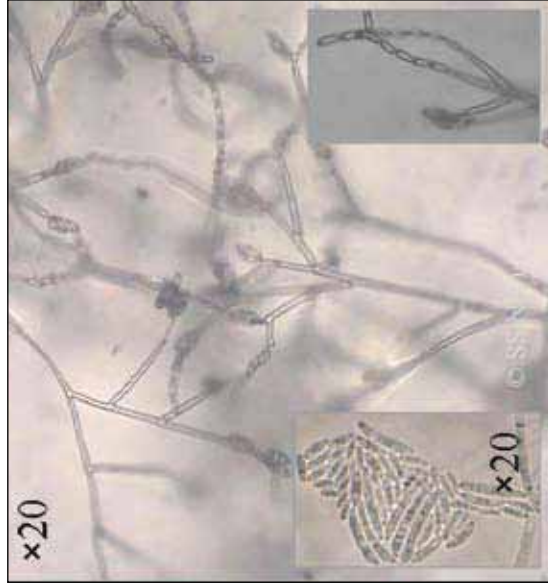
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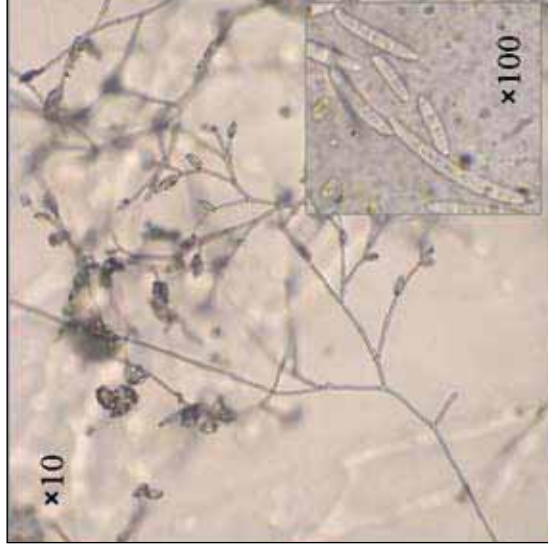
b



c



d



e



f

Fig. 7. Different species of *Fusarium* associated with sorghum grain mold complex: a) *F. andiyazi*, b) *F. proliferatum*, c) *F. sacchari*, d) *F. verticillioides*, e) *F. thapsinum*, f) *Fusarium* sp. (*unidentified*).

Mycelium penetrates the pericarp and ramifies throughout the cross and tube cells within 5 to 10 days. Placental sac offers a niche for fungal growth that subsequently invades the endosperm and sometimes the embryo as well (Little 2000). The time sequence of infection, colonization and sporulation by grain mold fungi in relation to flowering and grain developmental stages and the phases of grain molding and grain weathering are depicted in figure 8.

Anthesis is a critical stage at which the sorghum flower is most susceptible to infection and colonization by grain mold fungi. Early infection on the apical portions of flower tissues occurs on glumes, lemma, and palea and subsequently grains are covered by mycelial growth and sporulation.

5. Grain mold management

Because the host-pathogen-environment interaction is highly complex and variable in sorghum grain mold, no single control method has been found effective. Adjusting sowing dates to avoid warm and humid conditions during flowering to grain maturity does reduce grain mold severity, but it is not realistic in most environments due to the constraint of limited growing season. Several other methods, such as application of chemical fungicides and biocontrol agents have been shown to provide some degree of protection under experimental conditions, but their effectiveness and economic feasibility in on-farm situations have not been demonstrated. Host-plant resistance, therefore, forms the major component of grain mold management,

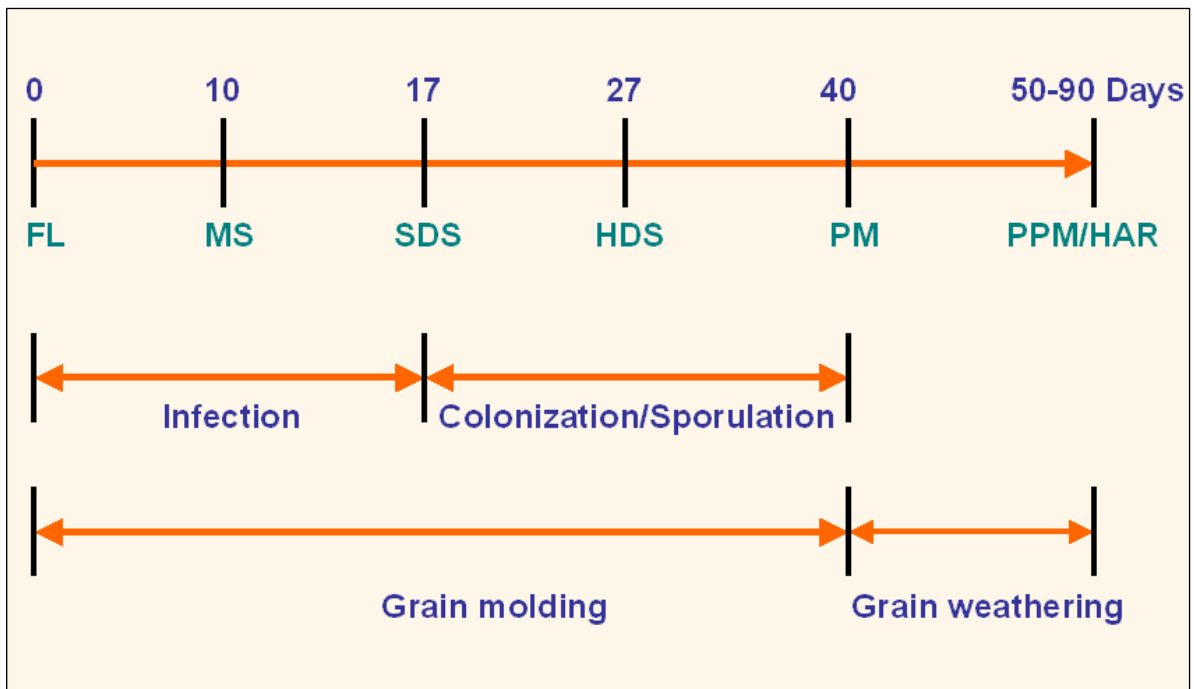


Fig. 8. A generalized time course (in days) of flowering stages and grain mold development in sorghum: FL = Flowering; MS= Milk stage; SDS = Soft dough stage; HDS = Hard dough stage; PM = Physiological maturity; PPM = Post physiological maturity; HAR = Harvesting.

and this could be complemented with other practices to help reduce the disease severity. Progress made in grain mold management through host-plant resistance and other methods is briefly discussed here.

5.1. Host-plant resistance

It is well known that variation exists for grain mold resistance in sorghum germplasm, but breeding to improve for resistance has had limited success. This is partly due to incomplete understanding of the genetics of resistance and the complex interaction of traits that influence grain mold resistance (Thakur et al. 1997; Stenhouse et al. 1998; Reddy et al. 2000). Grain mold incidence has been more severe on white-grain short-duration improved cultivars and hybrids than on colored-grain long duration cultivars. Accordingly, at ICRISAT, major research efforts have been on development of grain mold resistant hybrid parents and varieties (Reddy et al. 2005b). Screening techniques that clearly discern sorghum lines into different grain mold severity classes are critical for the success of a resistance breeding program.

5.1.1. Screening methods

Screening for grain mold resistance in sorghum has been done through field and laboratory procedures and have been modified over time. More recently, a greenhouse screening method has been developed at ICRISAT Patancheru that facilitates screening sorghum lines against individual mold pathogen under controlled conditions. These methods are described below.

5.1.1.1. Field screening

Screening for sorghum grain mold resistance at ICRISAT, Patancheru is done without artificial inoculation since sufficient natural inocula of mold fungi are present during the rainy season

over sorghum fields (Bandyopadhyay and Mughogho 1988; Bandyopadhyay et al. 1991). The screening method since has been further refined and the steps involved in screening are as follows:

- Group the test entries into different maturity groups for the convenience of comparison between entries in the same maturity groups.
- Plant each entry in 2 rows of 4m long in replicated/unreplicated designs in blocks of different maturity groups.
- Include susceptible and resistant/tolerant checks for each maturity group.
- Tag five plants with uniform flowering in each row of a 2-row plot.
- Provide sprinkler irrigation once or twice a day, 30-60 min each, on rain-free days, during the afternoon to provide high moisture levels (80–100% RH) for panicle wetness from flowering to physiological maturity (Fig. 9).
- Record the panicle grain mold rating (PGMR) at physiological maturity (*when most grains in the middle of the panicle develop black layer at the hilum*) on the tagged plants using a progressive 1 to 9 scale (see below).
- Record percent grain colonization by individual mold fungi on a panicle based on their symptoms (Figs. 4 & 6).
- Soon after PGMR recording harvest the panicles from the tagged plants, dry them under sun or in hot-air driers at 40°C for 48 h.
- Thresh each panicle and pool equal amount of grain from each panicle to make a bulk sample for each plot.
- Record the threshed grain mold rating (TGMR) by spreading the bulk grain in



Fig. 9. Sorghum grain mold nursery with sprinkler irrigation at ICRISAT, Patancheru.

the petridish with the help of a magnifying lens ($\times 10$) under proper lighting using the same 1 to 9 severity scale.

- Record percent grain colonization by individual mold fungi on threshed grain based on their symptoms (Fig. 6).
- Record both PGMR and TGMR on susceptible and resistant checks of the same maturity groups for comparison of test entries.
- Compute the data to calculate the mean PGMR and TGMR, and compare the mold severity of entries to classify them into resistant and susceptible classes.

As the grain mold severity scores are taken for each genotype at its right physiological maturity stage, this method provides a reasonable comparison between sorghum genotypes for mold reaction in the same maturity group and does not allow over scoring of early-maturing lines or underscoring of late-maturing lines. In addition, this method considerably saves the amount of water and timing of sprinkling, and

The 1 to 9 severity rating scale and disease reaction class

Severity rating scale	Percentage grain molded on a panicle	Disease reaction class
1	No mold	Highly resistant
2	1-5	Resistant
3	6-10	
4	11-20	
5	21-30	Moderately resistant
6	31-40	Susceptible
7	41-50	
8	51-75	
9	76-100	Highly susceptible

does not keep the field wet continuously for a long period, thus making the movement in the field much easy.

A significant positive correlation ($r = 0.9$) between PGMR and TGMR scores of 38 sorghum lines from 14 environments (Thakur RP et al. unpublished) suggested that grain mold severity ratings at one of the two stages,

preferably at physiological maturity—the PGMR, could be adequate to discriminate sorghum lines for resistant/susceptible reactions. The PGMR scores of sorghum lines provide more realistic reaction under field condition at the right stage of grain development than the TGMR scores, which are recorded about a week later until which time some saprophytic growth might occur on the grain. This is a significant step towards simplifying the evaluation process in terms of improving precision of data collection and economizing on time and resources. This procedure would also facilitate rapid screening of breeding materials and hence will improve the efficiency of the grain mold resistance breeding.

5.1.1.2. Laboratory screening

An *in vitro* laboratory screening technique developed at ICRISAT (Singh and Navi 2001) involves the following steps:

Isolation and multiplication of grain mold fungi:

- Surface sterilize the molded grains with NaOCl (1%) for 3 min and wash with several changes in sterile distilled water and dry these for a while.
- Transfer the sterilized grains aseptically onto oat meal agar plates.
- Incubate the plates at $28 \pm 1^\circ\text{C}$ for 7 days with 12h photoperiod.
- Isolate the most commonly occurring mold fungi, such as *Fusarium* spp, *Curvularia lunata* and *Alternaria alternata* separately onto oat meal agar for maintenance.

Multiplication of inoculum

- Soak 50g grains of a highly susceptible cultivar, such as SPV 104 in sterile distilled

water in a 150 ml conical flask for 4h and then autoclave it at 121°C and 15 psi for 15 min (Fig. 10a).

- Inoculate the autoclaved grains with each of the mold fungus separately and incubate at 28°C for 7 days (Fig. 10b).
- Shake the flasks every alternate day for uniform growth and development of fungi on the grains.

Inoculum preparation:

- Transfer 20-25 infested grains aseptically into a 50 ml beaker containing 15 ml sterile distilled water and vortex it for 2-3 min for dislodging the spores from the grains.
- Filter the spore suspension through a double-layered muslin cloth or a sterilized tea strainer (Fig. 10c).
- Adjust the spore concentration of the resultant suspension to 1×10^6 spores ml^{-1} using a haemocytometer and transfer this into an atomizer for inoculation (Fig. 10d)
- Prepare a mixture of spore suspension of the mold fungi by taking equal volume of spore suspension of each fungus and add 1 ml Tween 20™ (polysorbate 20) in 1000 ml spore suspension.

Inoculation of the test entries:

- Prepare humidity chambers in Petri plates by lining the lower lid of the petridish with a layer of absorbent cotton followed by two layers of blotting paper, and wet it with 15 ml sterile distilled water per plate.
- Sterilize the humidity chambers at 121°C and 15 psi for 15 min.
- Immerse about 100 clean and mature grains of the sorghum test lines in a 10ml mixture of spore suspension, prepared above, for 1 min.

- Pick up 25 inoculated grains and place them in a pre-sterilized Petridish humid chamber (Fig. 11a). Maintain 25 grains per plate for each of 2-3 replications for each test line.
- Incubate the inoculated grains contained in the humidity chambers at $28\pm 1^{\circ}\text{C}$ for 5 days with 12h photoperiod.

- Record mold severity of fungi colonizing individual grains (Fig. 11b) using a progressive scale of 1 to 9 as mentioned above, and also record seed germination.

The advantages of this method are that seed can be evaluated against individual fungi under controlled conditions and the evaluation



Fig. 10. Preparation of inoculum: a) autoclaved sorghum grain; b) infested grain; c) spore suspension; and d) spore suspension in an atomizer.

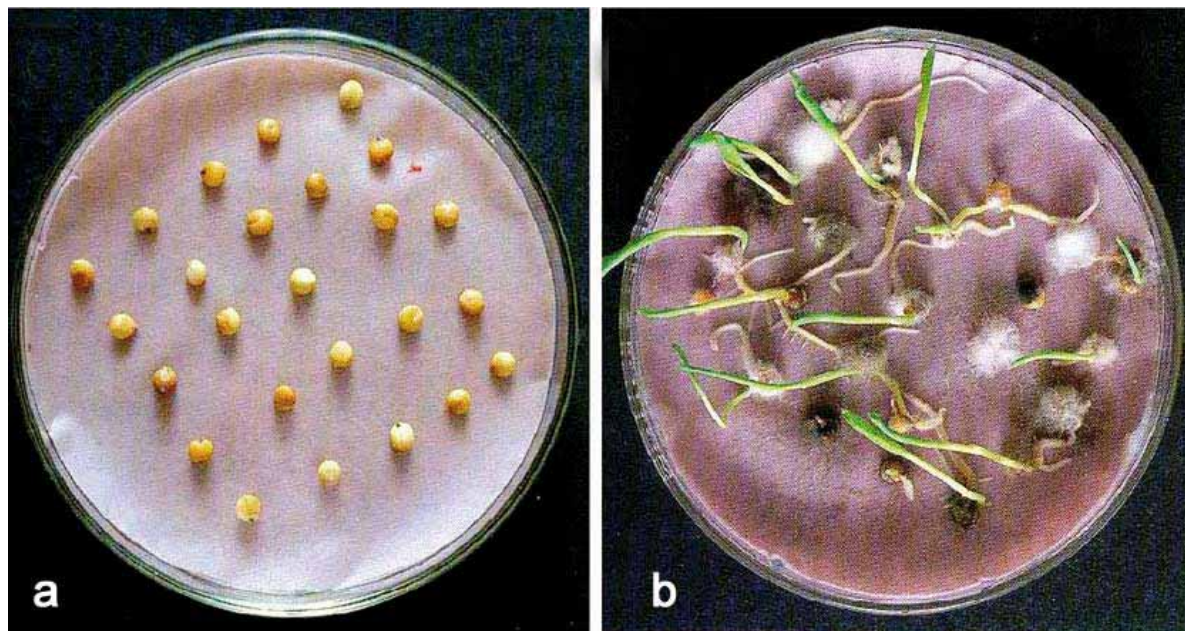


Fig. 11. In vitro evaluation for sorghum grain mold: a) inoculated grains on a blotter plate and b) grains colonized by different pathogens.

is not dependent on seasons. Using this technique several photoperiod-sensitive sorghum genotypes have been evaluated, which otherwise is difficult to test under field conditions (Singh et al. 1993). This method could be particularly useful for screening germplasm and breeding lines against specific grain mold fungi. Resistance identified by this method, however, needs confirmation on grown up plants in the field grain mold nursery or in the greenhouse by artificial inoculation.

5.1.1.3. Greenhouse screening

Identification of genetic resistance to a complex of fungal pathogens is difficult under field conditions. Histological studies have revealed variable infection patterns by different mold fungi (Forbes et al. 1992) and accordingly resistance to different fungi differ (Menkir et al. 1996). Therefore, it is necessary to identify resistance in sorghum genotypes to individual fungi, and subsequently resistances can be pyramided into a single sorghum cultivar using an appropriate breeding method. The recently developed a greenhouse screening technique at ICRISAT, Patancheru (RP Thakur and VP Rao, unpublished) offers this opportunity. This screening technique involves:

Inoculum preparation

- Soak grains of a highly susceptible line, such as cv Bulk-Y overnight and drain the excess water in the morning.
- Keep 80 g of soaked grain in each of 250 ml conical flasks and plug tightly.
- Autoclave the grains in the conical flasks at 121°C and 15 psi for 15 min.
- Inoculate the autoclaved grains with a specific mold pathogen isolate and incubate at 28±1°C for 10 days under 12h photoperiod.

- Shake the flasks on every alternate day to promote uniform growth and development of the fungus on the grains as mentioned earlier.
- 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 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 the surface sterilized seed of test lines and appropriate check lines in autoclaved potting mix (soil + sand + farm yard manure) 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/replication in a replicated experiment.
- Apply fertilizer, irrigate the pots regularly, and protect plants from insect damage by insecticide sprays as and when required.
- Transfer the pots in the greenhouse (25±2°C) when the plants are at pre-flowering stage.

Inoculation of panicles with mold fungus

- Tag uniformly flowering plants at full anthesis (>80%) in each line (Fig. 12a).
- Spray-inoculate the tagged panicles at full anthesis with the fungal suspension (Fig. 12b).
- Allow the inoculated panicles to dry for 2-3h and then provide wetness (>95% RH) by overhead foggers for 48h (Fig. 12c).

- Keep the individual fungus-inoculated plants in separate greenhouse chambers to avoid cross contamination.
- Again provide panicle wetness for 72h at physiological maturity to promote fungal growth and grain colonization.
- Record mold severity at physiological maturity (PGMR) on the tagged panicles using the 1 to 9 rating scale as mentioned earlier.
- Record percent grain colonization by individual fungi based on their symptoms (Figs. 4 & 6).
- Harvest the panicles, dry them and thresh, and record TGMR as mentioned earlier.
- Plate 25 grains per plate per replication in pre-sterilized humid chamber (blotter method), and incubate at $28\pm 1^{\circ}\text{C}$ for 5 days under 12h photoperiod to promote grain colonization.
- Record grain colonization by individual fungi (Figs. 4 & 6).
- Compute and analyze the data for comparing the resistance level of test lines against each fungal isolate.

5.1.2 Resistance sources

Following the field screening of more than 13,000 photoperiod-insensitive germplasm lines, Bandyopadhyay et al (1988) identified 156 lines, including one white-grain line having tolerance/resistance to grain mold. Further, Singh et al (1995) identified four white-grain lines as mold-resistant out of 66 *guinea*-based sorghum accessions. However, the factors associated with mold resistance in *guinea* sorghums are difficult to incorporate in white-grain lines because of poor-agronomic traits and low grain yield of *guinea* sorghums (Mukuru 1992). White-grain agronomically elite, mold-resistant lines in non-*guinea* background have



a



b



c

Fig. 12. Greenhouse screening for sorghum grain mold: a) panicles at full anthesis, b) spray inoculation of panicle and c) wet panicles after inoculation.

been reported by some workers (Singh et al. 1995; Audilakshmi et al. 1999). However, the resistance levels in these elite lines needs validation. Sorghum lines IS 14384 and CGM 39/17-2- 2 exhibited consistently high levels of resistance both to head-bugs and grain molds over years and localities in western and central Africa (Ratnadass et al. 2003).

Resistance has been found mostly in colored grain sorghums with and without tannins and also in very few white-grain sorghums (Bandyopadhyay et al. 1988; Audilakshmi et al. 2000, 2005b). However, the level of resistance is invariably more in colored grain genotypes with tannins, followed by colored grain genotypes without tannin, and much less in white- and hard-grain sorghums (Reddy et al. 2000).

Using a laboratory-based screening method white-grain photoperiod-sensitive lines, which could not be reliably screened using field-screening, resistance has been identified in *guinea* lines (Table 1) (Singh et al. 1993). However, the photoperiod-insensitive conversions of these lines have not shown the desired levels of resistance in the field test (Thakur RP unpublished). Some of the colored-grain resistance sources are IS 21599, IS 8614, IS 14388 and IS 14384 (Table 1). In a more recent study (Thakur et al. 2003a; Thakur RP, unpublished) through a multilocation, multiyear evaluation of 34 elite mold resistant B-lines, 8 B-lines with stable grain mold resistance across 14 test-environments have been identified (Table 2), which could be used in breeding grain mold resistant hybrids.

Table 1. Grain mold resistance of sorghum germplasm lines at ICRISAT, Patancheru.

Germplasm lines	Origin	Grain mold score ^a	Agronomic traits				
			DTF	Glumes color	Grain color	Grain hardness (kg seed ⁻¹)	Panicle type
IS 7173	Tanzania	1	151	Black	Straw	12.36	Loose
IS 23773	Malawi	1	112	Black	Straw	12.16	Loose
IS 23783	Malawi	1	108	Red	Straw	12.12	Loose
IS 34219	India	1	109	Straw	White	8.80	Loose
IS 7326	Nigeria	2	151	Straw	White	10.72	Semi loose
IS 4963	India	2	96	Straw	White	9.20	Loose
IS 5726	India	3	108	Straw	White	8.56	Loose
IS 4011	India	2	100	Straw	White	9.56	Compact
IS 5292	India	2	147	Straw	White	8.40	Loose
IS 21599	Malawi	1	68	Light brown	Light red	6.44	Semi loose
IS 8614	Uganda	1	56	Purple	Reddish brown	6.20	Semi compact
IS 14388	Swaziland	1	55	Straw	Reddish brown	3.72	Semi loose
IS 14384	Zimbabwe	1	57	Purple	Light red	10.64	Loose
SPV 104	India	5	67	White	White	5.08	Compact

^a Based on a 1–5 scale, where 1 = no mold; 2 = 1-10%; 3 = 11-25%; 4 = 26-50% and 5 = >50% moldy grain. DTF = Days to flowering.

5.1.3. Inheritance of resistance

The genetics of grain mold resistance has been reported to be complex, governed by major and minor genes, additive and epistatic effects with significant $G \times E$ interactions (Stenhouse et al. 1998; Audilakshmi et al. 2000).

In a recent study Audilakshmi et al (2005b) showed that grain mold resistance in the white-grain was polygenic, and additive \times additive gene interaction were significant implying that simple recurrent selection or backcrossing could accumulate the genes for resistance. The complex genetics of mold resistance is due to the presence of different mechanisms of inheritance from various sources. Evaluation of segregating population for resistance and selection for stable derivatives in advanced generations in different environments could be effective.

5.1.4. Mechanisms of resistance

Three basic mechanisms known to be involved in resistance are flower and panicle structure, grain hardness, and association of phenolic compounds.

(a) Flower and panicle structures. The morphology of flower and panicle provides a partial mechanical barrier to the entry of fungal spores into ovules and thus reduces mold severity. For example, glumes coverage and lax panicles have been shown to contribute to reduction in mold severity (Glueck et al. 1977; Mansuetus et al. 1988). However, this relationship may not be strong enough to prevent mold infection under conditions conducive for mold development (Williams and McDonald 1983; Audilakshmi et al. 1999). A strong association was found between glumes color and grain mold resistance and it could be

Table 2. Grain mold resistance stability in some agronomically elite sorghum hybrid parental lines.

Line	PGMR	DTF	Growth traits					
			Plant height (m)	Panicle type	Glumes color	Glumes coverage (%)	Grain color	Grain hardness (kg seed ⁻¹)
SGMR 08	2.2	71	1.9	SC	White	50	White	7.1
SGMR 10	2.4	79	1.8	SC	Red	25	White	5.6
SGMR 13	2.4	76	1.7	SC	Red	50	Red	4.9
ICSB 352	2.4	67	1.4	SC	Red	25	White	5.8
ICSB 363	2.1	67	1.6	C	Red	50	White	5.8
ICSB 380	2.5	72	1.7	SC	Red	50	White	6.9
ICSB 391	2.1	66	1.5	SC	Red	50	Red	6.9
ICSB 403	2.1	73	1.6	SC	Brown	50	Brown	4.5
IS 14384 R-check	1.0	73	2.6	L	Brown	90	Red	8.7
296B S- check	3.9	76	1.3	C	White	25	White	4.9
Trial mean	2.5	73	1.7	-	-	-	-	6.0
LSD ($P < 0.05$)	0.3	1.6	5.4	-	-	-	-	1.6

PGMR= Panicle grain mold rating; DTF= Days to flowering; SC= Semi compact; C= Compact, L= Loose.

possible to enhance grain mold resistance in white-grain sorghum by incorporating colored-glumes character (Audilakshmi et al. 1999; Reddy et al. 2005b).

(b) Grain hardness. Direct relationship between grain hardness and mold resistance has been shown (Menkir et al. 1996; Ghorade and Shekar 1997; Audilakshmi et al. 1999; Reddy et al. 2000). As grain mold develops under wet conditions, a key factor to consider is the ability of the sorghum genotype to retain grain hardness under prolonged wet condition. However, high degree of grain hardness is not compatible with traits required for food quality (Audilakshmi et al. 1999). Therefore, an appropriate level of grain hardness necessary for mold resistance is required in sorghum genotypes without compromising on its food quality.

(c) Phenolic compounds. A pigmented testa, where condensed tannins are present, is the most important trait conferring grain mold resistance (Esele et al. 1993). Red pericarp, where flavan-4-ols are located, also confers resistance to grain mold, but not as strongly as pigmented testa. Pigmented testa and red pericarp, when combined, provide additive effects on resistance (Esele et al. 1993). However, not all sorghums with red pericarp are resistant to grain mold. The associations of flavan-4-ols and tannins with grain mold resistance have been demonstrated in cultivars with colored pericarp and with pigmented testa (Jambunathan et al. 1990; Martizen et al. 1994; Melake-Berhan et al. 1996). However, neither flavan-4-ols nor tannins are found in white-grain sorghum cultivars grown extensively in India for food. Therefore, grain hardness, and perhaps colored glumes are the only factors that contribute to mold resistance in white-grain cultivars.

Alternatively, removal of red pericarp from testa-less cultivars by dehulling may allow the use of red sorghum as food since the dehulled sorghum product can be milled into white flour. Mold-resistant red sorghums can be readily used as animal feed since they do not contain condensed tannins, are equally nutritious as white sorghum, and are likely to be safe with respect to mycotoxins because of mold resistance.

5.1.5. Breeding for grain mold resistance

Breeding for grain mold resistance at ICRISAT and elsewhere began many years ago but the progress has been rather slow because of the association of several plant traits with resistance.

Several breeding methods, including pedigree selection, backcrossing, and population breeding have been tried to generate grain mold resistant inbreds and varieties and populations. Analysis of efficiency of various crossing methods indicated the superiority of biparental matings followed by pedigree selection in breaking linkages between undesirable plant traits and grain mold resistance (Reddy et al. 2000). At ICRISAT, Patancheru breeding for grain mold resistance has received major emphasis for white-grain sorghum as white grain is most preferred for human consumption in Asia. A number of populations, varieties and inbred lines have been developed at ICRISAT that are briefly described below.

Development of varieties and R-lines. White-grain *Zerazera* germplasm accessions from Sudan and Ethiopia that were less susceptible to grain mold under natural conditions and possessed desirable food quality were used to develop a variety CSV 4. This variety was

subsequently used as a restorer parent of several hybrids (CSH 5, CSH 6 and CSH 9) and as one of the parents in the development of varieties (CSV 9, CSV 10 and CSV 11). This variety is still being used as one of the parents in crossing program at ICRISAT to generate genetic variability for grain mold resistance.

Several *Zerazera* germplasm accessions and their derivatives, and high yielding and adapted lines, such E 35-1, CS 3541, SC 108-3, SC 108-4-8 and SC 120 were used extensively in crosses to widen the genetic base of grain mold resistant lines (Murthy et al. 1980). Pedigree selection and multilocation testing resulted in identification of a number of elite grain mold tolerant lines some of which were used to develop high yielding grain mold resistant varieties. Notable among these are: SEPON 77 (released as Nica-Sor) in Nicaragua in 1985; M 90038 (released as SEPON 82) in Niger in 1993; SEPON 82 × S 34 (released as Sorvato 1) in Togo in 1998.

A number of grain mold resistant dwarf early lines with semi-compact heads and *guinea* grain and glumes traits were developed through pedigree breeding (Stenhouse et al. 1998). Some of the *guinea* type selections, such as ICSV 95055, ICSV 95046, ICSV 95068 and ICSV 95068 had good level of grain mold tolerance and comparable grain yield of a commercial variety ICSV 112 (Reddy et al. 2000).

A grain mold resistant population was developed by incorporating white-grain mold resistant breeding lines, color-grain mold resistant lines, and high yielding improved lines into *ms*₃ (genetic male-sterility inducing gene). Two bulks of this population US/R and US/B maintained at ICRISAT have undergone half-sib family selections and two

cycles of random mating with several *guinea* lines incorporated (Reddy et al. 2006). This population is currently being maintained at ICRISAT, Patancheru and is shared with NARS partners for deriving mold resistant hybrid parents and varieties.

Development of grain mold resistant hybrid seed parents. Several grain mold resistant germplasm lines, such as IS 2501, IS 2815, IS 3436, IS 10288B, IS 10475B, IS 10646, IS 21599, and IS 23585 that were mostly photoperiod sensitive and had colored grains were crossed with grain mold susceptible, but high-yielding maintainer lines (*A*₁ cytoplasm) such as ICSB 11, -17, -37, -42, -51 and -70. Selections were carried for high heritable traits, such as days to flowering, plant height, and grain color in *F*₂, *F*₃s and advanced generations. Family-based selection for grain mold resistance under high mold pressure and individual plant-based selection within the resistant families allowed to identify grain mold resistant lines and those with maintainer reaction were converted into male-sterile lines (Fig. 13). Of 58 grain mold resistant seed parents with *A*₁ cytoplasm developed of which 35 had white grain, 20 red-grain, and three brown-grain (Reddy et al. 2005a). Of the eight resistance sources used, IS 2815 contributed to nearly 50% of the derivatives. For detailed data on various agronomic characters and pedigree of these grain mold resistant seed parents, visit the website: <http://www.icrisat.org/text/research/grep/homepage/sorghum/breeding/main.htm>. Also, the grain mold resistance sources, IS 9470 with *A*₁ (*milo*), *A*₂, *A*₃, and *A*₄ (*maldandi*), and IS 15119 with *A*₃ and *A*₄ (*maldandi*) cytoplasm have been converted into male-sterile lines.

Since 2000, efforts have been made to develop grain mold resistant hybrid seed parents in bold white-grain and elite agronomic background.

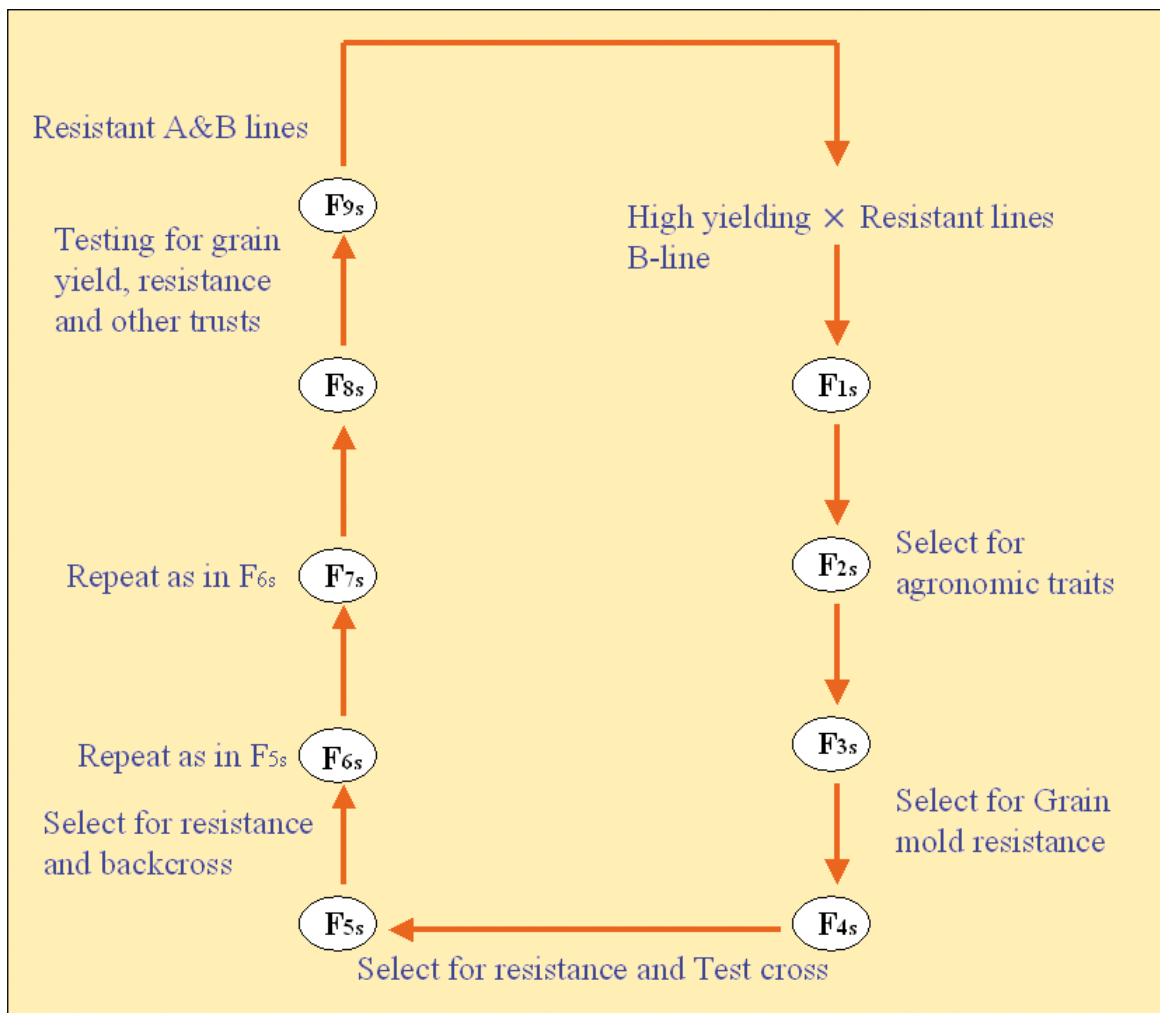


Fig. 13. Scheme of a breeding method used for developing grain mold resistant hybrid parental lines in sorghum at ICRISAT, Patancheru.

Pedigree selection for grain mold resistance among segregating progenies derived from the crosses between resistant lines (ICSB 383, ICSB 392, ICSB 403, IS 8614, IS 13817, IS 10646, IS 25060, IS 21599 and IS 23585) and high yielding bold grain B-lines (ICSB 11, ICSB 37, ICSB 42, ICSB 70 and ICSB 101) and subsequent conversion of resistant lines with maintainer reaction into male-sterile lines led to the development of 43 promising A/B-lines. Evaluation of 28 single-plant selections from these 43 promising B-lines during 2003

rainy season resulted in identification of three white-grain lines with pigmented (red) glumes - SGMR 3-2, SGMR 7-1 and SGMR 7-2 with moderate resistance levels (Reddy et al. 2005b). These could be used in breeding programs in developing grain mold resistant hybrid parents and varieties.

Several high-yielding mold resistant A-/B-lines in white-grain background and with white glumes, such as ICSB 353, ICSB 362, ICSB 368, ICSB 379 and ICSB 402 and a variety, PVK 801 have been developed.

Development of grain mold resistant hybrids.

Resistance to grain mold has been shown to be either dominant or over dominant in crosses involving susceptible red-grain A-lines (from Texas A&M) and mold resistant white-, red-, and brown-grain R-lines (from ICRISAT, Patancheru). However, the resistance levels were lower in white-grain hybrids than those in colored-grain hybrids. In some cases, the hybrids were resistant even though the parents themselves were susceptible indicating the possibility of different resistance mechanisms and dispersal of favorable genes in different parents with complementary effect in their hybrids. These inferences are supported by recent studies on the relationship between resistant (R)/susceptible (S) hybrid parents and their hybrids. The study indicated a greater possibility of producing grain mold resistant hybrids from crosses of R × R, R × S, followed by S × R than those from S × S (Table 3). It is speculated that the diverse and complementary mechanisms, each with small effects, may be acting synergistically in hybrids leading to higher levels of resistance in some crosses. Thus, it may be worthwhile to breed both the hybrid parents for grain mold resistance for developing resistant hybrids.

Way forward to breeding for grain mold resistance. Present day breeding for disease resistance is a collaborative effort of pathologists, molecular biologists, and breeders working across locations and regions. In parts of Africa, where hard, white-grain is preferred in food preparation the traits associated with *guinea* sorghums, such as hard corneous endosperm, open panicles, and extensive glumes coverage could be exploited. Colored grain sorghums could increasingly be used for feed and beverages as being used in western Africa. Greater emphasis is needed on both nuclear genetic and cytoplasmic diversification

Table 3. Distribution of grain mold resistant sorghum hybrids in different cross categories.

Cross category	No of hybrids	No of hybrids with mean PGMR ¹ and TGMR ¹ score ≤4.0
R × R	24	24 (100) ²
R × S	60	42 (70)
S × R	24	16 (67)
S × S	60	13 (22)

¹Panicle grain mold rating (PGMR) and threshed grain mold rating (TGMR) scored using a 1–9 scale, where 1 = no mold and 9 >75% grain molded.

²Percent of hybrids.

of hybrid parents for grain mold resistance address the concerns on narrow genetic base of the improved hybrid parents and varieties. The modern tools of biotechnology, such as QTL analyses and marker-assisted selection provide new opportunities to enhance grain mold resistance. Identification of DNA markers for antifungal proteins could be useful if they are deployed against fungal pathogens with which they have not evolved. Flavan-4-ols in red colored grain (without testa), and tannins and flavan-4-ols in colored grains (with testa) are strong and stable mechanisms that could be exploited.

5.2. Other methods of management

5.2.1. Biocontrol

Among the bioagents tested for antagonistic property against the grain mold pathogens *Trichoderma viride*, *T. harzianum* and *Pseudomonas fluorescens* showed promising results both at laboratory and at field level. Other bioagents *T. hamatum* and *T. koeningii* performed fairly well in checking the growth of the major mold pathogens (Indira et al.

2004). The bioagents *P. fluorescens*, *T. viride* and *T. harzianum* enhanced the germination in the mold infected seeds, and the effect was superior to chemical seed treatment. The bioagents were equally efficient in enhancing the vigour of the seedlings obtained from mold infected seeds, which otherwise would be stunted, abnormal and infected (Indira et al. 2004). However, its economic and technical feasibility for on-farm application is very limited.

5.2.2. Chemical control

Application of chemical fungicides appears to provide some degree of protection against grain mold, but it is neither practical, because of wet weather conditions during grain maturity, nor economical except for small fields with valuable sorghum lines that need to be protected. Most studies on the efficacy of fungicide sprays in controlling grain mold have been limited to research stations (Somani et al. 1995) and no practical use has been demonstrated.

5.2.3. Avoidance

Avoidance of grain mold has been the most important traditional control strategies. In areas where photoperiod-sensitive cultivars are grown, grain mold is avoided because flowering and grain filling occur after the end of rainy season. Grain mold is avoided in seed production plots as the crop is grown with irrigation in dry season. Avoidance can be practiced either by delaying sowing dates or by growing medium- to late-maturing cultivars such that the grain filling and maturity stages occur after end of the rains. Sowing date adjustments may require irrigation, which is not always available to resource-poor farmers. Delayed flowering can also lead to complications, e.g., severe attack by insect pests such as head bugs (*Calocoris angustatus*)

and midge (*Stenodiplosis sorghicola*). Head bugs accentuates the mold incidence (Sharma 1993; Ratnadass et al. 2003). Seed germination is influenced by earhead bug and grain mold (Indira et al. 1991).

5.2.4. Timely harvesting and drying

Harvesting sorghum at physiological maturity (black layer formation at the hilar end of the grain) helps prevent saprophytic growth of mold fungi and reduces damage. Drying grain immediately after harvest to about 10% moisture level prevents mold development in storage. However, drying will be only practical if economics favor such a method. Commercial drying of sorghum will become important only if the demand increases and there is a premium for clean grains.

In a recently conducted study on farmers' fields (Audilakshmi et al. 2005a) it has been shown that harvesting of rainy season sorghum at physiological maturity and artificially drying significantly reduced grain mold infestation and grain deterioration compared to those harvested at normal maturity and dried under sunlight. Produce from harvest at physiological maturity and artificially dried crop had much better quality and fetched a market price 55% more than that harvested at normal maturity. A minimum of 15 ha of sorghum crop area was necessary to cover the cost of the artificial drier in a season. The advantage of the technology is that it gives a remunerative price to the farmer for the better grain quality and facilitates early arrival of the sorghum in the market. This also helps early planting of the second crop in the post rainy season to better utilize the residual moisture.

5.2.5. Dehulling

In several sorghum hybrids that have endosperm resistance to mold fungi, the pericarp becomes discolor under moderate mold pressure.

Mechanical dehulling can be used to remove pericarp to get rid of grain discoloration to enhance the market price (Geervani and Vimala 1993). The value addition to moldy grains with linkages with sorghum processors is a researchable issue and needs immediate attention.

5.3. Integrated disease management

Integrated management of grain molds in sorghum involves: growing mold resistant/tolerant cultivars; harvesting the crop at physiological maturity and quick drying to bring down the grain moisture content to around 10%; storing grains under proper storage conditions; adjusting sowing time, if feasible, to avoid flowering and maturity in heavy rains; and need based sprays with tilt (chemical) and biocontrol agents where feasible.

6. Alternative uses of moldy grain

Due to price advantage, moldy grains find application as raw material in several industrial sectors. Brewing industries use moldy and germinating sorghum for making beer and whisky (Sheorain et al. 2000) thus offer new market opportunities for molded sorghum. Mold infection during germination can exhibit slightly higher α -amylase activity compared to healthy grain suggesting that moldy grain may be better suited for malting (Satish Kumar et al. 1992). As a follow up of the Government of India policy announcement of blending conventional fuel with ethanol from sugarcane and other alternate sources, efforts have been made at ICRISAT to identify inbred lines and develop hybrids that can provide high levels of alcohol (Reddy et al. 2005c). Uptake of molded grain by the industrial sector helps

generate cash income for farmers, and reduces entry of molded sorghum grain directly into the diets of the poor. The research process to enhance the efficiency of industrial use of grain and to encourage adoption of other post harvest methods to reduce grain mold should consider the socioeconomic, operational, and institutional framework of the target group in addition to the technical aspects.

7. Technology transfer

A number of scientists from National Agricultural Research System (NARS) from Asia and Africa have been imparted short- and long-term training in sorghum grain mold screening and breeding for grain mold resistance. This has been done by training research scholars, research fellows, visiting scientists, and apprentices. In addition to training and sharing strategic and applied research information and technologies ICRISAT has been supplying seed of grain mold resistant/tolerant lines, breeding materials, trials/nurseries on request to scientists and researchers world over. Grain mold field screening method has been widely adopted in India and in several countries in western and central Africa.

Through the ICAR-ICRISAT partnership sorghum grain mold nurseries conducted at several locations in India for several years, sorghum scientists in India have immensely benefited by selecting the lines suitable for local adaptation. These nurseries have also helped identify major grain mold causing fungi predominant at a given location and select stable grain mold resistant lines for use in breeding programs, which in turn have improved the efficiency and pace of both in-house and NARS grain mold resistance improvement programs.

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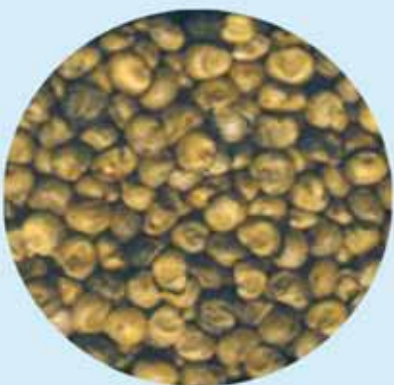
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Addendum

For details on identification of different species of *Fusarium* one can refer to:

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The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) is a nonprofit, 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 Future Harvest Centers of the Consultative Group on International Agricultural Research (CGIAR).

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