Sorghum Grain Mold: Current Status

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Introduction

Grain mold is an important biotic constraints of sorghum, and seriously compromises the grain yield and quality gains obtainable from improved cultivars. This disease gained worldwide significance after the widespread introduction and cultivation of short-duration sorghum cultivars that replaced longer-duration landraces and local cultivars. The newer cultivars matured and set seeds under wet and humid conditions that were favorable for the development of grain mold fungi. Damage caused by grain mold includes losses in seed yield, quality, market value, storage quality, viability, and food and feed processing quality of seed. In addition, several mold-causal fungi produce mycotoxins in grain. Mycotoxins in feed slow the growth rate, predispose animals to other infections, and are teratogenic and carcinogenic.

Due to its importance, grain mold research has been an integral part of sorghum research in several national programs and at ICRISAT In addition, sorghum researchers in CLAN member countries established a working group on grain mold in 1993. ICRISAT's research on grain mold has evolved over time and can be described in three phases: 1973 to 1990, 1991 to 1996, and 1997 and beyond.

Research Progress up to 1990

During the first phase (1973 to 1990), the major mold-causal fungi were identified — *Fusarium moniliforme, F. pallidoroseum, Curvularia lunata,* and *Phoma sorghina.* These mold fungi were shown to infect and colonize grain from flowering until grain maturity. The deleterious nature of moldy grains in food processing was quantified in collaborative studies by biochemists and pathologists. Initially, resistance-screening activities were conducted in the field using sprinkler irrigation and inoculation and bagging of panicles.

Several lines with putative resistance to grain mold were identified (IS 18758 and IS 14332). These lines were used in a breeding program to develop high-yielding, mold-resistant sorghum cultivars. However, high levels of mold resistance in high-yield backgrounds could not be combined. Later, the field screening technique was simplified to evaluate mold resistance in germplasm and breeding lines. The modified technique involved wetting panicles of plants from the flowering stage up to two weeks after grain maturity. Panicle inoculation was not necessary since aerobiological studies showed that abundant spores of mold fungi were naturally available to infect developing grains. Studies on headbug grain mold interaction showed that headbug damage increases mold damage, even in mold-resistant genotypes. Using the field

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screening technique, more than 13 000 germplasm lines were screened and 156 lines identified as mold resistant. All mold-resistant lines had colored grain with the exception of IS 25017 (white grain). Most of the colored-grain lines had a testa layer, but five colored-grain lines were devoid of testa and were red in color. Nearly 50 of these resistant sources were evaluated in the International Sorghum Grain Mold Nursery for 7 years. Most of these test lines showed stable resistance across locations and years. Tannins, flavan-4-ols, and grain hardness were shown to be the three major factors associated with resistance. Tannin and flavan-4-ols were associated with resistance in genotypes with a colored pericarp and pigmented testa. Flavan-4-ol was the primary determinant of resistance in the colored-grain genotypes that lacked a testa layer. In the white-grain genotypes, tannins and flavan-4-ols were absent, and grain hardness was the major factor associated with resistance. Several of these lines were used in another mold resistance breeding program to determine whether resistance from the colored grain could be transferred to elite, white-grain backgrounds. While it was possible to obtain white-grain, mold-resistant lines, the yield levels of these new breeding lines were not high.

Research Progress, 1991-96

One drawback of field screening is that it can be conducted only once in a year during the rainy season. Therefore, an in vitro screening technique was developed in which mature threshed grains were inoculated with a spore suspension of individual mold fungi, then incubated in a moist chamber for 5 days, and evaluated for mold incidence and severity. Using the in vitro screening technique, mold evaluations can be separately done for individual mold fungi at any time of the year. This is in contrast to field screening, where it is difficult to partition the severity of individual mold fungi. In this phase, a major effort was directed towards identification of sources with high levels of mold resistance in white-grain backgrounds. A large number of lines from an ICRISAT sorghum conversion program and photoperiod-sensitive lines were screened using field screening and/or *in* vitro methods. Several photoperiod-sensitive sorghum lines with high levels of resistance and converted lines with moderate levels of resistance were identified. The likely mechanism associated with resistance in the photoperiod-sensitive lines is the presence of antifungal proteins. The mold-resistant converted sorghum lines had short grain-filling periods. These lines were used in another mold resistance breeding program. Also, a separate seed parent breeding program was initiated to develop high-yield potential, mold-resistant, red-grain (without testa) A, B, and R lines using colored-grain mold resistance sources identified during the first phase. Because of their poor agronomic characteristics, the photoperiod-sensitive lines were difficult to use in the breeding program. However, it was possible to develop the desired red-grain F_1 hybrid seed parents and varieties with mold resistance. In mold-endemic areas these cultivars have potential for use as feed, locally or for export.

Research Progress in 1997, Future Strategies

Two major research gaps that still remain are: inadequate levels of resistance in high-yielding white-grain sorghum and lack of epidemiological information on the relation-

ship between climatic factors and grain mold incidence and severity. Research on these two areas began in 1997. Bioassay methods to determine toxicity of antifungal proteins have been developed using purified antifungal protein extracts from mold-resistant genotypes. Relative humidity above 98% was essential for sporulation of *F. moniliforme,* but the humidity requirement and duration varied for different mold fungi. In field experiments, it was found that panicle wetness at or after maturity is favorable for mold development.

Conventional breeding methods have not been successful in developing white-grain, mold-resistant lines in high-yield backgrounds. This is because grain mold is caused by multiple pathogens and variable fungal species. Also, the environment heavily influences the expression of resistance. Several quantitative traits influencing resistance have been identified. Therefore, it is desirable that a combination of conventional and biotechnology approaches is followed to understand the mechanisms of resistance and then use molecular tools to transfer resistance. Antifungal proteins defend plants against plant pathogens. Among the antifungal proteins, chitinases, ß-glucanases, sormatins, and ribosome-inhibiting proteins are noteworthy in inhibiting fungal growth. Chitinases have been found in mold-resistant sorghums and their overexpression or production of more effective forms in grains may be beneficial. Genes encoding chitinases have been identified. Transformation of sorghum with engineered promoters and coding regions for chitinases may assist in the development of resistant varieties. Initial work on antifungal proteins, in collaboration with Texas A&M University, USA, will center around the insertion and expression of chitinase and glucanase genes from *Trichoderma* species into *E. coli,* extraction of these proteins, and testing of fungitoxicity against the predominant mold fungi. Later, transformation systems with several candidate genes will be developed. A second approach will focus on the development of molecular markers for several quantitative trait loci (QTL) that can increase the efficiency of conventional breeding methods. Research on this aspect has begun at Texas A&M University, but there is scope for using different and multiple parents to develop mapping populations for the marker study. Several candidate parents are being identified and there is excellent scope for partnerships between the national programs, advanced research institutes, and ICRISAT.

Epidemiological information on the role of climatic factors in mold development would assist in two ways. Firstly, to refine in vitro screening techniques, particularly to assess mold resistance under variable disease pressure. Secondly, to develop risk assessment models that can aid the breeding process and the deployment of cultivars of desired maturity that can escape high mold pressure on one hand and terminal drought on the other. Future epidemiology research needs are to determine the need of free water for infection; study the effect of temperature, relative humidity, and wetness on sporulation; and determine the effect of wetness at different post-flowering stages on mold development. Using the data, it should be possible to develop models to predict mold development and to characterize sorghum-growing regions for mold risk. There is scope for collaborative research in this area, particularly for model development and risk assessment.