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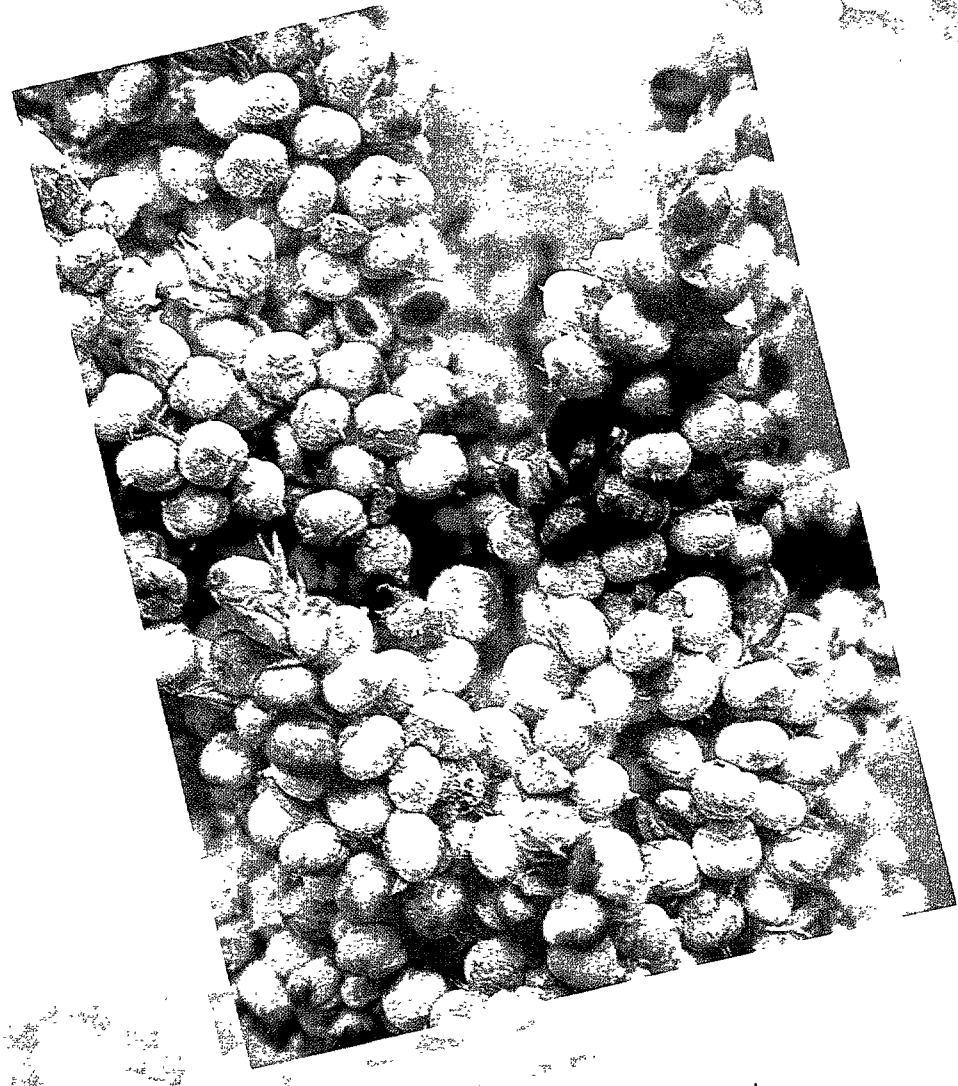
Technical and Institutional Options for Sorghum Grain Mold Management



International Crops Research Institute for the Semi-Arid Tropics

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Abstract

Sorghum grain mold constitutes one of the most important biotic constraints to sorghum improvement and production worldwide. It is estimated that annual economic losses in Asia and Africa as a result of grain mold are in excess of US$ 130 million. The poverty implications include loss of access to food, exposure to health risks through contaminated food, and income losses through lower prices. The papers in this volume review recent advances in biochemical and genetic studies, as well as recent institutional developments in the sorghum utilization sector. The opportunities these developments present and the potential for impact on the poor is discussed. Recommendations are discussed under 3 themes. The first theme recommends further work to de-link grain hardness and antifungal properties and identify resistance genes. This needs to be underpinned by the development of molecular markers. The second theme recommends that grain mold needs to be tackled as part of a cluster of quality related issues that are important to industrial users of the crop. New marketing institutions, such as contract growing, also need to be explored. The third theme focuses on the need for networking activities to link together public research with private sector activities related to market development. Stronger markets for sorghum are essential to maintain the crop in the farming systems that the poor depend on for food, fodder, and employment. An approach that combines technical and institutional innovations could provide enormous benefits by bringing the power of science to bear on the livelihoods of the poor.

Résumé

Options techniques et institutionnelles pour la lutte contre la moisissure des grains de sorgho: comptes rendus d'une consultation internationale, 18–19 mai 2000, ICRISAT, Patancheru, Inde.
La moisissure des grains de sorgho constitue la plus importante contrainte biotique au développement et à la production du sorgho à travers le monde. On estime la perte économique annuelle en Asie et en Afrique résultante de la moisissure des grains à plus de 130 millions de US$. Les incidences sur la pauvreté incluent la perte d'accès à la nourriture, l'exposition aux risques alimentaires à travers la nourriture contaminée, et les pertes économiques dues à la chute des prix. Les articles de ce volume dressent le bilan des récentes avancées dans les recherches génétiques et biochimiques, ainsi que les récents développements institutionnels dans le secteur d'exploitation du sorgho. Les opportunités que ces progrès représentent et le potentiel d'impact sur la pauvreté sont ici examinés. Les recommandations sont discutées selon trois thèmes: Le premier thème recommande des travaux supplémentaires pour confondre la dureté de la graine des propriétés anti-fongiques, et d'identifier les gènes résistants. Cela a besoin d'être étayé par l'utilisation des marqueurs moléculaires. Le second thème recommande d'inclure la moisisssure de la graine dans l'ensemble des critères de qualité qui importent aux utilisateurs industriels de cette culture. De nouvelles institutions de marketing, ainsi que de nouveaux contrats de culture, ont également besoin d'être explorés. Le troisième thème se focalise sur la nécessité pour les activités en réseau de lier recherche publique et secteur privé afin d'améliorer le marché. De plus solides marchés pour le sorgho sont essentiels au maintien de la culture dans les systèmes d'exploitation dont la masse pauvre dépend en nourriture, fourrage et emploi. Une approche qui combine les innovations techniques et institutionnelles pourrait apporter d'énormes bienfaits, en faisant s'appuyer les moyens d'existence des pauvres sur la science.

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Technical and Institutional Options for Sorghum Grain Mold Management

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ICRISAT Research Strategy in the Medium Term

Rodomiro Ortiz[1]

Looking to the future, the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) believes that any center of the Consultative Group on International Agricultural Research (CGIAR) needs to refocus on its comparative advantages as an international, nonprofit, apolitical, scientific organization. We think that ICRISAT researchers are uniquely positioned to bridge technology gaps between north and south, and south-south. They are well suited as trusted brokers in mediating complex science-based tradeoffs, e.g., for genetic resources and natural resources, and they are ideal catalysts of new scientific initiatives that address international problems, which lie beyond what individual national agencies can handle alone.

Like all CGIAR centers, ICRISAT also recognizes the need to more directly link its work to impacts on reducing poverty for the poorest of the poor. We describe this approach as 'Science with a Human Face'. We are increasing our investment in socioeconomics research to more fully understand the nature of poverty in the semi-arid tropics (SAT), and how technological interventions can more effectively relieve it. We are emphasizing an impact culture so that all scientists recognize the urgency of following their work through until it benefits the poor.

As per the CGIAR mission to alleviate poverty, sustain food security, and protect the environment, ICRISAT does targeted research-for-development (R-D) with a very broad range of partners in:

- Genetic conservation and enhancement;
- Natural resources management; and
- Socioeconomics and policy.

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Ortiz, R. 2000. ICRISAT research strategy in the medium term. Pages 1–6 *in* Technical and institutional options for sorghum grain mold management: proceedings of an international consultation, 18–19 May 2000, ICRISAT, Patancheru, India (Chandrashekar, A., Bandyopadhyay, R., and Hall, A.J., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

Our research aims to help the most disadvantaged people living at the beginning of the new millennium in this modern but contrasting world. Hence, we expect that investments for R-D in SAT will allow the rural poor of today to have a brighter future with the adoption of emerging technology ensuing from ICRISAT research. Our philosophy in R-D considers a "small landholder development trajectory" from subsistence to commercial scale (Fig. 1), in which we consider that the farmers are not homogeneous and that research products should help them to move along the trajectory. Hence, scientists of the Genetic Resources and Enhancement Program have an array of products to offer. Low input environments require a yield stabilizing technology, whereas matching technology to achieve high yield potential should be developed for high input environments. Such a moving target needs to be addressed by a heterogeneous, but dynamic moving strategy, which often changes at a given point of time. Our scientists along this trajectory are using ICRISAT research tools for development. In this way SAT farmers may move from marginal agriculture to an improved system.

ICRISAT has been allocating resources for developing its research agenda using a demand-driven method that looks for efficiency, equity, sustainability, and impact. Targeting the poverty domain remains a problem in R-D aiming poverty alleviation, especially if the selected geographic area

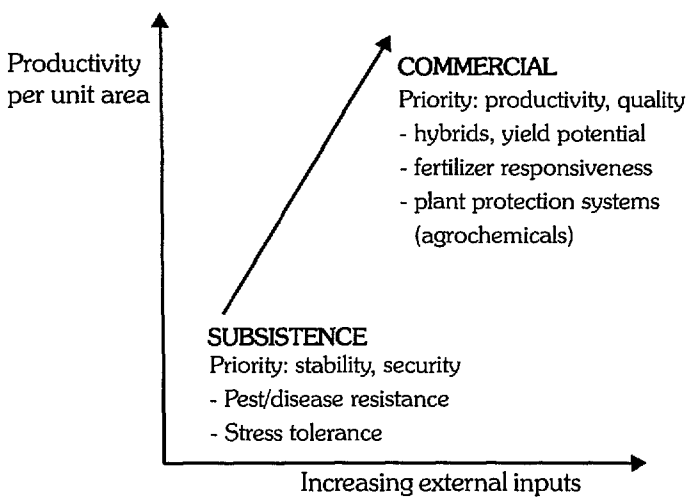

Figure 1. Smallholder development trajectory.

does not match 100% with ICRISAT "preferred poor clients", i.e., non-target clients may obtain benefits or the poor people living in SAT may be missing these benefits. ICRISAT should define few areas because large domains are ineffective for targeting poverty alleviation. Targeted investment in a few areas seems to be more rewarding than large funding for many areas.

To achieve success in its endeavor, research at the Genetic Resources and Enhancement Program of ICRISAT is demand-driven and has a problem-solving orientation. The keys for success, keeping in mind the people who are working or will be working with us are:

- Enhanced communication and interaction;
- Clear and decentralized organizational structure;
- Appropriate working and corporate culture;
- Research planning and transparent budgets;
- Staff accountability through milestones or agreed standards such as publication outputs, fund raising, technology exchange, resource management, and public awareness campaign; and
- Incentives for performers.

Partnerships

While bringing new partners [(e.g., private sector or non-government organizations (NGOs)] to implement together our R-D agenda, ICRISAT researchers are still building synergy with national agricultural research systems (NARS) and keeping joint strategic research with advanced research institutes. A proactive role was taken by ICRISAT Management since 1999 to attract the investment of the private seed sector to support the genetic enhancement of cereal crops such as sorghum (*Sorghum bicolor* (L.) Moench) and pearl millet (*Pennisetum glaucum* (L.) R. Br.). Already, support from the largest Indian seed company, the Maharashtra Hybrid Seeds Company Limited (MAHYCO) was obtained few years ago to support breeding of pigeonpea (*Cajanus cajan* (L.) Millsp.) hybrids. In the first quarter of 2000, we received positive responses from the private seed sector (about 50% of those who were approached by ICRISAT) for breeding sorghum and pearl millet and some of them have already provided their funding as per

signed project document, in which clearly it was agreed that any product ensuing from the research they are supporting will remain as international public goods.

Information Management and Knowledge Sharing

In this era of globalization and mass flow of information, any center of excellence needs to be perceived as freely sharing knowledge with their stakeholders. ICRISAT has started its work in this field to become in the short-term the organization that provides the right information timely and share knowledge freely about SAT agriculture. Likewise, ICRISAT has taken a pro-active role in "learning-by-doing" as the new means of providing training to our partners in R-D.

Applied Genomics for Research-neglected Crops of the Semi-arid Tropics

Molecular markers are descriptors that offer reproducible results for characterizing genotypes. Genomics refers to the investigations of whole genomes by integrating information provided by molecular markers with informatics. Applied plant genomics improves the understanding of crop gene pools, which are being enlarged by including transgenes and "native" gene pools that are becoming available through comparative analysis of plant biological repertoires. Nowadays, molecular markers are being used to tag specific chromosome segments bearing the desired gene(s) to be transferred (or incorporated) into the breeding lines (or populations). Furthermore, finding new genes adds value to traditional agricultural products. Genetic resources available in gene banks are still the best source for a routine gene discovery but this work will be facilitated by gene databases assembled with the aid of applied plant genomics, which can also accelerate the utilization of available genes through transformation or meiotic-based breeding methods.

Genomic research, which began receiving major emphasis at ICRISAT in 1999, continues with its implementation. A new staff joined in April 2000 to lead the Applied Genomics Laboratory at Patancheru, India and to coordinate the new center-project in the subject, which was included in

ICRISAT's Medium-Term Plan (MTP) 2001–03. A significant capital investment was allocated for 2000 to acquire high-throughput lab equipment for genomics at ICRISAT. New polymorphic markers are being identified or mapped in pearl millet, sorghum, chickpea (*Cicer arietinum* L.), and groundnut (*Arachis hypogaea* L.), and gene flow will be investigated with molecular markers in pigeonpea.

ICRISAT has attracted restricted funding for pearl millet genomics and gene flow investigations with deoxyribonucleic acid (DNA) markers in pigeonpea with partners in the UK, for mapping drought tolerance taking advantage of sorghum-rice (*Oryza sativa* L.) gene synteny with Indian partners, and very likely from 2001 onwards for the rapid crop improvement of sorghum, groundnut, and chickpea with DNA-marker technology with NARS in Asia. ICRISAT with partners in USA will start in 2000 a new project for the identification of molecular markers for cultivated and wild groundnuts. We will be also working in mapping genes for fodder and stover quality in cereal and legume crops of the SAT. A new initiative to obtain funding from Aventis, USA for chickpea genomics was undertaken together with the International Center for Agricultural Research in the Dry Areas (ICARDA), Syria and with the assistance of the CGIAR Secretariat. Discussions are under way between Aventis and the three research partners that include Avesthagen Graine, a not-for-profit biotechnology provider from Bangalore, India.

Sorghum Grain Mold Management and the Potential Impact on the Poor

The introduction of high-yielding rainy season (kharif) sorghum hybrids in India over 20 years ago has led to dramatic increases in grain yield (from 587 kg ha^{-1} in 1970 to 1407 kg ha^{-1} in 1996/97). High levels of adoption of hybrids by farmers occurred in the major sorghum-growing state Maharashtra (about 80% of rainy season acreage). Despite this achievement, grains of hybrid sorghum suffer from infection and colonization by several fungi towards the end of the rainy season. This infection results in moldy growth often referred to as "blackening". The fungi are *Fusarium moniliforme* Sheld., *Alternaria alternata* (Fr.) Keissler, and *Aspergillus flavus* Link. These fungi have been shown to produce such harmful mycotoxins as zearalenone, fumonisins, aflatoxins, alternaria toxins, and ochratoxins.

ICRISAT in partnership with Indian researchers, and with the funding support from the Department for International Development (DFID), UK started a project aiming to assess the potential for developing integrated pre- and postharvest strategies for sorghum grain mold management in the semi-arid tropical production and utilization systems, and to suggest recommendations to address priority needs. The immediate beneficiaries will be donors and policy organizations engaged in planning and funding strategies to deal with sorghum grain mold, which will also include the private brewing, poultry, and seed sectors.

ICRISAT and its partners expect that the key stakeholders and ultimate beneficiaries of this research will be the resource-poor producers and consumers of rainy season sorghum whose access to safe and affordable food will be assured and whose produce will find expanding markets. We believe that sorghum needs from the industry will raise the demand of a commodity that is primarily grown by small and marginal farmers. The increased demand from the private sector will commercialize the crop and enhance the livelihood of the poor by raising cash income.

Technical and Institutional Options for Sorghum Grain Mold Management and the Potential for Impact on the Poor: Overview and Recommendations

Andrew J Hall[1], Ranajit Bandyopadhyay[2], Arun Chandrashekar[3], and Peter R Shewry[4]

The introduction of photoperiod-insensitive, short- and medium-duration, high-yielding rainy season (kharif) sorghum (*Sorghum bicolor* (L.) Moench) hybrids in India more than 20 years ago has led to dramatic increases in grain yield (from 587 kg ha^{-1} in 1970 to 1407 kg ha^{-1} in 1996). This was accompanied by high levels of adoption (80% of rainy season crops) by farmers in the major sorghum-growing state of Maharashtra. Despite this achievement, hybrid rainy season sorghum grains suffer from infection and colonization by several fungi towards the end of the growing season, often associated with late rains. This infection results in grain mold often referred to as "blackening". There is little doubt that in its broadest sense grain mold constitutes one of the most important biotic constraints to sorghum improvement and production worldwide (Frederiksen et al. 1982; Louvel and Arnoud 1984; ICRISAT 1987). Certain grain mold pathogens have

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Hall, A.J., Bandyopadhyay, R., Chandrashekar, A., and **Shewry, P.R.** 2000. Technical and institutional options for sorghum grain mold management and the potential for impact on the poor: overview and recommendations. Pages 7–33 *in* Technical and institutional options for sorghum grain mold management: proceedings of an international consultation, 18–19 May 2000, ICRISAT, Patancheru, India (Chandrashekar, A., Bandyopadhyay, R., and Hall, A.J., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

repeatedly been associated with losses in seed mass (Castor and Frederiksen 1980; Hepperly et al. 1982), grain density (Castor 1981; Ibrahim et al. 1985), and percent germination (Castor 1981). 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. Several mold fungi are producers of potent mycotoxins that are harmful to human and animal health and productivity. The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India has estimated US$ 130 million as total losses due to grain mold in the semi-arid tropical areas of Asia and Africa (ICRISAT 1992). The poverty implications of grain mold are associated with loss of access to food, exposure to health risks through contaminated food, and income losses through lower prices.

While the cause, scope, and implications of the problem are clear, what possible avenues are open to address this problem? On the whole results have not been promising. Several approaches to control grain mold have been attempted. These include fungicidal control and integrating adjustments to sowing dates with high-yielding and relatively less susceptible hybrids (Forbes et al. 1992). However, disease control through input and management intensive approach has been found to be incompatible with the resource endowments of farmers and their complex cropping strategies (Williams and Rao 1981). Efforts to produce sorghum genotypes with tolerance to grain mold by conventional breeding methods have yielded partially successful results (Stenhouse et al. 1998). However, a high degree of resistance could not be incorporated. The tolerance is operative only under moderate disease pressure. Farm-level processing of molded grains (dehulling) has been suggested as an approach to improve the quality of molded grain (ICRISAT 1986; Stenhouse et al. 1998). This approach is yet to be tested. However, it is unclear whether it would lead to reduction in mycotoxin contamination.

Two recent developments have prompted a reappraisal of the prospects of successfully addressing the grain mold problem. Firstly, a series of recent biochemical and genetic studies (Sunitha et al. 1992, 1994; Mazhar and Chandrashekar 1993, 1995; Sunitha and Chandrashekar 1994a, 1994b; Sunitha Kumari et al. 1996; Bandyopadhyay et al. 1998) has examined grain mold resistance or tolerance in relation to grain hardness, starch structure, invasion response mechanisms, and the genetic elements controlling the production of antifungal compounds and properties. These studies have

shown considerable promise in understanding the mechanisms that confer mold resistance. Secondly, a recent United Kingdom Department for International Development (DFID) Crop Post-Harvest Programme project (Factors Affecting the Utilisation of Sorghum in India) has highlighted the importance of sorghum to resource-poor farmers and the emergence of non-food uses as a major market for the crop (Hall and Yoganand 2000). Sixteen percent of total production of rainy season grain and 40% of marketed sorghum are used for industrial utilization, mainly in the poultry and grain alcohol sectors (Klieh et al. 2000) and this utilization scenario seems set to accelerate.

Taken together these biological advances and related institutional developments warrant a reappraisal of approaches to dealing with the grain mold problem. The papers contained in this proceedings are the result of this reappraisal. The remainder of this overview summarizes the salient points of these papers, the discussion at the meeting at which they were presented, and the recommendations. We begin our overview with a discussion of the relevance of sorghum to the livelihoods of the poor. We feel it is important to begin with this as our background. It provides the development significance of the grain mold problem. It also highlights the way the livelihoods of the poor and the commodity that they produce and/or depend on are inextricably associated with the fortunes of the market, the private sector, and the technical and policy factors that pattern the behavior of these institutions.

The Poverty Context of Sorghum

The identity of the rural poor

Hall et al. (2000) argue that in sorghum production systems it is no longer valid to consider farmers as the rural poorest. At best they are marginal farmers; at worst and most frequently they are landless and reliant on agricultural employment. Numerically the poor are the dominant group in rural society. Their greatest concern continues to be access to food. If the poor have land, sorghum production contributes to this, but never more than partially. Often, the main contribution of agricultural production to their livelihoods is through the creation of employment opportunities. The best that agricultural development can offer them is cheap food, but more important, the means to access it (Hall et al. 2000).

Sorghum-based livelihoods

Sorghum, particularly rainy season sorghum, provides the poor with cheap food. The converse is often true for the postrainy season sorghum due to the high price. However, it is farming systems—including sorghum production—that is the greatest means of accessing food via the employment opportunities that it creates. The most important and rather intangible—and therefore underplayed—importance of sorghum is as an input into these farming systems. The relative importance of sorghum as a source of fodder for livestock is of crucial importance. It is the integrated nature of these crop livestock systems that underpins the sustainability of the farming systems in the semi-arid tropics of India. Sorghum therefore underpins the system that is most important in allowing the poor access to employment and therefore food. This importance, regardless of any other concerns is a fundamental reason for sustaining the presence of sorghum in the cropping patterns of the Indian semi-arid tropics (Hall et al. 2000; Hall and Yoganand 2000).

Future prospects for sorghum-based livelihoods

What are the likely mechanisms for maintaining the position of the crop in farming systems? Agricultural and trade policy reforms will at worst be neutral and at best marginally supportive of the crop. Increasing rural labor shortages will also tend to favor the crop due to its relatively low labor demands. The competitive position of the crop—profitability—will need continued support from demand for the commodity at a level at least equal to that at present. Rural household demand for grain will at best remain stable and at worst show a steady decline. The same is also true for urban demand for food, with declines likely to be more substantial. However, even though cropping patterns have witnessed the decline of sorghum, it seems that the fodder demands of mixed crop livestock systems are such that the crop has reached a floor level of production. Its relevance in the cash-based rural economies would therefore be enhanced through the development of stronger markets (Hall et al. 2000).

Having made a case that sorghum is important to the livelihoods of the poor and therefore that grain mold affects these sorghum-based livelihoods, how do we reconcile the need for better markets for sorghum with the need to

solve the grain mold problem? The papers in this proceedings tackle the problem by first laying out in some detail the biological nature of grain mold and then reviewing management options. We have summarized this below, before introducing the discussion of the emerging utilization scenarios and the opportunities these have for operationalizing some of the recent scientific advances.

Pathology and Implications of Sorghum Grain Mold

Basic biology

Bandyopadhyay et al. (2000) elaborate in detail the complexities of mold infection of grain, explaining that this can take place in the early stages of grain formation or at the post-maturity stage. For the purpose of this review, grain mold refers to a condition resulting from associations of mold fungi with sorghum flower tissues occurring from anthesis to harvest.

More than 40 genera of fungi are associated with molded grains. However, only a few species infect sorghum flower tissues during the early stages of grain development. These are (in approximate order of importance) *Fusarium moniliforme* Sheld., *Curvularia lunata* (Wakker) Boedijn, *Fusarium semitectum* Berk. & Rav., and *Phoma sorghina* (Sacc.) Boerema, Dorenbosch, & van Kesteren. *Fusarium moniliforme* and *C. lunata* are of significance worldwide. If mature sorghum grains are incubated on non-selective agar, the above fungi may be isolated in low frequencies relative to many other fungi. Williams and Rao (1981) listed the species most frequently isolated in studies of mycoflora associated with sorghum grain. Subsequent studies list much the same spectra of fungal species. Prominent among these are species of *Alternaria*, *Drechslera*, *Cladosporium,* and *Olpitrichum*. A pictorial guide of fungi commonly associated with sorghum grain has been recently published (Navi et al. 1999). The importance of several of these mycoflora is not well known.

Gibberella fujikuroi (Sawada) Ito (*Fusarium* Section *Liseola*) is an important mold fungus infecting sorghum grain. Several vegetative compatibility groups (VCGs) have been reported in the fungus. All the 11 mating populations of the fungus have been isolated from sorghum from different parts of the world. Among these, mating population F is more

prevalent on sorghum compared to mating population A. The population structure of *Fusarium* Section *Liseola* on sorghum has not been studied in India. Differences in pathogenicity have been reported in *C. lunata* isolated from different geographical areas in India (Somani et al. 1994). There is a need to determine the frequency of occurrence of different mating populations in India, primarily from mycotoxin point of view. Researchers in Australia have described *Fusarium nygami* Burgess & Trimboli in association with sorghum grain (Burgess and Trimboli 1986). This species resembles *F. moniliforme*, but produces chlamydospores. Its role in the etiology of grain mold is unknown.

Mycotoxins

Several fungi involved in the grain mold complex produce toxins and secondary metabolites. Several chemically and biosynthetically diverse mycotoxins such as fumonisins, moniliformin, fusaproliferin, fusaric acid, fusarins, beauvericin, and gibberellic acids are produced by fungi belonging to *Liseola* section of *Fusarium* genus (Leslie 1999). Fumonisins (B1, B2, and B3) are a family of mycotoxins produced by the molds *F. moniliforme* and *Fusarium proliferatum* (Matsushima) Nirenberg. Of the toxins produced, aflatoxin is considered to be the most potent, naturally occurring carcinogen known. It has been linked to various health problems in both humans and animals. Aflatoxin is a byproduct of mold growth in a wide range of commodities including sorghum. Two molds that are major producers of aflatoxin are *Aspergillus flavus* Link and *Aspergillus parasiticus* Speare. These fungi can be found virtually everywhere in the world.

Bhat et al. (2000) describe the analysis of sorghum samples collected from markets and households in sorghum-growing regions of Andhra Pradesh in India. They show that the extent of fumonisin contamination is higher in rain affected and moldy samples compared to that in normal samples. Though aflatoxins were found to "co-occur" no correlation was observed between fumonisin B1 and aflatoxin B1 indicating that both the toxins exist independently in the sample. The outbreak of fumonisin toxicity in the human polulation during October 1995 in parts of the Deccan Plateau in India was associated with unseasonal rains. Most of the crops that were harvested during this period became visibly moldy.

Results of a rapid epidemiological survey in Andhra Pradesh and Karnataka states of India indicated that (1) only adults who were consuming the moldy grains were affected; (2) the outbreak was self-limiting, non-contagious, and non-fatal; (3) people consuming grains harvested during the previous year, or harvested before or after the rains, or those who had access to other staples were not affected; and (4) children and pregnant women were not given sorghum in their diet and so were not affected. People were aware of the moldy nature of the grain and had little alternative but to consume it. The affected people mostly belonged to the lower socioeconomic groups who did not have access to other staples.

Samples taken during the outbreak indicated that in the mycological profile *Fusarium*, *Aspergillus,* and *Alternaria* species were the dominant mycoflora in sorghum. The extent of fungal contamination as assessed by ergosterol content was detected in the samples in quantities up to 120 mg kg^{-1}. Fumonisin B1 was present at higher levels in samples obtained from the affected households. The levels of aflatoxin detected were extremely low.

Bhat et al. (2000) discuss the potential of decontaminating or detoxifying molded sorghum grain. While a number of chemical treatments and mechanical processing approaches have been suggested, both their efficacy and their social and economic feasiblity seem far from clear.

Mycotoxins diagnostics

One important area where advances are being made is in the area of mycotoxin diagnostic tools. Thirumala-Devi et al. (2000) describe the recent advances in serological methods for the quantitative estimation of mycotoxins and the application of these as practical, robust, and cost-effective field tools. They have also stressesed that application of such immunochemical methods require high quality antibodies and associated methodologies if estimations are to be accurate and consistent. A key component of the serological methods has been associated with development of methods for producing the antibodies themselves.

The traditional method of producing so called polyclonal antibodies involves the use of laboratory animal, with antisera derived from the blood products of animals in which a suitable immunoresponse has been stimulated. Monoclonal production methods have started to supersede the traditional animal approaches. Although this approach has disadvantages in

terms of cost, effort, time, and the requirement for tissue culture facilities, monoclonal antibodies tend to exhibit much smaller inter-assay variations and yield more highly reproducible results than polyclonal antibodies. Thirumala-Devi et al. (2000) have produced and characterized monoclonal antibodies for aflatoxins that are highly sensitive and additionally with varying specificities. Recent advances in the field of recombinant antibody technology have opened up new possibilities for serological diagnosis because it is possible to engineer low-cost antibodies with desirable affinity and specificity essentially by manipulation of the basic domain structure of the immunoglobulin molecule.

Such serological advances, coupled with appropriate development in the field of immunoassay, will be important in reducing the cost and ease of mycotoxin detection. The development of such tools will underpin the development of quality control standards. Hall et al. (2000) argue that this will not only help monitor contamination-free food and feed chains but also provide a way of enforcing quality levels in contract growers' schemes, and will underpin the development of price premiums in marketing chains for contamination-free product. These price incentives for poor producers will be key factors in helping the adoption of approaches to reduce the grain mold problem.

Management of Grain Mold

Mold epidemiology

Although grain mold is associated with sorghum that matures during conditions of wet weather, published reports on quantitative relationships between weather variables and mold are scarce. The reason for this may partly be the complex nature of grain mold, caused by various fungal species, which may infect the grain at different growth stages. A better understanding of this complex interaction coupled with the predictive power of modern simulation model may contribute to one of the most important traditional control strategies—avoidance. This usually involves manipulating sowing dates or the growing duration of cultivars to ensure that grain filling and maturity stages occur after the end of the rains.

Bandyopadhyay et al. (2000) review recent ICRISAT epidemiology studies and conclude:

- There is good potential for the development of risk assessment models. These would be of value in assessing the risk of sorghum grain mold in different locations, which could be used to advise on suitable sorghum varieties for planting. Such decision-support approaches have proved successful in developed world agriculture. However, one needs to be more cautious in advocating this for the developing world and be aware of the type of institutional infrastructure that may be required to support such an approach.
- Such a forecast system can aid market intelligence to determine the availability of sorghum of different quality standards. This can be subsequently verified by rapid market appraisals. Such a method may reduce cost of sorghum grains with a predetermined standard and this may be important in the continued commercialization of the crop.
- Information on the relationship between weather and grain mold could be used to improve the technique to screen for resistance (the use of mist to provide good infection conditions has already been used in ICRISAT). The effect of canopy structure (height and density) and panicle architecture will both affect the microclimate of the developing grain and periods of surface wetness in the head. Attempts should be made to combine these physical phenotypic characteristics with other forms of resistance such as antifungal proteins.

Drying and processing of infected grain

Untimely rains and the consequent dampness of sorghum grains both in the field and at harvest and storage are a major cause of grain mold. Improved drying of grains after harvest would undoubtedly reduce the mold growth. Although considerable research has been done on drying of grains, no information is available on technical and economical feasibility of drying sorghum grain for reducing grain mold. As a mold management tool, grain 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.

Considerable variation exists for the extent of invasion of mold fungi within the endosperm. A few widely grown sorghum hybrids have the

capacity to withstand mold invasion in the endosperm, but the fungi discolor the pericarp even when the invasion is moderate. If the pericarp is removed, such grains appear almost as clean as non-moldy grains. Removal of the pericarp will be sufficient to remove most of the fungus and to reduce mycotoxins, as has been shown in rice (*Oryza sativa* L.) (Vasanthi and Bhat 1998) and α-amylase levels. Removal of the pericarp is possible by several means including mechanical dehulling. Research at ICRISAT has shown that the appearance of moldy grain can be improved by dehulling (ICRISAT 1986).

Despite the technical possibilities of both these strategies one needs to be cautious in advocating either of these approaches as a major focus of grain management efforts. These approaches may be technically robust (but even this needs further clarification). However, Bandyopadhyay et al. (2000) have warned that such approaches need to consider the presence of a supporting socioeconomic and institutional framework that would allow these types of technical interventions to be operationalized. In the current production and utilization environment this framework is absent. Hall et al. (2000) discuss the way the changing utilization environment may be altering this position, particularly with respect to quality enhancing measures such as drying and the price premiums that may emerge if new industrial users of the crop start to intervene in the sorghum market directly.

Resistance Breeding

In most cases, avoidance or chemical control in farmers' sowings is impractical. Similarly processing of molded grains does not seem a practical option for most producers and rural households at this stage. Host plant resistance is the most preferred method of control and is the core of integrated disease management strategies. For this reason, major research efforts have focused on development of resistant cultivars. Determination of factors associated with resistance and improvement of screening techniques are major efforts in this research.

Resistance mechanisms

In the last 15 years there has been a great deal of research directed toward determining resistance mechanisms. Chandrashekar et al. (2000), Reddy et al. (2000), and Waniska (2000) review this work in this proceedings. In

summary, three basic mechanisms are involved in resistance. These are panicle and flower structure, grain hardness, and 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 sorghum lines with red pericarp are resistant to grain mold. The association of flavan-4-ols and grain mold resistance has been demonstrated in Asia (Jambunathan et al. 1990), Argentina (Martinez et al. 1994), and USA (Melake-Berhan et al. 1996). The role of phytoalexins, such as apigeninidin, in mold resistance needs further research (Schutt and Netzly 1991). While apigeninidin is inhibitory to mold, flavan-4-ols is not. Schutt and Netzly (1991) suggested that flavan-4-ols accumulate in sorghum grains as a biosynthetic precursor of apigeninidin.

Direct relationship between grain hardness and mold resistance has been shown by different independent research (Jambunathan et al. 1992; Menkir et al. 1996; Ghorade and Shekar 1997; Audilakshmi et al. 1999). Since grain mold develops under wet conditions, a key factor to consider is the ability to retain hardness by mold-resistant grain. Resistance to pre-harvest sprouting is likely to be a factor that helps grains to retain hardness under wet conditions. In most circumstances, a high degree of hardness is not compatible with grain characters required for food quality (Audilakshmi et al. 1999). Therefore, an appropriate level of grain hardness necessary for mold resistance is required in grains without compromising on its food-making properties.

There are conflicting reports on the role of panicle morphology and flower structure in grain mold resistance. Glueck et al. (1977) and Mansuetus et al. (1988) concluded that glume coverage and lax panicles contribute to reduction in mold severity. However, other research involving fairly large number of test genotypes does not indicate a relationship between the extent of glume cover on grains or panicle compactness and grain mold resistance (Menkir et al. 1996; Audilakshmi et al. 1999). The latter conclusion is also reinforced by early reviews on grain mold (Williams and Rao 1981; Williams and McDonald 1983). Audilakshmi et al. (1999) found a strong association between glume color and grain mold resistance and believe that it is possible

to enhance grain mold resistance in white-grained sorghum by incorporating colored-glume character.

The current understanding of the mechanism of resistance in different types of sorghum cultivars is therefore as follows:

- Flavan-4-ols is the factor associated with resistance in cultivars with colored pericarp. Tannins are involved in resistance in cultivars with pigmented testa.
- Neither flavan-4-ols nor tannins are associated with resistance in white-grained sorghum since these compounds are absent in white-grained cultivars.
- Grain hardness, and perhaps colored glumes, is the only factor that contributes to mold resistance in white-grained cultivars that are used for food in India.

Limitations to breeding for grain mold resistance and new ways forward

Murty (2000) and Reddy et al. (2000) outline the progress to date and the limitations to further advances in breeding for grain mold resistance. They concluded that conventional breeding for resistance exploited grain hardness in white-grained sorghum. Programs in India and ICRISAT developed several high-yielding hard-grain restorer lines and male-sterile lines. While white grain is the preferred type in India, hard grains are not preferred for food purposes. Furthermore, while new sources of resistance can still be exploited the progress from such programs is expected to be slow because grain mold resistance is expressed late in the life cycle of the crop, is difficult to measure, has complex inheritance, and is significantly influenced by the environment. New initiatives should be complemented with marker technology by identifying markers for genes contributing to resistance.

These conclusions echo those of Chandrashekar et al. (2000) and Rooney and Klein (2000) in this proceedings. The theme of these papers is that in the short term new sources of resistance could be introduced to sorghum, possibly via a transgenic route. However, they stress that this is a short-term solution. For the longer term, research is necessary that builds on current understanding and recent advances in biochemistry of grain mold resistance, and the genetic factors that control it. The focus will be on studies

that allow the separation of grain hardness from antifungal proteins. This work will have to be underpinned by the development of molecular markers for the sorghum genome.

Industrial Utilization

So far we have seen that although complex, sorghum mold management may respond to recent advances in biochemistry and molecular biology. We have also seen in a number of instances that the relevance of some technical options for mold management is bound-up with features of sorghum markets and the presence (or usually absence) of incentives (price premiums) in these markets for mold-free grain. Much of the preceding discussion has also been based on the assumption that the utilization of sorghum in India will continue to be as a human food crop. However, our opening remarks concerning the poverty context of sorghum clearly identify the development of new (alternative) markets as a priority. Furthermore, we know that the utilization scenario is changing. We now review these changes and consider the challenges and opportunities these represent for future work to address the grain mold problem.

Scope of industrial utilization

Demand for energy sources for poultry feed continues to grow in India. This is in line with annual growth rates of 10% for layers and 15% for broilers. Current demand for compounded poultry feed is 12 million t per year, of which 30–35% is maize (*Zea mays* L.). Projections suggest that by 2020 maize demand will rise from current levels of 3.5 million t to 31 million t. In view of this the poultry industry recognizes the urgent need to develop alternative feed sources. Sorghum is already widely used, albeit at low inclusion rates. The industry anticipates that sorghum will need to replace most of maize currently used. Access to reliable supplies of cheap sorghum of a consistent and adequate quality is identified as a problem by the poultry industry (Seshaiah 2000). Significant sorghum utilization is also taking place in grain alcohol sector, mainly using molded grains (Sheorain et al. 2000). The brewing industry expresses some interest in the use of sorghum as adjuncts.

The technical viability of sorghum in "alternative" utilization niches, particularly poultry feed, is amply demonstrated by Reddy and Rao (2000) and Subramanian and Metta (2000). Similarly, Sheorain et al. (2000) present the technical viability of using sorghum for alcohol production, based on the experience of the techniques developed by the inhouse research and development (R&D) facility of a major international drinks company setting up whisky production using sorghum. Beyond the technical possibilities that these papers demonstrate, a clear message is that the utilization environment is starting to change quite rapidly and that this change is being initiated by the private sector.

Grain mold as part of a quality constraint cluster

Industrial users of sorghum view grain mold as only one of a cluster of quality-related issues that need to be dealt with. Unless these problems are addressed as a cluster, sorghum will be less competitive than maize. The potential leverage that the private sector might be able to bring to the grain mold problem rest on the resolution of these problems as a group or cluster. To illustrate the point, the poultry industry could quite easily be instrumental in popularizing specially adapted grain mold resistant varieties. But it could only do that if these same varieties had a similar calorific value to maize; if it could procure these through contract growers' schemes; if these schemes allowed farmers to adopt productivity enhancing technology; and if this made sorghum cheaper. In addition it would need to be helped to undertake commercial scale feeding trials with new genotypes, probably at an early stage in the plant breeding program.

Partly this clustering of constraints relates to the transition from food use to industrial use. However, it is not the case that just two distinct markets are developing (food versus non-food). Rather an increasingly complex set of markets with potentially more sophisticated and varied grain quality requirements are starting to emerge and this is probably why scarce public sector institutional resources are finding difficulty covering all the potential components necessary to achieve competitive utilization of sorghum in new utilization niches.

Recommendations

The recommendations for further research arising from this review are quite diverse in their scope. For clarity we have divided the recommendations into three broad themes: (1) recommendations for basic research addressing the pathology, biochemistry, and genetics of grain mold; (2) recommendations for more applied types of research relating to developing industrial utilization of molded grain; and (3) recommendations concerning ways that these research themes can address from an operational point of view, keeping in view a developmental agenda.

Theme 1: Pathology, biochemistry, and genetic studies

The review and consultative discussion concluded on the relative merits of different biochemical and genetic approaches to grain mold resistance as follows:

Pigmented testa. Pigmented testa containing sorghums do not currently occur in India. This character is associated with high tannin and poor food quality and should be avoided.

Red pericarp. Sorghums containing red pericarp are acceptable for some end uses but are not favored. Hence it may be selected as a short-term goal but must be eliminated in the longer term.

Grain hardness. Grain hardness is clearly related to resistance and sufficient variation is present in the germplasm to provide breeding material. However, hard texture is not favored for all end uses; medium or soft texture is preferred for food use. In addition, hardness is currently associated with high levels of antifungal proteins and this linkage needs to be understood and then broken. Hardness appears to result from high levels of kafirin. The biochemical basis for this effector needs to be understood and the existence of modifier genes (as in Quality Protein Maize) established.

Antifungal proteins. A link between antifungal proteins (AFPs) and resistance is clearly established, although only partial protection is obtained. In the short term it will be possible to select for high levels of characterized AFPs (sormatin, ribosome-inactivating protein, β1-3 glucanase, endochitinase) in developing and mature infected grain by direct analysis or by using antibodies

or molecular markers. In the long term more information is required on the endogenous AFPs of sorghum to:

- Identify new components;
- Determine activity and synergism;
- Determine location;
- Determine initiation of synthesis and amounts in developing and mature tissues; and
- Relate the above to resistance.

Marker-assisted selection. The development of molecular markers for the sorghum genome (e.g., simple sequence repeats) is essential to underpin the selection of resistance and the combination of resistance with yield and end use quality. It will also facilitate dissection of the various aspects of resistance; for example, the separate effects of hardness and AFPs. A more directed approach can also be adopted using markers based on characterized AFPs (e.g., thaumatin) and putative resistance genes (based on other species).

Transformation. Transformation is an essential prerequisite for long-term improvement and must continue to be supported. Transformation should initially express sormatin, and β1-3 glucanase/endochitinase under a strong endosperm specific promoter. This will act as "proof of concept". Foreign genes could be used, but these should preferably be from maize or other cereals. Genes encoding proteins expressed in non-food tissues should be avoided due to potential problems of acceptability and allergy/intolerance. Further transformation may require specific promoters to control the level and tissue specificity of expression; e.g., glume, developing endosperm, and ovary wall/pericarp. These could be derived from maize or isolated from sorghum using homologs from maize.

Resistance genes. There is a long-term need to identify and characterize resistance (R genes) in sorghum. These genes would be expected to ultimately regulate the manifestation of different phenotypes which confer resistance to grain mold. In the short term the conserved sequences of the R genes may be used to design primers, which can be used to generate polymorphism in marker-assisted selection.

Theme 2: Industrial utilization and public-private sector partnerships

Market institutions. A review of New Institutional Economics (NIE) approaches suggests that innovations in marketing institutions offer the scope for reducing the transaction costs associated with procuring quality grain and in finding adequate and consistent supplies. Experimentation to test different institutional innovations (cooperatives, farmers' associations, contract growers' schemes) would be useful. It would also be an opportunity to assess their value to both the farmer and the client industry. For example, contract growing arrangements could be linked to the supply of appropriate mold-resistant varieties and associated production and postharvest handling. Part of such a study would be the need to understand the reasons these types of institutional innovations had not developed already.

Technology and institutional innovations. There is a potentially large list of useful technical research that could be done to underpin sorghum utilization and that is related to the grain mold problem and its associated quality cluster. This includes:

- Grain mold resistance through genetic markers (multiple public and private sector partners, particularly seed companies).
- Screening of genotypes for specific private sector applications—starch content, oil content, recovery rates (jointly with industry).
- Feeding trials with molded sorghum (commercial scale jointly with poultry and cattle feed industry).
- Developing sorghum adjuncts (jointly with industry).
- Sorghum malting trials (jointly with industry).
- Improved knowledge base for forecasting grain mold in different market catchment areas (jointly with market institutions and industry).

A linked element may be the need to develop grain quality standards that are practical to operationalize. Work on mycotoxin diagnostics would contribute to this. This would probably be a prerequisite for developing any type of contract growing arrangement. Standardization of the various grades of grain mold damage would be a priority. This in turn may require a reexamination of technical options to support quality assurance such as

manipulation of planting and harvesting dates, and improved approaches to grain drying. It is recognized that such approaches for dealing with grain mold have found little success in current production and marketing systems. However, the introduction of marketing institutions to link farmers to industry could provide the price incentives (and penalties) as well as the institutional routes for technical backstopping, and so provide a supportive context for such technical approaches to mold management. Innovative mold management options need to be tested in this new institutional environment.

Processing of molded grain for food or industry was advocated by several workshop participants. Two issues emerge. Firstly, if this is to be part of an approach where the objective is value addition of partially damaged grain, then the market and associated institutional factors will be critical to success. At this stage it is not clear the extent to which either the for-profit or the non-profit private sector would be willing to develop this context. Secondly, it is not feasible to process grains with heavy mold damage due to technical reasons, let alone the economic and social feasibility considerations. Such investigation should be of limited scope.

Theme 3: The proposed structure of future work and the scope of public-private sector partnerships

The discussion of the scope of research needs implies three features. Firstly, there is quite a large range of technical and non-technical components of research that are necessary to address or contribute to the sorghum grain mold problem. These components range from basic research to applied. Secondly, there are elements where the poor will clearly be the direct beneficiaries. On the other hand some elements of the research will concern "enabling" intermediary organizations to engage more successfully in activities that will provide livelihood opportunities for the poor, both directly and indirectly. These organizations may be either the private for-profit sector or non-government organizations. Thirdly, these technical issues are intimately bound-up with this institutional context and partnership of various types will be key in addressing them. Put another way, solving the grain mold problem, requires an appreciation of the institutional context of the problem, and the constraints and opportunities this contains. Institution innovations both in terms of research practice and in output markets will be as important as technical advances in addressing the grain mold problem. So, for example,

the poverty impacts that will arise from new research are strongly linked to the long-term market prospects for sorghum as an industrial raw material and the potentially important role of the private sector in developing this market.

With these features in view, and cognisant of the fact that a number of private organizations have already pledged their willingness to commit resources into collaborative efforts, the following recommendation is made: *A project framework is required which can link together a cluster of technical and non-technical research projects. The scope of the latter has been outlined above. The purpose of this framework would be two-fold. Firstly, to ensure that value addition is achieved from communicating findings of different pieces of sorghum-related research to the relevant stakeholders. Secondly, and potentially more importantly, the framework would perform a networking function to start and build relationships between key stakeholders, particularly public and private sectors.*

At this stage the purpose of the framework project is not to establish a formal "sorghum association"—the private sector has already declined this option. Rather the purpose of the framework would be to provide resources and focus, allowing a more organic pattern of institutional development to take place over the course of three years of project support. In the medium to long term the development of supportive networks of scientist, entrepreneurs, and development advocates from the public and private sectors will be key in ensuring that technical advances in grain mold management find functioning uptake pathways and are operationalized in ways that provide livelihood opportunities for the poor.

Conclusion

The papers presented at this meeting have demonstrated that despite the complexities of the grain mold problem, real opportunities are emerging to address its management. Technological advances will drive these opportunities, particularly marker-assisted breeding. However, the changing institutional environment, and particularly the potentially pivotal role of the private sector, provides enormous scope for advancement. The recommendations outlined in this paper detail the way in which these two types of opportunities can be exploited by careful attention to the institutional framework of a new imitative in grain mold management. Potentially

enormous benefits could arise from this approach in terms of bringing the power of science and technology to bear on the livelihoods of the poor.

The challenge now lies in attracting sufficient commitment from a consortium of donors to put into action an ambitious program of research and action research activities, specifically a program of research:

- that will cut across conventional research sectors—crop protection, crop postharvest, and livestock;
- that will have both upstream strategic elements and downstream adaptive and developmental and action research elements;
- that will blur the conventional distinction between public and private "good" research;
- which may at times be more concerned with establishing networking relationship between key players and devising institutional innovations, than in producing conventional technical outputs; and
- which views the "research projects" as a way of addressing both technical and policy issues simultaneously stimulating institutional learning and disseminating the policy lessons this provides.

The next step forward will be to seek seed funds to develop this approach into a concept note with stakeholders to fund the framework project. Once established, this will act as focus for drawing in projects dealing with the grain mold problem, and providing them with an operational uptake mechanism.

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Biology, Epidemiology, and Management of Sorghum Grain Mold

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Grain mold is a major disease wherever sorghum (*Sorghum bicolor* (L.) Moench) is grown if moist weather conditions prevail after flowering until grain maturity and before harvest. Traditional sorghum cultivars often escape grain mold because they are photoperiod sensitive and of long duration with flowering so timed that grains mature only after the rains have ceased. Yield from these cultivars are generally low to modest. The cultivars have limited adaptation outside their natural habitat and often fail to produce grain if rains cease early. To increase yield potential and avoid terminal drought stress, most sorghum improvement programs have developed photoperiod-insensitive, short- to medium-duration cultivars. These have wide adaptability and mature before the end of rains but are commonly infected by grain mold. In spite of mold susceptibility, farmers have adopted the photoperiod-insensitive cultivars since these often yield more than twice than that of the photoperiod-sensitive cultivars.

This review attempts to summarize knowledge of the biology, epidemiology, and management of sorghum grain mold. Some parts of this review are based on a similar review by the senior author (Forbes et al. 1992). We have not attempted to review information on mechanisms and breeding for resistance, and the new molecular biology research underway since other authors have covered these topics in this proceedings.

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Damage Induced by Grain Mold

There is little doubt that grain mold in its broadest sense constitutes one of the most important biotic constraints to sorghum improvement and production worldwide (Frederiksen et al. 1982; ICRISAT 1987). Certain grain mold pathogens have repeatedly been associated with losses in seed mass (Castor and Frederiksen 1980; Somani and Indira 1999), grain density (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 (Table 1). As discussed later, several mold-causal fungi are producers of potent mycotoxins that are harmful to human and animal health and productivity.

Table 1. Threshed grain mold rating (TGMR), ergosterol content, and price of sorghum grain samples collected from different primary markets of two sorghum-growing states in India in November 1986[1].

Cultivar	TGMR[2]	Ergosterol ($\mu g\ g^{-1}$)	No. of samples	Price (Rs. 100 kg^{-1})		
				Minimum	Maximum	Mean
CSH 5	1	0.57	6	133	165	150.50
	2	1.53	8	120	160	139.20
	3	3.26	7	130	165	138.60
	4	4.10	1	123	123	123.00
	5	9.08	5	100	133	121.00
CSH 9	1	-	0	-	-	-
	2	1.49	6	133	175	156.70
	3	3.66	6	130	170	142.50
	4	5.24	8	110	160	133.10
	5	7.37	11	117	135	125.40

1. Bellary, Gadag, Hubli, Dharwar, Haveri, Ranibenoor, Davangere, and Belgaum markets in Karnataka; and Sangli, Latur, Bir, Jalgaon, Malkapur, Nandura, Akot, Akola, Washim, Hinganghat, Wardha, Yavatmal, Wadgaon, and Bhokar markets in Maharashtra.
2. Based on 1 to 5 scale, where 1 = no mold and 5 =>50% threshed grain surface area molded.

In spite of general agreement that grain mold is important, there have been few attempts to quantify losses resulting from the disease (Williams and McDonald 1983). Accurate information on losses caused by the disease is difficult to obtain since it involves the assessment of losses from production through to marketing and finally utilization of the crop. Nevertheless, the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) has estimated US$ 130 million as total losses due to grain mold in the semi-arid tropical areas of Asia and Africa (ICRISAT 1992).

Terminology and Definition of Grain Mold

Grain blackening, "black sorghum", and discolored sorghum are the most common terms used by sorghum farmers, traders, and users in the rural communities in India as often the molded grains appear black. Several and diverse terms have been used to describe fungal infection and colonization of sorghum flower and grain tissues (Williams and Rao 1981). There is now a consensus for the exclusive use of the term "grain mold" to describe the condition resulting from fungal spoilage of sorghum grain. Some divergence in terminology may continue, however, as a reflection of an even more fundamental level of dispute among researchers on definition (Forbes et al. 1992). Definitions of grain mold are only rarely given in explicit terms (Castor 1981; Williams and Rao 1981). Most definitions of grain mold appear to fit into one of two general concepts of fungal-related grain deterioration.

The first concept (A) (Fig. 1) describes a condition resulting from fungal infection and colonization of grains occurring any time between anthesis and harvest. Here grain mold can be broadly defined as a fungal component of preharvest grain deterioration, involving numerous fungal species interacting parasitically and/or saprophytically with the plant.

The second concept (B) (Fig. 1) restricts the definition of grain mold to infection and colonization of flower tissues prior to grain maturity. In this limited definition, only a few fungi may be involved. The complex of field fungi that colonize the grain after physiological maturity is not part of grain mold per se, but rather constitute a component of weather or general postharvest grain deterioration. Postmaturity mold development is most common during the rainy season, even when dew formation and high relative humidity occurs. Rainfall is not essential for the development of severe mold under such circumstances. Most of the mold growth is restricted to the

pericarp without much invasion of the inner grain tissues. Often, the result of late infection is superficial discoloration of the grain that reduces its appearance and market value. Other conditions such as spotting of grain, alternate wetting and drying, and insect damage can also cause grain deterioration.

Concept

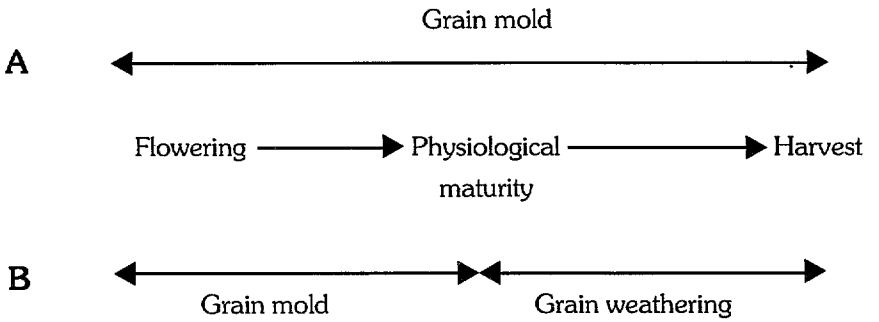

Figure 1. Concept of mold and weathering of sorghum grain (Source: Forbes et al. 1992).

The two concepts are practically similar. For example, early and late infections in concept A are comparable to the grain mold and grain weathering of concept B. Fungal-related grain deterioration, whether occurring before or after grain maturity, can cause important losses. The objective of plant improvement programs, therefore, is to develop sorghum cultivars resistant to all aspects of fungal-related grain deterioration.

These concepts differ mainly in the way that infections occurring before grain maturity are related to fungal colonization of the mature grain. In concept A, the difference is quantitative. The earlier the infection occurs, the greater the potential for damage and the fewer the fungal species involved. In concept B, infections occurring prior to grain maturity could be considered qualitatively different from postmaturity colonization. The early infections involve relatively few fungi acting as true parasites on living tissue. Postmaturity grain colonization involves many genera of field fungi that colonize primarily nonliving tissue. For the purpose of this review, grain mold refers to a condition resulting from associations of mold fungi with sorghum flower tissues occurring from anthesis to harvest.

Symptoms

Qualitative difference between early infections and postmaturity colonization determine the symptoms of grain mold. Symptoms of the two conditions can be very different. Early infection by a grain mold pathogen probably occurs on the apical portions of flower tissues such as glumes, lemma, and palea. One of the first visible symptoms following inoculation is pigmentation of the lemma, palea, glumes, and lodicules. This factor is highly cultivar dependent, and may be linked with mechanisms of resistance. Mycelial growth then proceeds toward the base of the flower, either in the flower tissues or in the spaces between these tissues. Infection of the grain itself occurs at the base, near the pedicel, and can interfere with grain filling (Frederiksen et al. 1982) and/or cause a premature formation of the black layer (Castor 1981). Either condition causes a reduction in grain size, a symptom often associated with grain mold. Visible superficial growth (the first signs of the fungus) occurs at the hilar end of the grain, and subsequently extends acropetally on the pericarp surface. Climatic conditions determine whether this growth will eventually spread to that part of the grain not covered by the glumes. Severe infection in the field results in grains with pink, white, or black fungal mycelium and sporulating structures depending on the pathogen (Bandyopadhyay 1986).

Fungal colonization of mature sorghum grain produces a different set of symptoms. Colonization occurs primarily on the exposed part of the grain and may be limited to that area. Postmaturity colonization is generally what produces the "moldy appearance" of grain maturing in humid environments. The color of the moldiness depends on the fungi involved. Several mold genera can colonize the pericarp and discolor it without penetrating deep inside the aluerone layer and the endosperm. Although grain integrity is not affected by such superficial molding, market value of such grains is reduced due to moldy appearance. Mold severity is often accentuated by preharvest seed germination. Sorghum grain can germinate on the panicles after black layer formation if wet conditions occur. When preharvest germination occurs, the embryo bulges to form the plumule and radicle that makes the hilar end to erupt and split. The split in the pericarp is often not visible unless the grains are removed from glumes. During the germination process, amylase digests the endosperm area adjoining the embryo and makes the grain soft, thereby predisposing the grains to colonization by mold fungi.

Differences between early infections and postmaturity colonization can be difficult to substantiate in the field. Both conditions occur together, and late-season colonization can mask symptoms of infection occurring during grain development.

Causal Fungi

Fungi belonging to more than 40 genera are associated with molded grains. But only a few fungi infect sorghum flower tissues during early stages of grain development. These are (in approximate order of importance) *Fusarium moniliforme* Sheld., *Curvularia lunata* (Wakker) Boedijn, *Fusarium pallidoroseum* Berk. & Rav., and *Phoma sorghina* (Sacc.) Boerema, Dorenbosch, & van Kesteren. *Fusarium moniliforme* and *C. lunata* are of worldwide significance. The pathogenicity of these fungi has been established by inoculation of plants in the field and in the greenhouse.

If sorghum grains after maturity are incubated on non-selective agar, the above fungi may be isolated in low frequencies relative to many other fungi. This is because the pericarp of sorghum routinely supports a rich and varied mycoflora that is not eradicated with conventional techniques of surface sterilization. Williams and Rao (1981) listed the species most frequently isolated in studies of mycoflora associated witn sorghum grain. Subsequent studies have listed much the same spectra of fungal species. Prominent among these are species of *Alternaria*, *Drechslera*, *Cladosporium,* and *Olpitrichum*. A pictorial guide of fungi commonly associated with sorghum grain has been recently published (Navi et al. 1999). The importance of several of these mycoflora is not well known. These fungi are generally thought to be restricted to the pericarp, but penetration into the endosperm can occur if the mature grain is exposed to high relative humidity or moisture for an extended period. Under severe climatic conditions, the endosperm can be completely colonized and partially degraded by field fungi (Glueck and Rooney 1980).

Fungal colonization of pericarp tissues of many cereal grains is common. Depending upon the timing and degree of penetration, these fungi are considered to be saprophytes or pathogenic weak parasites (Neergaard 1979).

Gibberella fujikuroi (Sawada) Ito (*Fusarium* Section *Liseola*) is an important mold fungus infecting sorghum grain. Several vegetative compatibility groups (VCGs) have been reported in the fungus. All the 11

mating populations of the fungus have been isolated from sorghum from different parts of the world. Among these, mating population F is more prevalent on sorghum compared to mating population A. However, these two mating populations do not differ in their pathogenicity on sorghum (Jardine and Leslie 1992). Resistance appears to be generally across the species and not strain specific (Mansuetus et al. 1997). However, Mansuetus et al. (1995) also reported that resistance to some strains of *F. moniliforme* could be overcome by other strains belonging to different mating populations and VCGs. The population structure of *Fusarium* Section *Liseola* on sorghum has not been studied in India. Differences in pathogenicity have been reported in *C. lunata* isolated from different geographical areas in India (Somani et al. 1994).

Damage Caused by Grain Mold

Evaluation of grain mold

To accurately assess the importance of grain mold, however, it becomes necessary to correlate the level of damage with the corresponding level of disease. Assessment of the importance of grain mold, therefore, is effective only to the degree of accuracy in measuring grain mold. Measurement of grain mold severity is also important for other areas of research, including epidemiology and host resistance (Forbes et al. 1992).

Visual rating has been the most common means of quantifying grain mold. Visual appraisal involves a complex of factors and can estimate severity (degree of colonization of a uniform sample indicated by signs or discoloration), incidence (proportion of grain affected), or damage (reduction in grain size), depending upon the method of assessment. Large numbers of samples have been screened using visual appraisal method since it is the quickest and easiest method (Bandyopadhyay and Mughogho 1988). Visual assessment of grain mold severity has been standardized using a common scale of well-defined units such as percentage of grain surface affected (Forbes 1986; Bandyopadhyay and Mughogho 1988). Advances in the search for resistance to grain mold achieved to date can be attributed to screening techniques based primarily on visual appraisal. Other measures of severity are closely correlated to visual estimation method. For example, visual appraisal and ergosterol concentration were significantly correlated (Seitz et al. 1983; Forbes 1986; ICRISAT 1986).

Several factors can bias visual appraisal. For example, light-colored grains show more grain mold than dark-colored grains with equal severity. There may be also variation between perceptions of different persons evaluating the same grain sample. To avoid these problems, and be more accurate, workers at ICRISAT, Patancheru, India compare grain samples with light-grained and dark-grained standards of known severity levels (Bandyopadhyay and Mughogho 1988). Comparing threshed grain is the most accurate method of visual assessment of grain mold (Frederiksen et al. 1982). Mold can be also assessed in intact panicles, but mold development on glumes and grain area covered by glumes make rating of panicles less reliable.

Since visual appraisal evaluates the superficial condition of sorghum grain, it cannot provide information on the internal colonization of grain. Assessment of the fungal mass and propagules within a given amount of grain tissue provide accurate information on the amount of the fungi within the grain at assessment time which considers all fungal growth events that had taken place. Most attempts to quantify grain mold pathogens in grain tissue have measured the proportion of grains infected with certain pathogens, i.e., incidence (Hepperly et al. 1982; Granja and Zambolim 1984; Gopinath and Shetty 1985). Infection frequencies are also measured by plating and incubating whole grain on blotting paper, or agar medium (both selective and non-selective). Whole-grain plating can be biased towards more competitive (fast growing) fungal component of the mycoflora (Neergaard 1979). Use of selective agar (Castor 1981) or chemical treatment of grain (Gopinath and Shetty 1985) can compensate this bias. Forbes (1986) spread suspensions of ground seed tissues on a *Fusarium*-specific agar to quantify colonization by *F. moniliforme*. This technique, proposed as an indicator of disease severity, estimates the amount of viable fungal tissue (propagules g^{-1} of seed tissue). Use of media in assessment of mold is time and resource consuming, and should be attempted in studies where accurate information of the internal and external status of mold is required in the grain.

Fungal biomass in sorghum grain can be also estimated by measuring the concentration of ergosterol, a sterol produced by fungi but not by plants (Seitz et al. 1977). Ergosterol measurements are routine at ICRISAT (1986) (Table 1) and have been used to distinguish levels of grain mold resistance (Jambunathan et al. 1991). The procedure is sensitive and has the ability to estimate total (viable and nonviable) fungal biomass. Differences in ergosterol concentrations are often found among grain samples with similar degrees of

superficial mold growth (Seitz et al. 1983). This suggests that the method can provide an indicator for the extent of internal mold colonization, which is not externally visible. Since ergosterol estimation cannot distinguish fungal components in a molded sample, assessment of severity of different fungi (visually or on agar) in a grain sample in conjunction with ergosterol measurement provides a fairly good idea of the identity of fungal complex and quantity in a sample.

Relationship of disease severity and damage

The relationship between grain mold severity and damage has been seldom quantified although the potential for grain mold to damage grain is often demonstrated. Severity appears to be more closely associated with viability than with yield. In a study, two measures of severity (ergosterol concentration and propagules of *F. moniliforme* g^{-1} seed tissue) were more highly correlated with percentage germination than with seed mass or grain density (Forbes 1986). Percentage germination as a measure of grain mold should be routinely followed while assessing grain mold.

Mycotoxins

Several fungi involved in grain mold complex produce toxins and secondary metabolites. Normally, short-season sorghums that mature during rains are prone to contamination by mycotoxins than long-duration varieties that mature after the end of the rainy season in Nigeria (Salifu 1981). The best protection against mycotoxins is to monitor their presence in feeds and foods by testing all along the pathway from initial harvest of grains to the finished product.

Several chemically and biosynthetically diverse mycotoxins such as fumonisins, moniliformin, fusaproliferin, fusaric acid, fusarins, beauvericin, and gibberellic acids are produced by fungi belonging to *Liseola* section of the genus *Fusarium* (Leslie 1999). Fumonisins (B1, B2, and B3) are a family of mycotoxins produced by the molds *F. moniliforme* and *F. proliferatum* (Matsushima) Nirenberg. In addition to sorghum, these molds commonly infect maize (*Zea mays* L.) and rice (*Oryza sativa* L.); hence the potential for presence of fumonisins in feed and foodstuffs is high. Fumonisins affect various animals differently and have been linked to esophageal cancer in humans. An outbreak of poisoning, characterized by abdominal pain and

diarrhea, caused by the ingestion of fumonisin-contaminated maize and sorghum has been reported from several villages in India (Bhat et al. 1997, 2000). Mating population F of *Liseola* section of *Fusarium*, isolated frequently from sorghum, is less potent in producing fumonisin B1 compared to mating population A, which is more prevalent on maize (Leslie and Mansuetus 1995) on which the problem of fumonisin B1 is more acute. There is a need to determine the frequency of occurrence of different mating populations in India, primarily from mycotoxin point of view.

Zearalenone is primarily produced by the mold *Fusarium graminearum* Schwabe which also commonly produces vomitoxin. Hence, there is evidence that if zearalenone is detected, there is a high probability that other fusarial mycotoxins may be present. Zearalenone is classified as an estrogenic mycotoxin because it frequently causes estrogenic responses in animals. Zearalenone-contaminated feed or grain when consumed can cause a wide variety of reproductive problems in livestock. In swine, it causes vulvovaginitis, low birth weights, fetal reabsorption, aborted pregnancies, reduced litter sizes, abnormal estrus, and feminization of males. Zearalenone can delay the breeding process and cost the producer significant economic and physical losses.

Vomitoxin, chemically known as deoxynivalenol (DON), is produced most commonly by *F. graminearum*. Outbreaks of acute gastrointestinal illness in humans may be linked to vomitoxin. In feed, vomitoxin can cause vomiting, feed refusal, immune suppression, diarrhea, and weight loss. Swine are extremely sensitive to vomitoxin. Vomitoxin levels as low as 0.5 ppm (500 ppb) have been reported to cause palatability problems, resulting in economic losses due to low feed intake. Once contaminated feed is removed from its diet, the animal may return to good health. Visible grain mold can indicate possible contamination by zearalenone and vomitoxin; however, absence of visible mold growth does not necessarily assure grain free from contamination by these mycotoxins (Bowman and Hagler 1991).

T-2 toxin, which is produced by several species of *Fusarium*, is the principal causal toxin in the human disease alimentary toxic aleukia. It also has an adverse effect on many animals. Impairment of protein synthesis is a significant aspect of T-2 toxicity. Poultry studies have shown that T-2 intoxication has led to a reduction in weight gain and other problems such as beak lesions, poor feathering, motor function impairment, and increased susceptibility to *Salmonella* spp. In many other animals, intestinal peristalsis,

emesis, and altered mitochondrial function have been documented. T-2 intoxication is compatible with widespread tissue and organ damage including hematological, hepatic, renal, pancreatic, muscular, and cardiac effects. Damage caused by T-2 to the digestive track is irreversible.

Ochratoxin, commonly produced by the molds *Aspergillus ochraceus* Wilhelm and *Penicillium viridicatum* Westling can be found in maize, barley (*Hordeum vulgare* L.), and sorghum. It may be present in conjunction with aflatoxin, one of the most potent naturally occurring carcinogens. In fact, ochratoxin is a suspected carcinogen. It affects kidneys in animals exposed to naturally occurring levels of this mycotoxin. Turkeys and other poultry exhibit lower productivity levels during field outbreaks of ochratoxicosis. Symptoms include retarded growth and decreased feed conversion. It has also been known to affect egg production in laying hens. About 10–20 ppb for commodities destined for human or animal consumption may cause health problems and economic losses. Some foreign markets have set regulation limits ranging from 5 ppb to 50 ppb. In a recent preliminary study at ICRISAT, ochratoxin exceeding 300 $\mu g\ kg^{-1}$ grain was detected in 6 of 14 sorghum grain samples intended for incorporation as one of the ingredients of chicken feed by a feed company (DVR Reddy, ICRISAT, India, personal communication).

Aflatoxin is considered by many to be the most potent, naturally occurring carcinogen known. It has been linked to a variety of health problems in both humans and animals. Aflatoxin is a byproduct of mold growth in a wide range of commodities including sorghum. Two molds that are major producers of aflatoxin are *Aspergillus flavus* Link and *Aspergillus parasiticus* Speare. These fungi can be found virtually everywhere in the world. They are soilborne, but like to grow on high nutrient seeds. Their toxins are produced before harvest in the field, and also after harvest in storage. In both cases, damage due to insects, mishandling, or environmental stress can enable the fungi to invade the seed. Consumption of aflatoxin-contaminated feed can cause many health and performance problems. Often it is not suspected as a cause of poor performance in livestock and poultry. In many cases, other diseases develop that are then diagnosed as the cause of poor performance. Because aflatoxin is not suspected as the primary cause, contaminated feed can continue being fed long after problems develop and substantial loss has resulted. The United States Food and Drug Administration has set a maximum allowable level of total aflatoxins at 20 ppb. Commodities

used for human and animal consumption must be tested to ensure that aflatoxin levels are below this number. The foreign markets regularly inspect loads and reject shipments of commodities with levels higher than 4–15 ppb. In general, sorghum is not considered as a good substrate for the production of aflatoxin compared to maize, groundnut (*Arachis hypogaea* L.), and other oil-rich seeds.

Relationships between Grain Mold and Weather

Although grain mold is associated with sorghum that matures during conditions of wet weather, published reports on quantitative relationships between weather variables and mold are scarce. The reason for this may partly be the complex nature of grain mold, caused by various fungal species, which may infect the grain at different growth stages.

Recent studies at ICRISAT use three approaches to investigate the effect of weather on the grain mold complex. One is to search for empirical relationships, by comparing naturally occurring grain mold in various locations and in different growing seasons, and analyzing the variation of grain mold severity in relation to weather patterns. Another approach is to carry out experiments in controlled environments to determine response curves for processes such as infection and sporulation with a range of values for individual weather variables. The third approach is to manipulate the physical environment in field experiments by the use of shelters and application of controlled wetting of the crop during critical periods in the growing season.

Empirical relationship

Empirical relationships were established from an international grain mold trial in West and Central Africa, which involved 15 sites in ten countries (Benin, Burkina Faso, Cameroon, Central African Republic, Chad, Côte d'Ivoire, Ghana, Mali, Niger, and Nigeria) (Ratnadass et al. 1999). The trials were undertaken in two years, which provided 26 site/season combinations, with daily weather data for the complete growing season. Grain mold was assessed visually at the time of harvest in plots with and without the control of head bugs (*Calocoris angustatus* Lethiery in India and *Eurystylus oldi* Poppius in West Africa) by repeated insecticide applications. Correlations between disease and weather variables were obtained with the "window" computer

program (Coakley et al. 1988), which calculates mean or totals of each variable over different periods (or windows of time). Windows were chosen to begin on each day from the start of the growing season and to vary in length (2, 5, 10, 15, 25, 30, 35, and 40 days) and thus weather data from the whole growing season was examined. The weather variables included precipitation frequency, total precipitation, the number of consecutive days with precipitation, the number of consecutive days without precipitation, maximum temperature, minimum temperature, temperature range, maximum relative humidity, and minimum relative humidity.

It is perhaps surprising that very few highly significant correlations ($P < 0.001$) were obtained between rainfall-derived variables and grain mold scores. However, large numbers of highly significant correlations were found with the mean maximum relative humidity. The greatest number was 965 for a susceptible sorghum genotype (S 34). No significant correlations were found with grain mold resistant genotypes (e.g., ICSV 1079). When head bugs were controlled, there was a dramatic decrease in the number of highly significant correlations.

Scatter diagrams of grain mold score versus maximum relative humidity revealed non-linear relationships. When the maximum relative humidity (RHmax) was<90%, the mean grain mold score for 21 genotypes was consistently low, but a marked increase in grain mold was found at around 95% RHmax and above. Similar patterns were observed for individual genotypes.

Controlled environment experiments

Recent experiments in ICRISAT were carried out to elucidate the effects of wetness duration on infection by various grain mold fungi (*C. lunata, Drechslera australiensis* M.B. Ellis, *P. sorghina, F. moniliforme,* and *Cladosporium oxysporum* Berk. and Curt.). Sorghum panicles were inoculated at flowering, milk stage, soft dough stage, hard dough stage, and physiological maturity, and immediately after inoculation they were placed in a dew chamber for different periods of time (0, 16, 24, 40, 48, and 72 h) to keep them wet. Subsequently the plants were kept in a greenhouse until harvest, and samples of 50 grains from each plant were incubated in moist conditions to assess grain mold incidence.

Results from all the fungal species examined showed a generally similar pattern. For *C. lunata* and *P. sorghina*, infection was much less at flowering than at any other growth stage. At milk stage, a longer wetness period (72 h) was needed for maximum infection than at soft dough, hard dough, or maturity. At hard dough and maturity, some infection occurred with *P. sorghina* even with no wetness treatment. The result for *F. moniliforme* was similar to *P. sorghina* except that the infection was less at milk stage with 72 h of wetness.

Mist and shelter experiments

Field experiments have been carried out at ICRISAT in recent years in which small areas of a sorghum crop have been covered from milk stage until harvest. Under some of the shelters, the crop was kept wet each night by applying mist from 18:00 hours to 06:00 hours. In different treatments, mist was applied at different growth stages. Grain mold was assessed (overall score and individual fungi) at regular intervals between flowering and harvest.

Maximum grain mold was observed in the natural control (no shelter or mist) in 1998 (a wet year), but not in 1999 (a dry year). Very little grain mold was found in the dry treatment (with shelter and no mist) and in 1998, although the increase in mist treatments was more gradual than the natural control, final mold scores were similar. In the control treatment of 1998 season, the dominant grain mold pathogen was *Colletotrichum graminicola* (Cesati) Wilson. In 1999, grain mold scores in the control and mist treatments were similar and the dominant fungal species were *C. graminicola* and *C. lunata*. In the natural control, severe anthracnose infection was observed in the peduncle and the panicle.

Future prospects

The results of recent work outlined here indicate that there is good potential for the development of risk assessment models. These would be of value in assessing the risk of sorghum grain mold in different locations, which could be used to advise on suitable sorghum varieties for planting. If the risk of grain mold is low, high-yielding susceptible varieties may be most suitable, whereas resistant varieties (which may have other disadvantages) should be sown in high-risk areas.

Information on relationships between weather and grain mold could be used to improve techniques to screen for resistance (the use of mist to provide good infection conditions has already been used in ICRISAT). The identification of partial resistance (tolerance) may require moderate disease pressure, which could be achieved by manipulating the environment. The effect of canopy structure (height and density) and panicle architecture will both affect the microclimate of the developing grain and periods of surface wetness in the head. Attempts should be made to combine these physical phenotypic characteristics with other forms of resistance such as antifungal proteins.

More information is needed on sorghum grain mold epidemiology. The experiments carried out so far in ICRISAT are just a start of potential investigations. Effects of temperature on grain mold have not been investigated, and controlled environment studies on sporulation (this determines the grain mold symptoms) are urgently needed.

Improved knowledge on disease epidemiology and information on tolerant varieties will be valuable tools for advising on management options in sorghum production and marketing. For example, a mold risk model based on weather can forecast the occurrence and severity of mold in different sorghum-growing areas. Such a forecast system can aid market intelligence to determine the availability of sorghum of different quality standards that can be subsequently verified by ground-truthing. Such a method may reduce search cost of sorghum grains of a predetermined standard in markets.

Head Bug x Grain Mold Interaction

Nymphs and adults of head bug feed on developing grains, which are also infected by mold fungi. Head bugs suck the sap out of developing grains, which become tanned, shriveled, and under severe infestation, become completely invisible outside the glumes. Head bug injured grains are more prone to damage by mold fungi (Sharma et al. 2000). Bug injury provides a ready site for entry of mold fungi and further colonization inside the grain. Grain sap exuded from bug-injury site provides a ready substrate for the mold fungi to grow on grain surface. As a result, bug injury makes most mold-resistant sorghum genotypes susceptible by breaching their resistance. Insect damage can significantly increase the number of fungal colonies associated with grain and modify their relative abundance. For example, Marley and

Malgwi (1999) in Nigeria observed that following insect damage, *G. fujikuroi*, *P. sorghina*, and *Cochliobolus lunatus* Nelson and Haasis were the most common fungi in decreasing order of frequency in comparison to the normal order of *P. sorghina*, *G. fujikuroi*, and *C. lunata* (imperfect stage of *C. lunatus*). Grain mold damage can be accentuated to a large extent due to mild injury by head bugs. Some factors that are associated with grain mold resistance also confer resistance to head bugs. For example, grain hardness contributes resistance to grain mold as well as to head bug. Therefore, it should be possible to develop combined resistance to grain mold and head bugs in the same cultivar to avoid losses due to grain mold and head bug during the rainy season (Sharma et al. 2000).

Management of Grain Mold

Sorghum grain mold is a complex disease. Several options have been shown to provide adequate protection against the disease under experimental conditions, but their actual utility in on-farm situations has not been demonstrated. Often, research on grain mold has dealt only with a single method of control at a time, be it chemical control, avoidance, resistance, or timely harvest. Almost all the research on grain mold control has been conducted under on-station conditions, away from the socioeconomic, marketing, organizational, and institutional framework under which the farmers operate. Therefore, adoption of most grain mold control methods on farm is far from satisfactory.

Avoidance

Avoidance of grain mold has often been described as one of the most important traditional control strategies (Castor 1981; Williams and Rao 1981). In areas where photosensitive cultivars are grown, grain mold is avoided because flowering and grain filling occur in the dry season. Avoidance is still one of the most important control strategies still in commercial seed production. Most seed is produced with irrigation in dry season or regions to avoid grain mold and other problems. 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 (*C. angustatus*) and midge (*Contarinia sorghicola* Coquillett). Nevertheless, several farmers in Nanded area of Maharashtra in India have used avoidance successfully when the economic opportunity arose to replace sugarcane (*Saccharum officinarum* L.) with sorghum during the summer crop season. During the last two years, sugarcane in the irrigated Nanded tract has become less remunerative due to low market price. On the other hand, the price of clean sorghum grain has increased two-fold. As a result, farmers in Nanded area now grow irrigated sorghum in >50,000 ha from February to May under dry summer environment and harvest clean mold-free grain that fetch a significant premium in the market.

Chemical control

Chemical control appears to provide some protection against grain mold, but it is neither practical nor economical except for small fields with valuable sorghum lines that need to be saved. Literature abounds with reports on the efficacy of fungicide sprays in controlling grain mold under experimental conditions in research stations (Patil et al. 1978; Naik et al. 1981; Deshpande et al. 1987; Singh and Agarwal 1992; Somani et al. 1995). Most studies involving fungicides and grain mold-related fungi, however, deal with the efficacy of seed dressings for improving seedling emergence and vigor (Munghate and Raut 1982; Vidyasekharan 1983; Patil et al. 1986; Gopinath and Shetty 1992). Hot water treatment can eradicate some fungi from sorghum grains (Bhale and Khare 1982).

Resistance

In most cases, avoidance or chemical control in farmers' sowings is impractical. Host plant resistance is the most preferred method of control and is the backbone strengthened by other methods in integrated disease management strategies. For this reason, major research efforts have focused on development of resistant cultivars (Murty 2000; Reddy et al. 2000). Determination of factors associated with resistance and improvement of screening techniques are major efforts in this research.

Resistance mechanisms. In the last 15 years there has been a great deal of research directed toward determining resistance mechanisms. Reddy et al. (2000) and Waniska (2000) have reviewed this work elsewhere in this

volume. In summary, three basic mechanisms are involved in resistance. These are panicle and flower structure, grain hardness, and 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 sorghum lines with red pericarp are resistant to grain mold. The association of flavan-4-ols and grain mold resistance has been demonstrated in Asia (Jambunathan et al. 1990), Argentina (Martinez et al. 1994), and USA (Melake-Berhan et al. 1996). The role of phytoalexins, such as apigeninidin, in mold resistance needs further research (Schutt and Netzly 1991). While apigeninidin is inhibitory to mold, flavan-4-ols is not. Schutt and Netzly (1991) suggested that flavan-4-ols accumulate in sorghum grains as a biosynthetic precursor of apigeninidin.

Direct relationship between grain hardness and mold resistance has been shown by different independent research (Jambunathan et al. 1992; Menkir et al. 1996a; Ghorade and Shekar 1997; Audilakshmi et al. 1999). Since grain mold develops under wet conditions, a key factor to consider is the ability to retain hardness by mold-resistant grain. Resistance to pre-harvest sprouting is likely to be a factor that helps grains to retain hardness under wet conditions. In most circumstances, high degree of hardness and grain characters required for food quality is not compatible (Audilakshmi et al. 1999). Therefore, an appropriate level of grain hardness necessary for mold resistance is required in grains without compromising on its food-making properties.

There are conflicting reports on the role of panicle morphology and flower structure in grain mold resistance. Glueck et al. (1977) and Mansuetus et al. (1988) concluded that glume coverage and lax panicles contribute to reduction in mold severity. However, other research involving fairly large number of test genotypes have found lack of relationship between the extent of glume cover on grains or panicle compactness and grain mold resistance (Menkir et al. 1996b; Audilakshmi et al. 1999). The latter conclusion is also reinforced by early reviews on grain mold (Williams and Rao 1981; Williams and McDonald 1983). Audilakshmi et al. (1999) found a strong association between glume color and grain mold resistance and believe that it is possible

to enhance grain mold resistance in white-grained sorghum by incorporating colored-glume character.

In summary, following is the current understanding of the mechanism of resistance in different types of sorghum cultivars. Flavan-4-ols is the factor associated with resistance in cultivars with colored pericarp. Tannins are involved in resistance in cultivars with pigmented testa. However, neither flavan-4-ols nor tannins are associated with resistance in white-grained sorghum since these compounds are absent in white-grained cultivars. Grain hardness, and perhaps colored glumes, are the only factors that contribute to mold resistance in white-grained cultivars that are used for food in India.

Histological basis of resistance. Several histological studies indicate similar patterns of initial infection, induction of pigmentation of glumes and seed parts, and subsequent colonization of sorghum flower tissues (Castor 1981; Bandyopadhyay 1986; Forbes 1986). Forbes et al. (1992) have reviewed colonization events in mold-resistant and susceptible cultivars in detail. Infection events differ in *C. lunata* and *F. moniliforme*. During the initial period of flower invasion, *C. lunata* can infect the apical part of the ovary wall from the colonized lemma, palea, lodicules, filaments, pollen grain, and decaying style (Bandyopadhyay 1986). Mycelium penetrates the pericarp and ramifies throughout the cross and tube cells within 5 to 10 days. Generally, endosperm is not directly colonized. Placental sac offers a niche for fungal growth that subsequently invades the endosperm and sometimes the embryo as well. Differences between the infection patterns of *C. lunata* and *F. moniliforme* may partially explain why resistance to the two fungi occasionally differs (Castor 1981; Louvel and Arnoud 1984). Sorghum genotypes free from colonization of at least one fungal species have been identified suggesting that it should be possible to identify sources of resistance to specific fungi (Menkir et al. 1996b). Subsequently, resistance to several mold species can be pyramided into a single sorghum cultivar using an appropriate breeding program.

Screening techniques. Screening at ICRISAT is currently done without artificial inoculation (Bandyopadhyay and Mughogho 1988) since natural inocula are present during the rainy season over sorghum fields (Bandyopadhyay et al. 1991). High moisture levels are assured by sprinkling on rain-free days from flowering to harvest. Sprinklers are used as necessary

throughout the period of grain development as well as after physiological maturity. The long duration of sprinkler irrigation was required due to the lack of information on role of wetness at different grain development stages on mold development. Materials to be screened are compared with grain samples with known levels of grain mold severity. However, there is a need to improve the screening technique with artificial irrigation since such a method induces very high disease pressure obliterating minor differences in resistance and is unsuitable to identify cultivars with moderate levels of mold resistance. Ideally, the screening technique should be able to induce variable mold pressure on a set of sorghum lines used as standard against which test lines are evaluated. The degree of resistance in a genotype is dependent on the grain mold pressure it can withstand. Lines that are more mold resistant are able to withstand higher mold pressure compared to lines that are less mold resistant. Therefore, the breakdown point beyond which mold resistance of a genotype is overcome is a good indicator of the level of mold pressure that the line can withstand.

A laboratory-based screening technique has been developed to evaluate photoperiod-sensitive sorghum genotypes that are difficult to test under field conditions (Singh et al. 1993). The method involves dip-inoculating mature seeds with either individual or a mixture of mold fungi and incubating the grains for 5 days in moist chambers. The advantages of this method are that grains can be evaluated against individual fungi and the evaluation is not dependent on seasons (Singh and Navi 2000).

In Argentina a seed company uses a method to separately screen for resistance to grain mold fungi occurring before grain maturity, those colonizing seed tissues after grain maturity, and those causing head blight. The different types of resistance are identified by screening at different stages of plant development and in the case of head blight, on the basis of symptom development in the peduncle. Screening with this technique has shown that resistance to early-season grain mold pathogens is not always associated with resistance to fungi causing damage late in the season.

Statistical approach has been used for the identification of resistance by some researchers in northern Africa. Multivariate statistical techniques were followed to determine mold severity in test cultivars based on incidence of important grain mold pathogens, grain quality, germination, and seedling viability, and visual assessment of moldiness (Louvel and Arnoud 1984).

Sources of resistance. Extensive search for sources of resistance has been the topic of several research papers. The methodology used while screening for resistance differed widely from evaluation under natural conditions (Hiremath et al. 1993; Menkir et al. 1996a, 1996b); under artificial wetness provided by sprinkler irrigation (Rao and Williams 1980; Bandyopadhyay et al. 1988; Rao et al. 1995; Singh et al. 1995); by multilocational testing (Indira et al. 1991; Narayana et al. 1997; Rodriguez et al. 1997); and using an in vitro technique (Singh et al. 1993). Resistance has been found in different grain types such as white-grained sorghums (and colored grain sorghums with and without tannins. However, the level of resistance is invariably more in colored grain genotypes with tannins, followed by colored grain genotypes without tannin, and finally white- and hard-grained sorghums. After screening more than 13,000 photoperiod-insensitive germplasm lines, Bandyopadhyay et al. (1988) identified 156 grain mold-resistant lines out of which only one had white grain. On the other hand, four white-grained mold-resistant lines were identified among 66 guinea-based sorghum accessions (Singh et al. 1995) suggesting that it is possible to obtain resistance in white-grained lines. However, the factors associated with mold resistance in guinea sorghums are difficult to incorporate in white-grained lines without adversely affecting yield-contributing factors (Mukuru 1992). White-grained, agronomically elite, mold-resistant lines in non-guinea background have been reported by some workers (Meckenstock et al. 1993; Singh et al. 1995; Audilakshmi et al. 1999). However, other workers have not verified resistance levels in these elite lines. There is a need to assemble all reported sources of resistance and reevaluate them together to confirm their resistance.

Significance of pericarp in mold resistance. Moldiness of grain is visualized on its surface. Therefore, the outer layer of grain, i.e., the pericarp, influences appearance of grain mold on grain surface. Mold growth is less perceptible in grains with red pericarp compared to grains with white pericarp. Mold-resistant cultivars with red pericarp contain phenolics that contribute to reduced growth of mold fungi on grain surface. On the other hand, mold can grow readily on the pericarp of white-grained sorghum since there are no inhibitory factors present in the pericarp. Therefore, grains with corneous hard endosperm, though resist fungal colonization internally, cannot suppress late infection and sporulation of fungi such as species of *Cladosporium*, *Alternaria,* and *Curvularia* on the pericarp. However, less grain mold

develops on thin pericarp compared to thick pericarp (Glueck and Rooney 1980) although, this association is not universal. Since it is difficult to inhibit late fungal infection and sporulation on pericarp, physical methods (e.g., polishing) to remove moldiness from grain surface can be of value (see section on polishing later in this chapter).

Red-grained cultivars are not consumed as food in India since white grains are preferred as food. As a result, no commercially available sorghum has red grains in India. Removal of red pericarp from testa-less cultivars by dehulling may allow use of red sorghum as food since the dehulled sorghum product can be milled into white flour. The greatest potential of mold-resistant red-grained sorghums is in the animal food sector. Mold-resistant red sorghums can be readily used as animal feed since they: (1) do not contain condensed tannins, (2) are equal to white sorghum nutritionally, and (3) are likely to be safe with respect to mycotoxins due to mold resistance.

Pre-harvest sprouting and mold resistance. Internal colonization of grain is often accentuated by incipient sprouting of grains in the field. Under wet conditions, the embryo may germinate before harvest by initiating radicle and plumule. Although the radicle and plumule may or may not be visible outside, the pericarp splits over the hilar end. Such pre-harvest sprouted grains become soft due to the digestion of parts of the endosperm by α-amylase and are predisposed to extensive colonization by mold fungi, primarily species of *Fusarium* and *Curvularia*. Pre-harvest sprouting can occur as early as 15 days after pollination (Maiti et al. 1985; Steinbach et al. 1995) suggesting that significant internal grain colonization, predisposed by pre-harvest sprouting, can begin to occur before the hard dough stage. It is likely that the extent of internal colonization of grains can be minimized in genotypes that have seed dormancy, which extends for a month after maturity. It is necessary to determine the relationship between resistance to pre-harvest sprouting and resistance to grain mold. If a strong relationship exists, search for resistance to grain dormancy in sorghum germplasm would be valuable. Molecular approaches can aid incorporation of resistance to pre-harvest sprouting since quantitative trait loci (QTLs) that determine resistance to pre-harvest sprouting in sorghum have been mapped (Lijavetzky et al. 2000).

Timely harvest

Sorghum is considered to be physiologically mature when a black layer forms at the hilar end of the grain. The black layer isolates the grain from the rest of the plant and therefore, photosynthates are no longer transported to the grain. After black layer formation, the grains begin to lose moisture and dry. Incidence and severity of grain mold increases after physiological maturity of the grains, particularly when harvesting is delayed and wet conditions persist (Table 2). Therefore, reduction in mold damage is possible if the crop is harvested at physiological maturity when moisture levels range from 15% to 18%. However, grains need drying to 10–12% moisture to avoid molding during storage and further processing.

Table 2. Mold rating of threshed sorghum grains harvested at different days after physiological maturity (PM), ICRISAT Center, Patancheru, India.

Genotype	Date of flowering[1]	Date of PM	TGMR[2] 0 days[3]	7 days	14 days
IS 14384	23 Aug	2 Oct	1.5	1.8	1.8
IS 14332	28 Aug	7 Oct	1.6	3.4	3.7
ICSV 2	21 Aug	30 Sep	2.0	3.3	5.0
Local FSRP	23 Aug	2 Oct	1.9	4.3	5.0
SE±				0.21	

1. Seedling emergence date: 10 June.
2. Threshed grain mold rating (TGMR) on a 1 to 5 scale where 1 = no mold and 5 = >50% threshed grain surface area molded.
3. Time of harvest (days after PM).

Processing of sorghum in relation to grain mold management

In spite of all precautions and measures to manage grain mold, the mold fungi often damage sorghum grains during late unseasonal rains that have become common during sorghum harvest in most years. Moldy grains, being cheaper, are often consumed by the poor in spite of inferior quality of such grains from the point of view of food preparation and health hazards due to mycotoxin contamination. Due to price advantage, moldy grains find application as raw material in several industrial sectors (Reddy and Rao 2000; Seshaiah 2000; Sheorain et al. 2000; Subramanian and Metta 2000). Uptake of moldy grain by different industrial sectors not only generates cash income for farmers, but also reduces entry of moldy 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 postharvest methods to reduce grain mold should consider the socioeconomic, operational, and institutional framework of the target group in addition to the technical aspects.

Drying

Mold fungi infect sorghum grains in the field. Sorghum can be harvested at physiological maturity but often farmers delay harvesting after maturity to dry grains in the field. The problem of grain mold is most acute when the mature crop is then exposed to wetness and rain. Harvesting at physiological maturity is a method of reducing mold severity and is feasible if farmers have grain-drying opportunities. In addition vivipary may occur, with sorghum grains germinating after maturity in the field. Grains may then be harvested wet and need to be dried; in the traditional Indian situation the grain is dried under the sun. Wet grain may also be dried artificially but this may not be economical for the farmer unless drying is performed using community-owned dryers or simple indigenous methods that can be practiced by small groups of farmers. It may also be possible to use mold inhibitors such as propionic acids during the drying process to reduce the risk of mold development (Shetty et al. 1995). Although considerable research has been done on drying of grains, no information is available on technical and economical feasibility of drying sorghum grain for reducing grain mold. Grain drying as a mold management tool 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.

Polishing

Damage to grains by mold fungi depends on the time of infection and grain structure. The three major parts of sorghum grain are the embryo, outer pericarp, and inner endosperm. Early infection causes considerable colonization of internal grain tissues resulting in digestion of protein-starch matrix that offers substantial integrity to the grains. The result of early infection is soft grains that are often unsuitable for preparing food. On the other hand, mold growth due to late infection is often restricted to the pericarp with little internal colonization of endosperm. Such grains superficially appear moldy and fetch low price in the market.

Considerable variation exists for the extent of invasion of mold fungi within the endosperm. A few widely grown sorghum hybrids have the capacity to withstand mold invasion in the endosperm, but fungi discolor the pericarp even under moderate mold pressure. If the pericarp is removed, such grains appear almost as clean as non-moldy grains. Removal of the pericarp will be sufficient to remove most of the fungus and to reduce mycotoxins, as has been shown in rice (*Oryza sativa* L.) (Vasanthi and Bhat 1998) and α-amylase levels. Removal of the pericarp is possible by several means including mechanical dehulling. Research at ICRISAT has shown that the appearance of moldy grain can be improved by dehulling (ICRISAT 1986). Village-level dehulling of molded grains has been suggested to improve the quality of molded grain (Stenhouse et al. 1998). It may be possible to decorticate the sorghum using rice polishers (Raghavendra Rao and Desikachar 1964) or the decorticator developed by the International Development Research Centre (IDRC), Canada (Reichert et al. 1982), which leave the germ intact. The commercial value of dehullers and dehulled products has been demonstrated with clean sorghum grains (Geervani and Vimala 1993). Further research is required on several aspects of value addition to superficially molded grains for which linkages with sorghum processors and consumers are necessary. These research areas include identification of suitable cultivars with adequate mold resistance and grain quality characters; optimization of the dehulling procedure and equipment for grains with different levels of moldiness, grain hardness, grain size, and mold resistance; limits of grain moldiness under which the technology is operable; economics of value addition and its effect on consumer acceptance; nutritional, keeping quality, and safety of dehulled grains; and other related

areas. However, before extensive research can begin, there is a need to determine the consumer acceptability and marketability of the dehulled products and to identify the market opportunities in the rural and urban sectors. Therefore, a pre-feasibility study is required for dehulling technology that appears to be one of the few short-term solutions to the most important food quality problem of sorghum consumed by the poor.

Feed

Fishing and shrimp farming on one hand and poultry on the other account for most of the meat produced by the organized sector in India. There may be increased demands for beef and pork in the future while milch cattle demand diets rich in protein. Thus, it is only the poultry industry that would routinely use sorghum. In USA, sorghum grain for cattle is often processed into flakes (Hulse et al. 1980), which increases the digestibility (Osman et al. 1970; Fredrick et al. 1973). Sorghum is also used to make silages, which involves a process of germination and fermentation (Hulse et al. 1980). These treatments have been shown to increase the availability of protein and starch from sorghum with soft grains making better feeds. The use of processed sorghum for poultry could be explored. Moldy sorghum may be detrimental to the growth of chicken and the removal of mycotoxins would have to be addressed prior to the use of sorghum in poultry feed (Vasanthi and Bhat 1998). During the flaking process, decortication may occur that would remove some of the toxins, which might be present if moldy sorghum was used. However, processing of sorghum for poultry feed will increase the cost of the feed, rendering it non-competitive at present. Mold-resistant red-grain sorghum is a safe energy source in poultry feed. Mycotoxin-free less moldy whole grains are acceptable to poultry industry for which institutions are required for quality assurance against mycotoxin contamination. Sorghum is as nutritious for cattle and poultry feed as maize (Mitzner et al. 1994; Reddy and Rao 2000; Subramanian and Metta 2000).

Alcohol

Liquor manufacturers making grain-based products may use moldy and germinating sorghum (Sheorain et al. 2000) thus offering new market opportunities for moldy sorghum. The use of externally added enzymes

would enable the use of harder and more mold-resistant sorghums for this purpose (El Khalifa et al. 1999). 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).

Starch

There is very little difference between sorghum starch and maize starch (Norris and Rooney 1970; Chandrashekar and Desikachar 1984). However, there are differences in the yield of starch, which may be attributed to lack of concerted breeding and selection for this trait.

Recommendations

Considerable progress has been made in understanding the biology of grain mold fungi and mechanisms of resistance to the disease during the last 30 years. Efforts to produce sorghum genotypes with tolerance to grain mold by conventional breeding methods have yielded partially successful results. High degree of resistance could not be incorporated. The tolerance is operative only under moderate disease pressure. Research areas that need emphasis in future include:

- Determine the relative distribution of fungi belonging to *Fusarium* Section *Liseola* on sorghum, primarily from mycotoxin point of view.
- Determine the extent of occurrence of mycotoxins in sorghum grains and identify factors that are associated with mycotoxin contamination.
- Develop an improved knowledge base for grain mold epidemiology for forecasting the disease.
- Develop a screening system to evaluate sorghum genotypes under variable disease pressure.
- Use limited postharvest grain dormancy trait as a means to reduce postmaturity grain mold in the field and in storage.
- Intensify grain mold management research in two areas, each for a different time-frame:
  - For the long term, host plant resistance with emphasis on use of molecular biology tools to understand and integrate mechanisms of resistance in mold-resistant cultivars.

- For the medium to short term, determine the technical feasibility, consumer acceptability, and economic viability of alternate mold management methodologies such as timely harvest, grain drying, and grain processing. More importantly, encourage use of grains for feed and other industries that can tolerate mold to some extent through private-public partnership research.

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Structure, Phenolic Compounds, and Antifungal Proteins of Sorghum Caryopses

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Sorghum (*Sorghum bicolor* (L.) Moench) is a cereal of remarkable genetic variability, which makes it difficult to classify. A few names of sorghum are milo, *jowar*, kafir corn, guinea corn, and *cholam*. The plant originated in equatorial Africa and is distributed throughout the tropical, semi-tropical, and arid regions of the world. The seed or caryopsis of sorghum provides a major source of calories and protein for millions of people in Africa and Asia. Grain characteristics of sorghum have been documented by Rooney and Miller (1982). Appearance and quality of sorghum are affected significantly by genetically controlled characters.

Grain is marketed, according to the US grain standards (USDA 1987), as sorghum, tannin sorghum, white sorghum, and mixed sorghum. The sorghum grade does not contain more than 3% of sorghum with a pigmented testa or undercoat. The white grade contains sorghum with a white pericarp without a pigmented testa. It cannot contain more than 2% of sorghum with colored pericarp or testa. Mixed sorghum contains mixtures of sorghum with and without pigmented testa. Tannin sorghums contain proanthocyanidins as part of their phenolic compounds but do not contain tannic acid nor hydrolyzable tannins. Tannin sorghums have a pigmented testa on the innermost layer of the pericarp. The pigmented testa is seen as a dark layer between the light endosperm and the pericarp when the caryopsis is scraped to remove the pericarp. Bleaching using the bleach test causes the constituents in the pericarp and testa to oxidize and yields black pigments on

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the surface of the caryopsis (Waniska et al. 1992). Sorghums with a pigmented testa and tannins remain black longer during bleaching than do non-tannin sorghums.

Appearance and Genetics of Sorghum

Several interacting factors affect the color and overall appearance of sorghum caryopses. Appearance is mainly affected by pericarp color and thickness, presence of pigmented testa, and endosperm color (Rooney and Miller 1982). Secondary plant and glume colors, as well as, deterioration due to the environment, insects and molds, complicate the evaluation of pericarp color and other properties. Immature pericarp tissues, when damaged, respond with antimicrobial phenolic compounds, which form pigments that stain the pericarp and endosperm. Insect and mold damage of the pericarp commonly occurs together when caryopses mature in hot, humid environments.

Pericarp color is genetically controlled by the R and Y genes. The combination of these genes can produce white or colorless (R_yy or rryy), lemon yellow (rrY_), or red (R_Y_) color. The intensifier (I) gene increases the brightness of the pericarp color; this is especially prominent in red pericarp sorghums. The Z gene controls pericarp thickness. Sorghums with homozygous recessive (zz) genes possess a thick mesocarp. A thick pericarp contains small starch granules, which causes a chalky appearance that masks the color of the testa and endosperm (Earp and Rooney 1982; Rooney and Miller 1982). A pigmented seed coat or testa is present when both B_1_ and B_2_ genes are dominant. The tptp genes control testa color, which can be brown or purple. Caryopses with a pigmented testa (B_1_B_2_) and a recessive spreader gene (ss; type II) or dominant spreader gene (S_; type III) contain condensed tannins and are called brown or tannin sorghums (Hahn and Rooney 1986). Type III sorghums have more tannins than type II sorghums, whereas type I sorghums do not contain tannins or proanthocyanidins.

A black sorghum from Sudan, Shawaya, has very high levels of flavonoids that oxidize in the pericarp during caryopsis development and mask pericarp color. The black pigmentation is a recessive trait. Some sorghums have a dominant trait for intense pigmentation or blotching in the pericarp and peripheral endosperm when damaged. Blotching detracts from appearance and food utilization. Darker secondary plant and glume colors are dominant and increase the pigmentation of milling and food products.

Each secondary plant color (tan, red, and purple) has a range of glume colors (from very light to very dark). The lighter glume colors yield products that are less pigmented and are preferred in most foods.

Yellow endosperm cultivars contain high levels of carotenoid pigments. Endosperm color affects appearance, especially in caryopses with a thin pericarp and without a pigmented testa. Caryopses appear yellow when the pericarp is thin and colorless, the testa is absent, and the endosperm is yellow. A thick mesocarp and colorless pericarp cause a white or chalky appearance. Heteroyellow endosperm sorghum results when sorghums with yellow and non-yellow endosperm colors are hybridized (Rooney and Miller 1982). Bronze sorghums contain a thin, red pericarp with yellow endosperm color, while cream sorghums contain a thin, white pericarp with yellow endosperm.

Waxy endosperm cultivars contain three genes (wx) in the recessive form. Heterowaxy genotypes contain one or two of these genes in the dominant form whereas normal or non-waxy endosperm sorghums contain all three genes in the dominant form. Waxy cultivars contain nearly 100% amylopectin and the endosperm looks like candle wax (Rooney and Miller 1982).

The environmental conditions during caryopsis development and maturation greatly affect the appearance of sorghum because the panicle or head is readily exposed to biotic and abiotic agents (Castor and Frederiksen 1980; Glueck and Rooney 1980). An environment that is hot and humid during maturation negatively affects quality. Deterioration results from insects, molds, moisture, and sunshine. Physical damage resulting from insect attack activates a phenolic defense mechanism that produces discolored spots in the pericarp and the endosperm. Mold colonization discolors the pericarp surface initially, breaks down the endosperm composition and structure, and adversely affects processing quality (Waniska et al. 1987). Sorghums that have an open panicle structure and caryopses with thin pericarp, condensed tannins, corneous endosperm, and large, tight glumes are generally considered to be more resistant to grain molding and weathering (Waniska et al. 1989).

The high-lysine (hl) sorghum from Ethiopia has a soft, floury endosperm texture, a shriveled kernel structure, and is susceptible to deterioration in environments where it rains during caryopsis development (Rooney and Miller 1982). The improved protein digestibility sorghum (Weaver et al. 1998) and the chemically induced, high-lysine sorghum (Ejeta and Axtell 1987)

have intermediate-soft to soft endosperm textures; they have reduced grain yield and increased deterioration due to grain molds and weathering.

Food-type sorghums have tan secondary-plant-color, straw-colored glumes, white or clear pericarp, no pigmented testa, intermediate to hard endosperm texture, and increased resistance to grain weathering that mill into products with bland flavor, white color, and no off-colors.

Grain Morphology

The caryopsis consists of three distinct anatomical components (Fig. 1): pericarp (outer layer), endosperm (storage tissue), and germ (embryo). The outer layer or pericarp originates from the ovary wall (Saunders 1955; Glennie et al. 1984) and is divided into three histological tissues: epicarp, mesocarp, and endocarp (Earp and Rooney 1982). The outermost layer, or epicarp, is generally covered with a thin layer of wax. The epicarp is two or three cell layers thick and consists of rectangular cells that often contain pigmented material. Unlike most cereals, the sorghum mesocarp contains starch granules. A thick pericarp usually contains three or four mesocarp cell layers filled with small starch granules (Fig. 1). The inner pericarp tissue, endocarp, is composed of cross cells and tube cells. Pericarp thickness (the Z gene) ranges from 8 μm to 160 μm (Blakely et al. 1979; Earp and Rooney 1982) and varies within an individual mature caryopsis. The areas below the style and near the hilum are the thickest with the sides of the kernel being thinnest. The inner tube cells conduct water during grain germination whereas the outer cross cells form a layer that impedes moisture loss. A stylar area is located on the tip of the caryopsis, opposite the germ. The black layer, or hilum, is the colored placenta scar tissue that develops at the germ tip when the caryopsis reaches physiological maturity. Cell walls of sorghum pericarp, aleurone, and endosperm exhibit a blue autofluorescence, which is mainly due to esters of ferulic acid.

The seed coat or testa is derived from the ovule integuments. The thickness of the testa ranges from 8 μm to 40 μm and varies within individual caryopses (Blakely et al. 1979; Earp and Rooney 1982). The thickest area usually is observed below the style and the thinnest on the side. In sorghums with dominant B_1 and B_2 genes, the testa is pigmented and contains tannins (proanthocyanidins). Tannins are present in the testa and the pericarp in sorghum with dominant $B_{1_}$ $B_{2_}$ and spreader (S_) genes.

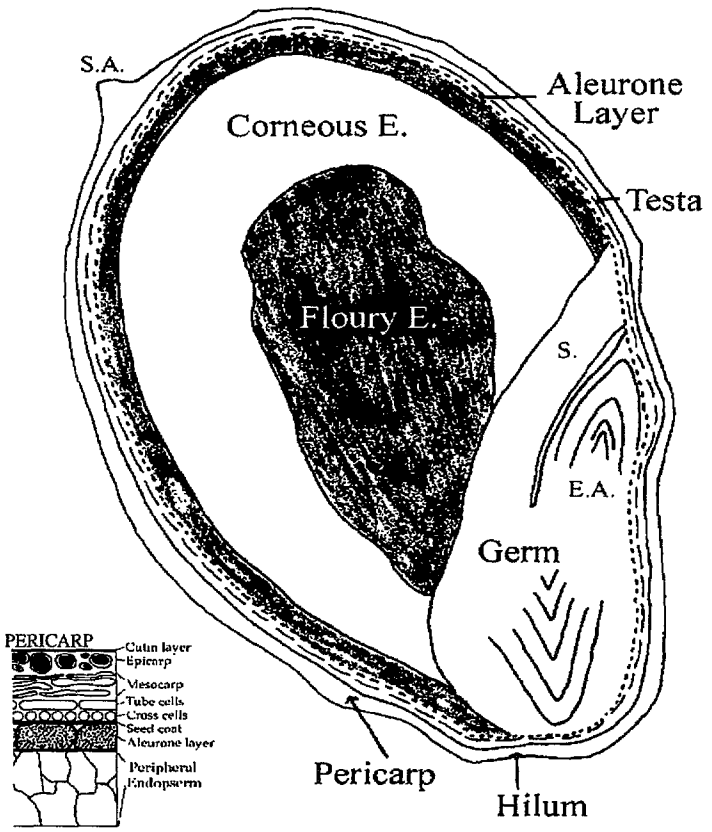

Figure 1. Diagram of sorghum caryopsis showing the pericarp [cutin, epicarp, mesocarp, tube cells, cross cells, testa, pedicel, and stylar area (SA)], endosperm (E) (aleurone layer, corneous, and floury), and germ [scutellum (S) and embryonic axis (EA)] (Source: Rooney and Miller 1982, with permission).

The endosperm tissue is triploid, resulting from the fusion of a male gamete with two female polar cells. It is composed of the aleurone layer, peripheral, and corneous and floury areas (Earp and Rooney 1982) (Fig. 1). The aleurone is the outer cover and consists of a single layer of rectangular cells adjacent to the testa or tube cells. The cells possess a thick cell wall, large amounts of proteins (protein bodies, enzymes), ash (phytin bodies), and oil (spherosomes). The peripheral area is composed of several layers of dense cells containing more protein and smaller starch granules than the corneous area. Both the peripheral and corneous areas appear translucent, or vitreous, and they affect processing and nutrient digestibility. Waxy sorghums contain larger starch granules and less protein in the peripheral endosperm than regular sorghums (Sullins and Rooney 1974, 1975).

The corneous and floury endosperm cells are composed of starch granules, protein matrix, protein bodies, and cell walls rich in cellulose, β-glucans, and hemicellulose. Starch granules and protein bodies are embedded in the continuous, protein matrix in the peripheral and corneous areas (Seckinger and Wolf 1973; Hoseney et al. 1974). The protein bodies are largely circular and 0.4–2.0 μm in diameter (Taylor et al. 1984). High-lysine cultivars contain fewer and smaller protein bodies than do regular sorghums, and thus contain significantly less alcohol soluble kafirins (Seckinger and Wolf 1973; Paulis and Wall 1979). The starch granules are polygonal and often contain dents from the protein bodies. Their size varies from 4 μm to 25 μm, the average being 15 μm. Granules present in the corneous endosperm are smaller and angular whereas those in the floury endosperm are larger and spherical.

The opaque, floury endosperm is located near the center of the caryopsis. It has a discontinuous protein phase, air voids, and loosely packaged, round, starch granules (Hoseney et al. 1974). The presence of air voids diffracts incoming light, which gives an opaque or chalky appearance.

The germ is diploid due to the sexual union of one male and one female gamete. It consists of two major parts: the embryonic axis and scutellum (Fig. 1). The embryonic axis contains the new plant and is divided into a radicle and plumule. Upon germination and development, the radicle forms primary roots whereas the plumule forms leaves and stems. The scutellum is the single cotyledon and contains reserve nutrients, i.e., moderate amounts of oil, protein, enzymes, and minerals, and serves as the bridge or connection between the endosperm and germ.

The kernel or grain is considered a naked caryopsis although some African types retain their glumes after threshing (Saunders 1955). Caryopses differ widely in weight (3–80 mg), test weight (708–785 g L^{-1}) and density (1.15–1.38 g cm^{-3}). Commercial US sorghums are generally 4 mm long, 2 mm wide, and 2.5 mm thick with a kernel weight of 25–35 mg, test weight of 747–772 g L^{-1} and density of 1.28–1.36 g cm^{-3} (Rooney and Miller 1982).

McDonough and Rooney (1990) monitored grain development of six sorghum cultivars using scanning electron microscopy. The pericarp begins to differentiate into distinctive layers at 3–6 days after anthesis (DAA). It is fully developed at 6–9 DAA and, thereafter begins to compress. Cells in the testa layer are apparent at 3–6 DAA, whereas the aleurone layer takes longer to develop (6–12 DAA). Ovary walls contain simple and compound starch granules at anthesis (Glennie et al. 1984; McDonough and Rooney 1990). Simple starch granules and protein bodies begin to develop in endosperm cells at 3–6 DAA. Protein bodies at this stage are covered by a thin filamentous webbing, which later develops into a distinctive protein matrix. During the milk stage (8–14 DAA), the endosperm cell walls contain a large number of holes to facilitate translocation of storage and biochemical components. The holes in the cell walls gradually disappear by the hard dough stage (20–25 DAA). The endosperm gains most of its storage material by the hard dough stage of caryopsis development, while moisture content decreases continuously from soft dough stage (15–19 DAA) (Glennie et al. 1984). Physiological maturity (or black layer) marks the end of nutrient delivery and the beginning of senescence and dessication of the caryopsis. The vitreous endosperm has a continuous protein matrix, which is attached to the starch granules, protein bodies, and cell walls (McDonough and Rooney 1990). The floury endosperm has a discontinuous protein matrix with many small voids between the starch granules. Rate of endosperm development is faster in sorghum with hard endosperm than that with softer endosperm.

Molds, Mycotoxins, and Grain Weathering

In many regions of the world, grain mold is a major disease of sorghum. Grain weathering is the added deterioration caused by the environment, sunshine, precipitation, and insects, during caryopsis maturation and exposure to

ambient conditions in the field. This reduces sorghum grain yield and quality, which affect physical properties, processing, and nutritional and market value (Glueck et al. 1978).

Mycotoxins are less of a problem in sorghum than in maize (*Zea mays* L.). Ergosterol levels correspond to mold colonization of weathered sorghum (Seitz et al. 1979) but have not been linked to animal health problems. Fumonisin has not been found at significant levels in sorghum probably because a different species that does not produce fumonisin, *Fusarium thapsinum* Klittich, Leslie, Nelson et Marasas sp. nov. colonizes sorghum (Klittich et al. 1997). Aflatoxin is not found at significant levels in the field but does appear on improperly stored, high-moisture sorghum (G.N. Odvody, Texas A&M University, Corpus Christi, USA, 1999, personal communication). These differences cause aflatoxin levels to be excessive in maize while sorghum is aflatoxin free in similar drought-prone environments.

Ergot, caused by *Claviceps africana* Fredrickson, Mantle, & de Milliano, has recently infested sorghum in the Western Hemisphere (Isakeit et al. 1998). The sclerotia contain alkaloids that may cause problems when fed to animals (Berde and Schild 1978). However, significant livestock or human health problems have not occurred in Africa and Asia where ergot has existed for centuries. This may change as more sorghum production occurs in cooler, moist environments. The hybrid seed industry must limit ergot infestation since the organism invades sterile florets and prevents seed production.

An array of fungal species, the most prominent being *Curvularia lunata* (Wakker) Boedijn, *Fusarium* spp, *Alternaria* spp, *Phoma sorghina* (Sacc.) Boerema, Dorenbosch, & van Kesteren, and *Dreschlera* spp, cause grain molding in the field (Castor and Frederiksen 1980). Wet, humid weather cause molding of caryopses after anthesis and post-physiological maturity. Limiting grain mold in sorghum has been very difficult. Chemical control is cost prohibitive, and biological control mechanisms have not been feasible. The most cost-effective and realistic method to control grain mold is by genetic resistance. However, selection for grain mold resistance is difficult because numerous genetic factors are reported to influence grain mold resistance.

Plant traits such as panicle shape, plant height, and glume structure have been associated with grain mold resistance (Castor and Frederiksen 1980; Rao and Rana 1989). Caryopsis traits such as endosperm hardness, a

pigmented testa layer, and red pericarp color are related with grain mold resistance (Glueck and Rooney 1980; Jambunathan et al. 1992; Esele et al. 1993; Audilakshmi et al. 1999). Although many sorghums with tannins and higher levels of phenol-based pigments are resistant to molding, these compounds cause dark colors, astringency, and/or decreased nutritional value in foods or feeds (Earp et al. 1983; Hahn et al. 1984). Accordingly, sorghums for food use are either grown in environments that are not conducive to deterioration, with a correspondingly lower yield, or cultivars resistant to grain mold are selected which yield less desirable food. Hence, grain mold resistance is necessary in food-type sorghums. Many food-type sorghums have been developed that have improved grain mold resistance; but none, currently, are resistant to grain mold in hot, humid environments. Many red pericarp sorghums have more resistance to grain mold than the white pericarp sorghums. None of these traits solely explains the variation in grain mold resistance found in sorghum. Additional factors necessarily underlie the visible factors in the expression of grain mold resistance. Continued efforts to overcome the limitations imposed by grain molds are required to facilitate increased production and utilization of sorghum in warm, humid environments. The following two sections discuss tannin and antifungal protein-based approaches to grain mold resistance in sorghum.

Tannins and Phenolic Compounds

Sorghum is unique among major cereals because some cultivars produce polymeric polyphenols known as tannins (Butler 1990). All sorghums contain phenols and most contain flavonoids; however, only cultivars with a pigmented testa, $B_1_B_2_$ genes, produce condensed tannins or pro-anthocyanidins. Most cultivated sorghums do not contain condensed tannins even though non-tannin, phenolic compounds are occasionally reported as tannins. Sorghums are classified as type I (without tannins), type II (tannins present in pigmented testa), or type III (tannins present in pigmented testa and pericarp). Tannins have antioxidant properties and are currently being considered as nutriceuticals (Hagerman et al. 1998).

Phenolic compounds have been divided into three major categories: phenolic acids, flavonoids, and tannins (Chung et al. 1998). Phenolic acids are derivatives of benzoic or cinnamic acid (Fig. 2). Flavonoids consist of two units: a C6-C3 fragment from cinnamic and a C6 fragment from

malonyl-CoA. The major groups of flavonoids in sorghum are the flavans—flavan-3-en-3-ols with double bond between C3 and C4 and hydroxylated at C3 are anthocyanidins. Tannins are polymers of 5–7 flavan-3-ol units (catechin) linked through acid labile carbon-carbon bonds (Hahn et al. 1984; Mehansho et al. 1987b; Butler 1990).

Sorghum containing tannin is called tannin or brown sorghum even though the pericarp color may be white, yellow, or red. Grain appearance is not necessarily related to tannin presence or content. Phenolic compounds

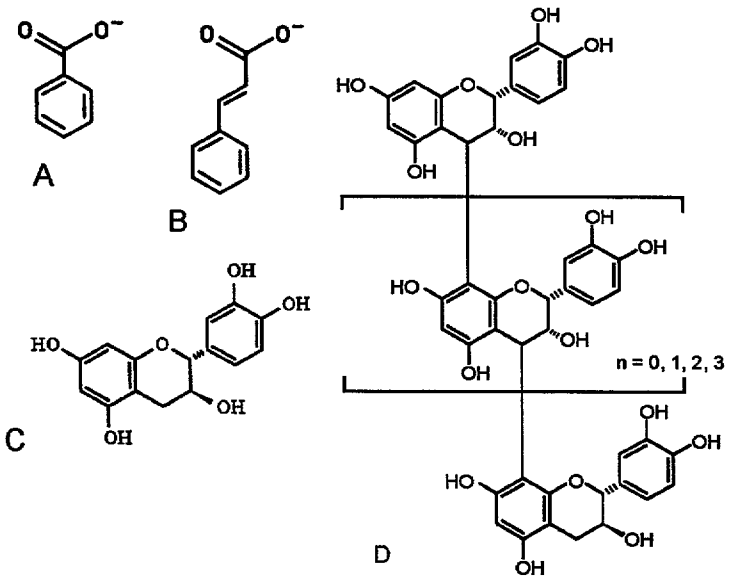

Figure 2. Basic structures of phenolic acids, flavonoids, and tannins (A = cinnamic acid; B = benzoic acid; C= the major flavonoid groups are flavanones (carbonyl at 4), flavonols (carbonyl at 4, hydroxyl at 3), flavones (carbonyl at 4, double bond between 2 and 3), and flavans (no carbonyl at 4); the major flavans are leucoanthocyanidins (hydroxyls at 3 and 4) and catechin (hydroxyl at 3, double bond between 3 and 4); and D = proanthocyanidin (tannin) polymer (n = 2–4) (Source: Rooney and Serna-Saldivar 2000, with permission).

and tannins have been reported in much higher levels (10- to 100-fold) in pericarp, glumes, and leaf sheaths than in the endosperm. Phenols isolated from the leaf sheaths (Sereme et al. 1993) and stems (Rey et al. 1993) can be used as pigments for clothes, pots, etc. Tannins (proanthocyanidins) apparently occur only in the pericarp, pigmented testa layers (Hahn and Rooney 1986), and glumes (Doherty et al. 1987) of some type II or type III sorghums but not in glumes of type I sorghums.

Hahn et al. (1983) separated, by reverse phase HPLC (high performance liquid chromatography), free and bound phenolic acids of sorghum. Eight main phenolic acids with different polarity were identified in extracts (Table 1). A typical brown sorghum contained the highest amount of free phenolic acids. Sorghum cultivars resistant to fungal attack contained both a greater variety and larger amounts of phenolic acids in the free form. Waniska et al. (1989) also partitioned sorghum phenolic acids and concluded that white cultivars without pigmented testa contained the lowest amount of phenolic acids. Brown cultivars contained higher levels of free phenolic acids and compounds and were more resistant to grain weathering. Phenolic acids and compounds increase during caryopsis development with a maximum at physiological maturity (and a decrease afterwards). This decrease may be due to decomposition, covalent linkage to structural polymers, or to entrapment with the solid endosperm matrix. Higher levels of phenolic compounds relate to improved grain mold resistance. This observation, however, may be the simple result of the strong correlation of phenolic compounds with tannins, since most tannin containing sorghums are more resistant to grain mold.

Table 1. Free and bound phenolic acid composition ($\mu g\ g^{-1}$) of sorghum.

	White sorghum (CS 3541)		Red sorghum (SC0630)		Brown sorghum (SC0719)	
Phenolic acid	Free	Bound	Free	Bound	Free	Bound
Gallic	-	19.7	-	46.0	-	26.1
Protocatechuic	7.4	133.9	13.0	83.0	8.0	15.8
p-Hydroxybenzoic	4.0	11.4	6.7	16.0	9.3	24.2
Vanillic	8.3	-	7.7	19.2	23.3	27.4
Caffeic	3.4	22.2	4.1	48.0	8.7	26.8
p-Coumaric	45.7	138.5	13.5	72.5	6.4	79.9
Ferulic	45.4	297.2	8.9	95.7	26.0	91.9
Cinnamic	9.4	-	10.7	-	-	19.7

Source: Hahn et al. (1983).

Tannins protect the grain against insects, birds, fungi, and weathering (Waniska et al. 1989). Rates of pre-harvest germination or early sprouting are lower in brown sorghums. These beneficial effects ensure that brown sorghums will continue to be produced in certain pest-ridden areas of the world (Butler 1990). These agronomic advantages are accompanied by nutritional disadvantages and reduced food quality. The antinutritional effects of tannin sorghums have been reviewed (Butler 1990; Butler and Rogler 1992; Chung et al. 1998; Hagerman et al. 1998).

The colors of sorghum grain and flour play an important role in its acceptance. In general, white sorghums produce the most acceptable colored food products. Food color is the result of factors such as grain color, pericarp color, pigmented testa, endosperm color, presence of tannins, degree of milling, and pH of the food system. Pigmentation in the pericarp and testa is primarily due to phenolic compounds. The color intensity greatly depends on pH. Anthocyanins are very unstable in acid medium and are readily converted to the corresponding anthocyanidin under slight acidic conditions (Hahn and Rooney 1986).

Pericarp color of sorghum appears to be due to a combination of anthocyanin and anthocyanidin pigments as well as other flavonoid compounds (Hahn and Rooney 1986). Kambal and Bate-Smith (1976) found no flavonoids in the pericarp of a white sorghum whereas Nip and Burns (1971) found four major anthocyanins in the pericarp of six white sorghums. Lutoforol was identified as the major pigment in red pericarp sorghums (Nip and Burns 1969). Cooking in alkali, i.e., alkaline tô and tortillas, usually promotes color formation especially in non-white sorghums. The glume or secondary plant color also affects the appearance of the caryopsis and food products. Lighter colored products are prepared using grain with straw-colored rather than darker glumes.

Several methods have been used to characterize phenolic compounds and tannins in sorghum (Maxson and Rooney 1972; Butler 1982; Hahn et al. 1984; Hagerman et al. 1997) (Table 2). Many tannin methods measure phenolic compounds, which may or may not be condensed tannins. The amount of tannin in the sorghum kernel is impossible to determine because a significant proportion cannot be extracted and assayed (Hahn and Rooney 1986; Butler 1990) and a suitable standard for sorghum tannins is unavailable. The highly polymerized or condensed tannin molecules are the most difficult to extract which may account for the decreased extractability of

tannins as caryopses mature. Different solvents and methods yield different tannin values because they extract and measure different chemical parts of the tannin molecule (Hagerman and Butler 1989). Methods that measure the reducing power of phenolic compounds are not specific for tannins. Protein precipitation methods do not differentiate between the types of tannins; they are sensitive to reaction conditions and the protein used. The acidic butanol method is specific for proanthocyanidins but other anthocyanidins in sorghum interfere with the reaction. The vanillin-HCl method using catechin as the reference with blanks subtracted indicate that significant levels of tannins are only present in type II and type III sorghums (Table 3). Tannins in type II sorghums are not extracted with methanol but with acidic methanol. Low values of tannin in type I sorghums are due to interfering compounds; hence these values are erroneous. Tannic acid is not present in sorghum, even though this laboratory and others have used tannic acid as a reference compound.

Table 2. Assays commonly used to determine sorghum phenols and tannins.

Method	Standard	Reagents	Extraction	Time	Assayed compounds
Vanillin	Catechin	4% HCl 1% vanillin in methanol	Methanol 1% HCl in methanol	24 h 20 min	Leucoanthocyanidin Proanthocyanidin
Prussian Blue	Catechin	$FeCl_3$ in HCl	Methanol	1 min	Phenolic compound
Folin-Denis	Tannic acid	Folin-Denis reagent	Water	5 h	All reducing compounds
Protein precipitation	Tannic acid	$FeCl_3$, alkaline detergent	Methanol	20 min	Proanthocyanidin
Folin-Ciocalteu	Catechin	Folin-Ciocalteu reagent	Methanol	1 h	Phenolic compound
	Gallic acid	Dimethylformamide	1% HCl in methanol	2 h	Phenolic compound
Acid butanol	Catechin	5% HCl in 2-butanol	–	1 h	Proanthocyanidin
Relative degree polymerization	-	4% HCl 0.5% vanillin in acetic acid 4% HCl in acetic acid	Methanol	20 min	Leucoanthocyanidin Anthocyanidin Proanthocyanidin

Source: Hahn et al. (1984); Hagerman et al. (1997).

Table 3. Effect of sorghum type on polyphenol levels using different assays and α-amylase inhibition[1].

	Polyphenol level (mg catechin 100mg^{-1})						
		Vanillin-HCl		Modified Vanillin-HCl			
Sorghum[2]	Prussian blue	With blank	Without blank	With blank	Without blank	24 h	α-amylase inhibition (%)
Type I	0.07	0.03	0.12	0.02	0.37	0.55	3.7
	(0.04–0.09)	(0.01–0.04)	(0.05–0.24)	(0.01–0.03)	(0.20–0.58)	(0.33–0.96)	(1.8–15.4)
Type II	0.07	0.03	0.19	0.07	0.52	0.98	6.0
	(0.05–0.09)	(0.02–0.04)	(0.09–0.52)	(0.02–0.19)	(0.34–0.88)	(0.68–0.98)	(2.3–15.8)
Type III	0.39	2.39	2.68	1.10	1.75	2.14	31.7
	(0.2–1.0)	(0.9–6.7)	(1.1–7.4)	(0.4–3.5)	(1.0–4.8)	(1.5–4.8)	(13.9–77.0)

1. Values in parentheses indicate range.
2. Type I sorghums do not have pigmented testa; Type II sorghums have pigmented testa without a dominant spreader gene; Type III sorghums have a pigmented testa and dominant spreader gene.

Source: Earp et al. (1981).

The antinutritional effects of tannins include diminished growth rate, protein digestibility and feed efficiency in rats, hamsters, swine, poultry, and ruminants (Jambunathan and Mertz 1973; Maxson et al. 1973a, 1973b; Cousins et al. 1981; Muindi and Thomke 1981; Mehansho et al. 1987a, 1987b; Knabe 1990; Mole et al. 1990, 1993). Tannins in sorghum reduce digestibility and efficiency of utilization of absorbed nutrients from 3% to 15%. Most animals consume brown or tannin sorghums at the same or slightly higher rates but do not gain as much weight.

Extracted tannins bind to proteins and inhibit many enzymes in in vitro assays (Hagerman and Butler 1994; Hagerman et al. 1997). Tannins from sorghum stimulate production and secretion of proline-rich, salivary proteins which bind tannins during mastication in many mammals (Asquith et al. 1987; Mehansho et al. 1987a); and some tannin-protein complexes are recovered unchanged in the feces (Butler and Rogler 1992). Tannin labeled with C^{14} was not absorbed by chicks; however, C^{14} labeled unknown polyphenols were absorbed and recovered in serum and other tissues. Apparently, lower molecular weight, absorbable polyphenols cause the systemic effects attributed to tannins (Butler and Rogler 1992).

Phenolic compounds (monomers, polyphenols, and tannins) do not interfere with absorption of iron in the gastrointestinal lumen. Iron absorption, however, is strongly inhibited by tannic acid (not present in sorghum) and

gallic acid followed by chlorogenic acid; whereas, no inhibition was observed when catechins or condensed tannins were added to the diet (Brune et al. 1989).

Several approaches for detoxification of brown sorghums have been proposed. The most common and practical ways are by decortication and malting. Decortication removes the pericarp and testa and, therefore, most tannins. However, decortication is inefficient because most brown sorghums have soft endosperm texture (Chibber et al. 1980; Nwasaru et al. 1988; Reichert et al. 1988). Parboiling of brown sorghum prior to decortication is a better alternative for tannin removal (Young et al. 1990). Chemical decortication with alkali solutions followed by neutralization with acid decreases the amount of assayable tannins (Kock et al. 1985; Butler 1990; Waichungo and Holt 1995). Steeping alone or steeping and malting effectively lowers measurable tannins up to 43% (Osuntogun et al. 1989). Sorghums treated with moisture and wood ashes lowers measurable tannins, up to 97%, improves protein digestibility and increases weight gains in rats (Mukuru et al. 1992). Similar effects are achieved when brown sorghums are treated with *Magadi* soda. Several experimental alkaline treatments to detoxify brown sorghums were proposed by Price et al. (1979), Butler (1990), and Waichungo and Holt (1995).

Antifungal Proteins

Proteins inhibitory to fungal growth have been identified in sorghum endosperm. Extracts of immature and mature hard and soft endosperm areas were inhibitory to growth of *Fusarium moniliforme* Sheld. (Kumari et al. 1992; Kumari and Chandrashekar 1994). Hard endosperm sorghums had more of three proteins that exhibited antifungal protein (AFP) activity. One of these proteins, a mycelia-hydrolyzing protein, probably is related to a cysteine protease inhibitor in pearl millet (*Pennisetum glaucum* (L.) R. Br.) (Joshi et al. 1998).

Seetharaman et al. (1996) identified three other AFPs, sormatin, chitinase, and glucanase, that increase during caryopsis development; they were high at physiological maturity and decrease at combine harvest maturity of the grain. Ribosome-inactivating protein (RIP) levels were high at 15 DAA, and then subsequently decreased. Effectiveness of sorghum AFPs in vitro was demonstrated when a mixture of sormatin, chitinase, glucanase, and RIPs

(110 $\mu g\ \mu L^{-1}$) inhibited spore germination and ruptured hyphal tips in both *C. lunata* and *F. thapsinum* (Tables 4 and 5), but only inhibited hyphal elongation for <36 h (Seetharaman et al. 1997). These AFPs are positively charged and water-soluble and elute from an anionic column with a salt gradient. The amount of AFPs in physiologically-mature sorghum caryopses was estimated to be approximately 260 ppm (30–35 mg in each caryopsis).

Table 4. Effect of protein fraction eluted from a Sephadex column using a linear salt gradient (10–500 mM) on spore germination of three fungi[1].

Fraction	Protein ($\mu g\ 100\mu L^{-1}$)	Antifungal protein (ppm)	*Fusarium thapsinum*	*Curvularia lunata*	*Aspergillus flavus*
1	306	0	++++	++++	++++
2	226	240	++++	++++	++++
3	208	260	++	++	+++
4	44	160	+	+	+

1. Scale: – = no germination, through ++++ = normal germination.

Table 5. Effect of concentration of proteins from fraction 4 eluted from a Sephadex column using a linear salt gradient (10–500 mM) on spore germination of *Curvularia lunata* and *Fusarium thapsinum*[1].

Protein ($\mu g\ 100\mu L^{-1}$)	Antifungal protein (ppm)	*F. thapsinum*	*C. lunata*
0	0	++++	++++
4.4	15	+++	+++
22	80	+	++
44	160	+	+
110	390	–	–
220	790	–	–
220, boiled	790	++++	++++

1. Scale: – = no germination, through ++++ = normal germination.

Sormatin is a thaumatin-like protein, i.e., a small, basic protein, approximately 22 kDa, which acts by causing membrane permabilization (Vigers et al. 1991). A β-1,3-glucanase of 30 kDa was identified in sorghum (Darnetty et al. 1993; Krishnaveni et al. 1999a, 1999b). Chitinases hydrolyze the N-acetylglucosamine polymer, chitin, a compound of fungal cell walls (Boller 1985; Yun et al. 1997). Several endogenous and stress inducible chitinases ranging from 21 kDa to 33 kDa were reported in sorghum (Darnetty et al. 1993; Krishnaveni et al. 1999a, 1999b). The RIPs range from 28 kDa to 31 kDa and inhibit protein synthesis in target cells by specific ribonucleic acid (RNA) N-glucosidase modification of 28s RNA (Logemann et al. 1992). Maize RIP antibodies cross react with a 30 kDa sorghum protein (Seetharaman et al. 1996). Results from these investigations suggest that understanding the interplay between AFP levels in caryopses, i.e., stage of maturity and environment (humidity/imbibition), and time of pathogen infection is important to maximize the bioactivity of AFPs in vivo.

Mobility of AFP within caryopses is a prerequisite for their possible role as a fungal growth inhibitor. Sorghum AFP leached from immature caryopses, but was retained in the pericarp of mature caryopses during water imbibition (Seetharaman et al. 1996). A similar study by Swegle et al. (1992) on the mobility of barley (*Hordeum vulgare* L.) chitinases is consistent with how AFPs help protect caryopses and seedlings from pathogenic fungi before and during germination. They found that AFPs were released from the endosperm into surrounding environment 24 h after water imbibition. If AFPs are mobile and 'bound' to the pericarp during imbibition, then AFPs could be concentrated in <10% of the caryopsis. This would increase AFP concentration about 10-fold, thereby, increasing their antifungal potential.

Antifungal protein and grain mold resistance (environment)

The AFPs are potentially important in fungal inhibition in the field; however, field data has been unclear about the relationships of AFP and grain mold resistance. Seetharaman et al. (1996) were the first to observe a significant inverse correlation coefficient between sormatin content in caryopses at 30 DAA with sorghum grain mold rating at harvest time. Subsequently, this was observed in seven environments with high levels of grain mold incidence (Rodriguez-Herrera et al. 1999). Levels of four AFPs were significantly affected by the 16 $F_{2:5}$ lines in all environments with higher levels of sormatin

and other AFPs observed in the resistant lines compared to the susceptible lines (Fig. 3). The data strongly suggest that sormatin is associated with grain mold resistance. Orthogonal contrasts showed that the β-1,3-glucanase levels were significantly higher in the resistant group in only three of seven environments. This suggests that β-1,3-glucanase may be associated with grain mold resistance in sorghum. However, its function on grain mold resistance may not be as direct as other AFPs or perhaps the β-1,3-glucanase level in plants is also a result of other mechanisms of response, different from a disease-resistance response. Chitinase and RIP concentrations in the resistant compared to susceptible lines were 1.5- to 14-fold higher and were associated with grain mold resistance.

Sormatin and chitinase contents were not significantly different ($P<0.05$) between resistant and susceptible lines at Halfway, Texas, USA in 1996. This supports the constitutive expression of sormatin in caryopsis. Sormatin and chitinase contents in caryopses of resistant and susceptible lines grown under a grain mold-free environment were higher than when grown in environments with grain mold incidence. There are several possible causes of this result. Resistant lines may maintain higher levels of sormatin for longer periods than susceptible lines under grain mold pressure and/or resistant lines may induce more AFP production than susceptible lines upon infection. Resistant lines had higher levels of sormatin one or two weeks after being stressed with grain mold fungal species compared to susceptible lines (Bueso et al. 2000). Resistant tissues accumulated chitinases more rapidly and in some instances to higher final concentrations than susceptible tomato tissues (Punja and Zhang 1993). Since AFP levels were affected by environment/fungal pressure and by degree of resistance, proteins that exhibit antifungal properties appear to have an important role in sorghum grain mold resistance.

Correlation estimates among the AFPs were positive and significant at most of the environments, except when grown in a grain mold-free environment. A significant and positive correlation indicates that under grain mold incidence, as one AFP increases in the sorghum caryopsis, so do the others. Co-expression of all AFPs seems to be a strategic biochemical action of the plant's resistance mechanism to grain mold. In vitro studies with *Trichoderma reesei* E. Simmons and *Fusarium sporotrichioides* Sherb demonstrated that a combination of barley RIP and chitinase inhibit fungal growth more efficiently than do either enzyme alone (Leah et al. 1991).

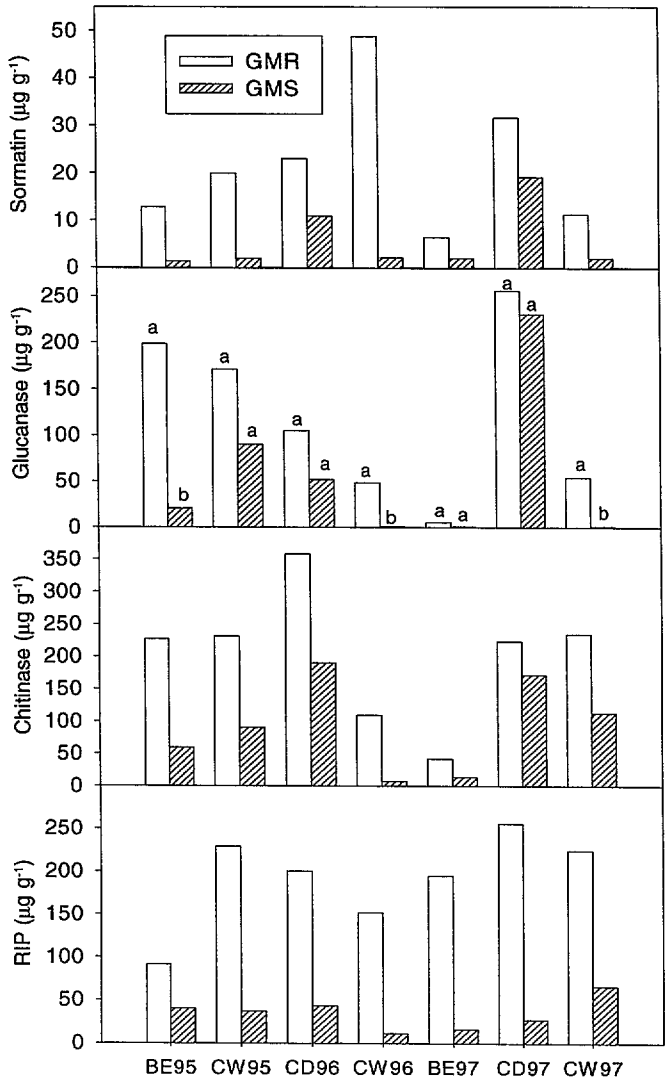

Figure 3. Mean antifungal protein levels (µg g^{-1}) in mature caryopses of eight grain mold resistant (GMR) and eight susceptible (GMS) lines grown in seven environments. Resistant lines have more AFP than susceptible lines except for glucanase. For glucanase means followed by the same letter are not significantly different (within environments).

Antifungal protein and mold resistance (inoculation and environment)

Variations between resistant and susceptible lines in wet and dry field environments and when inoculated with *F. thapsinum* and *C. lunata* at anthesis were studied. Mold resistance was based on the history of each cultivar during previous growing seasons (Table 6). We intentionally chose those differing in mold resistance and AFP content. Hence, there were

Table 6. Categorization of cultivars, plant maturity, caryopsis properties, and amounts of sormatin and chitinase in sorghums varying in mold resistance, Texas, USA.

Cultivar	AFP content[1]	Mold resistance[1]	Maturity[2]	Pericorp color	Pigmented testa	Spreader gene[3]	Endosperm hardness[4]	Sormatin[5]	Chitinase[5]	Mold rating at 50 DDA[6]
SC719-11E	Low	High	Early	Red	Yes	Yes	Medium	13	101	1 4
Malisor 84-7	High	Moderate	Medium	White	No	No	High	111	400	1.2
R9025	High	High	Medium	Red	No	No	Low	83	166	1.2
Sureno	High	Moderate	Medium	White	No	No	High	50	386	1 2
Hegari*Dobbs	High	Moderate	Medium	White	Yes	No	Medium	222	562	1.8
IS2319	Low	Low	Late	White	Yes	No	Low	83	456	5.0
E35-1	Low	Low	Late	White	No	No	Medium	17	271	4 8
RTx2536	Low	Low	Medium	White	No	No	Low	32	246	3.8
RTx430	High	Low	Medium	White	No	No	Low	153	528	3.0
BTx638	High	Low	Medium	Red	No	No	Medium	271	448	1.6

1. AFP= antifungal protein. Source: Seetharaman et al. (1996).
2. Early maturity = <60 days after planting, medium = 61–80%, late = >81%.
3. When spreader (S) gene is dominant, tannins accumulate in pericarp and testa.
4. Abrasion during decortication: hard = <30%; medium = 30–50%; and soft = >51%
5. Content (mg caryopsis^{-1}) in control caryopses at 30 days after anthesis (DAA). The coefficient of variation in sormatin and chitinase was 11–23% for the different cultivars, locations, and age of caryopsis.
6. Mold rating: 1=no mold to 5 = >50% of grain surface molded.

resistant-high AFP, resistant-low AFP, susceptible-high AFP, and susceptible-low AFP cultivars. Mold rating of sorghums grown at Halfway was 1.35±0.15 indicating that the environment was not good for grain mold. Mold ratings between the resistant and susceptible cultivars exhibited no remarkable distinctions at 30 DAA even with fungal inoculation and sprinkling treatments. Accordingly, mold resistance did not correlate significantly with mold rating scores or chitinase and sormatin levels at 30 DAA. Nevertheless after 30 DAA, mold growth was favored by a warm-humid environment in the field and

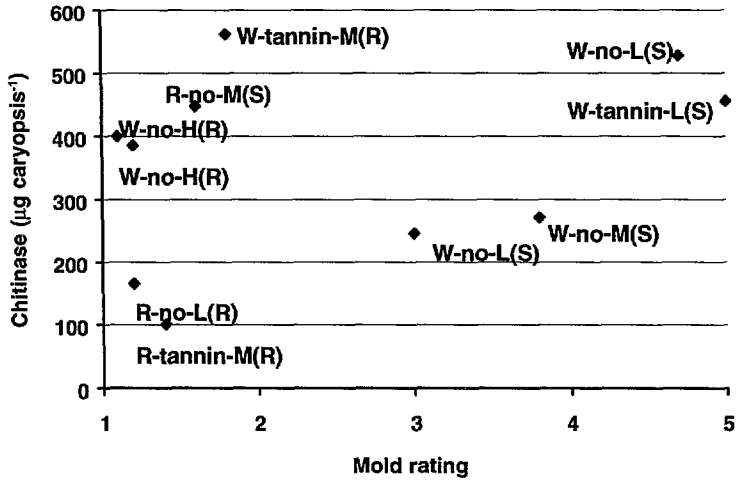

Figure 4. Scatter plot of chitinase in caryopsis at 30 days after anthesis (DAA) and grain mold rating of cultivars at 50 DAA. Labels are in the following format: pericarp color-presence of tannins-endosperm hardness (grain mold resistance); where W = white pericarp, R = red pericarp, tannins = tannins present, no = no tannins, H = hard endosperm, M = medium endosperm hardness, L = low endosperm hardness, S = susceptible, and R = resistant.

susceptible sorghums deteriorated. Hence, mold ratings increased considerably at 50 DAA and corresponded inversely with mold resistance.

The sorghums varied in maturity, pericarp color and composition, and endosperm hardness (Table 6), traits that have been associated with grain mold resistance (Bandyopadhyay et al. 1988; Esele et al. 1993; Menkir et al. 1996). At 50 DAA, two of three sorghums with pigmented testa, i.e., condensed tannins, had less deterioration but the third sorghum with pigmented testa molded (Fig. 4). Low mold ratings were observed in two sorghums with red pericarp and no tannins and in two sorghums with white pericarp and no tannins. Three other sorghums with white pericarp and no tannins exhibited much mold damage. So red pericarp color (vs white) was related to less molding, although the red pericarp sorghum, BTx638, exhibited more mold damage in previous years. Two sorghums with hard endosperm had the same low deterioration, as did sorghums with low and

medium endosperm hardness; whereas the four sorghums with the most deterioration had low or medium endosperm hardness. Hard grain had low mold ratings when stressed with sprinkling or inoculation with fungal pathogens and loss was less during decortication. This trend was also observed in control and the combined treatment but with $P \leq 0.1$.

Previous reports indicated that pigmented testa, red pericarp color, and hard endosperm confer mold resistance to sorghum (Castor and Frederiksen 1980; Hahn and Rooney 1986). Presence of proanthocyanidins but not flavan-4-ols and 3-deoxyanthocyanidins correlated with grain mold resistance (Melake-Berhan et al. 1996). Mold rating at 50 DAA was less when sorghum had red pericarp and/or hard endosperm traits. However, hidden factors, such as the presence of AFPs in the caryopsis might be involved. Seetharaman et al. (1996) noted differences in the mobility and extractability of chitinase and sormatin from sorghums of varying tannin contents. They found that Type I sorghums had variable levels of AFPs, Type II had increased sormatin and unchanged chitinase, while Type III had decreased AFPs. Therefore, they speculated that several mechanisms could interact to achieve grain mold resistance.

Amounts of AFPs in the caryopsis at 30 DAA were affected by field conditions of College Station and Halfway, Texas. Sormatin levels in caryopses were either higher (5) or unchanged (5) for sorghums grown in College Station compared to those in Halfway. Chitinase levels in resistant sorghums were either decreased (3) or unchanged (2) while chitinase levels in susceptible sorghums increased in three, decreased in one, and was unchanged in another cultivar when grown in College Station compared to those in Halfway. This suggests that less than ideal field conditions promote higher levels of sormatin and chitinase in susceptible cultivars and higher levels of sormatin but lower levels of chitinase in resistant cultivars.

The control caryopsis response between 30 and 50 DAA varied in AFP content and amount (Fig. 5). Sormatin increased in four resistant cultivars while chitinase increased in only one resistant cultivar. Sormatin and chitinase decreased in one resistant and all susceptible cultivars. It appears that elevated humidity induced AFP synthesis in some resistant cultivars but not in any susceptible cultivars. This partially contradicts the findings of Seetharaman et al. (1996) where AFPs decreased in caryopses after 30 DAA; however, the results could be evidence of an active defense response of AFPs in the caryopsis.

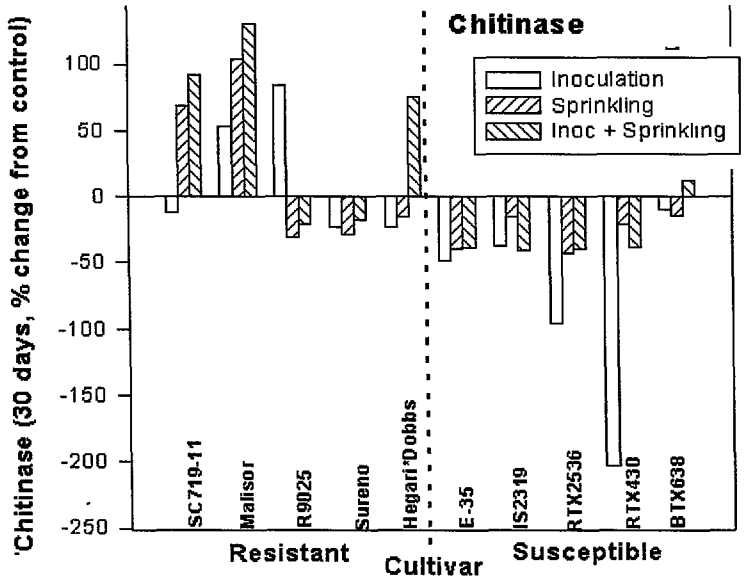

Figure 5. Changes in antifungal proteins as effected by sprinkling and/or inoculation in caryopses at 50 days after anthesis (DAA). (All values were compared to the control at 30 DAA in College Station, Texas, USA.)

The sprinkling treatment (intended to mimic brief showers) was conducted to determine mobility of AFPs in vivo. Sprinkling decreased sormatin in most cultivars at 30 and 50 DAA. Sprinkling increased sormatin in one susceptible cultivar, BTx638, at 30 DAA and one resistant cultivar, SC719-11E, at 50 DAA. Likewise, sprinkling decreased or did not change chitinase in most cultivars; however, sprinkling increased chitinase in two resistant cultivars, SC719-11E and Hegari*Dobbs, at 30 and 50 DAA respectively. Sprinkling caused mobility or a loss of AFPs in the caryopsis of most susceptible and several resistant cultivars.

Panicles were inoculated with fungal pathogens to determine whether AFPs were accumulated actively or passively. AFP levels decreased in most susceptible cultivars at 30 and 50 DAA (Fig. 5). Inoculation and fungal stress increased sormatin and chitinase at 30 DAA in two resistant cultivars (Malisor and R9025) and sormatin in two susceptible cultivars (IS 2319 and BTx638). Sormatin increased at 50 DAA in one susceptible and three resistant cultivars

and chitinase increased in two resistant cultivars. Sormatin levels at 50 DAA increased more consistently than chitinase levels in resistant cultivars. Fungal stress induced AFP synthesis more in resistant than in susceptible cultivars. Mold resistance appears to be related to the degree of AFP induction and not on the relative amount of AFP in the unstressed sorghum caryopsis.

The caryopsis response to inoculation and/or sprinkling between 30 and 50 DAA varied in AFP content and amount (Fig. 5). Most cultivars had unchanged or decreased AFP levels at 30 and 50 DAA. The resistant cultivar, SC719-11E, had increased sormatin and chitinase at 30 and 50 DAA. Susceptible BTx638 had increased sormatin at 30 DAA while two resistant cultivars, Malisor and Hegari*Dobbs, had increased chitinase at 30 DAA. The resistant-like behavior of BTx638, a susceptible cultivar, may be an anomaly of the growing season, misclassification of resistance, or due to other factors. The combined treatment of fungal inoculation and sprinkling resulted in AFP changes more similar to the sprinkling than to the inoculation treatment. Sprinkling generally caused reduced AFP in all cultivars, while inoculation increased AFP levels in some of the resistant cultivars.

The resistant cultivar that initially had low levels of AFP (SC719-11E) was induced by the treatments to accumulate more sormatin and chitinase at 30 and 50 DAA (Fig. 5). The other resistant cultivars, except Sureno, responded to one or more of the stresses with higher levels of sormatin or chitinase during caryopsis development. The susceptible cultivars, regardless of AFP level, responded with lower levels of AFP with only one exception. This implies that the amount of AFP in caryopsis is less important than induction of AFP accumulation in the caryopsis by stresses leading to grain molding.

Mold rating of sorghum caryopses did not correlate to AFPs (discussed earlier), but correlated with change (%) in AFP (Table 7). Significant correlation coefficients were obtained at 50 DAA but not at 30 DAA. Grain molding increased during 30 to 50 DAA. Mold rating at 50 DAA was negatively correlated to change (%) in sormatin after inoculation and in chitinase after inoculation and sprinkling. This means that less fungal infection occurred when AFP levels increased. The imposed stresses either diminished AFP content in some cultivars and/or induced AFP synthesis in other cultivars. Hence, inherent grain mold resistance was more clearly expressed in less ideal field conditions. Conversely, grain mold resistance was associated with the amount that AFP synthesis that was induced by the stress.

Effect of stress on tissues

We attempted to determine how anatomical tissues in the caryopsis respond to stresses (Table 8). We chose wounding the embryo by puncturing and by soaking as stresses to determine which tissues (embryo, endosperm, or pericarp) were active in the defense mechanism. Evidence of increased AFP induction in sorghum caryopsis in response to stress (wounding or soaking) was demonstrated using white sorghums without a pigmented testa [Dorado (resistant to grain mold) and RTx2536 (susceptible to grain mold)]. Soluble AFP in caryopsis (from caryopses 15, 30, and 50 DAA) were identified using antibodies for chitinase, glucanase, or sormatin. More chitinase was in the endosperm while more glucanase was in the pericarp of the controls. Most of sormatin was found in the endosperm of Dorado and in the pericarp of Tx2536. We observed differences in how each AFP accumulated after wounding and/or soaking of caryopses. Very little AFP leached from caryopses. AFPs were inducible in both the resistant and the susceptible cultivar. When caryopses were stressed, changes in AFP were greater in the endosperm where chitinase and sormatin increased, while AFP levels generally decreased in embryo and pericarp. Wounding caryopses (30 DAA) increased chitinase in embryo and endosperm and sormatin in endosperm. Hence, chitinase and sormatin levels were affected by these stresses. Much needs to be learned about what affects AFP levels, sequence of events, tissue location of accumulation, potency in vivo of AFP against fungi, etc.

Table 7. Correlation coefficients between mold rating at 30 and 50 days after anthesis (DAA) and percentage change in sormatin and chitinase in sorghum caryopses[1].

	30 DAA		50 DAA	
Treatment	Sormatin	Chitinase	Sormatin	Chitinase
Inoculation	0.09	–0.51	–0.63**	–0.50
Sprinkling	–0.02	–0.05	–0.58*	–0.56*
Inoculation + sprinkling	–0.16	–0.31	–0.58*	–0.66**

1. Values for control at 30 DAA were used as reference to calculate change due to treatment.
Note: $*P \leq 0.10$; $**P \geq 0.05$.

Table 8. Concentration of antifungal proteins (chitinase, glucanase, and sormatin) in tissues of sorghum at 30 days after anthesis.

	Dorado		RTx 2536	
Tissue	(mg g^{-1})	(µg $seed^{-1}$)	(mg g^{-1})	(µg $seed^{-1}$)
Pericarp	6.8	87	10.0	147
Endosperm	0.6	129	0.5	98
Embryo	4.4	84	2.3	56

Conclusions

Our long-term interest is to limit deterioration of sorghum to enable its use in food products. Our data suggests various phenolic compounds and AFPs can play roles in limiting fungal infection of caryopses. We know, from previous research, that many other chemical, structural, and biological mechanisms are involved in lowering grain molding and weathering. Caryopses resistant to grain molding respond to some stresses by accumulating chitinase and sormatin than did susceptible caryopses. Pyramiding the defense mechanisms (phenols, AFPs) with other attributes that contribute to grain mold resistance is a good strategy to increase grain mold resistance.

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Human and Animal Health Significance of Mycotoxins in Sorghum with Special Reference to Fumonisins

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Foodborne illness both in humans and animals are of considerable public health concern. They are also of economic significance in view of the productivity loss and other monetary losses. Unseasonal rains at the time of harvest, often leads to development of molds and subsequent formation of toxic secondary metabolites known as mycotoxins in various agricultural commodities. Risk analysis studies indicated that when these molds affect staple foodgrains, the significance to human and animal health is considerable because their quantum of consumption is higher than that of other foods (Bhat and Moy 1997).

The susceptibility of sorghum (*Sorghum bicolor* (L.) Moench) to various molds is well documented (Williams and McDonald 1983). Mycotoxigenic fungi such as *Aspergillus, Alternaria,* and *Fusarium* and mycotoxins such as aflatoxin and T-2 toxin have been reported in sorghum (Rukmini and Bhat 1978). Ergot of sorghum caused by *Sphacelia sorghi* McRae has recently been observed to cause considerable crop losses in various parts of the world (Bandyopadhyay et al. 1996).

During the last decade, fumonisins, a group of mycotoxins produced by *Fusarium moniliforme* Sheld. and related species, have received worldwide attention. These mycotoxins, discovered in 1988 in South Africa, have been

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shown to exhibit unique toxicological properties that differed with different animal species (Marasas 1996). An attempt was made to study the occurrence of fumonisins in sorghum and its health implications in India.

Natural Occurrence of Fumonisins in Sorghum

Among the various naturally occurring fumonisins produced by *F. moniliforme*, fumonisin B1 is the major metabolite and is most toxic. Analysis of sorghum samples collected from markets and households in sorghum-growing regions of Andhra Pradesh, India showed that the extent of fumonisin contamination was higher in rain-affected and moldy samples compared to that in normal samples (Table 1). Though aflatoxins were found to co-occur no correlation was observed between fumonisin B1 and aflatoxin B1 indicating that both the toxins exist independently in the sample.

Table 1. Natural occurrence of fumonisin B1 and aflatoxin B1 in sorghum in Andhra Pradesh, India.

Sample	No. of samples analyzed	No. of samples positive			Concentration ($\mu g\ g^{-1}$)	
		Fumonisin B1	Aflatoxin B1	Fumonisin B1 and Aflatoxin B1	Fumonisin B1	Aflatoxin B1
Normal sorghum	44	2	9	2	0.15–0.51	Traces–0.03
Rain-affected sorghum	25	25	24	24	0.07–7.80	0.002–0.830

Isolation of toxigenic *Fusarium* species from sorghum and production of fumonisin under laboratory conditions

Among 35 *Fusarium* species isolated from normal and rain-affected sorghum samples, six isolates were identified as *F. moniliforme* and one isolate as *Fusarium proliferatum* (Matsushima) Nirenberg. However, only 2 of the 6 isolates were found to produce fumonisin at levels of 5.8 and 27.4 $\mu g\ g^{-1}$.

Sorghum was found to support good fungal growth as indicated by ergosterol production (3.88 mg g^{-1}) and fumonisin (125.31 µg g^{-1}) under laboratory conditions (Table 2).

Table 2. Growth of *Fusarium moniliforme* and production of fumonisin B1 in sorghum grain at different incubation periods under laboratory conditions[1].

Incubation period (days)	Fumonisin B1 (µg g^{-1})	Ergosterol (mg g^{-1})
10	8.25±0.65a	0.72±0.04a
20	19.57±0.60b	1.02±0.04b
30	45.32±0.66c	2.33±0.08c
40	117.91±0.76d	2.88±0.08d
50	122.21±0.76e	3.56±0.11e
60	125.31±1.83e	3.88±0.10f

1. Values with different letters are significantly different at 5% level.

Health implications of fumonisins

Widespread natural occurrence of fumonisins at varying levels in agricultural commodities and disease outbreaks in different farm animals such as horses and pigs has been reported (Norred 1993). The first report of fumonisin toxicity to human and poultry came from India (Bhat et al. 1997).

Acute Foodborne Disease Outbreak in Humans

Environmental factors that led to the outbreak

During October 1995, unseasonal rains in certain parts of the Deccan Plateau in India damaged much of the rainy season (kharif) sorghum crop in these regions. The sorghum crop that was being harvested at that time was left heaped in the field. The high moisture prevailing inside the heap provided a conducive environment for mold growth and fumonisin B1 production. Most of the crops that were harvested during this period became visibly moldy.

Epidemiological investigations

A rapid epidemiological survey was carried out in 50 villages belonging to 6 districts of Andhra Pradesh and Karnataka states of India. From the survey, it was observed that the outbreaks affected 1424 people representating 27 villages (Table 3). In these villages, the sorghum crop was harvested and left in the field during the unseasonal rains and became moldy. The disease was observed only in adults who were consuming the moldy grains. The affected people mostly belonged to the lower socioeconomic groups such as marginal farmers and landless agricultural laborers who did not have access to other staples. The disease was self-limiting, non-contagious, and non-fatal. It did not affect populations consuming grains harvested during the previous year, or harvested before or after the rains, or those who had access to other staples. It was not observed in children and pregnant women who were not given sorghum in their diet. People were aware of the moldy nature of the grain and its loss of rheological properties due to moldiness. Difficulties were experienced in the preparation of unleavened bread from moldy sorghum flour as the dough became sticky resulting in unevenly cooked, dark colored, and sticky bread.

Mycological profile indicated that *Fusarium*, *Aspergillus,* and *Alternaria* species were the dominant mycoflora in sorghum (Table 4). The extent of fungal contamination as assessed by ergosterol content was detected in the samples in quantities up to 120 mg kg^{-1}. Fumonisin B1 was present at higher levels in samples obtained from the affected households. The levels of aflatoxin detected were extremely low.

Feedborne disease outbreak in layer hens

Mold damaged grains frequently get channelized into animal feeding stuffs. An outbreak similar to the one that occurred in humans also occurred in some poultry farms during the same period. However, the implicated feed ingredient was fumonisin contaminated maize (Prathapkumar et al. 1997). The disease was characterized by black sticky diarrhea, reduced weight grain, drastic drop in egg production, lameness, and mortality. While antibiotics and vitamin supplementation had no effect, feed withdrawal showed improvement. The feed upon analysis contained 8.5 $\mu g\ g^{-1}$ fumonisin B1. The disease could be reproduced in 1-day-old cockerels with fumonisin B1 contaminated sorghum.

Effect of household cooking procedures on fumonisin B1 content in sorghum

Sorghum is consumed mostly in the form of unleavened flat bread or *roti* and porridge. Studies with fumonisin B1 spiked sorghum samples showed an insignificant reduction of 6.4% in the fumonisin level during the baking of *roti*. In porridge prepared with spiked samples the reduction in the fumonisin B1 content was only 11.9% (Table 5).

Decontamination/detoxification of moldy sorghum

Various physical and chemical methods have been investigated for their capability of detoxifying mycotoxins from foods and feed constituents. However, many of these methods could not find practical application due to problems of residual toxicity and decreased nutritive value. Recently, biotechnological methods based on microbial fermentation to detoxify mold-contaminated foods is achieving much significance. Some of the advantages of these methods over chemical methods include use of milder processing conditions without the need for aggressive agents thus preserving the nutritive value as well as absence of residual toxicity (Bol and Knol 1992). Such methods have particular relevance to the Indian context because microbial fermentations form the basis for the preparation of many traditional products from cereals and pulses. Studies are now focusing on certain species of lactic acid bacteria that have been particularly effective in controlling mold growth and mycotoxin production (Gourama and Bullerman 1995). Surveys have shown that the poorer segment of population who do not have access to alternate food sources consumes moldy sorghum.

Survey on Utilization of Moldy Sorghum

A preliminary survey has been conducted in certain sorghum growing and consuming regions of Andhra Pradesh (Mahbubnagar, Nalgonda, and Ranga Reddy districts) to study the socioeconomic aspects of utilization of moldy sorghum at the household level. The results of the survey showed that the quality and yields of sorghum depended on general environmental conditions. Under conditions of unseasonal rains and drought, the yields are less than half of the normal yields and sorghum grain is frequently mold damaged. Under such conditions the mold damaged sorghum is consumed

Table 3. Households and population affected by the sorghum foodborne disease outbreak in the villages surveyed in Andhra Pradesh, India.

Village	Households		Population	
	Surveyed (number)	Affected (%)	Surveyed (number)	Affected (%)
Atkur Thanda	34	97.0	303	91.7
Atkur	80	72.2	438	73.0
Kalkoda	50	78.0	287	81.5
Noorlapur	38	84.2	249	91.9
Thatepally Thanda	39	100.0	241	100.0
Yacharam	52	44.2	355	34.3
Total	293	76.1	1873	76.0

Table 4. Mycoflora and mycotoxin content of sorghum samples involved in the foodborne disease outbreak in India.

Sample source (no.)	Mycoflora (% grains infected)			Mycotoxins ($\mu g\ kg^{-1}$)		Ergosterol ($mg\ kg^{-1}$)
	Fusarium sp	*Aspergillus* sp	*Alternaria* sp	Fumonisin B1	Aflatoxin B1	
Affected households (20)	44–84	12–72	32–76	0.14–7.80	Trace–0.163	3.76–100
Unaffected households (5)	28–64	40–68	76–92	0.07–0.36	Trace–0.080	10–120

Table 5. Effect of household cooking on fumonisin B1 content in sorghum.

Preparation	Fumonisin B1 level ($\mu g\ g^{-1}$)		Reduction in extractable fumonisin B1 (%)
	Before cooking	After cooking	
Unleavened bread (*roti*)	1.25	1.17	6.4
Porridge	1.68	1.48	11.9

either by directly milling the entire grain into flour, or by first washing and sun drying and milling or by blending with good sorghum flour. An attempt was made to assess the modus operandi of collection and harvesting of moldy sorghum and the cost involved. It was observed that under normal conditions of considerably good yield, part of the sorghum harvested is sold at the rate of Rs. 4–5 kg^{-1}. When 50% of the crop becomes moldy, part of it is sold at the rate of Rs. 2–3 kg^{-1}, mostly for animal feed. When less than 10% of the crop is unaffected by mold, the sorghum stalks are left in the field without harvesting. The cost of labor involved in harvesting either normal or moldy sorghum is Rs. 25–50 day^{-1} or distribution of 10 kg of good sorghum per person. The number of people employed for harvesting depended upon the acreage, the quantity to be harvested, and the capacity of the farmer to pay. Under these conditions, it was observed that the monetary benefits of utilization of moldy sorghum for animal feed could be improved by detoxifying the grain by processes such as fermentation and also supplementing with a protein source.

Microbiological Analysis of Moldy Sorghum

Moldy sorghum samples were collected from the rural households and screened for presence of lactic acid bacteria. On the basis of biochemical tests and colony morphology, lactic acid bacteria species, namely *Leuconostoc*, *Lactobacillus,* and *Pediococcus* have been isolated from the moldy sorghum samples. These bacteria have been shown to possess mold-detoxifying capacities (Gourama and Bullerman 1995). The effect of the above lactic acid bacteria isolated from moldy sorghum on mold and mycotoxin in sorghum is presently being studied.

Use of Legume Source for Fermentation of Sorghum

During the survey in Nalgonda district it was observed that farmers used moldy sorghum for cattle feed in the form of *kudithi* (a traditional semisolid cattle feed) along with starch water, maize (*Zea mays* L.), and pigeonpea (*Cajanus cajan* (L.) Millsp.) or horse gram (*Macrotyloma uniflorum* (Lam.) Verdc.) as protein supplements. An attempt was made to find out locally available legume sources which are not used by humans for consumption. Different regions in Bhongir and Ranga Reddy districts were visited to assess

the availability of wild legumes belonging to *Cassia* family, namely *Cassia tora* L. and *Cassia occidentalis* L. These legumes have been found to contain high amounts of protein and essential amino acids. However, due to presence of antinutritional factors such as trypsin inhibitors, polyphenols, and haemoglutinins, their use for animal feeds has not been investigated so far. Studies have shown that such antinutritional factors frequently found in legumes are reduced considerably under fermentation conditions. The seeds of *Cassia* can be used as support base for fermenting moldy sorghum, and a detoxified product with improved nutritive value can be obtained. Such a product can become a value-added feed thus giving monetary benefit to the farmers. It was observed that *C. tora* and *C. occidentalis* grow wildly in Nalgonda and Ranga Reddy districts. However, these legumes are not eaten by animals due to their unpleasant odor and not consumed by people and hence not collected.

Conclusions

Consumption of moldy sorghum can have significant health and economic implications both for humans and animals. Susceptibility of sorghum to mold damage is difficult to control under problems of uncertain weather conditions during grain development, maturation, and harvesting. Education and extension and communication measures on appropriate agronomic practices can be an important step in preventing mold damage of sorghum. On the other hand, supplementation of moldy sorghum with *C. tora* seeds for animal feeding can be a cost-effective approach to increase the utilization potential of moldy sorghum and means of income generation to the farmer.

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Recent Advances in Mycotoxin Diagnostics

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Mycotoxins are a group of chemically diverse secondary metabolites of fungi that have a wide range of toxic effects on humans and animals. It is important to be able to detect and quantify mycotoxins in foods and feeds so that such contaminated materials can be handled so as to protect human and animal health. A recent report from India (Katiyar et al. 2000) showed clearly the risk of liver cancer caused in the population in the Indian subcontinent as a result of either infection by Hepatitis virus B or ingestion of aflatoxin B1. When mycotoxins occur in agricultural commodities they occur in concentrations ranging from micrograms (ppb) to milligrams (ppm) per kilogram. Many different methods are available currently for the estimation of mycotoxins. Biological detection methods are based on the toxic effect of mycotoxins and use death, pathological lesions, changed biochemical events, or immunotoxic effects as indices for toxicity. But these methods are not specific and their sensitivity is generally low compared to those of other methods. Thus these are often used only as a tool for screening for general toxicity. Physicochemical assay methods such as thin layer chromatography, high performance liquid chromatography, gas chromatography, and mass spectrometry are laborious and require expensive instrumentation and clean-up of the samples. Thus these methods are of limited use for making routine safety and quality control screening for mycotoxins in agricultural

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commodities. To overcome the difficulties with biological and physico-chemical methods, new immunochemical methods have been developed. Serological methods that use specific antibodies are gaining wide acceptance for quantitative estimation of mycotoxins because of their sensitivity and specificity (Chu 1986). They are also less expensive to perform than the majority of analytical methods. This paper describes recent advances in serological methods, which should prove simple, robust, and cost effective for the quantitative estimation of mycotoxins.

Immunological Methods

The two major requirements for the application of immunochemical methods are high quality antibodies and methodologies to use the antibodies for the estimation of mycotoxins.

Antibodies

Polyclonal antibodies. Polyclonal antibodies are produced as a result of injecting antigens into an animal. They always contain a mixture of antibodies to various epitopes. The most frequently used method for the production of antibodies to mycotoxins is multiple-site immunization of rabbits with mycotoxin conjugated to a protein, followed by a booster at 5- to 6-week intervals. This procedure often does not yield high titer antibodies. A modification is to use several subcutaneous injections at multiple sites followed by a non-immunization rest period of six to eight months. Booster subcutaneous injections after this rest period resulted in the production of high titer antibodies for ochratoxin A and aflatoxins. Polyclonal antibodies for aflatoxin B1 often recognize all the four aflatoxins, B1, B2, G1, and G2. Interestingly, polyclonal antibodies raised against ochratoxins have been found to be specific to ochratoxin A.

Monoclonal antibodies. An alternative approach to the production of polyclonal antibodies is the development of stable hybridoma cell lines that secrete reagent quality monoclonal antibodies to mycotoxins. In monoclonal antibody production, the short-lived B lymphocytes from the spleen are individually immortalized by fusion with an immortal myeloma cell line to give a range of clones of B lymphocytes (hybridomas), each of which

produces its own antibody. This is achieved by isolating splenic lymphocytes that produce a single antibody, which reacts with a single epitope, and is of a defined class (IgG) and subclass, from an immunized mouse and fusing these with a myeloma cell line. Following a series of selection and screening steps, an "immortalized" clone that constantly produces antibodies of the desired affinity, specificity, and performance characteristics can be isolated. Although this approach has disadvantages in terms of cost, effort, time, and the requirement for tissue culture facilities, monoclonal antibodies tend to exhibit much lower degree of inter-assay variation, and yield more highly reproducible results, than do polyclonal antibodies. Using a novel immunization schedule, we have produced and characterized monoclonal antibodies for aflatoxins that are highly sensitive and that also differ in their specificities (Devi et al. 1999).

Recombinant antibodies. Recent advances in recombinant antibody technology have opened up new possibilities for serological diagnosis because it is possible to engineer low-cost antibodies with desirable affinities and specificities essentially by manipulation of the basic domain structure of the immunoglobulin molecule. It is possible to obtain deoxyribonucleic acid (DNA) that encodes the V_H and V_L fragments of the antibody molecule by specific amplification in a polymerase chain reaction (PCR). The resulting cDNA (complimentary DNA) can then be cloned into a vector such that expression of the cDNAs (from V_H and V_L genes) produces a single chain variable fragment (scFv) molecule. The expression vector is constructed so that when expression of the cloned gene is induced in an appropriate strain of *Escherichia coli* L., the scFv is produced, either as part of a fusion protein with the product of gene III that encodes the minor coat protein pIII of a filamentous phage (phage-display), or as a soluble molecule (Ziegler 1999). Because antibody fragments are displayed on the surface of infective bacteriophage, it is possible to select and clone an individual phage-antibody from a large population (Harper et al. 1999). Furthermore, it is possible to change the binding affinities and specificities of scFv fragments using techniques such as site-specific mutagenesis and the exchange of V_H and V_L segments between different scFv molecules. Once clones are made, it becomes possible to produce unlimited quantities of scFv at low cost in relatively low technology facilities (Torrance 1999). A current project funded

by the UK Department for International Development (DFID) is exploring these possibilities.

Development of the immunoassay

Once the appropriate antibody has been produced and characterized, it is possible to develop an immunoassay for specific mycotoxin determination. Two types of ELISA (enzyme-linked immunosorbent assay) format have been used for the assay of mycotoxins. We have concentrated on the development of indirect competitive ELISA and on the various parameters required to obtain optimum results in quantitative estimations of mycotoxins.

Other Novel Methods

Mimotopes

The food and feed industries, which routinely test ingredients and products for mycotoxins, are facing pressures to increase the amount of testing done. All the assay procedures used involve the handling of pure mycotoxins, which are toxic. Consequently, safety is an increasingly important issue for workers in these industries. Therefore, the use of an alternative form of mycotoxins in immunoassays would be of great value, as this could reduce the risks caused by handling pure toxins and also could provide a reliable substitute for pure toxins when performing immunoassays.

Peptides are known to be able to mimic the reaction between epitopes in antigens and the antibodies they elicit. These peptides have been termed mimotopes (Geysen et al. 1987). Random peptide libraries displayed on phage particles have been shown to be a powerful tool for obtaining mimotopes for epitopes recognized by monoclonal antibodies (Smith 1991; Cortese et al. 1994).

To search for an alternative substance to mycotoxins as immunochemical reagents, phage-displayed random peptide libraries have been used as a source of peptides that mimic the epitope(s) on mycotoxins. In phage display, a peptide or a protein is fused to one of the coat proteins of fd bacteriophage (e.g., pIII, pVIII), which means that the fused protein is displayed on the exterior surface of the phage particle (Parmley and Smith 1988; Felici et al. 1991; Smith 1991). Libraries consist of phages that contain

all possible amino acid sequences in the surface-exposed peptide. These are screened in order to select peptides with specific affinities or activities (Cwirla et al. 1990; Devlin et al. 1990; Scott and Smith 1990). Using peptide libraries, it is possible to select peptides that bind to an antiprotein antibody. Random peptide libraries have been used in a wide variety of applications (Scott and Smith 1990; Cortese et al. 1994; Katz 1997). However, very few workers have used this technology to select peptides that mimic non-proteinaceous chemicals such as biotin (Weber et al. 1992) and carbohydrates (Hoess et al. 1993). To our knowledge only a single report described the use of this technology. This concerned the mycotoxin deoxynivelenol (Yuan et al. 1999).

Phage-displayed mycotoxin epitope should provide a safe, convenient, cost-effective, and precisely defined standard for serological testing for mycotoxins. Also, they could possibly be used to coat ELISA plates in the place of mycotoxin or mycotoxin conjugated to a carrier protein. In a current DFID-funded project, we have selected phage-displayed peptides that can bind strongly to each of three monoclonal antibodies that vary in specificities (Devi et al. 1999). The applications of these mimotopes are currently under development.

Capillary zone electrophoresis

While the ultimate aim of mycotoxin testing is the correct and accurate identification of particular mycotoxins, methods that can detect simultaneously more than one mycotoxin are widely used as an intermediate step in the screening of potentially contaminated samples. Only those samples found to contain toxins are then subjected to a range of methods designed to facilitate the quantitative analysis of a particular mycotoxin. Intact mycotoxins can be separated from other components by dissimilar charge using electrophoretic techniques. The advantages of capillary zone electrophoresis include the use of much smaller sample volumes and the generation of substantially smaller volumes of hazardous waste, while the major disadvantage of this method is the long derivatization time. The method holds considerable promise and further developments to improve sensitivity and time taken for analysis are expected.

Conclusions

Serological tests have immense potential for the development of novel diagnostic tests which can help meet crop protection challenges in this millennium. The methods being developed are simple and safe to perform and are robust and cost-effective to carry out on a wide scale in a variety of laboratory circumstances.

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Some Solutions to the Problem of Grain Mold in Sorghum: A Review

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Grain mold of sorghum (*Sorghum bicolor* (L.) Moench) results from colonization of fungi in the developing grain towards the end of the growing season, being often associated with late rains. The major colonizing fungi [*Fusarium thapsinum* Klittich, Leslie, Nelson et Marasas sp nov., *Curvularia lunata* (Wakker) Boedijn, *Alternaria alternata* (Fr.) Keissler] also produce mycotoxins which are harmful to humans and livestock (Anasari and Shrivastava 1990; Forbes et al. 1992; Bhat et al. 1997). These fungi invade the developing grain but at different stages (Bandyopadhyay et al. 2000). Molded grains are unfit for most of the food uses. The accompanying discoloration brings down the marketability of the grain. Attempts to produce resistant genotypes by plant breeding have led to only moderate success (Stenhouse et al. 1998) while control by fungicides and crop management strategies may be beyond the means and abilities of many farmers (Williams and Rao 1981) and would lead to enviornmental problems. Consequently a review of novel approaches to improve the intrinsic resistance of sorghum to grain molds is timely.

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Plant Resistance Mechanisms

Plant resistance mechanisms can be broadly divided into two types which relate either to constitutive features of the structure or composition of the plant organ or tissue or to inducible systems which are only switched on when the plant is challenged by infection, damage, or treatment with a chemical elicitor. In some cases, such as antifungal proteins (AFPs), the same type of component may be present constitutively in some tissues and form part of an inducible response in others.

Constitutive resistance includes structural features, which prevent penetration into the host tissues and cells. For example, the thickness of the cuticle and cell wall may limit penetration into the cells while texture (hardness) may hinder the progress of the fungi within the cells themselves. A further level of constitutive resistance is conferred by the presence of AFPs peptides, and other compounds, either in the apoplasm or within the cells (usually the cell vacuoles or protein bodies).

In contrast, inducible systems are only switched on when the plant is challenged and can be considered to have three components: **receptor proteins** or other components that recognize the challenge and activate **signal transduction pathways** which lead to effects on **response genes**. Recent work has provided exciting information on receptor genes (as discussed below) while response mechanisms are also understood in some detail and include the generation of active oxygen species, changes in cell wall polymers, and synthesis of AFPs and low molecular weight anti-microbial compounds such as phytoalexins. The responses can lead to hypersensitive cell death and can be localized to the site of challenge or expressed systemically in other organs and tissues. However, we currently know little about the signal transduction pathways, which connect receptors to response pathways especially in grain.

One particularly well-studied aspect of plant resistance is the pathogenesis-related (PR) protein response. These proteins were initially identified in tobacco (*Nicotiana tabacum* L.) leaves responding hypersensitively to tobacco mosaic virus (TMV) (Van Loon and Van Kammen 1970) and have since been identified in a range of other species where their synthesis may be induced by microbial infection (viruses, viroids, fungi, or bacteria) or by chemical elicitors, notably salicylic acid and acetyl salicylic acid (aspirin). The latter is now known to relate to the role of salicylate in

pathogen signaling in the host plant (Doares et al. 1995; Mur et al. 1996). At least ten PR proteins are present in tobacco (Van Loon 1985; Bowles 1990), which are now known to have various biological activities, including enzymic activity as β-glucanases and chitinases, chitin binding, and membrane permeabilization (Shewry and Lucas 1997).

Both constitutive and inducible resistance mechanisms may play roles in the resistance of sorghum grain to mold and may also form the basis for future attempts to confer resistance by genetic engineering. However, the characteristics of the developing and mature sorghum grain must be taken into account when deciding on strategies. It is likely that the constitutive mechanisms may be more important than inducible mechanisms in the protection against grain mold.

We would like to distinguish between AFPs and disease resistance proteins. The former are directly involved in attacking or killing fungi and reducing their growth. The latter are signal mechanisms that activate different resistance mechanisms including the levels of AFP genes.

Grain Structure and Development in Sorghum

The structure of the mature grain of sorghum is shown in Figure 1. As in other cereal grains it comprises two main tissues, the triploid storage endosperm and diploid zygotic embryo, surrounded by the pericarp (a maternal tissue) and testa. The testa in some sorghum species is pigmented and contains condensed tannins. These may confer resistance to birds but also affect the nutritional quality. Tannins also confer a very high degree of resistance to molds (Harris and Burns 1973; Menkir et al. 1996) but Indian sorghums are virtually tannin free (Radhakrishnan and Sivaprasad 1989; Chavan and Salunkhe 1984). The thickness of the testa has been reported to vary from 8 μm to 40 μm (Earp and Rooney 1982) with the thickest area being below the style and the thinnest on the side of the kernel (Blakely et al. 1979).

The endosperm has a single outer layer of rectangular aleurone cells, which have thick walls and contain oil and protein bodies. Three layers of starchy endosperm cells can be recognized under the aleurone layer. The peripheral tissue comprises several layers of dense cells that are rich in protein but contain only small starch granules. Below the peripheral tissues are outer corneous (horny) cells, which contain starch granules embedded in a continuous proteinaceous matrix and inner floury cells in which the protein is

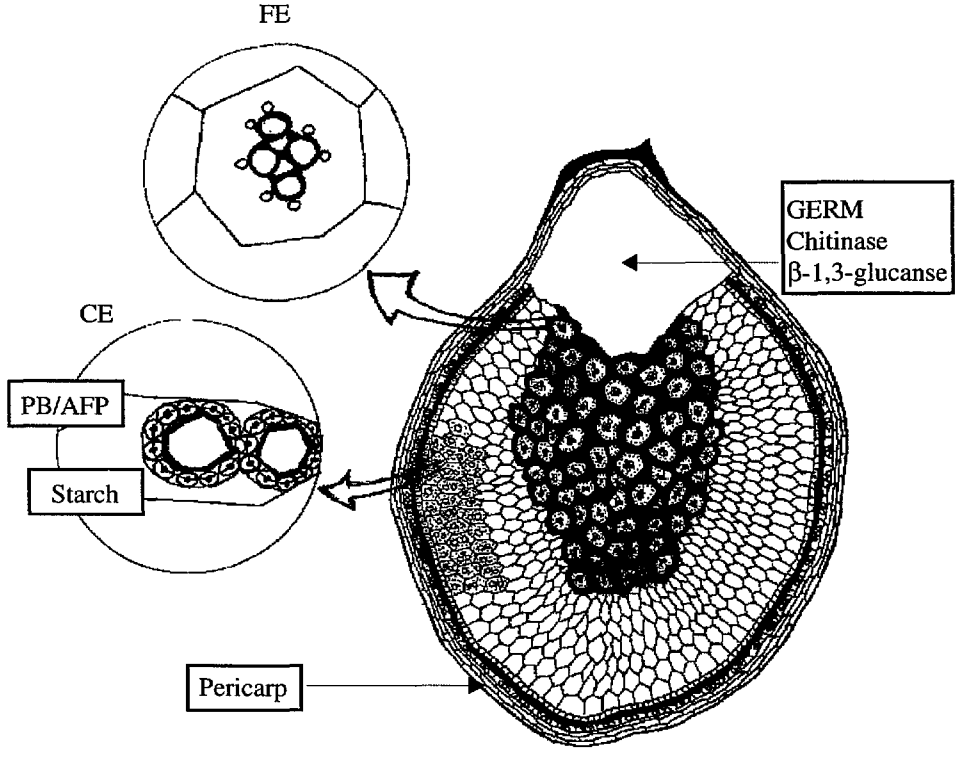

Figure 1. Diagrammatic representation of sorghum grain depicting the cells of the outer corneous endosperm and those of the inner floury endosperm. [Note: The cells of the corneous endosperm (CE) are elongated with polygonal starch granules and are filled with protein bodies (PB) and are rich in antifungal proteins (AFPs). Cells of the floury endosperm (FE) are round with round starch granules. These cells contain lower amount of proteins than do cells of the outer endosperm.]

loosely packed with air spaces and the starch granules are lenticular in shape (Serna-Saldivar and Rooney 1995).

As in other cereals, the starchy endosperm cells become disorganized and die during the later stages of grain maturation while the embryo and aleurone layers remain alive. This must be taken into account when designing strategies to provide resistance to pathogenic fungi, which infect during grain maturation.

Grain Texture and Resistance to Grain Molds

The endosperm of sorghum consists of an outer translucent layer (also called glassy, horny, vitreous, or corneous) and an inner opaque, white area (also called soft or floury). The proportions of these areas vary between cultivars (Kirleis et al. 1984). Grains are therefore described as hard or soft depending on the relative areas of corneous or floury endosperm (Kirleis et al. 1984). Glueck and Rooney (1980) reported that hard grains are more resistant to fungal infection during grain development, showing lower incidence of grain molds. This finding has been supported by more recent studies (Jambunathan et al. 1992; Sunitha et al. 1992) and has been reviewed elsewhere (Chandrashekar and Mazhar 1999).

A clue to the mechanism determining grain texture comes from studies of mutant lines of maize (*Zea mays* L.). The opaque-2 mutant is rich in lysine but has a soft texture and high susceptibility to infection by molds (Singh and Asnani 1975). The opaque-2 mutation results in reduced levels of the α-zeins and of a M_r 32,000 protein, which has since been identified as a ribosome-inactivating protein (RIP) (see below). The levels of both α-zein and the RIP are regulated by a deoxyribonucleic acid (DNA)-binding protein encoded by the opaque-2 locus (Schmidt et al. 1990, 1992; Lohmer et al. 1991; Bass et al. 1992). It is possible that the decreased level of the RIP may also contribute to susceptibility to molds as the homologous RIP from barley (*Hordeum vulgare* L.) has antifungal properties (see above and Table 1). However, this has not so far been demonstrated and indeed Dowd et al. (1998) suggest that maize RIP may be insecticidal. The development of hard-textured types of opaque-2 maize has been achieved by using genetic modifiers, and the resulting varieties are called Quality Protein Maize (QPM). Analysis of the QPM showed that the levels of α-zein are similar to those in the unmodified opaque-2 lines, but that the level of γ-zein is increased by 2- to

Table 1. Characteristics of some cereal grain ribosome-inactivating proteins.

Species of origin	M_r	Inhibition	Minimum inhibitory concentration	Reference
Maize	30,000	In vitro protein synthesis	0.70 $\mu g\ mL^{-1}$	Coleman and Roberts (1982)
Wheat		In vitro protein synthesis	3.0 $\mu g\ mL^{-1}$	Coleman and Roberts (1982)
Barley	30,000	In vitro protein synthesis	0.83 $\mu g\ mL^{-1}$	Coleman and Roberts (1982)
			3-30 nmoles	Leah et al. (1991)
Barley	30,000	*Trichoderma reesei; Fusarium sporotrichoides*	0.5 $\mu g\ well^{-1}$	Leah et al. (1991)
Maize		In vitro protein synthesis	Protein must be proteolyzed for activity	Hey et al. (1995)

3-fold (Wallace et al. 1990). Furthermore, the modifier genes behave semi-dominantly and a correlation is observed between the dosage of modifier genes, the level of γ-zein, and the degree of modification (Geetha et al. 1991). This is supported by several studies, which showed high amounts of γ-zein in either hard endosperms or in the hard outer layers of endosperms of maize (Pratt et al. 1995).

Similar results have been reported for sorghum with vitreous kernels and the outer translucent layers of these being rich in kafirins, particularly in γ-kafirins (Sunitha et al. 1992; Sunitha and Chandrashekhar 1994a; Mazhar and Chandrashekhar 1995). The γ-kafirins are rich in cysteine and form extensive intra-chain disulfide bonds. This may contribute both to hard texture and to resistance to fungal infection (Mazhar and Chandrashekhar 1993; Mazhar et al. 1993).

These data indicate, therefore, that high levels of γ-kafirin may confer resistance to fungal infection by contributing to hard texture. However, hard grain may also contain higher levels of AFPs which are located together with the kafirins and appear to be concentrated around the cell walls (Sunitha et al. 1992; Sunitha and Chandrashekhar 1994b).

Antifungal proteins

A range of proteins "synthesized" constitutively in developing seeds have been demonstrated to have antifungal properties, either in vivo or in vitro. The major protein types are briefly described below, focusing on those present in cereals.

Chitinases and glucanases

Chitinases and β-glucanases are part of the PR protein complex of tobacco discussed above. β-glucans and chitin are present in the cell walls of many plant pathogenic fungi (Bowles 1990) and it is therefore not surprising that chitinases and glucanases may have antifungal properties. Chitinases were among the first proteins to be implicated in resistance to fungal infection (Molano et al. 1979). They have been studied in wheat (*Triticum aestivum* L.) (Molano et al. 1979) and have been cloned from barley and maize while the rye (*Secale cereale* L.) protein has been sequenced at the protein level (Leah et al. 1991; Huynh et al. 1992b; Yamagami and Funatsu 1993, 1994). Most of the seed chitinases are basic (class I) chitinases which contain an N-terminal

cysteine-rich chitin-binding domain. Huynh et al. (1992b) reported that maize kernels also contain bean-type acid chitinases. Wu et al. (1994) reported the presence of acidic chitinase induced by *Aspergillus flavus* Link in the aleurone layers (pericarp) and the germs of developing maize. This protein is expressed during germination. A number of antifungal chitinases from cereal grain are summarized in Table 2.

Plant endochitinases may also exhibit activity as lysozyme, the two enzymes having similar three-dimensional structures (Holm and Sander 1994) which is consistent with the factor that both hydrolyze β-1,4-links in polysaccharide chains. Furthermore, one endochitinase from the cereal Job's tears (*Coix lacryma-jobi* L.) has been shown to inhibit α-amylase from gut of the locust (Ary et al. 1989) while another protein from pearl millet (*Pennisetum glaucum* (L.) R. Br.) appears to function as a cysteine protease inhibitor (Joshi et al. 1998). Plant endochitinases vary in their activity against pathogenic fungi and were more effective against fungi than bacterial chitinases (Roberts and Selitrennikoff 1988) but most act synergistically when combined with β-1,3-glucanases from the same tissues (Leah et al. 1991). This has been demonstrated in vivo for the barley endochitinase which acts synergistically with barley β-1,3-glucanase or barley RIP in conferring resistance to the soilborne fungal pathogen *Rhizoctonia solani* Kühn in transgenic tobacco plants (Jach et al. 1995).

Lectins and other chitin-binding proteins

Chitin is a β-1,4-linked polymer of N-acetylglucosamine, which is present in the exoskeletons of arthropods and nematodes and in the cell walls of fungi except Oomycetes. Several groups of anti-microbial proteins contain a chitin-binding domain of 30–43 amino acids, including the class 1 endochitinases (discussed above), lectins and hevein (Broekaert et al. 1995).

Hevein is a 43 residue antifungal peptide from latex of the rubber tree (*Hevea brasiliensis* (H.B.K.) Muell. Arg.). Related proteins are present in potato (*Solanum tuberosum* L.) (*win1* and *win2*) (Stanford et al. 1989) and in barley (*barwin*) and wheat (*wheatwin*) grains (Svensson et al. 1992; Caruso et al. 1993, 1996). *Wheatwin* is inhibitory to fungi (Caruso et al. 1996). A wound-inducible homolog from wheat (WPR4) is also inhibitory to fungi (Huh et al. 1998).

Table 2. Characteristics of some cereal chitinases.

Species of origin	Fungal species inhibited	Iso forms/size	Reference
Barley	*Trichoderma reesii* *Fusarium sporotrichoides*	pI 8.0	Leah et.al. (1991)
Wheat	*Fusarium* spp	pI 7.5–8.2	Molano et al. (1979)
Rye		M_r 30,000	Yamagami and Funatsu (1993)
		pI 9.7	Yamagami and Funatsu (1994)
Sorghum	*T. reesei* *F. moniliforme*		Krishnaveni et al. (1999a)
Maize		pI 5.9/4.7	Neucere et al. (1991), Huynh (1992b), Cordero et al. (1994), Wu et al. (1994)
Pearl millet[1]	*T. reesei* *F. moniliforme*	M_r 24,000 (250 ng)[2] pI 9.8 (800 μg mL^{-1})[2]	Joshi et al.(1998)
Jobs tears		M_r 26,400 pI 8.5–9.0	Ary et al. (1989)

1. The pearl millet protein is a cysteine protease /chitinase double-headed inhibitor, and the Job's tears protein is a α-amylase /chitinase double headed inhibitor

2. Effective dose.

Lectins bind to chitin and related polysaccharides and also agglutinate red blood cells. A large number of individual lectins have been characterized, notably from cereals, legumes and solanaceous plants, which are classified into various groups (Peumans and Van Damme 1995).

The most widely studied graminaceous lectin is wheat germ agglutinin (WGA) which is a M_r 36,000 protein comprising two chains of 171 amino acids. Although early work with WGA and other plant lectins demonstrated antifungal activity, it is now considered that this may have been due, in at least some cases, to the presence of contaminating endochitinases (Shewry and Lucas 1997). However, Ciopraga et al. (1999) have recently confirmed that a pure sample of WGA isolated by affinity chromatography was capable of lysing hyphal walls of *Fusarium* despite lacking endochitinase activity.

Type 1 ribosome-inactivating proteins

Type 1 ribosome-inactivating proteins (RIPs) are single chain proteins (M_r 26,000–32,000) which cleave the N-glycosidic bond of adenine in a specific sequence of eukaryotic ribosomal ribonucleic acid (RNA), making it unable to carry out protein elongation (Stirpe et al. 1992; Endo et al. 1987, 1988). RIPs may have antiviral properties, as in the well-characterized protein from pokeweed (*Phytolacca americana* L.) (Lodge et al. 1993).

Type 1 RIPs have been characterized from barley, wheat, and maize seeds, exhibiting a high level of sequence homology and I_c50 values ranging from 0.83 to 2.13.

The type 1 RIP of barley grain inhibits the growth in vitro of a number of fungi (*Trichoderma reesei* E. Simmons, *Botrytis cinerea* Pers., *R. solani*) and this activity is enhanced synergistically by ß-1,3-glucanase or endochitinase (Leah et al. 1991). Expression of RIP in transgenic tobacco results in increased resistance to *R. solani* and this is again enhanced by the co-expression of barley endochitinase (Logemann et al. 1992; Jach et al. 1995). Bieri et al. (2000) were unable to obtain much protection against *Erysiphe graminis* DC in transgenic wheat plants expressing the barley RIP gene.

There is evidence that the transcription of the maize RIP depends on the expression of the α-zeins (Di Fonzo 1988; Bass et al. 1992). Schmidt et al. (1990, 1992) showed that the opaque-2 (O-2) protein that activates the upstream of the α-zein is a transcriptional activator which also binds to a related sequence in a second gene called b-32. The b-32 protein is absent

from the opaque-2 mutants (Hartings et al. 1990; Lohmer et al. 1991) and has been shown to be a RIP. Walsh et al. (1991) showed that the RIP was synthesized as a proprotein with a region being removed during germination while Hey et al. (1995) reported that the connecting hinge between the two domains in the maize RIP inhibited enzymic activity on ribosomal RNA and was removed during activation. Endo et al. (1987) reported that a chain of the RIP from castor bean (*Ricinus communis* L.) (ricin) modifies the 28S RNA without cleavage and that the adenine at position 4324 is cleaved from the ribose by a specific N-glycosidase activity. They also showed that barley RIP acted in the same way (Endo et al. 1988). The cleavage site was GAGGACC, a sequence to which the elongation factor binds. Roberts and Stewart (1979) suggested that wheat germ RIP required ATP (adenosine triphosphate) and t-RNA (transfer RNA) for action while Carnicelli et al. (1992) observed that ATP and the post-ribosomal supernatant S-140 were required for the activation of isolated ribosomes by wheat and barley RIP. The characteristics of antifungal cereal RIPs are summarized in Table 1.

Thionins

Thionins are low M_r (5000) proteins which are rich in cysteine residues (Colilla et al. 1990; García-Olmedo et al. 1998). They were first isolated from wheat flour and shown to inhibit the growth of bacteria and fungi (Stuart and Harris 1942; Frenandez de Celeya et al. 1972). They have since been characterized from seeds of barley and oat (*Avena sativa* L.) and leaves of barley.

Thionins have well-defined activities against bacteria, yeast, and filamentous fungi (see García-Olmedo 1998). For example, Molina et al. (1993a,b) demonstrated that a range of purified thionins were toxic, to various extents, to bacterial pathogens and to the fungal pathogens *Rosellina necatrix* Hartig, *Colletotrichum lagenarium* Pass. *Fusarium solani* (Martius) Sacc., and *Phytophthora infestans* (Mont.) de Bary. In addition, the activity may be enhanced synergistically by the presence of non-specific lipid transfer protein, 2S albumin or trypsin inhibitors (Molina and Garcia-Olmedo 1993; Terras et al. 1993). More direct evidence for a role in plant defense comes from expression of thionins in tobacco resulting in reduced lesion size when the transgenic plants were challenged with the bacterial pathogen *Pseudomonas syringae* van Hall (Carmona et al. 1993) while over-expression of an endogenous thionin in *Arabidopsis* resulted in increased resistance to *Fusarium oxysporum* Schlecht. (Epple et al. 1997).

Thionins have been shown to have a range of biological activities in vitro, including inhibition of protein and nucleic acid synthesis, induction of membrane leakiness, and participation in redox reactions, the latter being perhaps the most likely explanation for their antifungal properties.

Thionins are also toxic to animals when injected intravenously or intraperitoneally. This, combined with their other potential in vivo activities, may limit their ultimate acceptability in transgenic plants.

Cereal grains also contain proteins with activity against α-amylase, which were originally termed γ-thionins. These are discussed later.

PR Protein 1

The PR Protein 1 (PR1) is the most abundant of the PR protein complex synthesized in tobacco leaves and has been estimated to account for up to 2% of the total leaf proteins (Alexander et al. 1993). The PR1 proteins from tobacco and tomato (*Lycopersicon esculentum* Mill.) are inhibitory to Oomycete fungi (*Peronospora tabacina* D.B. Adam and *P. infestans*) either in vitro and/or when expressed in transgenic plants (Alexander et al. 1993; Niderman et al. 1995). The mechanism of this activity is not known. Homologous proteins have been reported to occur in barley (Mouradov et al. 1994) but their activity has not been determined.

Thaumatin-related proteins

Protein related to the sweet protein thaumatin form part of the PR response in tobacco and are induced by osmotic stress in other species, where they are called osmotins. They also occur constitutively in seeds of cereals, with the best characterized example being zeamatin of maize. Zeamatin is an M_r 22,000 protein (Huynh et al. 1992a) which is active against a range of fungi (*Candida albicans* (C.P. Robin) Berkhout, *Neurospora crassa* Shear and Dodge, *T. reesei*, *F. oxysporum*, and *Alternaria solani* Sorauer) (Roberts and Selitrennikoff 1990; Huynh et al. 1992d). It appears to act by permeabilization of the hyphal membrane, leading to leakage and rupture (Roberts and Selitrennikoff 1990; Sunitha et al. 1994). Hence the name permeatins has been proposed for zeamatin and related AFPs from barley, oat, wheat, sorghum, and flax (*Linum usitatissimum* L.) (Roberts and Selitrennikoff 1990; Hejgaard et al. 1991; Vigers et al. 1991). Antifungal activity also appears to be a general property of the thaumatin-related

proteins, being exhibited by osmotins and PR-5 (see Shewry and Lucas 1997). Membrane-active proteins which may belong to the thaumatin family have been reported in sorghum (Sunitha et al. 1994). The characteristics of antifungal thaumatin-related proteins from cereals are summarized in Table 3.

Antifungal and anti-microbial peptides

Several types of small sulfur-rich antifungal peptides and anti-microbial peptides (AMPs) have been reported notably by Broekaert et al. (1995) as part of a broad screen of various seeds.

1. *Mirabilis japonica* peptides, Mj-AMP1 and Mj-AMP2, consist of 36–37 residues and inhibit the growth of a range of fungi and gram-positive bacteria (Cammue et al. 1992).
2. *Amaranthus caudatus* peptides, Ac-AMP1 and Ac-AMP2, comprise 29–30 residues and are homologous with the chitin-binding domains of lectins and hevein. They have anitfungal and antibacterial properties, which are antagonized by cations (K^+, Ca^{2+}) (De Bolle et al. 1996).
3. Defensins (γ-thionins) are present in a diverse range of species including cereals (Broekaert et al. 1995). They inhibit the growth of a range of fungal pathogens with either morphogenic (affecting hyphal elongation and branching) or non-morphogenic effects. Cereal defensins show low activity against fungi but inhibit α-amylases from humans and some insect pests. Florack et al. (1994) have cloned a 5 kDa protein from barley. The recombinant protein had antibacterial action.
4. Purolodines and low M_r proteins from wheat which show lipid binding were antifugal (Gautier et al. 1994).
5. Maize MBP-1 is present in the germ (Duvick et al. 1992). It comprises 33 amino acid residues and inhibits fungi and bacterial pathogens including *Fusarium* spp that infect maize seeds.

Table 3. Characteristics of cere l grain thaumatin-like proteins.

Species of origin	Size	Fungal species inhibited	Location in grain (Tested level)	Reference
Maize	M_r 22,0)		Endosperm (0.1 μg)	Roberts and Selitrennikoff (1986) Vigers et al. (1991) Huynh et al. (1992b)
Maize	M_r 22,0)	*Trichoderma reesei*	Endosperm (0.10 μg)	Malehorn et al. (1994)
	pI 9.1	*Alternaria solani*	Endosperm (1% of total protein)	
Barley	M_r 24,0)	*Trichoderma viride*	Endosperm (3 μg mL^{-1})	Hejgaard et al. (1991)

2S albumins

The 2S albumins are a major group of storage proteins present in seeds of many dicotyledonous plants (Shewry 1995). They typically comprise two subuntis of M_r, about 9,000 and 4,000 associated by inter-chain disulfide bonds. Terras et al. (1993) demonstrated that 2S albumins of radish (*Raphanus sativus* L.) are inhibitory to a range of pathogenic fungi including *Fusarium*, acting synergistically with thionins. However, the concentration required was high and the activity strongly was antagonized by cations (Mg^{2+}, K^+).

Phospholipid transfer proteins

The phospholipid transfer proteins (LTPs) are able to transfer phospholipids between membranes in vitro but probably play a defensive role within the plant. Anti-microbial LTPs have been reported from leaves and seeds of a range of species including cereals and these vary in their activity against different pathogens (Terras et al. 1992; Molina et al. 1993a,b; Segura et al. 1993).

Proteinase inhibitors

A vast array of proteinase inhibitors are present in plants, notably in storage tissues (seed and tubers) and protective latex (Richardson 1991). Their major role may be to confer resistance to insects and other invertebrate pests (nematodes and molluscs), but a mixture of trypsin and chymotrypsin from tobacco leaves was shown to inhibit spore germination and germ tube growth of *Botrytis* and *Fusarium* (Lorito et al. 1994). Joshi et al. (1998) have also described a cysteine proteinase inhibitor from seeds of pearl millet which inhibited mycelial growth of a range of fungi including *T. reesei* and *F. oxysporum*. Chen et al. (1998b, 1999) reported that a M_r 14,000 trypsin inhibitor from maize seed is a potent inhibitor of *Aspergillus oryzae* (Ahlburg) Cohn.

Polygalacturonase-inhibiting protein

Polygalacturonase is one of a number of enzymes, which is used by plant pathogens to digest pectin, resulting in cell separation and the release of biologically active oligomers (Collmer and Keen 1986). Polygalacturonase-inhibiting proteins (PGIPs) are present in the cell walls of many plants and

may contribute constitutive resistance to soft rot pathogens in fruit. However, their most important role may be to activate plant defense pathways by releasing signaling molecules. This was first indicated by the work of Jones et al. (1994) in tomato and that of Wang et al. (1998) in rice (*Oryza sativa* L.). The latter workers found that one of the genes conferring resistance to rice blast disease was related in sequence to PGIP.

Proteins demonstrated to inhibit grain mold fungi or related species

Many of the AFPs discussed above have been shown to inhibit fungi, which cause grain mold of sorghum, or related species of the same genera (*Fusarium*, *Aspergillus*, and *Alternaria*). These are briefly summarized in Table 4, which also give information on their inhibitory activity (I_{50}) and amounts present in seed tissues. Brown et al. (1997) using recombinant *A. flavus* expressing *Escherichia coli* L. β-glucuronidase (GUS) showed that the extent of invasion of the germ in resistant lines may depend on resistance mechanisms in the endosperm. Lozavaya et al. (1998) reported higher levels of activity of β-1,3-glucanase in kernels and callus of maize lines resistant to *A. flavus* in response to infection while Chen et al. (1998b) reported the presence of higher levels of an M_r 14,000 trypsin inhibitor in maize cultivars resistant to *A. oryzae*.

Antifungal Proteins in Sorghum Grain

Sorghum grain has not been studied in as much detail for AFPs as more widely cultivated cereals. However, it is probable that homologs of many of the proteins discussed above, particularly those present in maize, are also present in sorghum. In addition, several types of AFPs have been identified and characterized. These are discussed briefly below and listed in Table 5.

Uncharacterized antifungal proteins

Ghosh and Ulaganathan (1996) identified four proteins which inhibited the growth of *A. flavus*. Proteins 1, 2, and 4 (M_r 20,500, 16,300, and 12,200 respectively) completely inhibited spore germination at 15 μg mL^{-1} while Protein 3 (M_r 13,900) showed only partial inhibition at the same concentration.

Table 4. Antifungal proteins from cereals other than sorghum.

Species of origin	Designation	Fungal species tested	Size	Tested level	Reference
Maize	β1-3 glucanase				Cordero et al. (1994)
Barley	β1-3 glucanase	*Trichoderma reesei*	35 kDa	1.5 μg well^{-1}	Leah et al. (1991)
		Fusarium sporotrichoides	35 kDa	1.5 μg well^{-1}	
Maize	Unidentified	*Aspergillus* sp			Neucere (1997)
Maize	β-1,3-glucanase	*Aspergillus* sp			Lozovaya et al. (1998),
					Brown et al. (1997)
Maize[1]	Arginine-rich antifungal peptide	*Fusarium graminearum, Escherichia coli*	4.1 kDa	5-30 μg mL^{-1}	Duvick et al. (1992)
Wheat[1]	Agglutinin	*Fusarium* spp			Ciopraga et al. (1999)
Barley	Hevin-like proteins				Svenson et al. (1992)
Wheat					Caruso et al. (1993), Caruso et al. (1996), Huh et al. (1998)
Maize	Trypsin inhibitor	*Aspergillus flavus*		33–124 μg mL^{-1} of recombinants proteins	Chen et al. (1998b) Chen et al. (1999)

1. Located in the germ.

Table 5. Antifungal proteins reported in sorghum grain.

Designation/ characterization	Type/Activity	Fungal species inhibited	Inhibitory activity	Notes	Reference
CH1	Chitinases	*Trichoderma viride*	1 μg		Krishnaveni et al. (1999a)
CH2		*Fusarium moniliforme*	5 μg		
CH3		*Rhizoctonia solani*	1 μg		
		Pyricularia grisea			
		Phytophthora nicotianae			
Protein 1			Spore germination at 15 μg mL^{-1}	M_r 20,000, 16,300, 13,900, and 12,200, not characterized in detail	Ghosh and Ulaganathan (1996)
Protein 2	-	*Aspergillus flavus*			
Protein 3	-				
Protein 4	-				
18 kDa	-	*Fusarium moniliforme*	6.8 μg	Possibly carbohydrase	Sunitha and Chandrashekhar (1994a)
26 kDa	-	*Curvularia lunata*	15 μg	Possibly permeatin	
30 kDa	-				
	Chitinases	*Aspergillus flavus*		Mixtures of components tested	Seetharaman et al. (1996)
	β-1,3-glucanase	*F. moniliforme*			
	Sormatin	*Curvularia lunata*	360 ppm		
M_r 32,000–34,000	RIP			Activity not determined	Hey et al. (1995)
γ-thionins	AFP			Inhibits insect α-amylases	Bloch and Richardson (1991) Nitti et al. (1995)

Sunitha and Chandrashekhar (1994a,b) and Sunitha et al. (1994) identified three proteins of M_r 18,000, 26,000, and 30,000 which affected hyphal growth of *Fusarium moniliforme* Sheld. The M_r 18,000 component resulted in sloughing of cell wall polysaccharides while the other proteins resulted in leakage of cytoplasmic contents. It was concluded that the M_r 18,000 protein could be an enzyme acting on cell walls while the M_r 26,000 and 30,000 components could be related to permeatins (Sunitha et al. 1994). The proteins were synthesized at different points during development and were realized from bound form during germination (Sunitha Kumari et al. 1996). There were more AFPs in hard grains and in those resistant to fungal infection (Sunitha and Chandrashekar 1994b; Sunitha et al. 1992).

Proteinase inhibitors

Sunitha et al. (1992) showed higher levels of inhibitors of serine proteinases in developing hard grains, which were resistant to *F. moniliforme* than in developing soft grains. Chen et al. (1998b, 1999) have implicated the maize tryspin inhibitor in resistance to fungi such as *A. flavus*.

Ribosome-inactivating protein

Hey et al. (1995) showed that sorghum grain contained protein bands which reacted with antibody to maize RIP and had similar M_r. Their biological activity was not determined.

γ-thionins

Bloch and Richardson (1991) identified a new family of low M_r proteins in sorghum seed, which inhibited α-amylase from insects. These were subsequently called γ-thionins and defensins. Comparisons of three components showed that all comprised 47 amino acid residues with four disulfide bonds (Nitti et al. 1995).

Chitinase and β-glucanase

Krishnaveni et al. (1999a) described three chitinases from sorghum seed (CH1, CH2, and CH3 of M_r 24,000, 28,000, and 33,000 respectively), which inhibited the growth of *Trichoderma viride* Pers. at 1μg $10\mu L^{-1}$ and *F. moniliforme* at 5μg $10\mu L^{-1}$. Similarly, Darnetty et al. (1993) reported the

presence of one chitinase band of M_r 28,000 and two or three additional bands of M_r 21,000–24,000. They also identified one β-glucanase band of M_r about 30,000.

Cordero et al. (1994) and Krishanveni et al. (1999b) studied the induction during germination and response to fungal infection of chitinases and β-glucanases in maize and sorghum respectively.

Variability and relative importance of antifungal proteins

Seetharaman et al. (1997) showed that an AFP fraction containing permeatin (which they called sormatin), chitinase, glucanase, and RIP was inhibitory to spore germination of *F. moniliforme*, *C. lunata,* and *A. flavus*, all at 360 ppm. Hyphal rupture at the growing tips was observed for *Fusarium* at 70 ppm, and at 70–360 ppm for *Curvularia* but not for *Aspergillus*.

Further work compared the levels of these four proteins in eight mold resistant and eight susceptible lines derived from a single cross and grown in eight environments over three years (Rodriguez-Herrera et al. 1999). Infection with grain mold resulted in the induction and/or retention of more AFPs in the resistant lines, leading the authors to conclude that co-expression of all four proteins was required to confer resistance in lines with a non-pigmented testa.

Tannins and Other Secondary Products

The testa (seed coat) is pigmented in type II and type III sorghums with dominant B_1 and B_2 genes. Both these types contain condensed tannins with the greatest amount present in type III sorghums, which also have a dominant spreader (S) gene (Serna-Saldivar and Rooney 1995).

Tannins have antinutritional impacts on humans but provide resistance to birds (Butler 1989). There is also evidence that tannins and other phenols contribute to resistance to fungi. Assabgui et al. (1993) reported a good correlation between the content of *p*-ferulic acid in maize kernels with resistance to *Fusarium graminearum* Schwabe while Waniska et al. (1989) found greater levels of *p*-coumaric acid in some white pericarp lines which were resistant to grain weathering. It was also suggested that the conversion of *p*-coumaric acid to ferulic acid may be deficient in the susceptible cultivars. The importance of tannins in those varieties containing them in resisting grain mold infection is well documented (Harris and Burns 1973).

Red pericarp sorghums are more resistant to mold than are white pericarp sorghums and contain more flavan-4-ols (Esele et al. 1993; Menkir et al. 1996). Jambunathan et al. (1991) and Menkir et al. (1996) reported that flavan-4-ols were associated with resistance to grain mold in red-grained varieties. There has been no work relating to the level of flavan-4-ols to the enzymes involved in their synthesis.

No phytoalexins have been detected in the caryopsis of sorghum. Clive et al. (1999) have isolated cDNA (complementary DNA) clones from a sorghum mesocotyl library after infection with *Colletotrichum sublineolum* Henn, Kabat, and Bulak which appears to align partly with ribonuclease sequences in the database. The level of chalcone synthase, an enzyme which is involved in the synthesis of phytoalexin, was greater when mesocotyls were treated with *Cochliobolus heterostophus* Drechsler, which does not infect sorghum than when treated with *C. sublineolum*, which infects sorghum.

Resistance Genes

Resistance genes (R genes) present in the host plant may be activated on infection by pathogenic fungi, leading via signal transduction pathways to defense responses such as the production of phytoalexins and AFPs. The relationship between the R genes and pathogens may be extremely specific, resulting in responses only to specific species or races of pathogens. R gene-mediated responses clearly require that the infected tissue is metabolically active. Consequently, they could contribute to resistance to infection of sorghum with grain molds in the early stages of grain development rather than infections late in development or of the mature grain.

Much of the work on the identification of these genes arose from the mapping of resistance to certain loci on chromosomes using restriction fragment length polymorphism (RFLP) and other techniques. These techniques still hold promise for the identification of markers associated with disease.

The R genes have in recent years been cloned from a number of plant species, using either mapping approaches ("map-based" cloning) or transposable elements to facilitate gene identification and isolation. This has shown that resistance proteins (R proteins) share common features, most notably the presence of leucine-rich repeats which may be combined with protein kinase domains. Moreover, R genes map to clusters (Kanazin et

al. 1996; Ashfield et al. 1998). This has facilitated the isolation of further R genes, using easier polymerase chain reaction (PCR)-based strategies.

Comparisons of R gene sequences indicate that they are highly variable, duplicating and diverging over time. As a result, R genes often occur in clusters at complex loci, with subtle differences in their sequences relating to specificities for different pathogens or races of pathogen. Some of this variation may arise from the presence of transposable elements. For example, seven transposon-like elements have been reported in the Xa21 gene complex of rice, which determines resistance to *Xanthomonas* (Bureau et al. 1996; Song et al. 1997; Wang et al. 1998). Ellis et al. (1999) reported small differences in amino acid composition of thirteen alleles of the flax L protein which could account for differences in specificity of resistance to different rust strains between flax cultivars. They postulated that the differences may have arisen from intragenic recombination events. The disease-resistance genes map to areas very close to known genes for resistance (Yong et al. 1996; Collins et al. 1998). Mutations in resistance genes may also account for differences between susceptible and resistant varieties.

Exploitation of genetic markers and R genes in resistance of sorghum to grain mold

Studies of other cereals demonstrate that it is possible to identifiy biochemical or molecular markers, which can be exploited to follow resistance in breeding programs. Mingeot and Jacquemin (1997) found high polymorphism for one marker which was subsequently shown to encode a thaumatin-like protein, observing many patterns in about 48 varieties of wheat that were analyzed. Fariss et al. (1999) reported the use of 508 genetic markers including a large number of candidate genes in screening a population of 114 recombinant inbred lines between a hard red spring wheat and a synthetic hexaploid wheat (derived from *Triticum tugidum* L. and *Aegilops tauschii* Coss). They reported that the oxalate oxidase gene was a good marker for tan spot resistance using a pathotype avirulent to Lr23. A peroxidase gene was found to be linked to both resistance and to Lr23. A phenyl alanine ammonium lyase gene and a thaumatin gene both appeared linked to the resistance genes Lr27 and Lr31. The disease-resistance genes appeared clustered on the 7BL chromosome. Markers encoding chalcone synthase and a chitinase were associated with karnal bunt resistance. Itu et al. (2000) showed that resistance to fusarium

head blight was associated with the gliadin loci, Gli-B1 and Gli-D1, which is reminiscent of the positive relationship between prolamins and resistance to grain mold in sorghum. Similarly de la Pena et al. (1999) found quantitative trait locus (QTL) associated with resistance to fusarium head blight in barley.

The R genes have not so far been isolated from sorghum. However, their isolation by PCR-based technology should be facilitated by the availability of R gene sequences from other species including lettuce (Shen et al. 1998), wheat (Feuillet et al. 1998; Seyfarth et al. 1999), tomato (Ohmori et al. 1998), soy (*Glycine max* L.) (Kanazin et al. 1996; Yong et al. 1996); rice (Mago et al. 1999), potato (Leister et al. 1996), and maize (Collins et al. 1998). Both conserved and variable sequences are observed between different R genes and the former could be used to design PCR primers or oligonucleotides in order to isolate part or whole of the disease resistance genes from sorghum (Chen et al. 1998a). Once the genes or fragments thereof are available their relation with resistance to grain mold could be investigated by assaying for the expression of these genes in developing seed and their linkage with resistance in a breeding program.

Studies of other systems have shown that over-expression of R genes may lead to broad spectrum resistance with high levels of AFPs and other defense-related compounds (Tang et al. 1999). Over-expression of such genes in the developing head tissues of sorghum at the time of susceptibility to grain mold infection could lead to increased resistance.

Transgenic plants

Transgenic plants have been made with both the AFPs and with the kinases and leucine-rich repeat proteins that are involved in signal reception and transduction (Table 6). Transgenic plants made with specific AFPs may be able to resist certain fungi while a broader spectrum resistance may occur when kinases are used.

Over-expression of kinase-disease resistance genes

Over-expression of a kinase-disease resistance gene (Prf) in tomato increased resistance to three bacterial and one viral pathogen without any effect on fruit production (Oldroyd and Staskawicz 1998). Similarly, over-expression of Pto, another kinase, under control of constitutive promoter in three independent transgenic tomato lines elicited an array of defense responses including

Table 6. Characteristics of selected transgenic plants made to resist fungal infection.

Source of gene introduced	Type of gene	Introduction	Promoter	Resistance conferred	Reference
Kinase genes					
Prf	LRR	Tomato	Its own		Oldroyd and Staskawicz (1998)
Pto	Serine, threonine, kinase	Tomato	CamV		Tang et al. (1999)
Ndr				Broad spectrum	Cao et al. (1998)
N			Tomato	Necrotic	Whitman et al. (1996)
NPR	LRR protein			Broad spectrum	Cao et al. (1998)
AFP					
Barley thionin	AFP[1]	Tobacco			Huang et al. (1997)
Human lysozyme	ABP[1], AFP	Tobacco	CamV	Bacteria and fungi	Nakajima et al. (1997)
Pokeweed RIP	Protein synthesis inhibitor		Broad spectrum		Zoubenko et al. (1997)
Glucose oxidase	Producer of reactive oxygen		Broad spectrum		Wu et al. (1997)
Barley RIP	Protein synthesis inhibitor	Tobacco	Wound induced	Fungi	Logemann et al. (1992)
Bacterioopsin					Abad et al. (1997)
Trichoderma chitinase	AFP	Tobacco	CamV	Fungi	Lorito et al. (1998)
Rhizopus chitinase	AFP	Tobacco	Fungi		Terakawa et al. (1997)
Cecropin	AFP	Tobacco		Fungi	Huang et al. (1997)
Rice	Chitinase	Rice	CaMV	Fungi	Chareonpronwattana et al. (1999)
Rice	Chitinase	Rice	CaMV	Blast fungi	Nishizawa et al. (1999)
Nicotiana plumbaginifolia	β-1,3-glucanase	Tobacco	CaMV		Blandin and Castresana (1997)
Barley	β-1,3-glucanase, chitinase	Tobacco			Jach et al. (1995)

1. AFP = antifungal protein; ABP = anti-bacterial protein.

microscopic cell death, salicylic acid accumulation, and PR gene expression, and conferred resistance to *Xanthomonas*, *Pseudomonas*, and to the fungal pathogen *Cladosporium fulvum* Cooke (Tang et al. 1999). Expression of the NPR1 gene in *Arabidopsis* was manipulated to increase the level of its protein by 1.5- to 3-fold compared with wild-type plants (Cao et al. 1998). Plants with moderately increased levels of NPR1 protein exhibited significant increases in resistance to *Pseudomonas* and *Peronospora* pathogens. Defense-related genes (encoding AFPs) were expressed more strongly in plants over-expressing NPR1 but were not induced more rapidly during pathogen infection (Cao et al. 1998).

Over-expression of antifungal protein genes

Potatoes expressing the bacterio-opsin gene exhibited systemic necrosis, triggering systemic acquired resistance (SAR)-like responses that resulted in resistance to several pathogens (Abad et al. 1997). Similarly, plants expressing glucose oxidase produce active oxygen species, which appear to induce SAR as demonstrated by the activation of PR genes in potato (Wu et al. 1997). Expression of an inactive pokeweed antiviral protein induces fungal disease resistance in tobacco, concomitant with PR protein gene expression (Zoubenko et al. 1997).

Transgenic carrots (*Daucus carota* L.) expressing a basic chitinase from tobacco showed enhanced resistance to three out of five tested pathogens, but no increased resistance was detected when the chitinase was derived from petunia or when any one of three chitinases (including the tobacco chitinase) was expressed in transgenic cucumber (Punja and Raharjo 1996). Lysozyme was effective only for a few days (Nakajima et al. 1997). Nishizawa et al. (1999) showed that expression of intra- or extra-cellular chitinases from rice conferred resistance to *Magnaporthe grisea* (T.T. Heberet) Yaegashi and Udagawa (rice blast) in transfomed rice lines. There was no difference in the extent of resistance offered by either gene.

Thionins have been well studied and provide a good case study. Expression of barley-thionin in transgenic tobacco resulted in enhanced disease resistance to *P. syringae* (Carmona et al. 1993). Alteration of the amino acid sequence of cecropin, a toxic peptide from a toad by substitution of a single amino acid changed the half-life of the peptide significantly and resulted in reduced disease symptoms when expressed in transgenic tobacco (Huang et al. 1997).

Stark-Lorenzen et al. (1997) introduced the stilbene synthase gene into rice with the expectation that accumulation of a phytoalexin, resveratrol, would occur. Preliminary results indicated enhanced disease resistance to rice blast. These workers had previously introduced this gene into tobacco, tomato, and potato resulting in increased resistance to disease.

Silencing of heterologous genes

Many studies have shown that introduced genes may not be expressed in transgenic plants due to a phenomenon known as "co-suppression". However, recent studies have shown that this silencing is not universal and that these effects often may be reversed. Blandin and Castresana (1997) showed that a β-1,3-glucanase transgene was silenced in the leaves of 6-week-old tobacco but that activity was restored in the embryonic and endosperm cells of tobacco fruits. Chareonpronwattana et al. (1999) showed that silencing of a rice chitinase gene occurred mainly in homozygous plants and only in those plants that expressed large amounts of the recombinant protein.

Conclusions

Background

Current sources of resistance provide only partial protection against grain mold fungi, the most important of which are *Fusarium*, *Alternaria,* and *Curvularia*. Resistance can derive from a combination of characteristics (texture, red pericarp, pigmented testa, AFP), which act additively or synergistically. Some of the characters also have a negative impact on end use properties. Hence there may be a trade off between protection and quality. There is a need to identify short- to medium-term goals.

Recommendations

Pigmented testa. Pigmented testa containing sorghums do not currently occur in India. This character is associated with high tannin and poor food quality and should be avoided.

Red pericarp. Sorghums containing red pericarp are acceptable for some end uses but are not favored. Hence it may be selected as a short-term goal but must be eliminated in the longer term.

Grain hardness. Grain hardness is clearly related to resistance and sufficient variation is present in the germplasm that can be used to provide breeding material. However, hard texture is not favored for all end uses; medium or soft texture is preferred. In addition, hardness is currently associated with high levels of AFPs and this linkage needs to be understood and then broken. Hardness appears to result from high levels of γ-kafirin. The biochemical basis for this effector needs to be understood and the existence of modifier genes (as in QPM) established.

Antifungal proteins (AFPs). A link between AFPs and resistance is clearly established, although only partial protection is obtained. In the short term it will be possible to select for high levels of characterized AFPs (sormatin, RIP, β-1, 3-glucanase, endochitinase) in developing, mature, and infected grain by direct analysis or by using antibodies or molecular markers. In the long term more information is required on the endogenous AFPs of sorghum. There is a need:

- To identify new components.
- To determine activity and synergism.
- To determine location.
- To determine initiation of synthesis and amounts in developing and mature tissues.
- To relate the above to resistance.

Marker-assisted selection. The development of molecular markers for the sorghum genome [e.g., simple sequence repeats (SSRs)] is essential to underpin the selection of resistance and the combination of resistance with yield and end use quality. It will also facilitate dissection of the various aspects of resistance, for example, the separate effects of hardness and AFPs. A more directed approach can also be adopted using markers based on characterized AFPs (e.g., thaumatin) and putative resistance genes (based on other species).

Transformation. Transformation is an essential prerequisite for long-term improvement and must continue to be supported. It should initially express sormatin and β-1,3-glucanse/endochitinase under a strong endosperm specific promoter. This will act as "proof of concept". Foreign genes could be used but these should preferably be from maize or other cereals. Genes encoding proteins expressed in non-food tissues should be avoided due to potential problems of acceptability and allergy/intolerance. Further, transformation may require specific promoters to control the level and tissue specificity of expression, e.g., glume, developing endosperm, and ovary wall/ pericarp. These could be derived from maize or isolated from sorghum using homologs from maize.

Resistance genes. There is a long-term need to identify and characterize resistance genes in sorghum. These genes would be expected to ultimately regulate the manifestation of different phenotypes with resistance to grain mold. In the short term the conserved sequences of the R genes may be used to design primers which can be used to generate polymorphism in marker-assisted selection.

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Plant Responses to Early Infection Events in Sorghum Grain Mold Interactions

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Grain mold of sorghum (*Sorghum bicolor* (L.) Moench) is the greatest constraint for optimum grain yield where anthesis occurs in humid, warm, and rainy seasons (Forbes et al. 1992). Exposure under such conditions allows the floral niche to be exploited in a number of ways prior to grain development. Grain mold has remained a continuous problem for the semi-arid tropics of India, Africa, and the Americas.

Grain mold differs from grain weathering in that it is a condition occurring between anthesis and physiological maturity (Forbes et al. 1992). In sorghum, physiological maturity is denoted by the deposition of the black layer (Castor 1981). Grain weathering, on the other hand, is a situation in which fungi colonize the developing grain after physiological maturity, prior to harvest (Forbes et al. 1992). Though the means and mechanisms of these processes may differ, resultant reduction in seed viability and quality will often be quite similar.

It is useful to think of grain development as central to the entire plant's life cycle (Fig. 1). Seed health is the source of functional seedling emergence and adequate vigor resulting in robust tillering, booting, inflorescence emergence, and eventual heading (Fig. 1). Anthesis is a critical point at which the sorghum flower is most susceptible to infection and colonization by grain mold fungi (Castor 1981; Forbes 1986).

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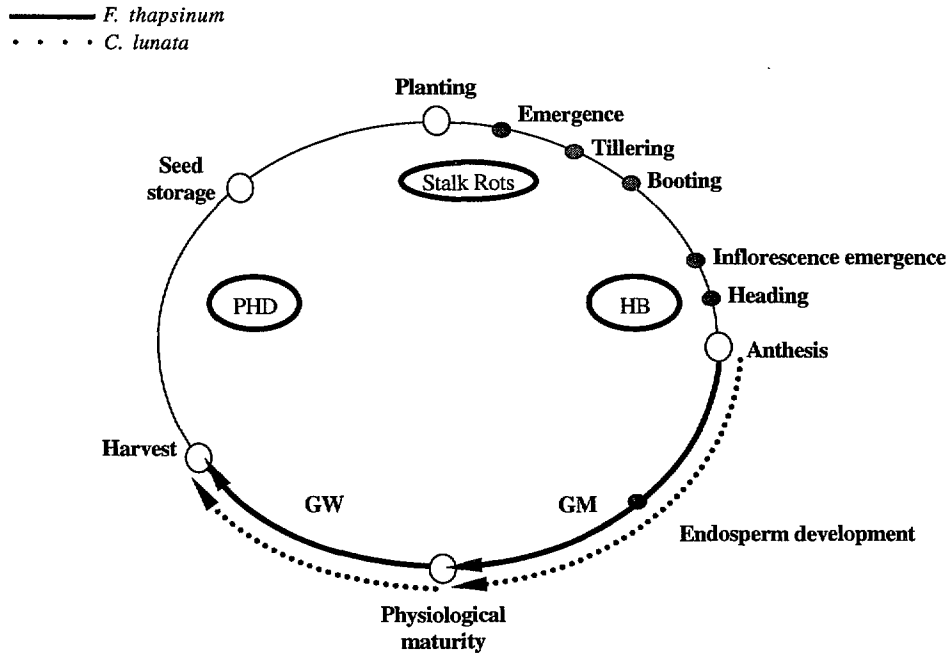

Figure 1. Interactions of Fusarium thapsinum and Curvularia lunata with various stages of the sorghum plant life cycle (GM = grain mold, GW = grain weathering, PHD = postharvest deterioration, HB = head blight).

Facultative Pathogens Associated with Developing Kernels

Fungi that are typically associated with early infection events are *Fusarium thapsinum* Klittich, Leslie, Nelson et Marasas sp nov. (Klittich et al. 1997), *Fusarium graminearum* Schwabe (*Gibberella zeae* Schw.), *Curvularia lunata* (Wakker) Boedijn, *Phoma sorghina* (Sacc.) Boerema, Dorenbosch, & van Kesteren, and *Alternaria* spp (Bandyopadhyay 1986; Menkir et al. 1996a). *Phoma sorghina* and species of *Alternaria* appear to be important in Maharashtra, India (Anahosur 1992). The majority of other fungi, which are seen in the literature, including *Aspergillus niger* van Tieghem, *Aspergillus fumigatus* Fres., *Cladosporium* spp (typically *Cladosporium sphaerospermum* Penz.; personal observation), *Bipolaris* spp, *Drechslera* spp,

Exserohilum spp, *Epicoccum* spp, *Fusarium semitectum* Berk. and Rav., and *Olpitrichum* spp are generally relegated to grain weathering.

In India, *Gonatobotrytis* spp and *Tricothecium* spp may have greater importance in Tamil Nadu, Gujarat, and Madhya Pradesh regions (Anahosur 1992). However, these two species are more rarely isolated in most geographies of North and South American sorghum production (personal observation). Fungi such as *Aspergillus* spp (especially *Aspergillus glaucus* Link ex S.F. Gray group) and *Penicillium* spp contribute to postharvest deterioration and toxin contamination of seed (Frederiksen 1986).

Fusarium thapsinum and *C. lunata* are the most important species causing grain mold worldwide (Fig. 1). Only *Fusarium* and *Curvularia* seem to be consistently associated with infection at early stages across most climates and geographies and are the subject of this paper. Both these species are especially important in Andhra Pradesh, India (Anahosur 1992). *Curvularia lunata* is one of the causal agents of "grain blackening" on kafir sorghums in the field where colonization not only occurs early in development but late on the mature grain surface. Both *F. thapsinum* and *C. lunata* may live as saprophytes in the field in soil or on plant debris. This indicates that inoculum is plentiful facilitating the initiation of a disease interaction at many different stages of the plant's life cycle (Fig. 1). In an appropriate niche, e.g., the sorghum floret, these fungi become effective facultative pathogens portraying an aggressive lifestyle on the most susceptible cultivars.

Damage resulting from these early infection events include: arrest of kernel development (pre- or post-fertilization), discoloration and mold, increased endosperm/germ colonization and degradation, decrease in kernel mass and grain density, decrease in germination, decrease in seedling vigor, and possible mycotoxin contamination. The concepts underlying endosperm degradation, which have been extensively studied and reviewed in the past, will not be treated in this paper.

Mechanisms of Resistance and Defense

Apparently there are three primary sources of resistance to grain mold fungi. These include the morphological or physical characteristics of the seed, glume, and/or panicle that do not promote introduction of fungal conidia into the infection court. Preformed structural attributes such as seed hardness or the presence of a testa layer may physically block fungal infection (Glueck

and Rooney 1980; Forbes 1986; Mansuetus 1990; Forbes et al. 1992; Esele et al. 1993; Menkir et al. 1996b). It is important to note here that these attributes primarily contribute to grain weathering resistance.

On the other hand, the involvement of secondary metabolites, whether preformed or induced may have a greater impact on defense against early infection (Rhodes 1994). Examples of preformed secondary metabolites include flavan-4-ols and proanthocyanidins (tannins) (Jambunathan et al. 1990). Flavonoid compounds induced in sorghum include the 3-deoxyanthocyanins. This group of compounds is characterized by a red pigmentation as seen in the sorghum phytoalexin apigeninidin (Nicholson et al. 1987; Snyder and Nicholson 1990; Snyder et al. 1991; Harborne and Baxter 1993).

It would be imprudent to discount the possible combined effects of hydrolytic enzymes and antifungal proteins in this early interaction. Hydrolytic enzymes such as chitinase and β-glucanase have been shown to be "upregulated" after plants are treated with fungal elicitor or infected (Seetharaman et al. 1997). Antifungal proteins and seed-storage proteins may play a role at this stage. The permatins (such as sormatins or homologs of zeamatin) and osmotins are closely related to the thaumatin-like proteins which turn-on in incompatible interactions (Lin et al. 1996). The permatins are literally capable of permeabilizing fungal membranes and may work in concert with the hydrolytic enzymes (Roberts and Selitrennikoff 1990). Yet another group, the ribosome-inactivating proteins (RIPs) inhibit protein translation of invading fungi. These RIPs may act as N-glycosidases that cleave adenine N-glycosidic bonds in rRNA (ribosomal ribonucleic acid) (Stirpe et al. 1992). Here it would be reasonable to think that host rRNAs would also be somewhat susceptible to glycosidase activity leading to inhibition of host translational machinery as well as that of the pathogen. In the past it has been thought that RIPs were mostly fungal specific, but this may not be the case.

Response appears to be quite rapid on the level of phenolic and flavonoid compound accumulation. Tx2911, a red seeded kafir-caudatum line produces large amounts of red-pigmented compounds in response to infection and colonization by *C. lunata*. This is best visualized by phenolic extraction of homogenized spikelet tissue 5 days after inoculation. The phenol layer reveals the accretion of what is most likely 3-deoxyanthocyanins

such as apigeninidin, luteolinidin, and related anthocyanins, whereas the extract from water-treated controls is typically a light green color (personal observation).

Plant defense responses to non-specific facultative pathogens generally take on four important phases. The first phase occurs when the fungal propagule makes contact with the surface of a plant structure, in this case the lodicule, lemmal tissue, and ovary base of the spikelet. Even before penetration has taken place, signaling mechanisms within the affected and surrounding cells detect the presence of the fungus (Collings and Slusarenko 1987; Graham and Graham 1991). As breach in the cell wall/membrane occurs a second phase is established, where generalized stress response/ infection recognition occurs. This activates an entire gamut of physiological activities including, but not limited to: (a) hydrogen peroxide generation and hydroxyl radical formation, leading to altered redox status and cell wall crosslinking, (b) influx of various anions and efflux of calcium cations, and (c) lipid peroxidation and enhanced phospholipase activity. The last two activities (b and c) also clearly contribute to the accumulation of salicylic acid, ethylene, and jasmonate in cells and plant tissues (Graham and Graham 1991; Knogge 1996).

The aforementioned activities participate, through complex signal transduction mechanisms, in the elicitation of defense response genes, which is the third and least understood phase. Transcriptional elicitation of a defense gene or a set of defense genes in and of itself is not sufficient to act as a defense mechanism. The mRNA (messenger RNA) products must be translated into protein products that act on biochemical intermediates in pathways that yield fungistatic or fungitoxic products or have a direct function, such as hydrolytic enzymes or permatins, in affecting the integrity and viability of the invading pathogen. Still, it is difficult to demonstrate a significant correlation between defense gene induction and fungistasis. Often the temporal and quantitative level of the response is correlated to achieving an enhanced resistance level as an end result (Joost 1993). Still, it is important to realize that it requires many different defense elements working in concert, often synergistically, to enable resistance (Knogge 1996).

Phenylpropanoid and Flavonoid Metabolism

The metabolism of phenylpropanoid and flavonoids are common events in most cell types of the majority of plants. These biochemical pathways are certainly enhanced during floral development, as has been observed in floral induction of *Arabidopsis thaliana* L. *and Antirrhinum majus* L. (van der Meer et al. 1993). In phenylpropanoid metabolism, a trans-animation event removes a keto group from phenylpyruvic acid (an end product of the shikimic acid pathway) and replaces it with an amino group producing the amino acid, L-phenylalanine (Goodwin and Mercer 1986). Phenylalanine ammonia-lyase (PAL) converts L-phenylalanine to trans-cinnamic acid via a deanimation step (Jones 1984: Dixon et al. 1994). This is the first keystone step in phenylpropanoid biosynthesis. Upregulation or induction of PAL mRNAs indicates a potential for increased biosynthetic flux into the pathway (Hahlbrock and Scheel 1989). PAL typically responds to light, UV, wounding, nutrient and salt stress, nitrogen starvation, fungal elicitor, and fungal infection (Jones 1984; Hahlbrock and Scheel 1989; Scott et al. 1990; Yamada et al. 1992; Dixon et al. 1994; Orczyk et al. 1996). These responses generally occur within a short time. Sorghum experiments by (Cui et al. 1996) showed accumulation of PAL mRNA within a few hours after BTx635 and RTx7078 seedlings were inoculated with *Bipolaris maydis* (Nisikado & Miyake) Shoem. (a non-pathogen of sorghum). *Perenosclerospora sorghi* (Weston & Uppal) Shaw (causes downy mildew of sorghum) induced greater levels of PAL mRNA in mildew resistant lines than in susceptible lines. *Sporisorium reilianum* (Kühn) Langdon & Fullert did not induce the accumulation of PAL messages in either meristematic or non-meristematic resistant lines. The clone used in this study was a 535 bp PCR (polymerase chain reaction) fragment, PAL1-1, which showed 70% homology to 40 gene/ cDNA (complementary deoxyribonucleic acid) sequences from 24 plant species. This clone's highest homology was with *gp*28 of rice (*Oryza sativa* L.) (Cui 1995; Cui et al. 1996). This clone is being employed in current analyses of the early grain mold interaction (Table 1).

Initial results suggest that PAL is active in Tx430 spikelets at a greater level when inoculated at anthesis with *F. thapsinum* when compared with water control. RT-PCR indicates the presence of several bands consistently appearing in inoculated and control treatments that correspond to PAL cDNAs, one of which hybridizes to PAL1-1 (unpublished data).

Table 1. Clones and primers.

Clones:	PAL 1-1 (sorghum phenylalanine ammonia lyase)[1]
	CHS2G (soghum chalcone synthase)[1]
Primers:	GLU 1/2, GLU 12/16 (β-1, 3-glucanase)
	CHT 1/2, CHT 1/2-99 (chitinase)
	PERM F/R (permatin)
	GS 1/2 (glutamine synthase)
	DFR 1/2 (dihydroflavonol reductase)
	c-OMT (c-O-methyltransferase)

1. Source: Cui (1995); Cui et al. (1996).

The downstream products of the flavonoid pathway include the flavones, flavanols, flavanones, anthocyanins, and deoxyanthocyanidins. The phenylpropanoid pathway gets "switched" to the flavonoid biosynthesis pathway via the condensation of three molecules of malonyl CoA with one molecule of *p*-coumaryl CoA to yield chalcone. This reaction is catalyzed by chalcone synthase (CHS). Cui et al (1996) obtained a 620 bp clone, CHS2G, that had 70% homology with 27 plant species. This clone's highest homology was with C2 of maize (*Zea mays* L.). Cui showed that CHS accumulated in sorghum (BTx635 and RTx7078) seedlings within 6 hours after inoculation with *B. maydis* (a non-pathogen of sorghum). *Sporisorium reilianum* did not induce the accumulation of CHS in either meristematic or non-meristematic resistant lines. *Perenosclerospora sorghi* induced higher levels of CHS in downy mildew resistant lines than in susceptible. CHS2G is being employed in current analyses of the early grain mold interaction (Table 1).

Preliminary RNA blot data suggest that CHS transcription occurs at a relatively constant level in both inoculated and control spikelets of resistant and susceptible cultivars. RT-PCR indicates the presence of a single band at about 600 bp that amplifies equally from control, *Fusarium*, and *Curvularia* treatments each at 48 h post-inoculation for Sureno (moderately resistant to grain mold) and Tx430 (susceptible) (unpublished data).

Antifungal Proteins

Chitinases and β-1,3-glucanases are the most commonly expressed hydrolytic enzymes in most plant-fungal interactions. Chitinases cleave β-1,4-N-acetylglucosamine linkages. In vitro studies have shown that fungal cell walls from several species are easily degraded by chitinase. Chitinases restrict the normal growth of *Trichoderma reesei* E. Simmons and *Phycomyces blakesleeanus* Burgeff, for example, in fungal growth inhibition assays (Roberts and Selitrennikoff 1986; Darnetty et al. 1993). A defense response in the plant is often mounted primarily in response to the soluble cell wall degradation products which act as elicitors. Two primer sets have been designed and synthesized to detect and amplify chitinase cDNA or gDNA (genomic DNA) fragments from sorghum (Table 1).

There are several major types of β-glucanases which are found in the walls of fungi. Cellulose (β-1,4) is found in the cell walls of Oomycetes and other members of the Chromista (~Stramenopila). Amyloid and xyloglucans (hetero-β-1,4), callose (β-1,3) and the β-1,6, β-1,3-1,6, and β-1,3-1,4-glucans are found in the majority of filamentous fungi including those responsible for grain mold (Burnett and Trinci 1979). As in the case with chitinase, glucan monomers, or dimers, the products of the enzyme act as elicitors of plant defense responses. When acting together, glucanases and chitinases operate synergistically to produce more than additive levels of antifungal activity. This was shown by Mauch-Mani and Boller (1988) who were able to demonstrate that in vitro mixtures of purified chitinase and β-1,3-glucanase would inhibit the growth of *Fusarium solani* f sp *pisi* Tones and *Trichoderma viride* Pers. Two primer sets have been designed and synthesized to detect and amplify sorghum β-1,3-glucanase cDNA or gDNA fragments.

Permatins are generally small proteins of approximately 22 kDa that exhibit antifungal activity against various fungi. These include *Neurospora crassa* Shear & B.O. Dodge, *Cercospora beticola* Sacc., *T. reesei*, and *Candida albicans* (L.) Mann (Darnetty et al. 1993). These proteins are found in particularly high concentrations in seeds; e.g., zeamatin in maize endosperm (Vigers et al. 1991). Sormatin is a homolog of zeamatin and has been investigated in relationship to grain mold and resistance of red- and white-grained genotypes in the field. Several genotypes have been identified

that "respond" to inoculation by increasing relative levels of sormatin after 30 or more days after inoculation (Rooney 1998).

Permatins function through permeabilization of fungal membranes. These proteins are related to the thaumatin-like proteins including some associated with pathogenesis, e.g., PR-R, PR-S, and osmotins from tobacco (*Nicotiana tabacum* L.). Darnetty et al. (1993) found that seed extracts of sorghum have high levels of permatin activity by use of gel activity assays. Also, western blots showed presence of a ~22kDa protein in extracts which typifies this protein class. A primer set has been designed for use in amplifying cDNAs and gDNA fragments to obtain reliable permatin clones. This primer set was designed from conserved regions of several cereal permatin genes from rye (*Secale cereale* L.), wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), and maize.

It would be reasonable to assume that these proteins may not be able to act until chitinases and β-glucanases function to degrade cell walls of invading fungal hyphae. This raises an important question: Can 22 kDa permatin proteins permeate fungal cell walls to act directly on the invading hyphal membrane? Thus, these proteins may not be inducible but present at certain levels enabling them to act immediately in defense. This idea may be tested by screening cDNAs from the same pools of control spikelet tissue of Sureno (moderately resistant to grain mold). So far this type of experiment indicates the presence of several transcripts amplified by various sets of chitinase, β-1,3-glucanase, and permatin gene specific primers as early as 3 h post-inoculation to 120 h (unpublished data). Putative antifungal protein transcripts accumulate very early in the seed during development. Their constant presence may then reduce infestation, infection, and resultant colonization resulting in reduced quality, germination, and vigor after storage.

Chitinase, β-1,3-glucanase, and permatin PCR-fragment clones were obtained using the primers mentioned to screen RNA blots produced from treatment-time-point combinations of sorghum genotypes, Sureno, Tx2911, SC170, and Tx430.

Biotechnology Resources and Future Directions

Currently four genotypes are being screened for defense response attributes during early grain mold interactions. Sureno, Tx2911, SC170, and Tx430 have been inoculated with *F. thapsinum*, *C. lunata*, and water controls. Total

RNA extracts have been made from samples collected from 3 h to 120 h time-points post-inoculation. Northern blots and dot blots are being produced and screened with a number of defense response clones from previous work (Table 1) (Cui 1995; Cui et al. 1996) and will also be screened with clones obtained during our ongoing studies.

The cDNAs are also being produced from these treatment-time-point combinations for use in obtaining cDNA clones of defense response transcripts. Also, this type of screening provides some limited information on the qualitative nature of the population of mRNAs that may exist in the young tissues of the spikelet and developing kernel in control versus inoculated situations. Clones that are obtained in these studies will also be used to probe sorghum BAC libraries for map localization of genes and to obtain complete genes with intact promoter regions.

Efforts shall continue in the following areas:

- Identification of defense genes that are activated in sorghum spikelets at higher levels or more quickly in response to *F. thapsinum* and *C. lunata*.
- Seeking out highly expressed developmental mRNAs in spikelet tissues.
- Identification, isolation, and characterization of promoter regions from functional defense gene(s) that are "upregulated" during pathogen challenge.

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Potential of Marker-assisted Selection for Improving Grain Mold Resistance in Sorghum

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Grain sorghum (*Sorghum bicolor* (L.) Moench) is an important cereal crop grown throughout the world on approximately 48 million ha annually. While many pests limit the productivity of the crop, grain mold of sorghum is typically the greatest disease constraint in warm and humid environments. The term grain mold is used to describe the diseased appearance of sorghum grain resulting from infection by one or more parasitic fungal species (Williams and Rao 1981). Grain mold is most commonly caused by *Fusarium moniliforme* Sheld. and *Curvularia lunata* (Wakker) Boedijn (Castor and Frederiksen 1980). This disease is especially severe when grain development coincides with wet and warm weather conditions.

Attempts to reduce or minimize grain mold damage have had limited success. Avoidance of warm and wet or humid conditions at maturity does reduce grain mold pressure, but it is not realistic in most environments due to the constraints of growing seasons. Specific fungicides are very effective in reducing grain mold incidence, but chemical control is simply not cost effective. Sorghum geneticists have long known that variation exists within sorghum for grain mold resistance but breeding to improve grain mold resistance in sorghum has had limited success. The limited success is due in part to an incomplete understanding of the genetics of sorghum grain mold

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resistance and the complex interaction of traits influencing grain mold resistance. Sorghum geneticists have long suspected that both qualitative and quantitative loci influence grain mold resistance. Esele et al. (1993) showed that several qualitatively inherited pericarp traits such as color and pigmented testa influence the level of grain mold resistance. Menkir et al. (1996) determined that hard endosperm types were more resistant to grain mold. Menkir et al. (1996) and Esele et al. (1993) demonstrated that the presence of a pigmented testa layer is very effective in increasing grain mold resistance.

While several qualitative loci affect grain mold resistance, they do not account for all the variation observed for grain mold resistance in sorghum. Therefore, resistance to grain mold in sorghum is usually considered a quantitatively inherited trait. Using a diallel design, Dabholkar and Baghel (1980) found that general and specific combining ability components of variation for grain mold resistance were highly significant. Murty and House (1984), used generation means analysis for evaluation of resistance to sorghum grain mold and found that the F_1 hybrid was more resistant to *C. lunata* and *F. moniliforme* than the mid-parent, indicating that grain mold resistance exhibited dominance effects. In a recent review, Stenhouse et al. (1997) summarized the research in this area. More recently, Rodriguez-Herrera et al. (in press) conducted a generation means analysis of a population (Sureno × Tx430) for grain mold resistance in eight environments in Texas, USA. In the generation means analysis, they detected additive effects in all eight environments, and dominance effects were detected in seven of eight environments. Epistatic effects were detected in only two of eight environments, but combined analysis indicated that higher order interactions were important when evaluated across environments. Rodriguez-Herrera et al. (in press) concluded that selection in specific environments is useful for enhancing resistance to mold in those environments, but it may not provide grain mold resistance across a wide range of environments.

Further improvements in grain mold resistance are critical to maintain the yield and quality of grain sorghum. The advent of new biotechnology techniques such as QTLs (quantitative trait loci) analyses and marker-assisted selection provide new opportunities to enhance sorghum grain mold resistance. Rami et al. (1998) recently described QTLs for grain quality and grain mold incidence. The objectives of this paper are to report our program's

progress in mapping QTLs for grain mold resistance and then present an approach to use marker-assisted selection in collaboration with phenotypic selection to improve grain mold resistance in sorghum.

Materials and Methods

Population development and evaluation

The sorghum population used for mapping consisted of 125 F_5 recombinant inbred lines (RILs) from a cross between RTx430 (Miller 1984) and Sureno (Meckenstock et al. 1993). The 125 F_5 lines were developed by single-seed descent from a hand emasculated cross of RTx430 × Sureno. In the $F_{2:5}$, seed from 20 panicles were bulked to create the lines for replicated evaluation. The RILs along with the two parents were evaluated in the field in a randomized complete block design with two replications in each environment. The test was grown in a total of six environments in Texas, USA (Table 1). Throughout the growing season, data were collected on the test for traits that could be related to grain mold resistance. These traits included grain mold rating (Frederiksen et al. 1991), plant height, exsertion, grain hardness, and grain density.

Genotype characterization and molecular analysis

For each of the RILs and the parents, deoxyribonucleic acid (DNA) was isolated from leaf tissue as described by Klein et al. (2000). Microsatellites were obtained from several sources and analyzed for polymorphism between Sureno and Tx430. Amplified fragment length polymorphisms (AFLPs) were also generated for these populations. Each microsatellite and AFLP displaying a polymorphism was then used in genotypic analysis of the RILs. The linkage map was constructed using the MAPMAKER MacIntosh v 2.0 and MAPMAKER/EXP v 3.0 and QTL mapping was completed using single point regression analyses and interval mapping.

Table 1. A list of the locations and years in which the grain mold recombinant inbred lines were evaluated in Texas, USA.

Location (Code)	1997	1998
College Station, Texas with overhead sprinkling (CW97)	X	
College Station, Texas (CS97, CS98)	X	X
Beeville, Texas (BE97)	X	
Corpus Christi, Texas (CC97)	X	
Lubbock, Texas (LB98)		X

Results and Discussion

Trait variation and distribution

Significant differences existed between the parents for numerous traits that influence grain mold resistance. In most traits, the variation in the RILs was continuous, indicating that the inheritance of the trait is polygenic (Table 2). As expected, there were significant phenotypic correlations between several agronomic traits and grain mold resistance, but the most important relationship is the negative relationship between plant height and grain mold rating. The relationship between height and grain mold resistance is discussed in more detail later.

Genetic linkage map construction

A total of 44 polymorphic simple sequence repeats (SSR) markers and 157 informative AFLP markers were scored in the mapping population. The resulting LOD score >3.0 linkage map placed 130 loci (44 SSR, 85 AFLPs, and 1 morphological trait) on ten linkage groups (designated Linkage Groups A through J) spanning a genetic length of 970 cM with an average distance between markers of 7 cM. There are seven gaps of 25 cM or more. The linear order of the SSR markers for the present map is in good agreement with that of the high-density linkage map derived from the cross of BTx623 and IS3620C (Bhattramakki et al. 2000).

Table 2. Mean, standard deviation (SD), and range for measured traits related to grain mold resistance in sorghum genotypes, Sureno and Tx430, and recombinant inbred lines.

	Mean ± SD			
Trait	Sureno	Tx430	Range	Mean ± SD
Plant height (cm)				
LB98	71 ± 8	41 ± 4	32–88	52 ± 10
CS98	72 ± 12	45 ± 4	31–91	56 ± 12
Exsertion (cm)				
BE97	4.9 ± 0.2	3.0 ± 1.2	1–5	3.5 ± 1.3
CW97	4.9 ± 0.2	3.4 ± 0.5	1–5	3.0 ± 1.4
CS97	4.9 ± 0.3	3.0 ± 0.4	1–5	3.0 ± 1.6
Grain hardness (TADD)[1]				
B98	12.7 ± 0.2	16.6 ± 1.3	9.2–25.4	15.2 ± 3.2
Grain density (g cm^{-3})				
LB98	1.37 ± 0.0002	1.365 ± 0.0004	1.335–1.386	1.362 ± 0.011
Grain mold rating[2]				
BE97	1.6 ± 0.2	4.8 ± 0.3	2.0–5.0	3.2 ± 0.6
CC97	1.9 ± 0.2	4.3 ± 0.2	2.1–4.5	3.1 ± 0.5
CS97	1.8 ± 0.2	4.2 ± 0.3	2.1–4.5	3.0 ± 0.5
CW97	2.1 ± 0.1	4.5 ± 0.1	2.2–4.6	3.4 ± 0.5
CS98	2.3 ± 0.2	4.3 ± 0.1	1.9–4.4	3.0 ± 0.5

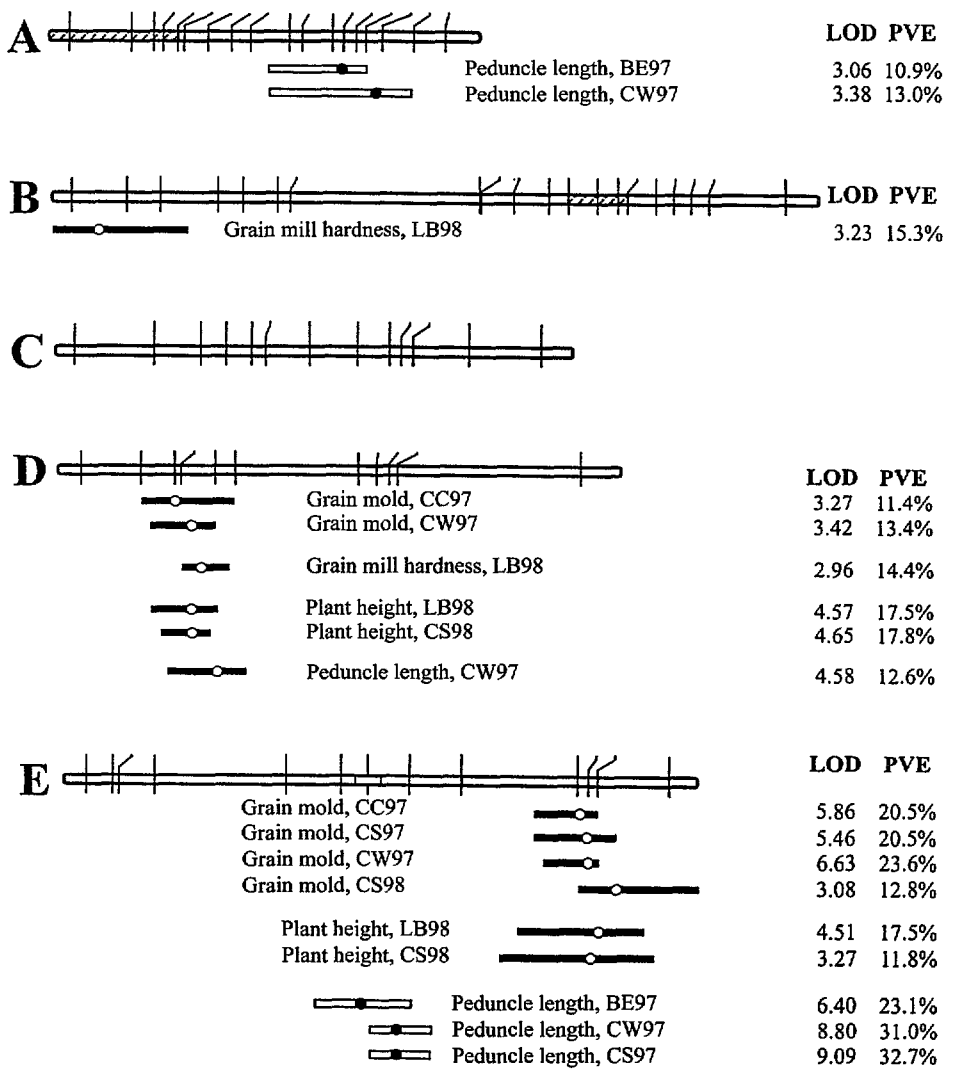
1 TADD = Tangential abrasive dehulling device.

2. Grain mold evaluated on a 1 to 5 scale where 1 = seed bright, free from mold damage; and 5 = very susceptible, embryos dead and endosperm deteriorated

QTL linkage analysis

Because single-point analysis and interval analysis gave very similar results, the results of the interval analysis are presented here. A total of five QTLs were identified with significant effects on grain mold incidence. The grain mold QTLs were present on linkage groups D, E, F, G, and I with each QTL

accounting for 10–24% of the phenotypic variation (Fig. 1). At each locus, reduced mold incidence was conferred with the Sureno allele. All these loci were highly significant and they account for a sizable amount of the phenotypic variation for grain mold. Due to the relatively small size of the mapping population, only QTLs having relatively large phenotypic effects were detected.



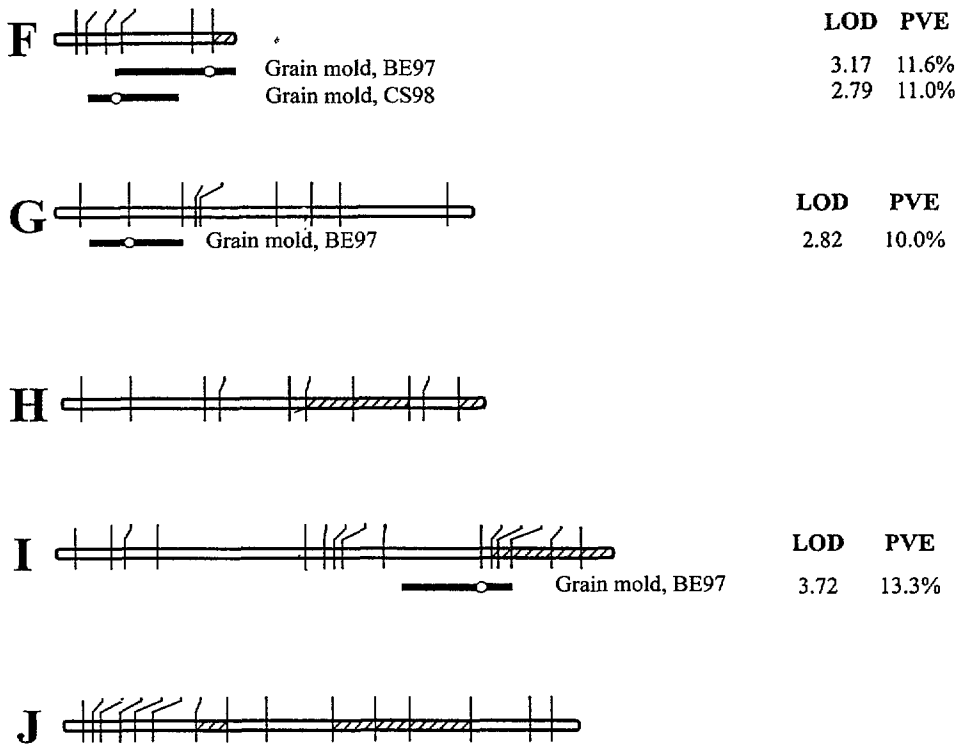


Figure 1. Sorghum linkage map and the locations of putative QTLs.
[Note: Centimorgan scale is given at the top with distances given in Kosambi centimorgans. Letter designation of each linkage group (A through J) is based on the notation of Peng et al. (1999). The specific designations of the genetic markers are not presented herein but they can be found in Klein et al. (submitted). Bars show the positions of QTLs with the peak LOD score identified by the symbol 'o'. QTL bars represent a one-LOD score confidence interval surrounding the peak score. Darkened bars represent trait QTLs affected by the Sureno allele while open bars represent QTLs affected by the RTx430 allele. Location and year of phenotypic evaluation mentioned in Table 1.]

It is important to note that these QTLs are not consistently expressed in every environment, indicating that the genotype by environment interaction is critical in grain mold resistance. For example, the grain mold QTLs on

linkage groups D, E, F, G, and I were present in 2, 4, 2, 1, and 1 of the five environments, respectively, in which grain mold was evaluated. These results are consistent with prior observations in that breeding for stable resistance to grain mold has been difficult and they support the conclusions of Rodriguez-Herrera et al. (in press), who indicated that grain mold resistance mechanisms are different in each environment and these mechanisms are likely controlled by different genetic loci.

As plant phenology has been implicated in contributing to grain mold incidence (Glueck and Rooney 1980; Esele et al. 1993), QTLs for plant stature (plant height, peduncle length) and kernel characteristics (grain milling hardness and grain density) were mapped to examine their relationship to QTLs for grain mold incidence. QTLs for plant height were detected on linkage groups D and E and these QTLs correspond closely with QTLs for grain mold on these linkage groups. The association between QTLs for plant height and grain mold incidence on linkage groups D and E accounts for the strong correlation between these traits that was mentioned previously. In each linkage group, the allele from the taller parent (Sureno) was associated with reduced grain mold incidence. Three QTLs for peduncle length were detected in this population, but only one overlapped a detected QTL for grain mold incidence (Fig. 1). This indicates that the association between peduncle length and grain mold resistance may be important but it is not nearly as important as the relationship between grain mold and plant height in this population. QTLs for grain milling hardness were detected on linkage groups B and D and the QTL on linkage group D overlapped the grain mold resistance QTL on linkage group D.

The results of the study do not indicate whether the grain mold QTLs that mapped to the same region as plant phenotypic characters are pleiotrophic effects of those loci or if they are truly unique and different grain mold resistance loci. Because of the consistent association of these traits with grain mold resistance, it is likely that some of the variation in these grain mold resistant QTLs on linkage groups D and E are influenced by plant height, peduncle length, and grain hardness. Additional studies with larger populations will be needed to fine map the loci controlling the traits in this region of the genome.

While two of the grain mold QTLs are related to phenotypic or kernel traits, the remaining three QTLs for grain mold resistance were not associated

with any phenotypic or other traits that was measured in the population. The potential function of these QTLs in conferring grain mold resistance in this population is not currently known, but there are several possible traits that influence these QTLs. Recently, Rodriguez-Herrera et al. (1999) evaluated the eight most grain mold resistant and susceptible RILs from the Sureno × Tx430 population for antifungal protein levels and found that the resistant lines had consistently higher levels of antifungal proteins than those in the susceptible lines. These results imply that antifungal proteins maybe important in conferring resistance to grain mold, but the whole RIL population has not yet been characterized for antifungal protein content. Even if these grain mold QTLs cannot be associated with any specific trait, they may still be used in a marker-assisted selection scheme for improving grain mold resistance.

Marker-assisted Selection and Future Studies

The goal of this research was to identify QTLs for grain mold resistance. Now that a set of QTLs has been identified, we are planning a program to use it for marker-assisted selection designed to enhance grain mold resistance in agronomically acceptable germplasm. We realize that marker-assisted selection per se for a quantitative trait has major limitations. Therefore, we have created a series of F_2 populations in which Sureno has been hybridized to several elite parental lines from the Texas A&M Experiment Station (TAES) sorghum improvement program. Our plan is to inoculate individual F_2 plants in segregating populations and select individual F_2 plants with high levels of grain mold resistance and acceptable agronomic characteristics. Once the initial selections are made, we will complete molecular analysis on the selected $F_{2:3}$ progeny to identify which of the selections have the greatest number of QTLs from Sureno for grain mold resistance. Therefore, this method ensures that we will select based on field evaluations first and then use the markers to further select for loci that are not possible to select for based on phenotypic characteristics.

This program is in the early stages. The F_2 populations will be evaluated and selected and molecular analysis will be completed in 2000 and this will be followed by additional generations of selection and evaluation to uniformity. We plan to compare grain mold resistance in progeny selected via field selection versus those that have been selected using both field and marker-assisted selection.

In addition to the marker-assisted selection project, more research is necessary to further understand the genetic inheritance of grain mold resistance in sorghum. In the near future, we plan to identify QTLs for antifungal protein content in sorghum and use this information to determine the relationship between antifungal protein content and grain mold resistance. As mentioned previously, the QTLs detected in this study were quite large in magnitude, presumably due to the relatively small population size. It is important to evaluate larger populations to identify QTLs of smaller magnitude for marker-assisted selection and to better understand the inheritance of this trait and its relationship with other phenotypic traits. Finally, sorghum breeders have long known that different sources of grain mold resistance are unique and the mechanism of resistance is probably just as unique. Therefore, it is critical to evaluate several different populations to identify QTLs for grain mold resistance in these sources and use this information to further improve grain mold resistance in sorghum. Generating and utilizing this information will only enhance our efforts to produce sorghum genotypes that are capable of resisting grain mold infection and damage in the future.

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Breeding Grain Mold Resistant Sorghum Cultivars

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Sorghum (*Sorghum bicolor* (L.) Moench) is the fifth important cereal crop after wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), rice (*Oryza sativa* L.), and barley (*Hordeum vulgare* L.) in the world. It is cultivated in about 46 million ha with a total production of 62 million t (FAO 1998). Asia and Africa account 86% of the total area under sorghum, but their contribution towards total production is only 58% (FAO 1998). Nearly 90% of sorghum is grown in rainy season while postrainy season sorghum in India accounts for the remaining area. Sorghum grain yield potential is high. However, there is a gap of about 3 t ha^{-1} in yield between research station yield trials and farmers' fields in India (AICSIP 1994). This reduction in the realized grain yield at farm level is due to several abiotic and biotic constraints.

Grain mold is an important biotic constraint of sorghum and seriously compromises the grain yield and quality grains obtainable from improved cultivars. Grain mold occurs throughout the humid tropical and subtropical climates particularly when improved, short- and medium-duration cultivars that mature before the end of the rains are grown (Bandyopadhyay et al. 1988). It is caused by several non-specialized fungi. These include: *Fusarium monliforme* Sheld., *Fusarium pallidoroseum* (Cooke) Sacc., *Curvularia lunata* (Wakker) Boedijn and *Phoma sorghina* (Sacc.) Boerema, Dorenbosch, & van Kesteren (Bandyopadhyay et al. 1988, 1998).

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Reddy, B.V.S., Bandyopadhyay, R., Ramaiah, B., and **Ortiz, R.** 2000. Breeding grain mold resistant sorghum cultivars. Pages 195–224 *in* Technical and institutional options for sorghum grain mold management: proceedings of an international consultation, 18–19 May 2000, ICRISAT, Patancheru, India (Chandrashekar, A., Bandyopadhyay, R., and Hall, A.J., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

Major efforts in breeding for grain mold resistance at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), and other places in India as well as USA have met with partial success in breeding together high resistance levels of grain mold and grain yield. Annual global losses due to grain molds have been estimated at US$ 130 million (ICRISAT 1992).

We have summarized in this paper the current efforts in breeding for resistance to grain mold with emphasis on ICRISAT work and outlined the future prospects.

Screening Techniques and Sources of Resistance

Following stringent techniques using sprinkler irrigation to maintain high humidity during the grain-filling period, with and without inoculation with conidial-mycelial suspension at Texas A&M University (USA) (Castor 1977) and at ICRISAT (Bandyopadhyay and Mughogho 1988), several sources resistant to grain mold were identified. These include both colored and white grain types. This method involved evaluating the materials grown under sprinklers, 14 days after physiological maturity after stratifying materials according to maturity groups—early, medium, and late (Stenhouse et al. 1998). A laboratory-based screening method has been developed recently and used to screen white-grained photoperiod sensitive materials, which could not be reliably screened using field-screening method. The photoperiod sensitive guinea lines identified by this method are IS 7326, IS 4963, IS 5726, IS 4011, IS 5292, and IS 27761 (Singh and Prasada Rao 1993). Other details of screening techniques have been provided by Bandyopadhyay et al. (1998).

Resistance Mechanisms

Understanding the mechanisms or characters associated with resistance and their interactions is useful in breeding for improved resistant cultivars. Several grain, panicle, and glume characters contribute to resistance in varying levels. Grain characters include hardness, structure of endosperm, pericarp thickness and color, presence of testa, and wax layer. Hardness of the grain is a significant mechanism of mold resistance (Jambunathan et al. 1992; Kumari and Chandrashekar 1992; Audilakshmi et al. 1999). Kernel hardness and pericarp color differentially contribute to grain mold resistance in white, red, and brown pericarp sorghum accessions (Menkir et al. 1996).

At ICRISAT, the F_4 and F_6 progenies evaluated under sprinkler irrigation were selected for threshed grain mold rating (TGMR) below 3 on a 1–5 scale, where 1 indicates no mold and 5 represents >50% of threshed grain surface molded. The resulting 166 white-grained, 105 red-grained, and 194 brown-grained selections were evaluated for grain hardness and grain mass. The characteristic features of white-, red-, and brown-grained groups of selections are given in Table 1, and the correlations among these traits are given in Table 2. The results showed that:

- White- and red-grained mold resistant selections (lacking testa) were harder than brown-grained selections.
- Red- and brown-grained selections had higher levels of grain mold resistance and greater 100-grain mass.
- Ratio of hardness to grain mass in white-, red-, and brown-grained selections suggested that resistance in white and red grains might be partially due to hardness (Table 1).

Correlations between hardness, grain mass, and TGMR (Table 2) generally supported the above conclusions (Reddy et al. 1992).

Table 1. Characteristics of grain mold resistant selections of sorghum at ICRISAT, Patancheru, India, rainy season 1991.

Grain color	TGMR[1]	Hardness[2](kg)	100-grain mass (g)
White	2.96±0.03	5.0±0.10	2.2±0.32
Red	2.40±0.03	5.2±0.09	2.4±0.05
Brown	2.60±0.02	4.6±0.08	2.5±0.04

1. TGMR = Threshed grain mold rating on a 1–5 scale, where 1 = no mold and 5 = >50% of threshed grain surface molded.
2. Pressure (kg) required for breaking the grain.

Table 2. Correlation coefficients among grain hardness, grain mass, and grain mold resistance in sorghum progenies of various grain colors at ICRISAT, Patancheru, India, rainy season 1991.

Grain color	Hardness vs grain mass	Grain mass vs TGMR[1]	Hardness vs TGMR
White	0.53**[2]	–0.03	–0.20*[2]
Red	0.54**	0.15	–0.01
Brown	0.25**	0.01	0.05

1. TGMR = Threshed grain mold rating on 1–5 scale, where 1 = no mold and 5 = >50% of the threshed grain surface molded.
2. * Correlation significant at $P = 0.05$. ** Correlation significant at $P = 0.01$.

Thin pericarp, thick and contiguous surface wax layer, and grain integrity were also linked to resistance to grain mold (Glueck and Rooney 1980). Corneous endosperm types were more resistant than floury endosperm types (Glueck et al. 1977).

Presence of testa has been shown to be the single most important trait conferring grain mold resistance, and red pigmentation of pericarp to a lesser extent (Esele et al. 1993; Menkir et al. 1996). Both mechanisms of resistance are found only in pigmented sorghums, and therefore are unsuitable for breeding for food quality sorghum for grain mold resistance (Stenhouse et al. 1998). Rapid grain filling also seems to result in resistance to grain mold (Singh et al. 1995).

Panicle and glume traits have been shown to be associated with grain molds. Open panicles (as in the guinea sorghums) and long glumes were reported to reduce grain mold incidence (Glueck et al. 1977). Four guinea sorghum lines with loose panicles and hard white grains were found resistant to molds (Rao et al. 1995). Glume cover, length, and area is related to mold resistance (Mansuetus et al. 1990).

Pigmented glumes (phenolic compounds) offer some protection from molds (Mansuetus et al. 1988; Audilakshmi et al. 1999). Several phenolic compounds (apigeninidin, flavan-4-ols, and tannin) in mature grains reduce grain molds (Harris and Burns 1973; Jambunathan et al. 1986; Menkir et al. 1996; Audilakshmi et al. 1999). However, a search for phenols in grains of white-grained cultivars with major differences in resistance and susceptibility has failed to identify any phenolics that could form the basis for selection for resistance (Stenhouse et al. 1998).

Recently, antifungal proteins have been identified in sorghum grains and appear to play a role in protecting the grains from fungal attack (Singh et al. 1995; Audilakshmi et al. 1999). Endosperm of hard grains (resistant lines) contained more protein and prolamine than that of soft grains (susceptible lines). The role of proteins in the resistance to fungal infection in low-tannin lines has been documented (Kumari and Chandrasekhar 1992).

Thus, the mechanisms important in breeding white, grain mold resistant sorghums are: hard corneous endosperm, thin pericarp, thick wax layer on pericarp, fast grain filling rate, large glume coverage, pigmented glumes, and open panicles. Antifungal proteins may also play an important role.

Genetics of Resistance

General combining ability effects seemed to be important for grain mold resistance (Rana et al. 1978; Dabholkar and Baghel 1983). Through P_1, P_2, F_1, and F_2 generation mean analysis it was shown that inheritance of resistance to grain mold was governed by additive or partial dominance type of gene action (Murty et al. 1980). Other investigations with F_1, F_2, and BC_1 populations showed that inheritance of resistance to grain mold was governed by four independently segregating genes—two with complementary (dominant) interaction and the other two with additive interaction (Shivanna et al. 1994).

A recent study suggested that there are five quantitative trait loci (QTLs) for grain hardness, four for flouriness, two for mold after harvest, and three for mold during germination (Rami et al. 1998). No QTLs for 100-grain mass could be detected.

By using four parental lines with distinct caryopsis characters in F_1, F_2, and BC_1 populations, it was demonstrated that presence of pigmented testa (B_1_ B_2_), a red pericarp (R_Y_), a thin mesocarp (Z_), and an intensifier gene (I_) were all dominantly inherited. A pigmented testa was the single most important trait conferring mold resistance. The effect of red pericarp was enhanced by the presence of an intensifier gene. The effects of both pigmented testa and red pericarp were additive (Esele et al. 1993). The inheritance of grain hardness, grain density, germination percentage, decreased water absorption rate and fungal load were studied in a 11×11 half diallel, and found that additive gene action and additive $\times$ additive epistasis were important. The parents CMRP 4, B 75219, B 50501, and SPV 775 were good parents for the above traits (Ghorade and Shekar 1996). In another study, grain density inheritance was reported to be non-additive (Rao et al. 1982).

Red grain color was dominant in F_1 hybrids of red-grained females and white-grained males (Reddy et al. 1992). It was found to be controlled by 2 to 3 interacting genes influenced by the type of cytoplasm (Khusnetdinova and El'konin 1987).

In another study, hardness was inherited dominantly in F_1 hybrids of soft red-grained females and hard white-grained males (Reddy et al. 1992). It was also demonstrated that both hardness in male parents and red color in the females inherited dominantly into F_1 hybrids, which were resistant to grain

molds while the parents themselves were susceptible (Table 3). In another investigation, additive × additive epistasis was important for hardness (Ghorade and Shekar 1996). Flouriness (the converse of corneous endosperm that is hard) had recessive inheritance (Hou et al. 1997). The inheritance of grain weight, a trait negatively correlated with hardness, was additive (Rao et al. 1982; Nguyen et al. 1998) or partially recessive (Biradar et al. 1996). Predominance of additive gene action for large grain was also reported (Borikar and Bhale 1982). However, other investigations suggest predominance of dominance or partial dominance for larger grain size (Chandak and Nandanwankar 1983).

Inheritance of high tannin content in grain is predominantly additive, but not dominant in sorghum grain (Dabholkar and Baghel 1982; Chang et al. 1987; Rodrigues et al. 1998). Generation mean analysis including parents, F_1, F_2, BC_1, and BC_2 of three crosses showed that additive effects with significant epistasis controlled tannin production in the grain (Kataria et al. 1989; Rodrigues et al. 1998).

Effect of B_1 Gene

A large proportion of tannins in the grain is found in the testa layer and its presence is due to the complementary genes B_1 and B_2 (the genotype $B_{1_}B_{2_}$ with brown testa, and the genotypes $b_1b_1b_2b_2$, $B_{1_}b_2b_2$, and $b_1b_1B_{2_}$ without brown testa). As a result, the grains are brown in the former group of genotypes and white in the latter group (Rooney et al. 1980). The white-grained male-sterile lines and restorer lines usually carry the B_2 gene; hence their hybrids are usually white-grained. Segregation of the B_1 and B_2 genes in the white-grained R-lines (ICSR numbers 35, 72, 160, 165, 172, 89058, 93032, and 93033) and in the A-line, ICSA 88020, enabled us to develop hybrids segregating for white and brown grain. Eight hybrids were screened in a randomized complete block design under sprinklers and the white- and brown-grained heads were scored separately for panicle grain mold rating (PGMR) on 1–9 scale, where 1 indicates free from molds and 9 represents >50% mold. Furthermore, white- and red-grained panicles were threshed separately, and scored for TGMR. The threshed grain from the hybrids multiplied in the previous postrainy season was sprayed with inoculum of *Fusarium* and *Curvularia* species during germination in petri dishes in the laboratory, and scored for germinating grain mold rating (GGMR).

Table 3. Performance of the selected red-grained sorghum hybrids and their parents at ICRISAT (Patancheru) and Bhavanisagar, India, rainy season 1991.

Pedigree	Grain yield (t ha^{-1}) IC[1]	Grain yield (t ha^{-1}) BS[2]	Days to 50% flowering	Plant height (m)	Agronomic score[3]	Grain mold rating[4]	Grain hardness[5] (kg)	Flavan-4-ols (A_{550} g^{-1} dry mass, H^+/Me extract)
Hybrids								
ATx2755 × ICSR 41	3.5	5.0	67	1.8	1.6	2	5.2	4.7
ATx2754 × ICSR 3	3.7	5.6	67	1.7	1.7	2	5.2	3.3
ATx2755 × ICSR 111	3.5	4.8	65	1.8	1.8	2	4.6	7.5
Parents								
BTx2754	2.2	3.2	69	0.9	3.4	3	2.8	6.2
BTx2755	2.2	3.0	68	1.0	3.3	4	2.7	5.2
ICSR 3	2.4	3.3	70	1.5	2.9	4	6.2	0.1
ICSR 41	2.6	3.2	72	1.6	2.3	3	4.8	0.2
ICSR 111	3.0	5.0	70	1.5	2.1	4	5.1	0.1
Control								
ICSH 153 (CSH 11)	4.0	5.3	66	1.9	1.3	4	3.4	0.2
SE ±	0.4	0.5	0.6	0.03	0.002	0.002	0.28	0.46
Mean	2.9	3.6	67	1.6	2.7	3.3	4.1	3.5
CV (%)	20	23	3	6	21	12	12	13

1 IC = ICRISAT; mean of several trials in high and low fertility fields, with and without sprinkler irrigation.
2. BS = Bhavanisagar; high fertility without irrigation.
3. Scored on a 1 to 5 scale, where 1 = most desirable and 5 = least desirable.
4 Based on threshed grain mold rating (TGMR) on a 1 to 5 scale, where 1 = no molds, 5 = >50% of threshed grain surface molded.
5. Pressure (kg) required to break the grain.

The brown-colored hybrids were highly resistant compared to white-grained hybrids for PGMR and TGMR, indicating the role of a single dominant gene (B_1) in the resistance under field conditions (Table 4). But the difference in GGMR in the laboratory between the white-grained and the red-grained hybrids was absent. The score for PGMR of the nine parents ranged from 5.3 to 7.7, whereas it ranged from 6 to 9 for TGMR, and from 4.5 to 9.0 for GGMR.

Partially dominant or dominant genes in F_1 hybrids of cytoplasmic male-sterile lines controlled flavan-4-ols (Reddy et al. 1992). Two genes with greater dominance effects and duplicate type of epistasis determined tannin content in the leaves (Chariya Srichantub 1988; Kumar and Singh 1998).

A single dominant gene controls reddish purple glumes (Mani 1986). Other studies showed a single dominant gene or two genes with inhibiting interaction for red glume over straw (Swarnalata et al. 1987), or one gene with partial dominance (Deshmukh et al. 1980). Two to three interacting genes influenced by cytoplasm control glume length and coverage (Khusnetdinova and El'konin 1987). Information on genetics of grain filling rate is scanty, although significant variation for grain filling rate was observed among sorghum genotypes (Toure et al. 1992).

Information on the genetics of resistance mechanisms is inadequate. The genetics depends on the type of materials used, mechanisms studied, the measurements taken, the level of humidity experienced, and the interference from head bug (*Calocoris angustatus* Lethiery) damage in the measurement of grain mold infection.

Breeding for Grain Mold Resistance

Varieties or restorer lines

The breeding efforts at ICRISAT and other breeding programs until 1990 aimed at the genetic improvement for mold resistance of varieties and restorer lines. The breeding program at ICRISAT can be divided into five phases:

1. White-grained lines from natural screening of white-grained introductions and populations.
2. White-grained lines from segregating white-grained populations screened under artificial conditions.

Table 4. Grain mold severity rating of the sorghum hybrids carrying white and red grain color genes evaluated at ICRISAT, Patancheru, India, rainy season 1992[1].

Hybrid	Panicle grain mold rating (PGMR) in field[2]			Threshed grain mold rating (TGMR) in field[3]			Germinating grain mold rating in laboratory[4]		
	White	Brown	Average	White	Brown	Average	White	Brown	Average
ICSH 93141	5.7	3.0	4.3	7.0	3.0	5.0	8.5	9.0	8.8
ICSH 93142	5.0	3.0	4.0	6.3	5.0	5.7	6.0	8.0	7.0
ICSH 93143	5.7	3.0	4.3	6.7	3.0	4.8	4.5	5.0	4.8
ICSH 93144	6.0	2.0	4.0	6.0	2.0	4.0	6.5	4.0	5.3
ICSH 93145	6.0	4.7	5.3	6.7	6.0	6.3	7.5	8.0	8.0
ICSH 93146	5.3	2.7	4.0	5.7	3.0	4.3	5.0	6.5	5.8
ICSH 93147	5.7	3.0	4.3	7.0	3.0	5.0	9.0	6.5	7.8
ICSH 93148	5.7	4.0	4.8	6.7	3.0	4.8	8.0	8.5	8.3
Mean	5.6	3.2	4.4	6.5	3.5	5.0	6.9	7.0	6.9
SE±									
Hybrids			0.05			0.07			0.05
Grain color			0.03			0.04			0.02
Hybrids × grain color			0.08			0.10			0.06
CV (%)			14			19			6

1. Split-plot design, under sprinklers in the field.
2 Panicles evaluated on a 1 to 9 scale, where 1 = <5% grain with mold, 9 = 80% grains with mold.
3. Threshed grains evaluated on a 1 to 9 scale, where 1 = no mold, 9 = 50% grain surface area molded.
4. Split-plot design with two replications; germinated seeds inoculated with mixed spore suspension of *Fusarium moniliforme*, *F. pallidoroseum*, and *Curvularia lunata*.

3. White-grained lines from segregating populations of white-grained high-yielding lines × red-grained mold resistance sources crosses.
4. Red-grained lines from segregating populations of white grained lines × red-grained sources crosses.
5. White-grained guinea lines improvement.

White-grained materials under natural screening. The earliest released early-maturing hybrid CSH 1, which was popular in the 1960s and 1970s in India, is highly susceptible to grain mold as it matures early in peak rainy season. This shortcoming led to the search for lines tolerant to grain molds. Several derivatives of zera zera germplasm from Sudan and Ethiopia were less susceptible to molds when screened under natural conditions. The approach was to select materials under multilocation testing for good food quality types with less grain deterioration in rainy season. As a result hybrids such as CSH 5 and CSH 6 and varieties such as CSV 4 that deteriorated less under rain were developed in India. The fact that derivatives of zera zera material were superior in resisting grain deterioration compared to earlier cultivars (CSH 1) confirmed their inherent tolerance. This program continued up to mid-1970s.

White-grained materials under artificial screening. A screening technique for mold resistance under sprinklers with artificial inoculation by grain mold fungi was developed by the mid-1970s. Several zera zera germplasm accessions and their derivatives, and high-yielding and adapted lines from various breeding programs were used extensively in crosses from mid-1970s onwards. In particular E 35-1, CS 3541, SC 108-3, SC 108-4-8, and SC 120 were used by ICRISAT breeders. The crosses were advanced to F_2 in which selection for height, days to flower, and agronomic desirability were practiced. The progenies from F_3/F_4 onwards were evaluated under artificial screening and the high-yielding lines with mold resistance were selected (Murty et al. 1980). This program continued up to late 1970s.

White-grained materials from colored-grain sources. The zera zera based program has been highly successful and widely followed at ICRISAT and other locations in India and in USA. Zera zera material dominates the elite improved germplasm. Serious concerns have been expressed about the narrowing of the genetic base. Therefore, concerted efforts were made to broaden the base of the parents used in developing high-yielding, white-

grained mold-resistant varieties. Grain mold resistant lines (mostly colored, with or without testa) (Table 5) were used in crosses with white-grained, high-yielding (zera zera) lines. For the first time, several F_1, F_2, and F_3 progenies derived from crosses between mold-resistant, colored-grain sorghum and susceptible white-grained sorghum were screened under an artificial environment to determine if white-grained segregates with high mold resistance could be identified (ICRISAT 1984). The F_1s from crosses between a mold susceptible colored line (Q 953) and mold susceptible white-grained lines (SPV 104 and SPV 351) had colored grain but were susceptible to mold. Identification of white-grained, mold resistant segregates in 1983 confirmed that grain mold resistance was not always associated with colored grain (ICRISAT 1984) and it could be transferred to white-grained lines. In the following rainy season, 597 mold resistant lines were selected based on TGMR, of which 2 had score 1, 134 had score 2, and 461 had score 3 (ICRISAT 1985). These were further selected for agronomic desirability. Thus, 36 white-grained mold resistant lines derived from parents were selected for further testing. The performance of some of the selected lines is given in Table 6. This program of breeding white-grained mold resistant lines was continued through the mid-1980s.

Red-grained lines. The white-grained selections showed only moderate levels of resistance, and their plant type was not appropriate. The white-grained mold resistance breeding program had limited success. Agronomic desirability, plant type, and resistance levels in the lines with red pericarp without testa (with flavan-4-ols) showed considerable advantage compared to white-grained selections (Table 7). However, red-colored grains are not acceptable for food preparation in some areas. Therefore, breeding for red-grained mold resistant lines, which was continued until the late 1980s, received reduced emphasis after the 1980s.

White-grained guinea lines. From early 1990s, the emphasis was given to exploit white-grained guinea sorghums, which were identified after laboratory screening as resistant to grain mold. Both pedigree and population breeding approaches have been used to develop derivatives that combine white grain color with plant type and yield levels of improved sorghum, yet retaining the guinea grain and glume characters (Stenhouse et al. 1998). Some success has been achieved and a number of dwarf early lines with semi-compact heads and guinea grain and glumes have been produced through pedigree

Table 5. Grain mold resistant sources of sorghum used as parents in breeding grain mold resistant varieties/restorers.

Source	Origin	Race	Grain color	Days to 50% flowering	Plant height (m)	Panicle grain mold score[1]	Threshed grain mold score[2]	Grain size (mm)	Grain mass (g 100^{-1})	Grain hardness
Parents contributed to white-grained varieties										
IS 2333	Sudan	Caudatum	Reddish brown	81	4.1	2.4	2.0	3.5	3.20	Partly hard
IS 18165	Lebanon	Caudatum	Light red	66	2.7	2.5	2.0	2.5	1.97	Partly hard
IS 20844	China	Caudatum	Reddish brown	54	1.4	2.0	1.8	2.8	2.53	Mostly hard
IS 25017	Sudan	Caudatum	Straw	73	3.7	2.3	2.7	2.8	2.39	Partly hard
Parents contributed to colored-grain varieties										
IS 7972	Nigeria	Guinea	Light red	127	3.7	-	-	4.0	5.64	Partly hard
IS 18165	Lebanon	Caudatum	Light red	66	2.7	2.5	2.0	2.5	1.97	Partly hard
IS 20708	USA	Bicolor	Light red	61	2.5	3.0	-	2.5	2.96	Partly hard
IS 20844	China	Caudatum	Reddish brown	54	1.4	2.0	1.8	2.8	2.53	Mostly hard
IS 21509	Malawi	Guinea caudatum	Light red	63	2.8	2.0	2.0	2.5	1.90	Partly hard
IS 24996	Sudan	Guinea caudatum	Reddish brown	78	4.1	1.8	2.0	4.0	4.12	Mostly hard
IS 25017	Sudan	Caudatum	Straw	73	3.7	2.3	2.7	2.8	2.39	Partly hard
IS 25032	Sudan	Guinea caudatum	Brown	78	3.5	2.3	2.1	3.5	3.57	Mostly hard
Parents contributed to guinea varieties										
IS 2825	Zimbabwe	Caudatum	Reddish brown	53	1.7	2.0	1.8	4.0	3.27	Soft
IS 7072	Sudan	Caudatum bicolor	Light brown	69	2.5	1.8	1.8	3.0	3.06	Partly hard
IS 14384	Zimbabwe	Guinea	Light red	57	3.4	2.2	2.1	1.8	2.46	Partly hard
IS 21059	Kenya	Caudatum	Light brown	120	3.7	-	-	3.0	2.46	Mostly soft
IS 25025	Sudan	Durra caudatum	Reddish brown	73	3.1	2.0	2.0	3.5	3.00	Mostly soft
IS 25032	Sudan	Guinea caudatum	Brown	78	3.5	2.3	2.1	3.5	3.57	Mostly soft

1. Panicles evaluated on a 1 to 9 scale, where 1 = <5% grains with mold, 9 = 80% grains with mold.
2. Threshed grains evaluated on a 1 to 9 scale, where 1 = no mold, 9 = 50% grain surface area molded.

Table 6. Selected entries from a yield trial of 36 white-grained mold resistant sorghum breeding lines at ICRISAT, Patancheru, India, rainy season 1996.

Entry	Pedigree	Days to 50% flowering	Plant height (cm)	Grain yield (t ha^{-1})	Mold score[1]
ICSV 96105	(IS 25017 × ICSV 233)-16-2-2-2-3-1	71	2.5	3.23	3.9
ICSV 96087	(IS 2333 × ICSV 88032)-3-1-1-2-1-1	69	1.7	3.21	4.9
ICSV 96102	(IS 25017 × ICSV 233)-6-4-2-1-1-2	72	2.4	3.06	4.8
ICSV 95010	(IS 25017 × ICSV 233)-16-2-2-2-2-1	73	2.8	3.04	4.3
ICSV 96089	[(IS 18165 × ICSR 33) x IS 18165]-2-1-1-1-1-1	70	1.7	2.77	4.3
ICSV 96094	(IS 20844 × ICSV 88022)-4-2-1-2-1-1	63	1.7	2.41	3.5
ICSV 112	Control	75	1.9	2.65	5.5
CSV 14	Control	73	2.5	3.42	6.1
CSH 13	Control	72	0.9	3.01	7.5
Trial mean		70	2.0	2.8	5.4
SE±		1.0	0.04	0.3	0.5
CV (%)		1.9	2.6	12.4	14.8

1. Grain mold evaluated on a 1–9 scale, where 1 = free from mold and 9 = >50% of the grains with mold.

Source: ICRISAT (1997).

Table 7. Selected entries from a yield trial of 39 colored-grain sorghum lines at ICRISAT, Patancheru, India, rainy season 1996.

Entry	Pedigree	Days to 50% flowering	Plant height (cm)	Grain yield (t ha^{-1})	Mold score[1]
ICSV 95023	(IS 25032 × ICSV 710)-11-1-2-1-1	72	2.1	3.24	3.3
ICSV 96127	(IS 20708 × ICSV 88041)-1-1-1-1	63	1.5	3.01	3.8
ICSV 95039	(IS 18165 × ICSV 88936)-1-1-1-1-1	63	1.5	2.74	2.4
ICSV 96141	(IS 24996 × ICSV 88035)-3-1-1-1-1-1	66	1.4	2.74	3.8
ICSV 96136	(IS 21509 × ICSV 430)-10-2-1-1-2	67	1.7	2.71	3.4
ICSV 112	Control	77	1.8	2.70	6.2
Trial mean		68	1.7	2.44	3.8
SE±		1.9	0.06	0.5	
CV (%)		4.0	5.1	12.5	21.9

1. Grain mold evaluated on a 1–9 scale where 1 = free from mold and 9 = >50% of the grains with mold.

Source: ICRISAT (1997).

breeding. Similar breeding efforts to produce improved guinea sorghums are underway in several programs in West Africa (Stenhouse et al. 1998). However, neither red-grained nor white-grained guinea products have been screened or selected for mold resistance under artificial screening. The data on grain yield, mold score, and agronomic traits for improved guinea lines are given in Table 8.

Population improvement

Development of random mating population was initiated in 1984 by incorporating white-grained, mold resistant breeding lines; colored-grain, mold resistant lines; and high-yielding improved lines into ms_3 US/R and US/B bulks. By the end of 1988, four random matings had been carried out (Stenhouse et al. 1991). Half-sib family selection was carried out for two cycles and then random mated. Several guinea lines were incorporated twice thereafter into this population, which underwent four random matings before selection. However, the population improvement program, though initiated in 1984, has not been fully exploited at ICRISAT.

Seed parents

Male-sterile lines available at ICRISAT before 1989 were not developed with any emphasis on resistance for pests or diseases. During ICRISAT's fifth phase of research from 1990 to 1997, efforts were made to develop male-sterile lines specifically resistant to different yield limiting factors. Several germplasm lines with putative resistance to grain mold were identified during 1980 to 1990. The grain color in all lines is brown (B), red (R), or light red (LR). The most promising lines are IS 2501 (B), IS 2815 (LB), IS 3436 (LR), IS 10288B (LR), IS 10475B (LR), IS 10646 (B), IS 21599 (LR), and IS 23585 (RB) (Table 9). These were tall and mostly photoperiod sensitive and were not suitable as male-sterile lines, because they are poor yielders in the rainy season. Hence, these resistant lines were crossed with high-yielding maintainer lines (A_1 cytoplasm) such as ICSB 11, ICSB 17, ICSB 37, ICSB 42, ICSB 51, and ICSB 70, which are susceptible to grain mold. Selection for highly heritable traits such as days to flower, plant height, and grain color was practiced in F_2 generation and the resulting F_3s were screened following the modified screening technique as outlined by Bandyopadhyay et al (1998). Grain mold resistance score (GMRS) on panicle and on threshed grain on 1 to 9 scale were taken. The panicle GMRS is based on the scale where 1 means below

Table 8. Selected entries from a yield trial of 11 derivatives of guinea × caudatum sorghum crosses at ICRISAT, Patancheru, India, rainy season 1996.

Entry	Pedigree	Days to 50 % flowering	Plant height (cm)	Grain yield (t ha^{-1})	Mold score[1]
ICSV 95055	(IS 7072 × ICSR 16)-4-3-4-3-1-1	69	1.6	2.43	2.3
ICSV 95043	(IS 21509 × ICSR 36)-1-3-2-1-1-1	73	1.9	2.14	6.5
ICSV 95052	(IS 25032 × ICSV 710)-4-5-1-2-1-1	71	1.3	2.13	4.7
ICSV 95046	(IS 2825 × ICSV 111)-1-3-2-1-2-1	72	1.8	2.00	4.0
ICSV 95068	(IS 14384 × ICSR 41)-1-1-1-1-1-1	71	1.8	1.97	4.3
ICSV 112	Control	76	1.9	2.38	7.3
Trial mean		73.9	1.7	2.05	5.6
SE±		1.2	0.05	0.23	0.05
CV (%)		2.3	4.7	16.1	15.8

1. Grain mold evaluated on a 1–9 scale, where 1 = free from mold and 9 = >50% grains with mold.

Source: ICRISAT (1997).

Table 9. Grain mold resistant sources used as parents in breeding seed parents at ICRISAT, Patancheru, India.

Source	Origin	Race	Grain color	Days to 50% flowering	Plant height (m)	Panicle grain mold score[1]	Threshed grain mold score[2]	Grain size (mm)	Grain mass (g 100^{-1})	Grain hardness
Phase I										
IS 2501	Sudan	Caudatum	Brown	83	2.0	-	-	4.5	1.65	Soft
IS 2815	Zimbabwe	Caudatum	Light brown	78	2.0	-	-	4.0	3.57	Mostly soft
IS 3436	South Africa	Kafir bicolor	Light red	57	1.6	-	-	4.0	3.45	Partly hard
IS 10288B	USA	Caudatum bicolor	Light red	52	1.3	5.0	-	2.8	2.80	Partly hard
IS 10475B	USA	Kafir bicolor	Light red	56	1.1	5.0	-	3.0	3.56	Partly hard
IS 10646	USA	Caudatum bicolor	Brown	67	1.3	5.0	-	2.5	2.00	Partly hard
IS 21599	Malawi	Guinea	Light red	74	3.7	2.4	1.8	2.8	2.02	Mostly hard
IS 23585	Ethiopia	Guinea caudatum	Brown	75	3.1	2.0	2.0	3.0	3.11	Mostly soft
Phase II										
ICSB 362	Bred line	-	White	64	1.8	5.7	5.1	-	3.30	Hard
ICSB 364	Bred line	-	White	64	1.7	6.6	5.3	-	3.10	Soft
ICSB 383	Bred line	-	Brown	67	2.0	5.4	3.8	-	3.10	Soft
ICSB 391	Bred line	-	Red	64	2.0	6.7	5.6	-	3.40	Hard
ICSB 392	Bred line	-	Red	68	1.6	6.5	5.3	-	3.80	Partly hard
ICSB 393	Bred line	-	Red	67	1.9	6.4	5.2	-	3.40	Hard
ICSB 403	Bred line	-	Brown	68	1.9	5.3	3.1	-	2.80	Hard
ICSB 405	Bred line	-	Red	64	1.6	6.5	5.8	-	3.60	Hard

continued

Table 9. *continued*

Source	Origin	Race	Grain color	Days to 50% flowering	Plant height (m)	Panicle grain mold score[1]	Threshed grain mold score[2]	Grain size (mm)	Grain mass (g 100 [1])	Grain hardness
IS 14375	Zimbabwe	Guinea	Red	69	3.0	2 2	2.1	1.8	2.10	Partly hard
IS 14380	Zimbabwe	Guinea	Light red	68	3.2	2.0	2.4	1.8	2.26	Mostly hard
IS 14384	Zimbabwe	Guinea	Light red	69	3 3	2.2	2.1	1.8	2 46	Partly hard
IS 14385	Zimbabwe	Guinea	Light brown	70	3 4	2 0	2.3	1.8	1.71	Mostly soft
IS 14390	Swaziland	Guinea	Light red	69	3.1	2.4	2.4	1.8	1.71	Partly hard
IS 25015	Sudan	Guinea caudatum	Gray	83	3.7	2.4	2.4	3.5	3.30	Partly hard
IS 25060	Ghana	Guinea caudatum	Reddish brown	79	4.2	2.0	2.0	3.0	3.00	Soft
IS 8614	Uganda	Caudataum	Reddish brown	56	1.1	1.6	1.8	3.0	3.58	Soft
IS 9308	South Africa	Caudatum	Reddish brown	56	2.3	2.2	1.7	3.0	2 87	Soft
IS 13817	South Africa	Caudatum	Reddish brown	56	2.2	1.6	1.8	3.0	2.85	Mostly soft
IS 13885	South Africa	Guinea	Light red	72	3.2	2.2	1.6	2 8	1.80	Partly hard
IS 14332	South Africa	Guinea	White	68	3.1	2.9	3.2	1.8	1.99	Mostly hard
IS 14388	Swaziland	Durra caudatum	Reddish brown	55	1 7	1.2	1.8	2.0	1.96	Soft

1. Panicles evaluated on a 1 to 9 scale, where 1 = <5% grains with mold, 9 = >80% grains with mold.
2. Threshed grains evaluated on a 1 to 9 scale, where 1 = no mold, 9 = 50% grain surface area molded.

5% mold infected grain, and 9 indicates >80% mold infected grain. The threshed grain GMRS is based on the scale where 1 represents no mold on the surface of grain and 9 indicates >50% surface of the grain molded. Selection among families was based on panicle and threshed GMRS and selection for high-yielding and agronomically desirable plants was practiced within the selected resistant families (Fig. 1). This procedure was continued from F_3 to F_7. Test-crossing with A_1 cytoplasm was followed simultaneously from F_4 onwards and the selected resistant progenies with male-sterility maintainers were converted into male-steriles through backcrossing. The A/B-lines obtained were evaluated for other characters.

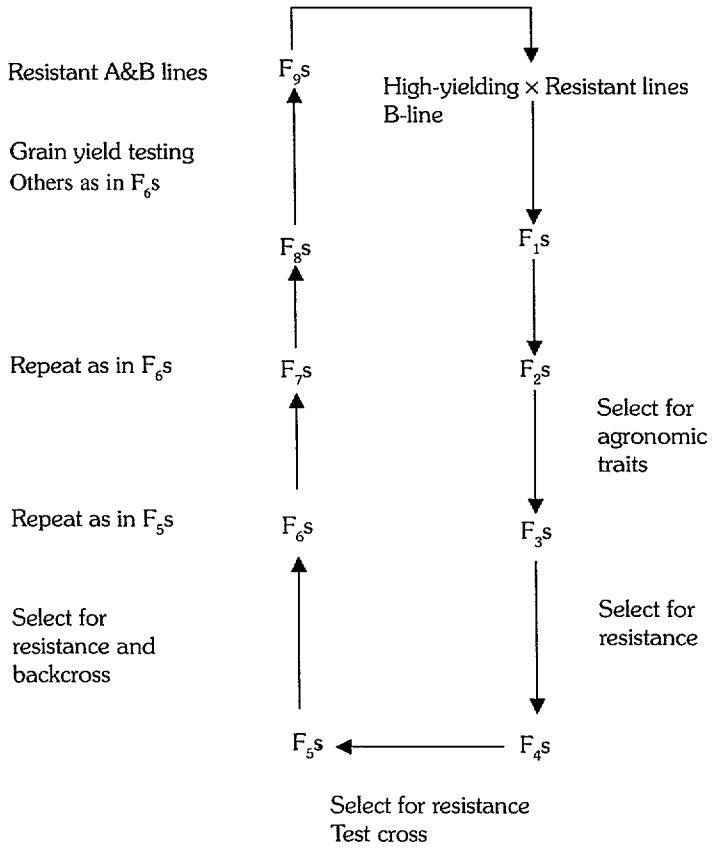

Figure 1. Method of breeding for grain mold resistant male-sterile lines in sorghum.

Fifty-eight seed parents with A_1 cytoplasm were thus produced at ICRISAT. Of these, 35 were white-grained, 20 red-grained, and three were brown-grained. The data on various agronomic characters, grain yield, and grain mold resistant sources were put in a web page (http://grep.icrisat.cgiar.org/). Performance of some selected A/B-lines is given in Table 10. Several resistant sources (IS 2815, IS 21599, IS 10288, IS 3436, IS 10646, IS 10475, and IS 23585) contributed to these 58 male-sterile lines. Of these, IS 2815 contributed to nearly more than 50% of the derivatives.

Various crossing systems were analyzed for their efficiency in producing desirable resistant lines in various grain colors. Biparental crossing system was superior to the backcross or three-way cross system both in terms of grain mold levels and number of desirable selections (Table 11). Also, the grain mold resistant sources, IS 9470 with A1 (milo), A2, A3, and A4 (Maldandi), and IS 15119 with A3 and A4 (Maldandi) cytoplasms were converted into male-sterile lines.

The above program was based on colored-grain resistant sources. Diversification of grain mold resistant male-sterile lines with guinea lines (Table 9) is being targeted in the second cycle of seed parents improvement, which began in 1999.

Hybrids

The selected grain mold resistant hybrids, ICSH 91200 (ATx2755 × ICSR 41), ICSH 91201 (ATx2754 × ICSR 3), and ICSH 91202 (ATx2755 × ICSR 111) (Table 3), obtained by crossing mold susceptible, red-grained female lines and white-grained susceptible male lines continued to maintain grain mold resistance in the third consecutive screening (Reddy and Singh 1993). They produced 5.0 to 5.6 t ha^{-1} grain yield while the mold susceptible control hybrid ICSH 153 yielded 6.4 t ha^{-1} (SE ±0.17).

Some more hybrids were derived by crosses between white-, red-, and brown-grained pollinators (bred by ICRISAT), and the red-grained but mold susceptible female lines developed at Texas, USA. Comparison of females, restorers, and their fertile hybrids for grain mold resistance is given in Table 12. Grain mold resistance was dominant in brown-grained hybrids of brown-grained males × red-grained females and in red-grained hybrids of red-grained males × red-grained females; and over dominant in red-grained hybrids of white-grained males × red-grained females. Red-grained but grain

Table 10. Performance of some selected grain mold resistant sorghum seed parents.

B-line	Pedigree	Threshed grain mold score[1]	Days to 50% flowering	Plant height (m)	Agronomic score	Grain yield (t ha^{-1})	Grain size (g 100^{-1})	Grain color
ICSB 353	(ICSB 11 × IS 2815)-2-1-2-1-2	5.1	70	1.7	2.3	3.8	3.1	White
ICSB 355	(ICSB 11 × IS 2815)-12-1-1	3.6	68	1.5	3.0	3.4	3.3	White
ICSB 376	(ICSB 11 × IS 2815)-43-1-2-1	5.1	75	1.7	2.0	4.2	3.5	White
ICSB 377	(ICSB 11 × IS 2815)-48-1-1	3.6	68	1.6	2.3	4.6	3.2	White
ICSB 395	(ICSB 37 × IS 10475B)-11-1-1-2	4.8	70	1.4	2.7	3.5	2.9	White
ICSB 402	(ICSB 42 × IS 23585)-1-7-1-1-1	3.3	69	1.5	2.0	4.0	3.6	White
ICSB 369	(ICSB 11 × IS 2815)-30-1-3-1-1	4.3	69	1.7	2.0	3.6	3.1	Red
ICSB 371	(ICSB 11 × IS 2815)-32-1-1-3-2-2	4.6	69	1.7	2.3	4.5	3.1	Red
ICSB 388	(ICSB 37 × IS 21599)-4-1-3-2-1-2	5.1	68	1.9	2.7	3.8	3.1	Red
ICSB 383	(ICSB 17 × IS 10646)-5-1-2	3.8	69	1.8	1.7	4.7	3.1	Brown
Control 296B		8.4	83	1.1	3.0	4.0	3.3	White

1. Threshed grains evaluated on a 1–9 scale, where 1 = no mold on the surface of grain and 9 = >50% surface of grain with mold

mold susceptible females and red- and brown-grained but resistant males, which were known to contain flavan-4-ols in quantities sufficient to maintain resistance, were the parents. The flavan-4-ols might have been inherited as a dominant trait resulting in resistant hybrids. Hardness in white-grained males could have contributed to the enhanced resistance expressed by white-grained hybrids.

Table 11. Panicle grain mold resistance scores in the progenies derived from different crossing systems, ICRISAT, Patancheru, India 1992 rainy season.

Cross	Score[1] Mean (SE±)	Minimum	Maximum	No.of progenies
White grain				
Biparental cross	2.8 (0.00)	1.7	3.0	259
Backcross[2]	2.9 (0.02)	2.6	3.0	15
Three-way cross[3]	2.8 (0.02)	2.4	3.0	24
Red grain				
Biparental cross	2.5 (0.02)	1.7	3.0	210
Backcross	2.6 (0.06)	1.7	2.8	13
Three-way cross	2.6 (0.05)	2.2	2.8	11
Brown grain				
Biparental cross	1.8 (0.03)	1.7	2.4	30
Backcross	1.9 (0.04)	1.7	2.0	2
Three-way cross	2.5 (0.04)	2.2	2.8	6

1. Grain mold evaluated on a 1 to 9 scale, where 1 = no mold and 9 = >50% surface of grain with mold.
2. Backcross to resistant parent.
3. Two susceptible parents and one resistant parent.

Table 12. Grain mold scores on panicles in hybrids and their parents at ICRISAT, Patancheru, India, 1992 rainy season.

Males		Score of	Hybrids	
Grain color	Score[1]	red-grained females	Grain color	Score
White	7.3 (4)[2]	7.0 (4)	Red	6.0 (4)
Red	5.1 (48)	6.9 (13)	Red	4.8 (48)
Brown	3.5 (5)	7.2 (5)	Brown	4.2 (5)

1. Score on 1 to 9 scale, where 1 = free from molds and 9 = >50% grain surface area molded.
2. Numbers in parentheses indicate the number of lines/hybrids on which the means are based.
Source: ICRISAT (1993).

The investigations at ICRISAT clearly demonstrated that it was possible to produce grain mold resistant hybrids from the existing materials if appropriate parents are crossed. However, grain mold resistance was low in white-grained hybrids compared to other grain color types.

Limitations

Several mechanisms are known to contribute to resistance. Breeding for white-grained mold resistant lines has exploited only hardness in the grain without affecting grain food quality. For example, hard grains are not suitable to make some foods such as *roti* (India), *injera* (Ethiopia), and *kisra* (Sudan). Further hardness limits the grain size, while the farmer looks for bold attractive grains. Glume pigmentation and glume cover can be used only to the extent that they do not lead to grain discoloration or threshing problems. Open panicle character of guinea are yet to be fully exploited in breeding white-grained mold resistant sorghum. Openness in panicles beyond a limit leads to poor productivity. Fast grain filling helps in increasing resistance levels, but measurement of this trait is difficult and limits the scope to evaluate large populations as required in breeding. Therefore, in areas where improved white-grained sorghums are important, such as in India, the options are numerous; but their effects on increase in grain mold resistance are meager especially in bold grains. In other parts of Africa, where hard white grain is preferred in food preparation (*tô*) the characters associated with guinea sorghums (hard corneous endosperm, open panicles, and extensive glume coverage) can be exploited.

Identification of markers for antifungal proteins is also expected to be useful. However, as antifungal proteins co-evolved with fungal pathogens in a particular plant species, it is doubtful if such proteins give complete protection from mold. The true potential of antifungal proteins is likely to be achieved only when they are deployed against fungal pathogens with which they have not co-evolved. Hence, transformation should be used for transfer of antifungal proteins between species, which is a difficult process.

Flavan-4-ols in red grain (without testa), and tannins and flavan-4-ols in grains (with testa) are strong and stable mechanisms, which may be combined with increased grain test weight. However, the areas where sorghum with a pigmented pericarp or testa are used are very limited in Africa.

Outlook

Conventional breeding exploited grain hardness in improving mold resistance in white-grained sorghum. Programs in India and ICRISAT developed several high-yielding hard-grain restorer lines and male-sterile lines. Recently US programs also started breeding hard white-grained male and female parents aiming to export markets in Africa and India, where white-grained sorghum is a staple food. In all these programs, zera zera sorghums have been used extensively. Limited efforts were directed in breeding red-grained mold resistant lines at ICRISAT. It is important that the genetic changes brought about by breeding for grain mold resistance in advanced lines over the years should be assessed. ICRISAT is planning such an experiment.

Further improvements in combining grain yield and mold resistance is possible through conventional breeding, which should include expanded systematic screening and selection in segregating progenies of specifically planned crosses. Such programs should involve guinea resistant sorghums in the crosses and exploit open panicles, large glume coverage in addition to grain hardness. However, the progress from such programs is expected to be slow because grain mold resistance expresses late in the life cycle of the crop, is difficult to measure, has complex inheritance, and is significantly influenced by the environment. Therefore, this program should be complemented with marker technology by identifying markers for genes contributing to resistance.

The proposed conventional breeding although helps to diversify grain sorghum for mold resistance and grain yield, it is unlikely that it would help in breeding high-yielding, bold, white-grained sorghum with complete protection against grain mold. Biotechnological approaches hold promise, but are resource intensive and may not provide new cultivars in the short term. The grain mold problem in sorghum may be assuaged but will not be solved in the foreseeable future by resistance breeding alone. Therefore, complementary methods at field and pre-consumption grain processing levels together with genetic enhancement are needed for a holistic mold management.

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Breeding for Grain Mold Resistance in Sorghum: Opportunities and Limitations

D S Murty[1]

Grain mold problem in sorghum (*Sorghum bicolor* (L.) Moench) is a complex of three phenomena: (1) infection of developing grains by parasitic and saprophytic fungi; (2) grain discoloration and weathering; and (3) loss of seed germinability or sprouting. Grain mold problem could be worse when there is a simultaneous attack by panicle feeding insects such as head bugs (*Calocoris angustatus* Lethiery). During the last few decades significant progress has been made in breeding for resistance to grain molds, but the problem still remains to be the major constraint to sorghum production and utilization. Certainly our knowledge of the disease and the associated factors has improved immensely with reference to the fungi involved and their individual and joint effects, the process of infection and fungal growth, the nature of damage to the grain, the toxins produced, screening techniques, mechanisms contributing to resistance, sources of resistance, and mode of inheritance in some of the resistant sources.

Since the 1970s, breeders have put in a major effort to improve grain mold resistance of sorghum while maintaining high yield and acceptable quality. Success is evidenced by the relatively low susceptibility and improved acceptability of some of the cultivars under test in the All-India Trials of the National Research Centre for Sorghum in the 1990s compared to those of the 1970s. The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India distributed superior F_5 lines from adapted ×

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mold resistant crosses through international nurseries such as SEPON (Sorghum Elite Progeny Observation Nursery) and some of the good grain selections made in these nurseries are recognized as improved cultivars in Asia, Latin America, and Africa. However, these cultivars represent only a limited improvement or success and are considerably susceptible to molds by pathological standards. There has always been a problem of subjective scores and comparable checks for maturity and resistance in the screening and evaluation nurseries. Starting with inoculation of panicles with parasitic fungi and bagging, followed by visual scores after grain maturity under the sprinklers, breeders and their collaborators have used several methods to assess grain mold resistance based on threshed grain mold score, germination, grain mass, grain water absorption, grain density, grain hardness as exhibited in various milling tests, and content of ergosterol, tannins, and phenolic compounds (e.g., flavan-4-ols). Several mechanisms of resistance to molds are known and applied in the breeding programs and almost all of them impose limitations to improve yield and/or grain/food quality. A few examples are discussed here.

Tannins present in the testa and/or pericarp are known to reduce molds and some high tannin and relatively hard-grain types resistant to molds have been identified. But such grains cannot produce acceptable foods or feeds in spite of suitable milling and detoxification. Red-grained types free from molds and without testa (and tannins) have been bred with improved yields but would require suitable milling technologies to make acceptable foods. Their yield superiority and net economic value to the farmer after milling loss compared to commercial white-grained checks is yet to be established.

Among large glume types that have been evaluated, accessions from the *membranaceum* group were susceptible to molds whereas many from the guinea race subgroup *margaretiferum* with involute glumes clasping the developing grains till physiological maturity were resistant to molds as well as head bugs.

The *margaretiferum* types thresh freely but have completely lax panicles with drooping branches and poor yield, while their glumes at maturity appear like a hat on the grain.Their grain mass is low (<2.0 g 100^{-1} grain) while hardness is very high. Correlations between grain size and hardness and grain number are negative while increased hardness and number are positively related and thus progress of selection for high yield with semi-lax panicles is

slow. Moreover, the open glumes at maturity may store water when they are erect on a semi-lax panicle. Another difficult problem in crosses with *margaretiferum* is combining free threshing mechanism and high yield. Experience with such crosses gave very few recombinants with high yield and free threshability. Very high degrees of threshability could as well pose problems of grain losses in the field. The loci controlling the threshing mechanism in the sorghum races need to be investigated in depth.

Among normal white-grained sorghums, such as caudatum, grain hardness appears to be the only mechanism contributing resistance. Breeders should also note that the location of germ in the caudatum types is disadvantageous and adds to the grain mold and sprouting problems. Grains with a deep-seated germ and symmetrical shape are preferred, although some large asymmetrical grains exhibit hardness.

There are several published reports on the genetics of some of the components of grain mold resistance. However, the inheritance patterns and conclusions in these studies possibly hold good only for the specific parents and crosses studied.

It is apparent that grain mold resistance in sorghum is contributed by several mechanisms and adaptations in different accessions. Different resistant sources found in the world collection represent varied combinations of such mechanisms and absolute resistance is not known. Therefore, the conventional breeding techniques are slow to deal with this situation and distinct progress could only be made in the long-term. Under the circumstances one could consider the biotechnological approach and search for suitable genes in alien species as candidates for a multiple gene transformation intended for protection of sorghum grain against the mold complex.

Ethanol Production from Sorghum

Virender Sheorain, Rakesh Banka, and Milind Chavan[1]

Sorghum (*Sorghum bicolor* (L.) Moench) is one of the main coarse cereal crops of India. India is second largest producer of sorghum in the world with production of about 10–11 million t from a total area of 12 million ha. This crop is ideally suited for semi-arid agroclimatic regions of the country and, it gives reasonably good yield with minimal requirement of irrigation and fertilizers (Maiti 1996). On the other hand, cereals such as wheat (*Triticum aestivum* L.) and rice (*Oryza sativa* L.) cannot withstand the harsh semi-arid climates. These crops also require fair amount of water and other inputs such as fertilizers and pesticides. Therefore, sorghum is one of the few cereals, which can be grown in semi-arid regions. However, demand for sorghum for human consumption is decreasing with enhanced socioeconomic status of population in general and easy availability of preferred cereals in sufficient quantities at affordable prices. Since sorghum must be cultivated in the semi-arid regions for fodder to feed the large cattle population of the country, industrial applications for this grain are needed so that sorghum cultivation becomes economically viable for marginal farmers.

Sorghum Cultivation in India

While India is the second largest producer of sorghum in the world, the yield of 840 kg ha^{-1} is the lowest amongst the major sorghum-producing countries in the world. The world average was 1435 kg ha^{-1} in 1994. Although yield of sorghum in India is much lower than the world average, it has been

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consistently increasing during the recent past. Between 1971 and 1995, the yield has increased from 504 kg ha^{-1} to 854 kg ha^{-1}. Maharashtra state has done specially well in terms of increasing the yield from 460 kg ha^{-1} to 910 kg ha^{-1} in the same period. At the same time, however, area under the crop in India has declined from 18 million ha in 1971 to 12 million ha in 1995. This trend of decline in area under sorghum cultivation has been observed in almost all the states. Therefore, increase in yield is primarily due to adoption of improved technology, primarily, F_1 hybrids. The overall area under hybrid sorghum has increased at a healthy rate in the 1990s. During 1989–90, the area under hybrid sorghum was 47% which increased to 60% by 1995–96. Yield of sorghum has steadily increased during the last two decades and is projected to increase up to 4250 kg ha^{-1} by 2010, but the area under cultivation is expected to reduce to 10.2 million ha (Table 1).

Table 1. Effect of high-yielding varieties on production of sorghum in India.

Year	Area (million ha)	Production[1] (million t)	Yield (kg ha^{-1})
1970	18.2	9.2	504
1996	11.4	9.8	854
2010 (Projected)	10.2	43.3	4250

1. About 60% production is in rainy season.

As mentioned earlier, sorghum is grown in the kharif (rainy season) and rabi (postrainy season) but the share of kharif is higher both in terms of area under cultivation and production. The kharif sorghum crop accounts for 55% of the total area under cultivation and 68% of the total production. In terms of yield, the performance of the kharif sorghum is better at 1100 kg ha^{-1} than 750 kg ha^{-1} recorded in the rabi season (Table 2). Produce of kharif season gets damaged to the extent of 20% and fetches a low price of Rs. 2500–4500 t^{-1} compared to Rs. 8000–12000 t^{-1} for rabi crop. One of the reasons for the low farm gate price of kharif sorghum is that there are several layers of traders between farmer and consumer whereas rabi crop has good demand and does not go through so many layers of traders. Rabi crop is almost entirely used for human consumption whereas kharif crop is not very popular for human consumption and largely is used for animal feed, starch, and alcohol industry.

Table 2. Salient features of kharif and rabi sorghum.

Parameters	Kharif (rainy season)	Rabi (postrainy season)
Cultivation	Poor farmers, small holdings, rain areas, no other inputs	Mostly rich farmers, irrigated land, use of fertilizers and pesticides
Variety	Hybrids (CSH 5, CSH 9, etc.)	Local selection, Gavaran, and Maldandi
Yield (kg ha^{-1})	1100	750
Marketing	Traders (almost 6 layers)	Informal (self consumption and sale to relatives, etc.)
Price (Rs. t^{-1})	2500–4500	8000–12000
Utilization	Food, animal feed, starch, and alcohol industry	Food
Damage	20–30%	Negligible

Only 5% of the area under sorghum in India is irrigated. Irrigation facility available to the crop is about 8% in Karnataka and about 6% in Maharashtra. However, it is barely 1% in Madhya Pradesh. Maharashtra, Karnataka, Madhya Pradesh, Andhra Pradesh, and Tamil Nadu are the top five sorghum-producing states and account for about 86% of the area under sorghum cultivation and about 90% of the total sorghum production in the country. Sorghum is a major crop of Maharashtra and a large proportion of cultivable land is under sorghum cultivation (Table 3). The other grains grown in the state are rice, wheat, and pearl millet (*Pennisetum glaucum* (L.) R. Br.). Over 48% of the area under sorghum cultivation in the country is in Maharashtra and more than half of the total production is from this state. Although area under sorghum has declined in the state, production has been increasing due to substantial gains in productivity.

Table 3. Area and production of principal cereal crops in Maharashtra, India in 1996.

Crop	Area ($\times 10^3$ ha)	Production ($\times 10^3$ t)	Yield (kg ha^{-1})
Wheat	799	2329	2915
Pearl millet	1947	1831	940
Sorghum	5692	6840	1096

Sorghum production in Maharashtra has much higher potential than is being realized at present. Yield of the rabi variety CSV 14R was 960 kg ha^{-1} in frontline demonstration plots compared to 740 kg ha^{-1} in the fields (Fig. 1). Kharif crop in the state is dominated by the hybrid CSH 9. The current yield of CSH 9 in farmers' fields is 1120 kg ha^{-1} whereas it was double in demonstration farms (Fig. 2). The state will be greatly benefited if the yield in farmers' fields could be increased to at least the level achieved in the demonstration farms. This will require proper training of farmers and application of good agricultural practices. This puts an onus on the state (Maharashtra) to develop industrial applications of sorghum to benefit the small and marginal farmers.

Industrial Applications of Grains

Grains are a rich and cheap source of starch. Starch finds applications in food, pharmaceutical, textile, and paper industries. Grain starch can be processed for production of dextrose, maltose, high fructose syrups, etc. Grains and grain starch are used for various fermentations such as ethanol, citric acid, lactic acid, erythritol, and sorbitol. In developed countries, large quantities of maize (*Zea mays* L.) are produced and processed for these applications. USA produced 260 million t of maize in 1998 of which around 20% was used for production of starch, high fructose corn syrup, glucose and dextrose, fuel and beverage alcohol, and breakfast cereals. In the same year, India produced only 9.5 million t of maize and had to import to meet its demand for similar applications, which could have been met by 11 million t of domestically produced sorghum. The main reason that sorghum is not being used is the absence of technologies for industrial uses of sorghum. Therefore, we need to develop industrial processes for sorghum. Seagram R&D Centre, Pune, Maharashtra has been working on optimization of process for ethanol fermentation using sorghum. This will be a major boon for the agricultural economy of the semi-arid tropics.

Use of Grains for Alcohol Production

Alcohol is produced by fermentation of any material that contains sugars either in the free form as in case of molasses or in the form of starch as in case of grains. Grain starch is hydrolyzed by a combination of enzymes.

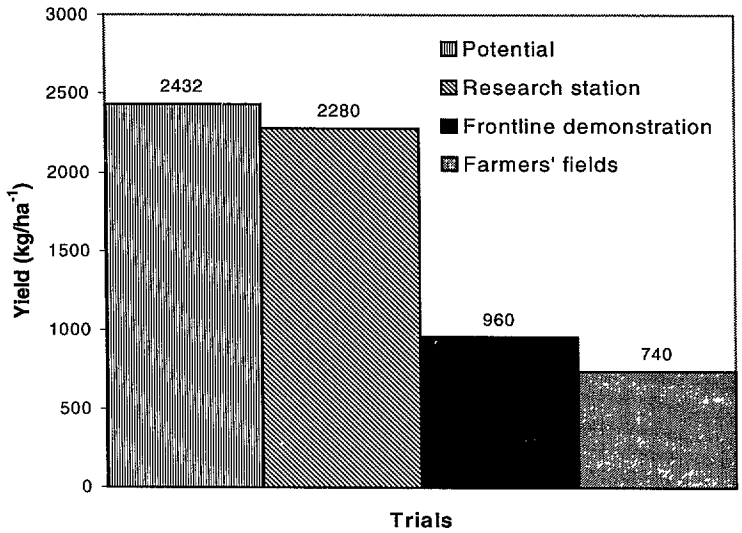

Figure 1. Scope for improvement in yield of rabi sorghum using the cultivar CSV 14R in Maharashtra, India.

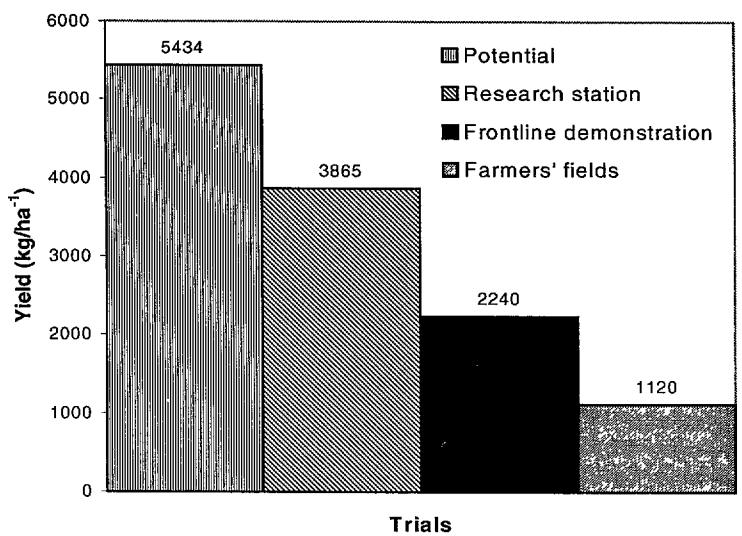

Figure 2. Scope for improvement in kharif sorghum using the hybrid CSH 9 in Maharashtra, India.

Hydrolysate containing free sugar is fermented to produce alcohol. Grain alcohol is much cleaner because of low sulfates and aldehydes. The former gives sulfurous odor and bad taste and later may have deleterious effects on health if consumed in large quantities. Therefore, grain alcohol is used for potable purposes in developed countries. However, in India, molasses alcohol is being used for manufacture of all types of alcoholic beverages despite its poor quality. Only rum is produced from molasses alcohol worldwide. The remaining material is used by chemical industries as raw material for production of approximately 200 organic chemicals and polymers including their downstream products. These products cater to a wide range of industrial and consumer products such as plastics, rubber, synthetic fibers, drugs and pharmaceuticals, pesticides, footwear, perfumes, flavors, dyes, and adhesives. Use of molasses alcohol for potable purposes makes it unavailable for industrial purposes and adversely affects alcohol industries.

Molasses contains sucrose, which is easily fermented by yeast to produce alcohol. However, for alcohol production from grains, the starch present in the grain must be hydrolyzed using costly enzymes and heat, to obtain fermentable sugars. This additional step of starch hydrolysis and low cost of molasses as compared to grains makes alcohol produced from molasses cheaper by 25–30%. However, treatment of byproducts generated by the process of fermentation of molasses into alcohol is difficult leading to pollution of the environment. Typical yield of alcohol from molasses is 225 L t^{-1}. Considering molasses contains 45% sugars, only 70–75% of the sugars get fermented to alcohol and the remaining sugars go into spent wash. Therefore, the effluent has high organic matter content. There are about 150 distilleries in India with installed capacity of 900 million L alcohol. They generate about 10000 million L of spent wash which has Biological Oxygen Demand (BOD) of 40–60 g L^{-1}. Most of this acidic spent wash is being disposed without proper treatment (Gandi et al. 1999). Although BOD and Chemical Oxygen Demand (COD) are reduced in the distilleries with modern waste treatment plants, the effluent remains black and has a foul odor.

On the other hand, in typical grain fermentation the alcohol yield is >360 L t^{-1} of grain, containing 60% starch which corresponds to 85% of conversion of sugars to alcohol. Moreover, the remaining sugars/starch do not go into the effluent because the stillage is dried. The dried residue is called

Distiller's Dried Grain and Solubles (DDGS), which is an excellent ingredient for animal feed. Seagram's state-of-the-art grain distillery at Nashik, Maharashtra is 'zero polluting'.

Process of Alcohol Production Using Grains

Ethanol production from grain involves milling of grain, hydrolysis of starch to release fermentable sugars, followed by inoculation with yeast. Chemically starch is a polymer of glucose (Peterson 1995). Yeast cannot use starch directly for ethanol production. Therefore, grain starch has to be completely broken down to glucose by a combination of two enzymes, viz., amylase and amyloglucosidase, before it is fermented by yeast to produce ethanol. The biochemical reactions and process involved in starch hydrolysis and fermentation are shown in Figures 3 and 4. Alcohol so produced is distilled from fermented broth. The remaining stillage is processed to produce DDGS (Fig. 5).

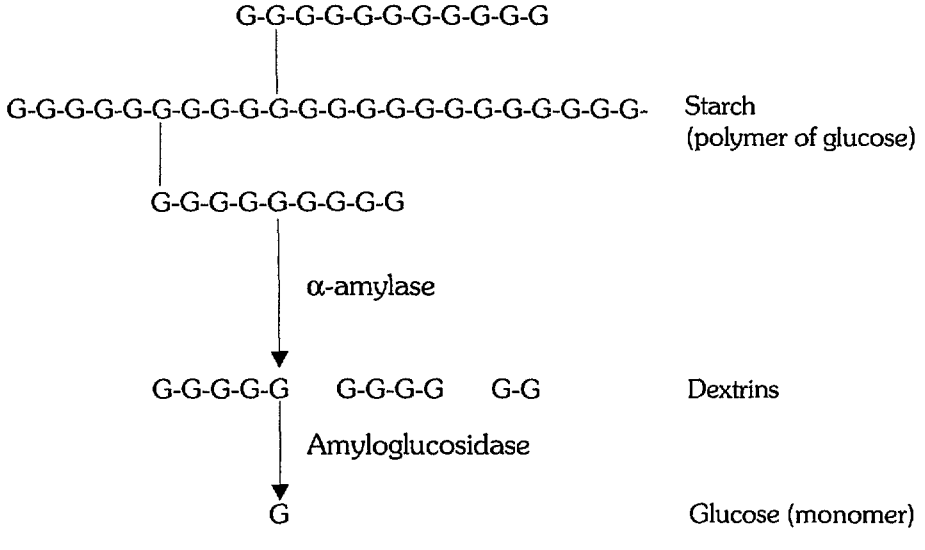

Figure 3. Enzymatic hydrolysis of starch to glucose.

$$(C_6H_{10}O_5)_n + nH_2O \longrightarrow nC_6H_{12}O_6$$

Starch Water Glucose

$$C_6H_{12}O_6 \longrightarrow 2C_2H_5OH + 2CO_2$$

Glucose Ethanol

Figure 4. Chemical equation for conversion of starch to glucose and glucose to ethanol.

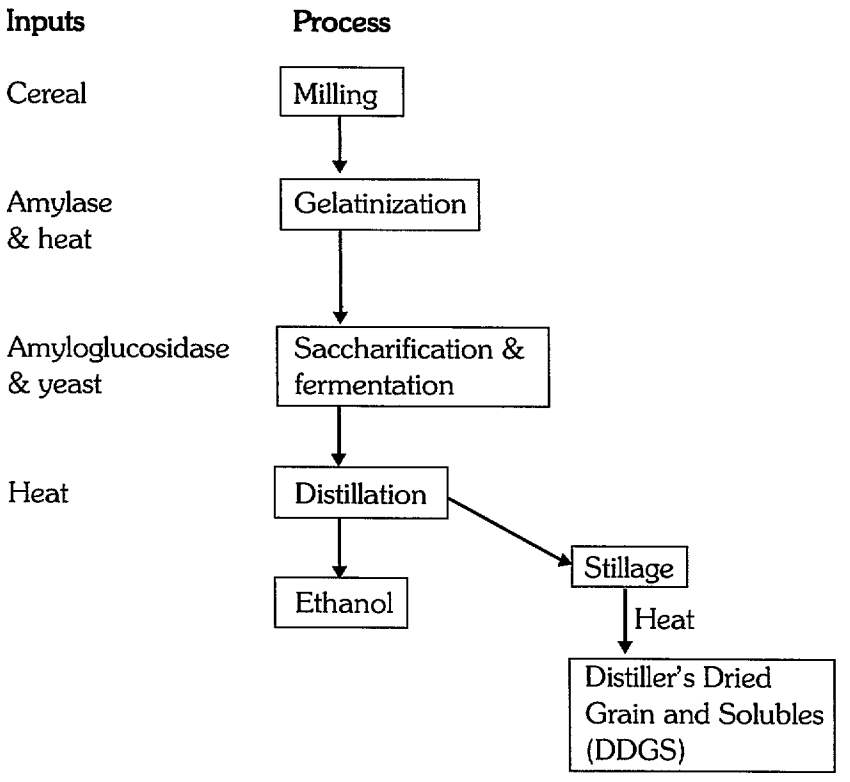

Figure 5. Flow chart of ethanol production from cereal grains.

Sorghum as Raw Material for Ethanol Fermentation

Availability

The major applications of sorghum are animal feed, ethanol production, and production of starch/starch derivatives. Sorghum grain fetches lower price when used for animal feed and the quantity used for starch production is very small. Therefore, use of sorghum for production of alcohol will help the poor farmers because they will get a good price for their produce.

Although maize is the most commonly used grain for alcohol production especially in USA, sorghum has several advantages over maize. Firstly, it has higher starch compared to maize (Table 4) (Lorenz and Kulp 1991). Secondly, sorghum is grown in both kharif and rabi seasons and the kharif crop is mostly F_1 hybrids, which have good fodder and grain yield. Lastly, out of 10–11 million t of sorghum produced annually in India, about 2–3 million t is wasted due to grain blackening following unseasonal rains. This grain is not suitable for human or animal consumption. Hence it is sold at a low price and thus gives low returns to the farmer. Thousands of marginal farmers will be benefited if such grains are used for alcohol production.

Table 4. Comparison of composition of sorghum and maize.

	Content (%)	
Component	Sorghum	Maize
Starch	63–68	60–64
Moisture	9–13	8–11
Proteins	9–11	9–11
Fats and oils	1–1.5	3–5
Crude fiber	1.5–2	1.5–2
Ash	1–2	1–2
Other organics	8–12	7–9

Higher yields

Although there are few distilleries in India already using sorghum, their alcohol yields are low. Sorghum can potentially give good yield of alcohol of about 380 to 390 L absolute alcohol t^{-1} of grain provided the process is optimized.

Value added byproducts

During fermentation grain starch gets utilized and the other components such as cellulose and protein remain unutilized in the stillage. The dried stillage, DDGS, is not only rich in protein and fat, but also rich in vitamins produced by yeast during fermentation. Approximately 240–260 kg DDGS t^{-1} of grain is produced as byproduct. DDGS makes an excellent animal feed. Therefore, all residues are utilized.

Value addition to damaged grain

Total installed capacity for potable alcohol production using both grain and molasses is 110 million L $year^{-1}$. If the average yield of alcohol production is 390 L t^{-1} grain, approximately 0.28 million t of sorghum will be required every year if all the potable alcohol was to be produced from sorghum. This will provide an uptake of 10–15% of available damaged grain annually and help create a market for damaged grain thereby considerably helping sorghum producers.

Contribution and Plans of Seagram for Use of Sorghum for Alcohol Production

Seagram has been producing international quality alcoholic beverages for the Indian market since 1994. All the products are made from grain alcohol. The capacity of grain alcohol manufacture in the country is very low as many companies, although licensed to produce grain alcohol, are not actually producing it. This is primarily due to low yield of alcohol production. Seagram has established the R&D center in Pune with the primary objective of improving the yield of alcohol production using indigenous raw materials such as grain, enzymes, and yeast. The research center is working on some aspects of use of sorghum for alcohol production.

Grain selection and process optimization

Coarse grains, which are not a dominant part of human food chain, were tried and sorghum turned out to be the best candidate. A process for production of alcohol using sorghum has been optimized. This process gives much higher yield than the industry average. Several hybrids of sorghum were tried for

their suitability for fermentation. The selected cultivars will be popularized among the farmers.

Backward linkages with farmers

Farmers will be encouraged to grow grains suitable for fermentation. The current yield of 1120 kg ha^{-1} in farmers' fields is very low compared to 2240 kg ha^{-1} in demonstration farms. Seagram along with seed companies and agricultural research organizations plans to educate the farmers to use good seed and agronomic practices to increase the yield in their farms. Seagram also plans to buy grains directly from farmers or their cooperatives to pass on maximum benefit to them.

Breeding of new varieties/hybrids

In an attempt to further increase the output of this cereal, a collaborative project with the National Research Centre for Sorghum (NRCS), Hyderabad, India and the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru has been initiated to undertake breeding programs.

Upgradation of key byproducts

The upgradation of key byproducts is important to reduce the gap between cost of grain alcohol and molasses alcohol. Seagram has already filed three patent applications for these processes.

Conclusions

Sorghum grain has good potential as a raw material for ethanol production. The process for alcohol production using sorghum, although a little expensive, is still preferable as it is environment-friendly compared to molasses. Use of sorghum for ethanol fermentation will use up to 10–15% of the mold-damaged grain which will help farmers realize a good price for their produce. Ethanol producing companies, research institutions, and the Government can coordinate with farmers to strategically develop value-added utilization of sorghum.

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Sorghum Grain in Poultry Feed

M P Seshaiah[1]

Poultry in India developed significantly during the last three decades. Poultry production has reached up to 150 million layers and 800 million broilers year^{-1} in addition to 1.5 million layer parents and 12.6 million broiler parents. This has placed enormous pressure on feed resources. The annual growth rate of layers is 10% while that of broilers is 15%. Andhra Pradesh is the largest poultry producing state accounting for one-third of the egg and 18% of broiler production in India.

Present requirement of total compounded poultry feed in the country is about 12 million t year^{-1}. Maize (*Zea mays* L.) is the main feed ingredient, which constitutes 30–35% of poultry ration. Other feed ingredients are deoiled (DO) soyabean (*Glycine max* L.) (10%), DO groundnut (*Arachis hypogaea* L.) (10%), and DO sunflower (*Helianthus annuus* L.) (10%); other constituents such as finger millet (*Eleusine coracana* (L.) Gaertn.), pearl millet (*Pennisetum glaucum* (L.) R. Br.), sorghum (*Sorghum bicolor* L.), broken rice (*Oryza sativa* L.), and fish meal constitute rest of the poultry ration.

The non-availability of cost-effective feed ingredients is a major factor inhibiting the growth of poultry industry. The growth rate of foodgrain production is nearly 2.5% year^{-1}. Though the production of wheat (*Triticum aestivum* L.) and rice has increased after the green revolution, the output of pulses and coarse cereals has remained almost stagnant. The yields also are very low.

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Maize gained importance in poultry field because of its high energy and absence of tannin. But its low availability and high cost are dwindling the profits of poultry farming. The production of maize in India is estimated to be about 10 million t year^{-1}. Poultry consumes 30% of it. To feed the anticipated poultry population by 2020, the requirement of maize for poultry will increase to 31 million t from the present level of 3.5 million t. It will be a very difficult task for our agriculturists to increase the maize production from the present level of 10 million t to 100 million t by 2020 to meet the need of poultry at 31 million t while maintaining a 30% share for poultry feed.

In view of the shortage of maize and huge requirement for poultry in the near future, it is necessary to develop alternative feed ingredients such as sorghum. But sorghum is used in limited quantity in poultry feed because its energy content is lower than that of maize and also it contains tannin (Table 1).[2] The price of sorghum is also higher than that of maize. If tannin-free sorghum is made available and is cheaper than maize, it can be used at 30% in poultry feed instead of maize.

Table 1. Energy level and constituents of sorghum and maize grains.

Particulars	Sorghum	Maize
Energy (kcal kg^{-1})	2650.0	3300.0
Proteins (%)	10.0	9.0
Fat (%)	3.0	3.9
Moisture (%)	9.0	10.0
Fiber (%)	4.0	3.0
Calcium (%)	0.2	0.2
Phosphorus (%)	0.3	0.4
Lysine (%)	0.3	0.2
Methionine (%)	0.3	0.2

2. Editors' note: Commercially important Indian sorghums do not contain tannin (see papers Reddy and Rao; and Waniska in this volume).

Sorghum Grain for Poultry Feed

V Subramanian[1] and V C Metta[2]

Sorghum (*Sorghum bicolor* (L.) Moench) is one of the staple cereals in several regions in India. Kharif sorghum occupies an important position in sorghum-growing areas and the grain is mostly used for human consumption. However, in times of heavy rain during grain-filling period, the grains are severely affected with molds making it difficult for human consumption. There is an ever-increasing demand for cereal grain for food and feed in India due to the growing population. Due to changing food habits and socioeconomic status, the consumption of sorghum for human food would reduce. Sorghum grain is as ideal as maize (*Zea mays* L.) for animal feed including poultry. There should be ways and means to utilize low-quality kharif (rainy season) sorghum grain to sustain income for the poor farmer.

Although sorghum has been used in poultry feed to a limited extent, there are apprehensions regarding use of sorghum in poultry feed. Farmers have the notion that sorghum has tannin and has low energy compared to maize grain. The commonly cultivated Indian sorghum grain does not have tannin. The total calorific value and nutritional composition of maize and sorghum are similar. Currently maize is the main cereal component in poultry feed although rice (*Oryza sativa* L.), sorghum, and pearl millet (*Pennisetum glaucum* (L.) R. Br.) are used to a very limited extent. Considering the nutritive value, cost, and availability, sorghum grain is the next alternative to maize in poultry feed. The use of kharif sorghum for poultry feed is the best

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viable option for utilization of sorghum grain, as the kharif grain may not be used in totality for human food. By educating the farmers on utilization of kharif sorghum, the market value of sorghum would increase, and thus benefit the farmers.

Two experiments were conducted on layer and broiler poultry in poultry farmers' holdings, using combinations of maize and sorghum in poultry feed. Experiments were conducted using two sorghum varieties from the market and the variety (ICSV 112) bred by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India. Both layer and broiler birds in two poultry farms were used. Maize was used for comparison. The main objective of conducting experiments in farmers' holdings was to convince the farmers to use sorghum for poultry. The results of the two experiments are discussed.

Materials and Methods

The feeding trial of the first experiment was conducted at a poultry farm in Hayat Nagar area, near Hyderabad, India. For layer experiments, white and yellow (*pacha jonna*) sorghum grains were obtained from the market. Commercial maize was used for comparison. Maize, sorghum, or combination of both was used at 45% of the total diet. Thus whole maize at 45%, sorghum at 45%, or 15% sorghum plus 30% maize was used. A total of 12 groups were formed with 39 layers in each group. The data on quantity of feed consumed and number of eggs were recorded for 21 days.

For the broiler experiment, the cereal component of the feed was 60% and mixed with 40% concentrate. Thus, maize at 60%, or sorghum at 60% (either white or yellow), or 15% sorghum plus 45% maize in feed was used. A total of 480 broiler birds (37 days old) of mixed sex were selected and 12 groups were formed. The feed consumed and growth of birds by weight was recorded for 21 days.

Based on the experience gained in farmers' holdings, a second experiment involving improved variety of maize (Deccan 103) and ICRISAT-developed sorghum (ICSV 112) grain was initiated. The experiment was carried out in a poultry farm in Patancheru. For layer experiments, 244 birds (54 days old) were selected. For layer feed, 50% of it was cereal component. Thus, feeds containing 50% maize (Deccan 103), 50% ICRISAT sorghum (ICSV 112), or 25% maize plus 25% sorghum were prepared. Farmers' feed

purchased commercially was used for comparison. The experiment was conducted for four weeks. The quantity of feed consumed and egg production were recorded for each group of feeds.

For the broiler experiment, 288 male broiler birds (21-day-old) were selected. The cereal component of the feed was 60%. Thus, maize (Deccan 103) at 60% and ICRISAT-bred sorghum (ICSV 112) at 60% or 30% maize plus 30% sorghum were used for making the feed. The balance 40% was made up with commercial concentrate. Farmers' feed was used as a comparison.

Proximate composition of maize (Deccan 103) and sorghum (ICSV 112) grain was determined in ICRISAT's quality laboratory. Taste panel of broiler meat was conducted by providing the meat from different treatments to 16 panelists. Mean taste panel scores were calculated.

Results and Discussion

Sorghum grain is used in poultry feed in small quantities, although maize is the major cereal for poultry feed. Sorghum can replace maize from 50% to 74% (Thakur et al. 1984; Rama Rao et al. 1995). Maize and sorghum grains have nearly similar chemical composition. Experiments with local sorghum obtained from market were used for layer and broiler experiments. The results on egg production using sorghum in feed are given in Table 1. Sorghum grain did not adversely affect egg production when maize grain was replaced by sorghum at 15% level, which is one-third of maize, as well as whole replacement of maize. This holds good for both white and yellow sorghum.

Table 1. Feed consumption and egg production in layer feed containing maize and sorghum grains.

Feed[1]	Feed consumed (g bird^{-1} day^{-1})	Egg production (%)
45% maize	117	95
15% white sorghum + 30% maize	118	94
45% white sorghum	116	94
15% yellow sorghum + 30% maize	118	96
45% yellow sorghum	116	94

1. Feed contained 55% concentrate and 45% cereal component

The performance of broilers using sorghum was also encouraging. Broiler birds receiving 60% maize (whole ratio) and sorghum at 15% or 45% level had the same feed intake. The growth rates of birds reared on the above feeds were also similar. The final bird weight and feed efficiency ratio did not vary among the feed that had varied levels of maize, and white and yellow sorghum (Table 2). The data suggest that sorghum can replace maize grain up to 45% without adverse effect on broilers.

Table 2. Broiler weight gain and final bird weight with maize and sorghum feed.

Feed[1]	Weight gain (g bird^{-1} day^{-1})	Bird weight (kg bird^{-1})	Feed efficiency ratio
60% maize	42.4	1.12	3.30
15% WS + 45% maize	42.3	1.18	3.22
45% WS + 15% maize	41.2	1.15	3.35
15% YS + 45% maize	39.5	1.11	3.87
45% YS + 15% maize	44.4	1.24	3.18

1 Feed contained 40% concentrate and 60% cereal component; WS = white sorghum; YS = yellow sorghum

With the data generated and experience gained on the use of local sorghum, another experiment was conducted with improved maize and sorghum varieties, for layers and broilers. The chemical composition of maize (Deccan 103) and sorghum (ICSV 112) grain were similar for several of the constituents (Table 3). The studies indicated that ICSV 112 is comparable to maize, either whole or mixed proportion up to 50% (Table 4). This conforms to the earlier observation on the use of sorghum for layers and broilers (Thakur et al. 1984; Asha Rajini et al. 1986). Feed consumption, egg production, and broiler weight did not show significant differences among the different feeds used. The organoleptic score for broiler meat was also similar among the treatments.

Table 3. Comparison of constituents in maize and sorghum grains.

Constituent	Maize (Deccan 103)	Sorghum (ICSV 112)
Protein (%)	9.8	8.9
Starch (%)	71.7	72.3
Sugars(%)	1.4	1.2
Fat (%)	5.2	3.7
Crude fiber (%)	1.4	1.2
Ash (%)	1.3	1.7
Gross energy (cal $100g^{-1}$)	414	412

Table 4. Comparison of sorghum and maize for poultry feed.

Feed[1]	Layer feed consumed (g $bird^{-1}$ day^{-1})	Layer egg production (%)	Broiler weight[2] (kg $bird^{-1}$)	Broiler taste panel score for chicken[3]
Maize (Deccan103)	102	83	1.63	3.9
Sorghum (ICSV 112)	99	82	1.53	3.8
ICSV 112 + maize (1:1)	102	84	1.60	3.5
Farmers' feed	97	81	1.62	3.4

1. For layers, feed contains 50% concentrate and 50% cereal component. For broilers, feed contains 40% concentrate and 60% cereal component.
2. Mean of 4-week-old birds.
3. Score: 1 = poor; 5 = excellent (mean of 16 panelists).

The experiment at farmers' holdings gave encouraging results both for layers as well as broilers. Further, the results were similar for white and yellow sorghum grain in comparison with maize. The results for the improved varieties of sorghum and maize grain also showed similar performance of layers and broilers. This suggests that sorghum can economically replace maize effectively thus enhancing its value as poultry feed. This will have great impact on the use of kharif sorghum grain.

Conclusions

Sorghum grain can replace maize in poultry feed to a great extent in view of the similarity in chemical composition of the grain. The results on egg production and broiler weight were similar in the two experiments, when sorghum or maize was fed as a source of energy. Local sorghum grain was as effective as high-yielding sorghum or maize. Thus, sorghum grain has high potential for use in poultry feed.

Acknowledgment

All these experiments were carried out when the senior author was at ICRISAT. The authors thank ICRISAT for the support to the project, and also the poultry units where the experiments were conducted.

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Sorghum in Poultry Feed

V Rama Subba Reddy and A Nageswara Rao[1]

Sorghum (*Sorghum bicolor* (L.) Moench) is the third important cereal crop cultivated in India. Its annual production was 11.08 million t during 1996–97 (Table 1). At the places of production, sorghum is one of the cereal crops for human consumption. The price of good quality sorghum is higher than that of maize (*Zea mays* L.). Only sorghum unfit for human consumption due to discoloration, small grain size, and rancidity is available for poultry consumption. Such sorghum is used in commercial poultry diets up to 100 g kg^{-1} diet. No decrease in production performance was reported with such inclusion level in commercial poultry diets.

Chemical Composition

Sorghum is quite variable in chemical composition (Table 2). The reported variation was: protein 10.0 to 14.1%, ether extracts 1.8 to 5.7%, crude fiber 1.5 to 5.9%, and ash 1.77 to 3.60%. Gowda et al. (1984) reported 0.55% tannins in sorghum while Sharma et al. (1979) observed no tannins. Much variability is seen in the values reported for energy. Gowda et al. (1984) reported an apparent metabolizable energy (AME) of 3257 kcal kg^{-1} for sorghum. Sharma et al. (1979) observed AME of only 2510 kcal kg^{-1} for sorghum, while in maize it was 3231 kcal kg^{-1}. The authors believe that varietal differences, sorghum quality (Table 3), methodology and experimental errors might have played a role for such a variation. Variation in the

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Table 1. Area, production, and productivity of sorghum in India and the state of Andhra Pradesh, 1996–97.

	India			Andhra Pradesh		
Season	Area (million ha)	Production (million t)	Yield (kg ha^{-1})	Area (million ha)	Production (million t)	Yield (kg ha^{-1})
Kharif	5.85	7.00	1196	0.388	0.465	853
Rabi	5.71	4.08	715	0.285	0.343	628
Total	11.56	11.08	958[1]	0.735	0.738	736[1]

1. Average

Source NRCS (1999).

metabolizable energy (ME) values of sorghum was also reported in the literature collected from other countries. The variation in the ME content was attributed to the tannin content. Jacquin (1985) estimated the ME content of sorghum by the equation ME (kcal kg^{-1}) = 3886 – (438 × tannin %). Fuller et al. (1996) reported a variation in tannin content from 0.2% to 2.0% and ME from 2617 to 3516 kcal kg^{-1} for sorghum. Sorghum protein appears to be slightly better in amino acid composition than maize protein.

Even though some physical and chemical treatments could reduce the tannin content, their value and practicability in poultry feeding is limited at present. Some of the treatments are:

- Treatment of grain with 20% solution of sodium hydroxide at 70°C for 8 minutes followed by washing with hot water (60°C).
- Soaking grain in 0.05 M sodium hydroxide and potassium hydroxide or sodium carbonate at 100°C for 20 minutes.
- Spraying ammonia solution (350 g kg^{-1}) at room temperature for 7 days.
- Treatment of sorghum to reduce dry matter to 70% followed by anaerobic incubation at 25°C for 48 h (Gualtieri and Rapaccini 1990).

Growth of Chicks

The information available on the growth of chicks fed with sorghum to replace maize, part per part or isocalorically, indicated that sorghum can be used to replace maize up to the maximum level tested, i.e., 400 g kg^{-1} (Singh and Barsual 1976), 600 g kg^{-1} (Dhamale et al. 1982), 532 g kg^{-1} (Sharma et al.

Table 2. Nutrient composition of sorghum[1] reported in the literature.

Composition[2]	1[3]	2	CSH 4 3	CSH 6 3	NS 4	HLS 4	5	6	7	8
Color	White	-	-	-	-	-	-	-	-	-
Dry matter (%)	89.80	90.20	-	-	-	-	88.40	-	-	89.88
ME (kcal kg^{-1})	2510	2593	-	-	-	-	-	-	3260	-
CP (%)	12.00	10.00	10.50	10.06	10.20	14.10	10.50	10.90	10.30	10.80
CF (%)	-	-	5.30	5.90	-	-	5.60	1.50	-	3.90
EE (%)	2.90	5.70	-	-	-	-	3.50	1.80	3.40	3.40
TA (%)	1.93	2.79	-	-	-	-	3.60	-	-	1.77
NFE (%)	-	-	-	-	-	-	65.30	84.20	-	8.13
Calcium (%)	0.36	0.12	-	-	-	-	0.01	0.08	-	0.06
Phosphorus (%)	0.33	0.69	-	-	-	-	0.20	0.13	-	0.16
Av. CHO (%)	59.80	56.00	-	-	-	-	-	-	63.00	-
Tannins (%)	Nil	-	Nil	Nil	1.81	3.13	-	0.50	Nil	-
Methionine (%)	-	-	0.12	0.13	-	-	-	0.16	-	-
Lysine (%)	-	-	0.20	0.26	-	-	-	0.27	-	-
Av. Lysine (%)	-	-	-	-	1.68	2.96	-	-	-	-

continued

Table 2. *Continued*

Composition[2]	1[3]	2	CSH 4	CSH 6	NS	HLS	5	6	7	8
			3	3	4	4				
Histidine (%)	-	-	0.18	0.21	-	-	-	-	-	-
Arginine (%)	-	-	0.42	0.55	-	-	-	-	-	-
Valine (%)	-	-	0.51	0.71	-	-	-	-	-	-
Threonine (%)	-	-	0.33	0.39	-	-	-	-	-	-
Phenylalanine (%)	-	-	0.54	0.55	-	-	-	-	-	-
Isoleucine (%)	-	-	0.36	0.40	-	-	-	-	-	-
Leucine (%)	-	-	1.21	1.40	-	-	-	-	-	-

1. CSH 4 and CSH 6 are varieties of sorghum; NS= Normal sorghum; HLS= High-lysine sorghum.
2. ME = metabolizable engery; CP = crude protein; CF = crude fiber; EE = ether extract; TA = total ash; NFE = nitrogen free extract; Av. CHO = available carbohydrates; Av. Lysine = available Lysine.
3. Source: 1 = Sharma et al. (1979); 2 = Sinha et al. (1980); 3 = Thakur et al. (1984); 4 = Eshwaraiah et al. (1990); 5 = Reddy and Reddy (1970); 6 = Reddy et al. (1976); 7 = Rama Rao et al. (1995); 8 = Hameed Pasha (1987).

1979), 200–215 g kg^{-1} (Nagra et al. 1987), 375 g kg^{-1} (Asha Rajini et al. 1986), and 580 g kg^{-1} (Rama Rao et al. 1998)(Table 4). Thakur et al. (1984) tested sorghum varieties CSH 5 and CSH 6 at 275 g kg^{-1} and 550 g kg^{-1} in starter rations and 300 g kg^{-1} and 600 g kg^{-1} in finisher rations and recommended sorghum at 275 g kg^{-1} in starter rations and 300 g kg^{-1} in finisher rations to replace 50% of the maize included in the reference diet.

Table 3. Protein content of promising hybrids and varieties of sorghum.

Genotype	Protein (%)	Genotype	Protein (%)
Hybrids		**Varieties**	
CSH 1	7.04	SPV 462	7.84
CSH 5	9.16	SPV 1231	9.32
CSH 6	9.44	SPV1328	10.4
CSH 9	8.31	SPV 1330	11.45
CSH 13	11.76	CSV 11	11.97
CSH 14	8.84	CSV 13	11.13
CSH 16	10.85	CSV 15	8.82
Sweet sorghum		RS 296B	11.39
SPH 821	9.33	Local	10.89
SPH 840	8.87	NSS 104	11.95
SSV 84	12.67		
MLSH 14	7.79		

Source: NRCS (1998).

Table 4. Performance of chicks fed with sorghum grain in diet compared to those fed with maize.

Ingredient	Quantity (g kg^{-1})	Experiment period	Weight gain (g)	Feed conversion ratio	Reference[1]
Maize	400	1–14 weeks	1238[2]	4.73	1
Sorghum	400	1–14 weeks	1304	4.63	
Maize	400	1–14 weeks	1381[3]	3.98	1
Sorghum	400	1–14 weeks	1395	4.02	
Maize	430	2–8 weeks	376	3.60	2
Sorghum	215	2–8 weeks	374	3.90	
Maize	400	9–20 weeks	568	9.62	
Sorghum	200	9–20 weeks	572	10.11	2
Maize	410	1–42 days	530	2.35	3
Sorghum	532	1–42 days	509	2.48	
Maize	600	1–56 days	1050	2.68	4
Sorghum	600	1–56 days	1021	2.58	
Maize	275	1–56 days	1371	2.63	5
Sorghum (CSH 5)	275	1–56 days	1273	2.83	
Sorghum (CSH 6)	275	1–56 days	1378	3.05	
Sorghum (CSH 5)	550	1–56 days	855	3.10	
Sorghum (CSH 6)	550	1–56 days	1296	2.87	
Maize	375	1–56 days	1185	2.53	6
Sorghum	375	1–56 days	1277	2.47	
Maize	580	8–49 days	1486	2.88	7
Sorghum	580	8–49 days	1383	2.94	

1 1 = Singh and Barsual (1976); 2 = Nagra et al. (1987); 3 = Sharma et al. (1979); 4 = Dhamale et al. (1982); 5 = Thakur et al. (1984); 6 = Asha Rajini et al. (1986); 7 = Rama Rao et al. (1998).
2. For White Leghorn breed.
3. For Rhode Island Red breed

Layer Performance

Egg production remained unaffected with sorghum in diet at 320 g kg^{-1} (Reddy and Reddy 1970), 550 g kg^{-1} (Narkhede et al. 1981), 400 g kg^{-1} (Gowda et al. 1984), 500 g kg^{-1} (Thakur et al. 1985), and 743 g kg^{-1} (Rama Rao et al. 1995). In these experiments, sorghum was used at a single level to replace maize part per part. Feed conversion ratio (FCR) was reported to be unaffected by inclusion of sorghum in the diet for layers (Table 5). Only in the experiments of Nagra et al. (1987) and Sharma et al. (1979), FCR was lower on sorghum diet than on maize diet. Thakur et al. (1985) reported reduced egg size with sorghum cultivars CSH 6 and HC 136 at inclusion level of 500 g kg^{-1} while others reported no differences (Reddy and Reddy 1970; Narkhede et al. 1981; Gowda et al. 1984; Rama Rao et al. 1995). Yolk color was reduced with inclusion of sorghum in diet (Gowda et al. 1984; Thakur et al. 1985). Shell thickness also was reported to have been reduced with sorghum in diet (Narkhede et al. 1981).

Table 5. Performance of layers fed with sorghum grain in diet.

Ingredient	Quantity (g kg^{-1})	Experiment period (days)	Egg production (%)	Feed $dozen^{-1}$ eggs (kg)	Egg mass (g)	Reference[1]
Maize	320	196	71.0	6.38	58.0	1
Sorghum	320	196	67.5	6.77	55.0	
Maize	550	100	75.8	2.03	52.2	2
Sorghum (CSH 5)	550	100	74.8	2.23	53.2	
Maize	400	75	87.5	1.49	44.2	3
Sorghum	400	75	84.9	1.54	45.0	
Maize	500	150	58.5	2.01	51.1	4
Sorghum (CSH 6)	500	150	58.3	2.06	45.8	
Sorghum (HC 136)	500	150	50.5	2.22	46.5	
Maize	720	224	68.3	2.23	54.1	5
Sorghum	743	224	67.9	2.21	56.0	

1. 1 = Reddy and Reddy (1970); 2 = Narkhede et al. (1981); 3 = Gowda et al. (1984); 4 = Thakur et al. (1985); 5 = Rama Rao et al. (1995).

Conclusions

Sorghum is the third important cereal crop in India. Sorghum is variable in nutrient composition. Varietal difference, methodology, experimental errors, and tannins present in some sorghum varieties might be responsible for such variation. The experiments on chicks and layers indicated that good quality sorghum could be used at fairly high levels even as the principal energy source in chick and layer diets. Discolored and small grain sorghum is only available for poultry feeding. Such sorghum may be used up to 100 g kg^{-1} in commercial poultry feeds.

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Sorghum Grain Mold: The Scope of Institutional Innovations to Support Sorghum-based Rural Livelihoods

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This paper reviews the scope of institutional innovations as means of tackling sorghum (*Sorghum bicolor* (L.) Moench) grain mold as an approach to support the livelihoods of the rural poor. By institutional innovations we refer to changes in the rules, policies and norms of various organizational structures and the way these can be manipulated or leveraged to produce desired outcomes[i]—in this case the alleviation of the financial and food security losses brought about by grain mold in rainy season sorghum. The grain mold problem is essentially one of pre-harvest fungal infection brought about by untimely rains towards the end of the rainy season. Hybrids for rainy season sorghum production are particularly susceptible to this problem. This is partly a result of their shorter duration, but also for reasons of plant habit and grain structure. The result is that grain becomes discolored and mold intensity may also increase during storage. Not only does the grain become unappealing for consumption, but it may also be dangerous to human health due to

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mycotoxin contamination (although there is conflicting evidence for this (Hodges et al. 2000; Bhat et al. 2000). Furthermore, blackening of grain causes price discounting in the market. Biological research alone has not yet been able to satisfactorily resolve this problem.

However, earlier studies on sorghum markets (Marsland and Parthasarathy Rao 1999, Klieh et al. 2000) indicated that institutional innovations in the marketing systems could potentially offer important opportunities for linking farmers more directly with new industrial users of the crop. The suggestion being that new marketing institutions were likely to have important backward linkages with respect to access and supply of quality enhancing technology to tackle issues such as grain mold. It was anticipated that some of the principles of New Institutional Economics (North 1990) with its focus on contractual arrangements, and the way these can be used to reduce search, quality enforcement and other transaction cost, might provide fresh insights into the way that producers could be linked more closely with the emerging new markets for sorghum and the new quality parameters that this implies. Grain mold in this context is particularly important, as although new industrial users such as the grain alcohol industry can use molded grain, this type of grain damage is the key source of concern for the poultry industry due to fears of mycotoxin contamination.

The earlier work of Hall (2000) on the sorghum utilization sector in India identified a further institutional issue. It was suggested that the expansion of sorghum into new industrial uses had occurred independently of the large body of public sector research on, for instance, sorghum processing technology or use as animal feed. The suggestion was also that further research was required to understand the institutional reasons why this was so and to help devise institutional innovations to overcome this problem. This paper is in part a response to that need.[ii]

In fact, as a focus of study institutions have emerged fairly recently as a variable of developmental significance and were until now largely ignored in both technology development strategies and economic policy. This growing policy focus relates to a number of related factors.

1. The increasing recognition of the limited ability of neo-classical economics to account for institutional issues in the analysis of the process of technical and economic change.

2. The emergence of institutional constraints as a major restriction in client targeting and uptake of new technology, and the associated need to devise a more inclusive conceptual framework to understand technical change as a process.
3. The increasing importance of the public-private sector interface. Particularly, the way this has led to the reexamination of the traditional distinction between public and private goods and the implications of this for the validity of the widespread institutional segregation of public sector research and private sector enterprise.

In order to explore the potential of institutional innovations in the context of grain mold, this paper begins by reviewing theoretical concepts concerning institutions and their development significance. Then, after establishing the importance of sorghum in Indian rural livelihoods, the paper goes on to review current patterns, practices and perceptions of a number of key public and private sector organizations in the context of grain mold and sorghum utilization. The paper concludes with recommendations concerning practical ways that institutional innovations can be brought to bear on the grain mold problem.

The Role of Institutions in Technical and Economic Change

Institutions as a focus of analysis have only recently started to gain legitimacy. In the past, much of technology and economic policy in the context of third world development has relied for its conceptual underpinning on neo-classical economics traditions. However, recent debate has started to identify the institution as the key variable (Clark 1991, 1995, in press). The literature on these issues examines the concept of institutions in two contexts. Firstly, in the context of the market as an institution, and the way in which new market institutions (for example, contract growing) can be used to improve the exchange of commodities and services. Secondly, in the context of science and technology, where the institution refers to the set of rules and norms that govern the creation of new knowledge in (often public sector) specialized research organizations and the transfer and application of this knowledge in economic production (either by private organizations or individuals, for

example, farmers). The following reviews the key points of these current debates in some detail.

New Institutional Economics[iii]

The term New Institutional Economics (NIE) refers to a loosely related set of concepts concerned with institutions and their role in economic performance and change. Specifically, it is an attempt to fill gaps in the neo-classical economic model of the market economy. In the neo-classical economic theory, exchange, and therefore the rules of the market institution, is regulated by the price mechanism, which itself responds to forces of supply and demand. The relationship between supply and demand is such that they act on each other to come to equilibrium. In an efficient market, supply should always equal demand.

For the neo-classical economic model to provide an explanation of economic systems, it relies upon a number of assumptions that it uses to abstract away from reality. For example, it assumes that perfect competition exists in markets. Of course, in reality markets are rarely so. NIE recognizes this, and by way of evidence points to marketing institutions that have been developed to cope with reality.

In fact, NIE shares two fundamental assumptions with neo-classical economics. Firstly, the assumption that goods are not in infinite supply, and secondly, that individuals are motivated to maximize "utility" or satisfaction. However, NIE rejects other important assumptions, including: that the quality of goods can accurately be predicted by buyers; that each party has perfect information about exchange; that individuals make rational decisions about buying and selling; and that the price mechanism is sufficient to regulate supply and demand. NIE is actually quite a broad set of theories. In the discussion presented below we focus on the set of theories that deal with transaction costs. This stems from the work of North (1990)[iv]—"a transaction cost theory of exchange".

Our current study of sorghum grain mold provides a useful demonstration of the nature of transaction costs and the way in which these costs do not fit the neo-classical economic model. Grain quality varies due to production and storage conditions both from year to year and between

different farmers. In addition, damaged grain may not readily be detected. It is therefore not valid to assume that a buyer can accurately predict the quality of goods for sale. Since the levels of information between buyer and seller are asymmetrical, it provides the seller the opportunity to cheat the buyer—the so-called moral hazard. To avoid being cheated the buyer has to "invest" in a number of transaction costs. For example, screening the seller to see if he is trustworthy; enforcing the contract if the produce is subsequently found to be sub-standard; and so forth. These costs render the market an inefficient form of exchange unless there are formal and informal rules (for example, contracts and customs) to prevent cheating. It is these rules that NIE calls institutions.

It is the moral hazard problem along with the other transaction costs buyers have to incur in acquiring information, negotiating and enforcing contracts, that leads (among other things) to market failure—i.e., a situation when the market is unable to act as a basis for the exchange of commodities or services. NIE is basically a theory institutions or "arrangements" that explains the existence and nature of such institutions in terms of their ability to compensate for missing markets, to lower transaction costs, and to reduce the opportunities for cheating and free-riding. North's (1990) important contribution to NIE was that he recognized that economic, social, and political institutions are created to reduce transaction costs. He suggests that economies that have developed fastest have been those that have built institutional structures to reduce transaction costs. However, this position has not necessarily found mainstream acceptance.

Examples of these institutions are diverse and include: printing the prices on goods to reduce the cost of negotiating prices; developing standards for weights and measures; creating unified laws and associated sanctions that allow the terms of a contract to be enforced; the development of insurance to spread risk; the development of accounting procedures to monitor the activities of agents; and so forth.

These institutions can be developed between private parties. For example, contract grower schemes. In such a case, a fruit exporter may enter a contract with producers to buy all fruit within certain quality limits. This reduces the search costs of the exporter—he knows where to find his export quality fruit. Equally, it reduces the risk to producers of investing in quality enhancing measures. Since the producer knows that he has an assured market at a price premium, he has a greater chance of recouping the cost of expensive production inputs.

Institutions can also be legislated for. For example, food safety laws reduce the costs of the consumer in establishing whether a product is safe to eat. It also allows a market to develop in processed foods more easily. Strictly enforced quality standards for German beer go back to the 15th century!

There is increasing use of the NIE approach in the developing country context (for example, Dorward and Kydd 1996, 1998). However, the most successful examples appear to have been those instances where it has been applied to creating micro-level institutional innovations in marketing systems. For example, the development of contracts to ensure timely supply of commodities, or systems that help buyers identify the location of surplus in production systems where production is dispersed and highly variable. It would appear that the transaction costs principles that NIE builds on could form the basis for the design of action research approaches where experimentation with new market institutions is used to try and resolve the market failure that commodity prices alone struggles to explain.

However, as far as we are aware the NIE approach has not yet been developed to the extent that it can be applied to the institutions governing the creation, supply, demand and application of new knowledge, although there may be scope to do this. We shall now go on to examine the sets of debate that examine the technology and innovation issue more directly.

Institutional arrangements for R&D

The origins of institutional arrangements for science and technology in almost all countries reflect the needs of earlier and arguably simpler economic systems. They reflect the assumption that investments in research by the public sector were necessary to produce the new technology that private organizations needed for economic production. This was based on the belief that only by producing ever more (economically) efficient technology would rates of economic growth be maintained and that economic growth would be the only way of raising living standards. This implied two further important assumptions. Firstly, that the relationship between investments in research and changes in economic production was linear (i.e., the investments in research automatically leads to, and is directly correlated with economic production). As a result, institutional arrangements needed to be designed with a hierarchy of function and structure to match this linear conceptualization of the innovation process. The second related assumption,

was the apparent need to create a very strong division of labor between knowledge search (mainly the public domain) and knowledge application (mainly the private domain). As a result the hierarchical relationship between the two sectors tended to maintain a strong institutional distinction.

National agricultural research systems (NARS) are often characterized by this type of institutional arrangement. In these systems, a clear hierarchy of functions can be seen, namely: basic research creates new knowledge that is passed down to adaptive research organizations who in turn pass down technology packages to (usually separate) extension organizations for transfer to farmers—the private sector actor in this example. The model is often referred to a pipeline model, where investment in basic research mysteriously emerges at the other end for the pipeline transformed into new technology for the farmer. The difficulties that this type of institutional model has in creating a client focus in agricultural research has been well documented for more than 20 years (for example, Biggs and Clay 1981; Biggs, 1990; Hall and Clark, 1995; Hall et al. 2000a).

The Indian NARS is a good example of this model and it has remained remarkably resistant to change over the years (Hall et al. 1998; Hall et al. 2000b, in press a). However, there is also evidence that the private corporate sector is starting to emerge in India as an important source of research capability. However, the way that public sector parts of the national research systems will accommodate this is less clear. How do these developments relate to the principles of public and private goods that underpin current institutional arrangements?

Public and private goods in agricultural research

The respective roles of public and private sectors in agricultural research are neatly defined by economic theory. The key principle concerns the relative degree to which research outputs (knowledge, technology, and information) are of a public or private goods nature. The concepts of rivalry (in the supply of knowledge) and excludability (the capture of propriety rights to knowledge) are used to predict which areas, through market failure, will need to remain in the public domain (see Pray and Umali-Denenger 1998 for detailed discussion). So, for example, recommendations on fertilizer rates are non-rival (one person's use does not reduce its supply to others) and non-excludable (the benefits cannot easily be restricted to the person who initially pays for

recommendations) and therefore resemble a public good. On the other hand, a bag of a new type of fertilizer has precisely the opposite properties and therefore resembles a private good.

The argument here is that the private sector is unlikely to invest in agronomy research, but is quite likely to develop new types of more effective fertilizer that it can then produce and sell. Left to the market, therefore, no research would be undertaken on agronomy due to "market failure". The fact that even conservative estimates show high rates of return to investments in public sector agricultural research has been cited by economists as evidence of persistent under-investment by the private sector in research (Thirtle and Echeverria 1994). The implication is that there is indeed market failure in terms of investment in research and that some sort of collective action is required (usually by public sector R&D bodies) to fill this gap.

However, it is becoming increasingly apparent that these types of public/private distinction are changing and indeed may never have been as mutually exclusive as neo-classical economic theory would have us believe. There is growing recognition that the boundary between public and private sector research is variable between research sub-sectors; may allow significant overlap; is dependent on the general degree of institutional development in a specific country (particularly with respect to markets); and is highly dynamic and likely to change over time (Thirtle and Echeverria 1994).

This sort of thinking is not confined only to developing country agriculture. Around the world and in diverse research sectors, public and private sector roles are starting to change. This change concerns two related sets of factors. First, the fundamental ideological shifts that have taken place concerning the role of the State in society. This has had implications for the most appropriate role of the State in the funding and execution of research. Secondly, recent conceptual developments concerned with understanding the way that innovation, technical change and economic growth actually take place. Coupled to this is the realization that public and private sector roles in this process need to alter quite radically from the current position (Clark, in press).

National systems of innovation

As countries around the world have started to come to terms with the need for institutional reform in the science technology sector, concepts such as the

national systems of innovation (NSI) (Freeman 1987; Lundvall 1992) have started to become important as a policy tool. NSI is a concept that helps understand and analyze the ability of countries to produce innovations of economic importance. It supplements analysis of innovation in the neo-classical economics tradition where the focus is on inputs (such as expenditures on R&D) and outputs (such as patents). While these indicators remain important as sources of information about content and direction of technological endeavor, their ability to measure the "innovativeness" of an economy is small (OECD 1997). Critically, the indicators used fail to account for all the inputs and outputs in the process; the approach takes little account of the way in which the process works; and most fundamentally it has difficulty dealing with the dynamic, complex nature of the process (Clark 1991, 1995).[v] The most significant contribution of NSI is that it identifies the institution as the key variable in the innovation process, and therefore directly relates institutional arrangements to innovation performance.[vi]

A NSI is defined in slightly different ways (Freeman 1987; Lundvall 1992; Nelson 1993; Patel and Pavitt 1994; Metcalfe 1995). Broadly speaking, it can be described as the system or network of private and public sector institutions whose interactions produce, diffuse and use economically useful knowledge. The component parts of the systems and their interactions are determined by culturally defined norms, historically determined institutional developments, and national priorities and are defined by geographic borders and national policies. It is not necessarily suggested that national governments have explicitly developed innovation systems in this way, although some clearly have. However, in economies where such interactive systems have evolved successfully, the innovative performance of these economies has been strong and this has been reflected in rapid rates of economic growth (Freeman 1987, 1991). Analysis stresses the importance of these institutions as nodes in a system where their interaction and interactive relationship along with other contextual factors is key to these knowledge flows. Attempts to understand the structure and dynamics of such systems are the core of modern thinking about the innovation process (Clark, in press; Edquist 1997; OECD 1997).[vii]

Rather than presenting a blueprint for institutional reform, NSI is concerned with mapping and evaluating channels for knowledge flows, identifying bottlenecks and suggesting appropriate remedial action. In this sense, NSI presents a set of analytical principles for understanding the

innovation process in a national context, and identifying leverage points for enhancing innovative performance. The overriding concern of these principles is that of improving the connectivity between different institutional nodes, particularly between those concerned with research and those concerned with enterprise or economic production. In this sense, the debate of the new roles of the public and private sector organization in the context of science and technology provision over the last decade or so has not just been concerned with the growing costs of public research and the need to shed the burden—although this undoubtedly is important. But it has also been a response to the growing realization that the creativity of innovation owes its existence to a large degree to the synergy that comes from the blurring of traditional distinction between scientific research and enterprise.

In response to this type of thinking, a range of new and often experimental institutional forms have emerged to improve the innovativeness of national science and technology systems. Examples from Britain include the increased use of science directorates, such as the UK Biotechnology Directorate, designed to focus the combined strengths of industry, research, university and government on key generic technologies. Other examples are interdisciplinary research programs and schemes like the UK "teaching company scheme" that introduce joint academic/industry supervision of PhD students that are also employees of companies (see Senker et al. 1993, for detailed discussion).

Institutional innovations

Slowly these types of concepts are starting to creep into the NARS of developing countries. An enlightened view of future agricultural research systems recognizes a diverse array of potential participants in research funding and execution and a large number of points of intersection between funding sources and research executing organizations (Echeverria 1998). Institutional innovations are indeed starting to emerge (see Box 1 for examples). These are presenting new ways to focus resources and expertise on problems that have resisted the attention of either public or private sectors alone. The important point for our discussion of sorghum grain mold is that forming productive linkages between public and private sectors offers the opportunity to provide an alternative and potentially more effective institutional focus to efforts to address the mold problem. The next sections

present the institutional context of grain mold and the opportunities and limitations that this context contains.

Box 1. Institutional innovations in agricultural technology R&D and promotion.

Bold-seeded sorghum variety development. In India, a consortium of private sector seed companies have got together to fund the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) to develop genotypes of bold-grained rainy season sorghum. The private companies will conduct the adaptive testing and hybrid development work whereas ICRSAT will draw on its strengths in basic research and its large collection of genetic resources. There was initially some resistance from ICRISAT to enter into such an agreement. This was not from the scientist involved. Rather it was the senior administrators who were uncomfortable with an arrangement that was at the outer margins of the traditional mandate of an international public good research institute.

Biopesticide production. The International Institute for Tropical Agriculture (IITA) has entered into partnership with private commercial organizations to manufacture and distribute a biopesticide for the control of locusts in sub-Saharan African. IITA had initially hoped that enough of the biopesticide could be manufactured by its traditional partners—the African NARS. However, the production process demanded specialist facilities and quality control procedures that were best achieved by identifying commercial enterprise partners and transferring the production know-how to them (IITA 2000).

New institutional roles for growers' organizations. Recent UNCTAD (1999) review demonstrated the way that private organizations can start to play a much more strategic role in planning and coordinating public sector R&D resources. The role of Growers' Associations can now often be seen as a key-focusing device for R&D. In Colombia, for example, it was found that in crops as different as cut flowers, coffee and sugarcane, such associations (called *gremios*) now play a major role in both providing and managing R&D, extension, training, and the provision of linked services to farmers. And in so doing, they allow the State to take on more strategic tasks for the agricultural sector as a whole.

Sorghum-based Rural Livelihoods In India

All India figure for sorghum production indicates that 10.5 million t grain are produced annually (FAO 1996). It is the third most important cereal after wheat and rice in terms of total production. Its production area is 11.7 million ha out of a total of 186.4 million ha (Government of India 1997). However, this represents only 6.7% of the cropped area and sorghum represents only 6% of national cereal consumption, and this is falling. These figures alone underestimate the importance of the crop in specific regions of India (Dayakar et al. 1996). Nevertheless, significant changes have taken place in sorghum production over the last decade or so. Kelley and Parthasarathy Rao (1993) suggested that an underlying cause of changing sorghum production patterns relates to its utilization and is reflected in the strength (or lack of) of consumer demand in both the farm household and the market. This issue may be allied to crop competitiveness (in a profitability sense) and the cash cropping opportunities that have become available to farmers.

Historically, the majority of sorghum consumption has been at the farm household level, although some was marketed. It was also an important food grain staple for both the rural non-farm and urban poor. Data from successive rounds of the National Sample Survey (NSS) clearly shows that on average per capita consumption of sorghum has dropped steadily for several decades (based on analysis of national level data). This has happened in both rural and urban areas and amongst the poor as well as in high-income groups (Marsland 1997).

The decline in sorghum production and consumption in recent years has raised a number of questions about its continued relevance to the poor. Time series district agricultural production figures and state level NSS data illustrate the broad patterns of production and consumption change. However, they tend to be at a level of aggregation and abstraction from the social context, that they give very little feel of the processes that underpin these changes. Nor do they really substantiate the relative importance of sorghum in the more broadly defined livelihoods of the rural population. The findings of a recent exploration of these issues (Hall 2000) highlighted the following points.

The identity of the rural poor

In sorghum production systems, it is no longer valid to consider the rural poorest as farmers. At best they are marginal farmers, at worst and most frequently they are landless and reliant on agricultural employment. Numerically the poor are the dominant group in rural society. Their greatest concern continues to be access to food. If the poor have land, sorghum production contributes to this, but never more than partially. More usually the main contribution of agricultural production to their livelihoods is through the creation of employment opportunities. The best that agricultural development can offer them is cheap food, but more important, the means to access it.

Sorghum-based livelihoods

Sorghum, and certainly rainy season sorghum, does provide the poor with cheap food. The converse is often true for the higher priced postrainy season sorghum. However, it is farming systems—including sorghum production—that is the greatest means of accessing food via the employment opportunities that it creates. The most important and rather intangible—and therefore underplayed—importance of sorghum is as an input into these farming systems. The relative importance of sorghum as a source of fodder for livestock is of crucial importance. It is the integrated nature of these crop livestock systems that underpins the sustainability of the farming systems in the semi-arid tropics of India. Sorghum, therefore, underpins the system that is most important in allowing the poor access to employment and, therefore, food. This systemic importance, regardless of any other concerns, is a fundamental reason for sustaining the presence of sorghum in the cropping patterns of the Indian semi-arid tropics.

Future prospects for sorghum-based livelihoods

While the systemic importance of sorghum has been highlighted, what are the likely mechanisms for maintaining the position of the crop in farming systems? Agricultural and trade policy reforms will at worst be neutral and at best marginally supportive of the crop. Increasing rural labor shortages will also tend to favor the crop due to its relatively low labor demands. The competitive position of the crop—profitability—will need continued support

from demand for the commodity at a level at least equal to that at present. Rural household demand for grain will at best remain stable and at worst show a steady decline. The same is also true for urban demand for food, with declines likely to be if anything more substantial. However, even though cropping patterns have witnessed the decline of sorghum, it seems that the fodder demands of mixed crop livestock systems are such that the crop has reached a floor level of production. Its relevance in the cash based rural economies would therefore be enhanced through the development of stronger markets.

The Evolving Utilization Scenario of Sorghum

Sorghum has been traditionally viewed as a human food in India, although its use as an industrial raw material is widespread in other regions of the world, particularly as an animal feed. However, a recent study by Klieh et al. (2000) clearly demonstrated that sorghum utilization is evolving and that industrial utilization is starting to assume some importance. The key industrial sectors using sorghum are animal feed, alcohol, and to a lesser extent starch. Fieldwork carried out for the current study supported the findings of this earlier work, suggesting that, if anything, demand for non-food uses is strengthening. However quantification of the level of demand is beyond the scope of this study and even at best it tends to be difficult to measure because of the high level of clandestine use of the commodity. It is useful to briefly present the findings of Klieh et al. (2000) as this provides a conservative estimate of industrial demand and makes growth projections based on information collected from the key players in the sector in 1998.

Table 1 summarizes the current demand for sorghum and presents projections for the year 2010. These estimates are based on data collected from industrial users and their apex organizations. Projections are based on expected levels of growth in demand for the products of these sectors at current inclusion rates. These figures suggest that in recent years industrial utilization of sorghum has accounted for between 6.5% and 9.2% of total sorghum production (currently approximately 11 million t annually). However, bearing in mind the fact that only rainy season grain is used, this represents 10.2–14.5% of rainy season grain production. With relatively stagnant levels of sorghum production predicted, the projected demand of 2–4 million t by the year 2010 suggests that industrial consumption could then

Table 1. Summary of industrial demand (× 10^3 t) for sorghum in India.

Industry	1998[1]	2010[2]
Poultry feed		
Broilers	86–129	570–1150
Layers	312–468	1100–1830
Other	20–30	156–234
Dairy feed	160–240	290–570
Alcohol	90–100	200–500
Starch	50	30–80
Brewing	0	?
Food industry	0	0
Exports	0	?
Total	718–1017	2346–4364

1 These figures reflect average utilization of sorghum during the 1990s, based on past inclusion rates and current requirements of raw material, rather than specific data for 1998 itself. The poultry and starch industries use sorghum only when maize is expensive or not readily available.

2. Projection.

Source: Klieh et al. (2000).

account for 20–39% of total sorghum production. In terms of rainy season production alone, these figures suggest that industrial utilization could account for 32–61 % of production (Klieh et al. 2000).

The implications of a trend of increasing agro-industrial utilization are two-fold. Firstly, it needs to be recognized that sorghum (particularly rainy season production) is making the transition from a subsistence food crop to a traded agro-industrial input (although it is already a traded food commodity and will continue to act as a subsistence food crop). Secondly, the private sector is becoming an increasingly important stakeholder in the sorghum sector.

However, Klieh et al. (2000) also point out that there are a number of generic factors that need to be considered in approaches to increasing utilization. Firstly, prospects for increased utilization are linked to the demand for the relevant industrial products; for example, eggs and other animal products. Secondly, sorghum is—or is perceived to be—inferior to maize (*Zea mays* L.), and must be able to compete in terms of price[viii]. Thirdly, perceptions of inferior quality almost certainly lead to clandestine use, making estimates of utilization difficult. Fourthly, (low) inclusion rates of sorghum are

conditioned by a number of persistent misconceptions of the properties and composition of the grain. This has reinforced the impression of sorghum as an inferior raw material. Although some of the utilization constraints presenting are technical, they are also institutional in nature. Most critical is the gulf that exists between public sector sources of knowledge and information on sorghum utilization and (potential) industrial users in the private sector (Hall 2000).

It is also important to point out that, as Marsland and Parthasarthy Rao (1999) indicated in a recent sorghum marketing study, industrial utilization of sorghum draws grain from a very specific sector of the market, namely low quality rainy season grain, often damaged by grain mold and consequently of a low price. The same study also emphasized the following:

- It is clear that sorghum is far from being a crop that is produced, marketed and consumed within the same district, and it certainly cannot be described as merely a subsistence crop. This is particularly the case for rainy season hybrid grain.
- Typically, in the major sorghum areas, farmers and village traders will travel up to 60 km to sell their sorghum, although exceptionally they may travel up to 100 km. Wholesalers in these same markets may buy sorghum from other markets in other states—perhaps 500 to 700 kilometers away—at times of the year when there are local shortages.
- With increased industrial utilization of sorghum and the consequent segmentation of the market, current market institutions may be poorly suited to meeting the consistency of supply and quality that different industries require.

Almost paradoxically the incidence of grain mold, and its effect of lowering grain prices, has been one of the key reasons for its use as an industrial raw material—damaged grain is simply cheaper than competing carbohydrate sources such as maize! Marsland and Parthasarathy Rao (1999) point out that once the price of sorghum goes below a certain level in relation to the price of maize, there will be some degree of substitution of sorghum for maize[ix]. Price is related to quantity but also quality and a major factor here is the degree of grain blackening. Partly blackened grain is acceptable to some sections of the poultry industry and also to the cattle feed industry, but fully blackened grain appears to be acceptable only to the alcohol industry (Marsland and Parthasarathy Rao 1999).

Grain Mold in the Context of Industrial Utilization

The poultry and grain alcohol and brewing industry were revisited in May 2000. The key objective here was to further establish the extent of industrial utilization of sorghum; the implications of grain mold for the particular industry, and to examine the feasibility of both technical and institutional innovations that would simultaneously negate the grain mold problem and help strengthen the industrial demand for the crop. The key findings are summarized below.

Grain alcohol industry

As in previous studies, the grain alcohol industry provided the most positive attitude to the use of sorghum, indicating that it is the major raw material. Low quality rainy season grain is being used and this is currently being bought from open markets. In order to be able to compete with molasses-based industry cost savings are essential. The two options available are to reduce the cost of raw material; the other option is to find grain types with higher starch content. In fact, for alcohol production molded grain (within limits) is acceptable and the lower price is an advantage. With current procurement arrangements from wholesalers the industry sees a number of problems:

- Grain is approximately 25% more expensive from the wholesalers than it is directly from farmers.
- Three grades of molded grain are found in the market: A, B, and C, with C being the worst and includes molded grain of a small size and often insect damaged. Grade C is unacceptable due to the "dusting" caused by insect damage, which represents a loss during processing.
- Wholesalers tend to mix C grade grain with better quality grade to give it higher value.
- Poor storage facilities in the marketing system increase the likelihood of being given inferior grain. Moisture content of grain, along with insect damage are the main problems.

The alcohol industry is looking at the possibility of direct procurement from farmers. Farmers' associations have already made approaches to enter into contract type arrangements for supplying grain. This could be used as a way of ensuring a reliable supply of grain and of adequate quality.

Options such as the use of red, mold-resistant varieties were discussed with the industry. While no objection in principle was raised, it was recognized that the introduction of such non-food varieties would probably have to be organized via some type of contract growing scheme. The discussion of varieties for grain mold resistance raised the issues of preferred grain characteristics more generally. It was indicated that over and above the grain mold issue, higher carbohydrate content of the grain would be a key trait, probably coupled with low oil content.

Poultry and poultry feed industry

The poultry industry portrayed a more conservative approach to sorghum use. This partly reflected the high profile nature of the companies that were visited and the quality sensitivities associated with sorghum. The following discussion needs to be tempered by the knowledge that significant quantities of rainy season sorghum are being supplied to the poultry industry in Maharasthra and Andhra Pradesh (Marsland and Parthasarathy Rao 1999; also personal communication with Dr R.B. Somani[x]).

The main constraints to utilization of sorghum were stated as follows:

- The tannin content of Indian sorghum (although all Indian sorghum hybrids are tannin free).
- The presence of mycotoxin in sorghum.
- The price of sorghum—it needs to be 20% below that of maize.
- The difficulties of procuring adequate quantities and qualities of sorghum, particularly in Andhra Pradesh.
- The lower calorific value of sorghum compared to maize due to lower oil content.

A number of options were discussed with the poultry industry. For example, the option of adopting red, mold-resistant sorghum varieties. Also, the option of using lightly molded grain in broiler rations. The impression given was that even though laboratory testing had shown that Indian sorghum contains no tannins and that moldy grain could be fed safely, the industry was rather cautious about these results. However, willingness to participate in commercial scale feeding trials was indicated, but it was also

suggested that this would need to be allied to low price and consistent supply of adequate quality grain.

Brewing industry

Currently the brewing industry is not using sorghum, but would be willing to try as an adjunct. Due to the high price of malted barley, the brewing industry would be interested in experimenting with malting of suitably adapted sorghum varieties. Molded grain would not be acceptable and consistent quality of supply would be essential. For use as an adjunct, a supply chain would need to be set up that could supply flaked sorghum.

Private seed companies

The private seed industry recognizes grain mold as an important constraint in rainy season hybrid sorghum. The industry is supportive of innovative approaches to deal with this problem, both through novel breeding approaches as well as the institutional innovations this might require. One of the companies visited demonstrated its willingness to adopt such approaches, giving the case of its partnership with a US plant-breeding company. The genotype development work is conducted in India. The finished hybrids are then produced and sold in the US.

The seed industry indicated that it was interested in tripartite tie ups: public sector research developing mold-resistant genotypes; private sector industrial users testing the suitability of new genotypes for utilization and establishing contract growing schemes to ensure quantity, quality and timeliness of supply of new grain types; and private sector seed industry conducting regional adaptation tests of new genotypes and undertaking seed distribution and allied activities to support contract growing schemes.

Quality constraint clusters

The overriding feature of the discussion of grain mold with new industrial users was that grain mold was only one of a cluster of quality related issues that needed to be dealt with. Unless these problems could be dealt with as a cluster, sorghum will still have to compete with maize. Any potential leverage

that the private sector might be able to bring to the grain mold problem would therefore be lost without this unified approach.

To illustrate the point, the poultry industry could quite easily be instrumental in popularizing specially adapted grain mold resistant varieties. But it could only do that if these same varieties had a similar calorific value to maize, and if the industry could procure these new resistant varieties through contract-growers' schemes. This would only succeed if these schemes allowed farmers to adopt productivity enhancing technology and if, in turn, this made sorghum cheaper. In addition, they would need to be helped to undertake commercial-scale feeding trials with new genotypes, probably at an early stage in the plant breeding program. This is a large group of inter-connected tasks. However, none of it is beyond the technical capabilities of relevant scientific research organizations. Equally it is well within the logistical capacities of the private sector to implement and manage.

Partly this clustering of constraints relates to the transition from food use only. However, it is not the case that two distinct markets are developing (food vs non-food). Rather, an increasingly complex set markets with potentially more sophisticated and varied grain quality requirements are starting to emerge. This is probably why scare public sector institutional resources are finding difficulty covering all the potential components necessary to achieve competitive utilization of sorghum in new and emerging utilization niches.

We shall deal with the reasons that this is not happening in more detail later. First, the following research or action research tasks are probably relevant to improved sorghum utilization.

Market institutional innovations

The earlier discussion of NIE, suggested that innovations in marketing institutions offers the scope to reduce the transaction costs associated with procuring quality grain and in finding adequate and consistent supplies. There is clearly justification to undertake some experimentation to test different institutional innovation and assess their value to both the farmer and the client industries. Contract growing arrangements, probably linked to supply of varieties and associated production and postharvest handling would be most appropriate. Part of such a study would be the need to understand the reasons these types of institutional innovations had not

developed already. A linked element may be the need to develop grain quality standards that are practical to operationalize. This would probably be a prerequisite for developing any type of contract growing arrangement. Standardization of the various grades of grain mold damage would be a priority.

Technology innovations

There are several potentially useful areas of technical research related to grain mold problem and its associated quality cluster that could underpin sorghum utilization. These include:

- Grain mold resistance through genetic markers (multiple public and private sector partners, particularly seed companies).
- Screening genotypes for specific private sector application such as starch content, oil content, recovery rates (jointly with industry).
- Feeding trials with molded sorghum (commercial scale jointly with poultry and cattle feed industry).
- Developing sorghum adjuncts (jointly with industry).
- Sorghum malting trials (jointly with industry).

R&D Institutional innovations

The discussion of the scope of market and technical research needs implies that much of the activities would require public-private sector partnership. This type of arrangement would need to be adopted as core methodology and it would probably imply some degree of joint funding. However, while the private sector is keen to engage in these types of arrangement, there is currently no institutional forum by which this is likely to become a reality. Furthermore, much of the discussion with the key private sector players implies grave doubts as the ability of public sector research institutes to enter into meaningful partnerships. It must be noted that the hesitation is mutual, with the public sector slightly unsure of the motivations of commercial enterprise.

In fact if we look at the system in which new sorghum knowledge is developed and applied we see two unrelated sub-systems. For example, we see the public sector viewing sweet (stem) sorghum as the top priority for its

breeding program in the context of non-food utilization of the crop. In contrast, the industrial sector clearly has a need for grain genotypes suited to various new uses-animal feed types; high starch content for alcohol production; malting or adjunct quality for the brewing industry and so forth. Private sector seed companies report a strong element of competition with the public sector breeding programs, particularly with respect to validation by the public sector of private hybrids. This tends to suggest that these are not mutually supportive actors in an efficiently functioning innovation system—in fact, quite the opposite.

As we have discussed earlier, the difficulties that public and private sectors have in relating to one another reflects a long history of institutional development. Often arrangements were such that public and private sector were "designed" to be quite separate. This is the case in many countries and the specific research sector does not seem to be related to the extent of the problem. The example of sorghum utilization is a good illustration of the way that the growing complexity of technology needs is making these distinctions irrelevant to the modern economy.

In practical terms what this means is that the expansion of the utilization base of sorghum into varied non-food uses is making it almost impossible for the public sector to respond appropriately to the increasingly complex needs of its clients. It is certainly impossible to achieve this when research resources are allocated on the basis of scientific merit rather than developmental outcomes. Furthermore, unlike the public sector's farmer client, the private sector is a customer that will happily look elsewhere for the technology it needs. And they will find it in other parts of the private sector, such as the varieties and hybrids of the private seed companies, or manufacturers of processing equipment. Alternatively, they will substitute sorghum with maize and this would hurt the livelihoods of many in the semi-arid tropics of India.

It should be stressed, however, that the opportunities for interaction between the public and private sectors are limited, although increasingly the private sector is being invited to workshops and similar events sponsored by the public sector. Nevertheless, even though this sort of peripheral interaction is starting, there is no indication that any organization can see a clear way to go forward and start and interact more productively. Our argument is that some form of institutional innovation is necessary to start this process and allow the public and private sector partner the opportunity of devising

together new working arrangements. But what form should this institutional innovation take?

Institutional Alternatives

Our earlier discussion suggests that this type of problem is generic and that research systems around the world have been struggling to come to terms with such problems for the last decade or so. It relates to the need to re-evaluate the role of the public sector in innovation systems; the need to devise new institutional structures that transcend the traditional public-private sector divide and that reflect the reality of the interactive nature of technological innovation. To illustrate the way these principles could be put into practice to help resolve the sorghum grain mold and its related cluster of quality constraints, we present the example of science directorates in Box 2. The parallels with the sorghum story are striking.

The suggestion is not necessarily that the idea of the science directorate be applied to the task of solving the institutional constraints of sorghum R&D. The example we provide of the Biotechnology Directorate in the UK worked due to a number of very specific contextual issues, not least of which was the over-whelming generic importance of biotechnology. Nevertheless, the Biotechnology Directorate holds some key principles that may be of assistance. Notable was the use of public funds to create a common body or organization with members drawn from specialized research institutes, universities and the private sector. The Directorate was given the task of identifying priorities for research and associated activities and mobilizing funds from both the public and private sectors to undertake research and promotion activities that otherwise neither party would invest in. Over and above these types of strategic function, the Directorate was a devise to build relationships between public and private organization—often for the first time.

Box 2. Science directorates.

One of the key institutional innovations in Britain in the 1980s was the establishment of science directorates. A primary reason for this approach was the realization that inventions coming from the UK public sector research system were not being nationally exploited (by the private and public sector) and that the commercial benefits were going to other countries[xi]. The associated fear was that this pattern would continue unless the UK Government took countervailing action.

The science directorates that these concerns led to were conceived as alliances of ministries, research bodies, and private industry. They were designed to promote specially promising technological avenues like polymer engineering, marine technology, and biotechnology. They were specifically a response to the failure of universities and polytechnics to keep pace with a growing national need for underpinning these new and important fields (Senker and Sharp 1988).[xii]

In the field of biotechnology, the UK Government had set up the Spinks Commission to explore ways in which relevant commercialization could be encouraged. The commission recommended that public funds should be used to set up a research-based company, which would have preferential access to research emanating from UK Medical Research Council (MRC) facilities. Although the government's response was initially lukewarm, it established the Biotechnology Directorate in 1981 to foster and promote the British scientific base in biotechnology, specifically university researchers, and to build links between the scientific community and industry (Senker 1998). Key players here were the large pharmaceutical companies who became actively involved in the Biotechnology Directorate and as a result were able to influence the prioritization of R&D while at the same time only paying a proportion of the costs involved.

Senker (1998) shows how several things resulted from this initiative. First of all, different organizations began to interact, probably for the first time in a comprehensive sense. Industry began to appreciate what the public research sector could offer while research communities that had previously not really integrated (such as engineers with natural scientists) were forced into communicating purposefully. This was almost a pre-condition for receiving what were considerable sums of "targeted" research money. A related feature is that of learning how to interact. Since the traditional approach of academic researchers and industrialists was always to hold themselves apart, it took some time for them to learn new traits[xiii].

Another feature was the use of funding criteria (for example the need for joint public private proposals) to help build bridges between important nodal organizations. However, this relationship building was not just through joint research efforts, but also through general networking opportunities that the creation of such a body can create. As much as anything the directorate principle was actually a learning exercise: learning about how to enter into and work in a partnership or alliance with unfamiliar organizations and styles of working. For the private sector, this was concerned with learning about where to access the knowledge previously locked up in public research organizations[xiv]

We believe that there is strong evidence in the case of sorghum for investing public funds (hopefully with complimentary private support) in an institutional device built on similar principles. The precise details would need to be developed bearing in mind the specific institutional landscape of the Indian sorghum sector. From a donor's perspective, this would provide a useful focusing devise for the outputs of more specific pieces of technical work. As indicated, these technical projects would cut across traditional research disciplinary boundaries (postharvest, crop protection, plant science) because of the clustering effects discussed earlier. On their own, component research projects will have little development significance. The involvement of the private sector in such an institutional innovation offers real prospects of sorghum grain mold and other quality related technical research making a tangible difference to sorghum-based livelihoods in rural India.

Conclusion

If the industrial utilization of sorghum is to deliver its developmental potential, grain mold and a cluster of quality related issues would need to be addressed through public-private sector partnerships. The central message of this paper is that many of the pieces of the sorghum jigsaw already exist. Some of the technical research has already been undertaken and other issues are within the research capacity of nodal organizations (nationally and internationally). However, the various actors and institutions with the capacity to create and operationalize new knowledge are poorly connected. Similar problems have emerged in the research sectors of countries around the world. Contemporary policy analysis and lessons from international experience suggests that the

answer lies in generating new institutional structures that transcend the traditional public-private sector divide.

We build on these ideas to present a radical alternative approach to mobilizing science and technology in support of sorghum-based rural livelihoods. The key element of this is to invest public resources to create an institutional forum or network with the sole function of mobilizing resources to commission research and operationalize sorghum-based knowledge and technology. This would be established in such a way that the process of institutional learning would shape and evolve its role and structure in response to the needs of the sorghum utilization sector. We believe that without an institutional innovation of this type, well-intentioned public sector research on grain mold and other quality related issues would continue to add unconnected (and unused) components to the evolving sorghum economy of India. Only by linking these pieces together through an appropriate institutional mechanism will new technology benefit sorghum-based industries. Only in this way will technology have a useful role in developing new markets to support sorghum-based rural livelihoods.

A more general policy lesson concerns the need to give greater prominence to institutional context in the design of research projects addressing what are apparently technical issues. Institutional innovations have an important role to play in successful R&D programs. The combination of technical research with institutional learning and the policy lessons this provides, offers a powerful way of bring science and technology to bear on the livelihoods of the poor.

Endnotes

i. Examples of institutional innovations are diverse and includes: new market institutions, such as contract farming; taking advantage of the incentives of private enterprise to leverage quality control measure further down the supply chain; and probably most interestingly of all, the potentially powerful outcomes of bringing together and merging the working practices agendas and incentive structures of the public and private research and non-research sectors.

ii. This issue is currently being explored in detail by two of the authors in a project funded by the Crop Post-Harvest Programme of the Department for International Development, UK (see Hall et al. 1998; Hall et al. 2000a, b; Hall et al., in press a, b; Clark, in press).

iii. I am grateful to Karen Wilken for her advice and assistance with this section on New Institutional Economics.

iv. Other branches of the subject related to the institutional mechanisms that have been developed to deal with the asymmetries and incompleteness of information; to internalize externalities and the role of property rights in this context; and in the coordination of collective action in the context of common property resources.

v. Clark's (1991) explanation of the terms complex and evolutionary (as implied by dynamic) is useful here. The processes are "complex in the sense that they are composed of many agents whose interactive behavior is only predictable to a limited extent since it is conditioned continually by relatively unknown future events: evolutionary because the agents are continually shifting their identify (forming, dissolving and reforming) in response to the variable environments in which they are placed."

vi. Edquist (1997, page 3) provides a useful overview of the main elements of recent thinking concerning innovation as follows: "Innovations are new creations of economic significance. They may be brand new, but are more often new combinations of existing elements. Innovations may be of various kinds, e.g., technological as well as organizational. The process through which technical innovations emerge are extremely complex; they have to do with the emergence and diffusion of different knowledge elements, i.e., with scientific and technological possibilities, as well the 'translation' of these into new products and production processes. This translation by no means follows a 'linear' path from basic research to applied research and further to the development and implementation of new processes and new products. Instead, it is characterized by complicated feedback mechanisms and interactive relations involving science, technology, learning, production policy and demand."

vii. Edquist (1997) provides substantial discussion on the precise definition of national systems of innovation and different ways authors have interpreted the concept and its shortcomings. Carlsson (1995) discusses a similar concept using the term technological systems. See also Clark (in press) for a treatment that stresses formal information theory.

viii. The grain alcohol sector is slightly different. Its main competitor is molasses. Excise rules and associated policy is only licensing new distillers to use grain as a raw material. Grain quality and perceptions of quality are of far less importance, and in fact sorghum, particularly damaged sorghum, has price advantages over most other sources of carbohydrate.

ix. The nutritional value of sorghum in comparison to maize places a ceiling on the degree of substitution that feed manufacturers and poultry concerns are prepared to make. During discussions with several poultry owners, the maximum level of substitution was 15%. For one commercial feed manufacturer, the maximum level was 10%.

x. Dr R.B. Somani (Dr Panjabrao Deshmukh Krishividyapeeth, Akola, Maharashtra, India) bases this on informal monitoring of sorghum market departures from the Akola market in the heart of the hybrid rainy season sorghum producing area.

xi. The most famous biotechnology example is the failure to patent the discovery of monoclonal antibodies by the UK Medical Research Council.

xii. A second reason for the inception was the impact of the controversial Rothschild Report of 1971 that introduced for the first time the contractor client principle into the UK research system. This had long-term consequences because it introduced possibly for the first time to many, that science had to be accountable to economic or societal goals—even if these were ill defined in the early years.

xiii. See Senker and Sharp (1988) for a complete account of this. The failure of disciplines to interact is often discounted in policy analysis but is arguably, however, a major weakness in the effectiveness of public research.

xiv. The ability of institutions to "learn" in this way, and changes in practice that often result is viewed as a key feature of successful innovation systems. Senker (1998) makes the distinction between the know-how and the know-who to achieve a task—"the knowledge of knowledge".

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About ICRISAT

The semi-arid tropics (SAT) encompasses parts of 48 developing countries including most of India, parts of southeast Asia, a swathe across sub-Saharan Africa, much of southern and eastern Africa, and parts of Latin America. Many of these countries are among the poorest in the world. Approximately one-sixth of the world's population lives in the SAT, which is typified by unpredictable weather, limited and erratic rainfall, and nutrient-poor soils.

ICRISAT's mandate crops are sorghum, pearl millet, finger millet, chickpea, pigeonpea, and groundnut; these six crops are vital to life for the ever-increasing populations of the semi-arid tropics. ICRISAT's mission is to conduct research which can lead to enhanced sustainable production of these crops and to improved management of the limited natural resources of the SAT. ICRISAT communicates information on technologies as they are developed through workshops, networks, training, library services, and publishing.

ICRISAT was established in 1972. It is one of 16 nonprofit, research and training centers funded through the Consultative Group on International Agricultural Research (CGIAR). The CGIAR is an informal association of approximately 50 public and private sector donors; it is co-sponsored by the Food and Agriculture Organization of the United Nations (FAO), the United Nations Development Programme (UNDP), the United Nations Environment Programme (UNEP), and the World Bank.