SORGHUM GRAIN MOLD

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PREFACE

Sorghum has great potential to contribute to national food security and to achieve sustainable livelihood security in dryland ecosystems. It is being utilized for various purposes such as food, feed, fodder and bio-fuel. Although, its productivity has increased from 522 kg per ha in 1969 to 907 kg per ha in 2014-15, area under sorghum cultivation, especially kharif sorghum is going down gradually. With changing needs of the people and time, it is facing strong competition from commercial and vegetable crops. Grain mold during rainy season cultivation has been another huge constraint that has affected the farming with this crop. Losses are so severe that all the organizations involved in research and development of sorghum across the world allocated major share of their resources to tackle the problem. Since nineteen-seventies, extensive research works have been carried out to understand the intricacies of the disease and its management feasibilities. Huge amount of literature is available that includes half-a-dozen good reviews, latest being in 2000. New advancements have taken place in grain mold research and development during twenty-first century. The information is scattered and need to be compiled and synthesized with in the form of a book for future researchers and planners. With this background the book on "SORGHUM GRAIN MOLD" has been written to celebrate the completion of 50th year of All India Coordinated Sorghum Improvement Project. Authors are hopeful that the book will serve its purpose for sorghum community in India and abroad.

(Authors)

ABSTRACT

Grain mold is a major yield and quality limiting constraint of rainy season sorghum in many countries in Asia, Africa, North and South America, where grain filling period of the crop coincides with rainy weather. The disease reduces grain yield as well as quality due to discoloration by infection and growth of many fungi. Improved short- and medium-duration cultivars that mature during rainy season in humid, tropical and subtropical climates are more prone to mold development. The disease is most severe in India, where white grain hybrids and varieties are grown. Multi-dimensional losses include yield, quality, marketability, food and feed safety, and diminishing cultivation areas. Concerted efforts to develop mold tolerant cultivars yielded results at slow rate. This book focuses on recent advancement taken place during twenty-first century at the same time analyses previous works and their implications. Grain mold history, pathological advancements, economic significance, plant breeding efforts to improve resistance, mechanisms to understand the disease development and management possibilities have been discussed, with color photograph and charts wherever required. In addition to this alternated uses of molded grain have been discussed for ethanol production. A separate section has been included to chalk out future research priorities with particular reference to India.

CONTENT

1. INTRODUCTION

Sorghum and grain mold

Sorghum (*Sorghum bicolor* L. Moench) is one of the most important crops in semi-arid tropics covering an area of around 40.67 million ha across the globe with total production of 57.60 million tones. Major areas are in Africa (66.6%) followed Asia (18.5%) and Americas (13.3%). Top ten countries in terms of acreage (million ha) are India (5.86), Nigeria (5.82), Sudan (5.41), Niger (3.82), USA (2.04), Ethiopia (1.80), Burkina Faso (1.67), Mali (1.59), Mexico (1.43) and Chad (1.15) [1] (Fig. 1). India has the largest acreage in Asia followed by China (0.62 million ha) and Yemen (0.43 million ha). An analysis of the trends in area and yield gains and associated changes in yield stability in the top 10 sorghum-producing countries from 1970 to 2009 revealed that the Asian countries and the USA recorded the largest drop in harvested area [2]. Grain yield levels, however, increased substantially in all the countries except Sudan.

Grain mold is a major disease of *kharif* season sorghum and is common in many countries in Asia, Africa, North America, and South America [3]. The disease is severe in Asia and Africa where white grain sorghum are grown widely. Improved short- and medium-duration sorghum cultivars that mature during the *kharif* season in humid, tropical, and subtropical climates suffer more. Late-maturing photoperiod sensitive sorghums generally escape grain mold as they flower and fill grain during dry weather. Colored grain sorghum which is grown for feed purpose in the United States, Mexico, Argentina, and Australia suffers relatively less from this disease. The disease is most severe in India where the high yielding white grain hybrids are grown during *kharif* season (Fig 2).

In India, sorghum is an important staple food for people living in the dry tracts of the country. It has also gained importance as feed, fodder and biofuel crop. It is grown during both rainy (*kharif*) and winter (*rabi*) seasons. During seventies sorghum was the second most widely grown crop in India only after rice and covered around 18 million ha in the states of Maharashtra,

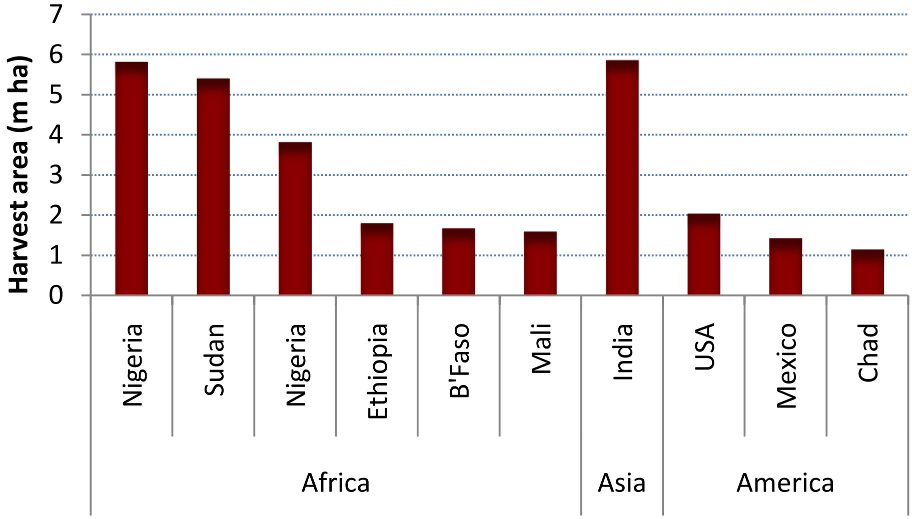

Fig. 1. Top ten countries in terms of sorghum acreage (Source: FAOSTAT, 2017)

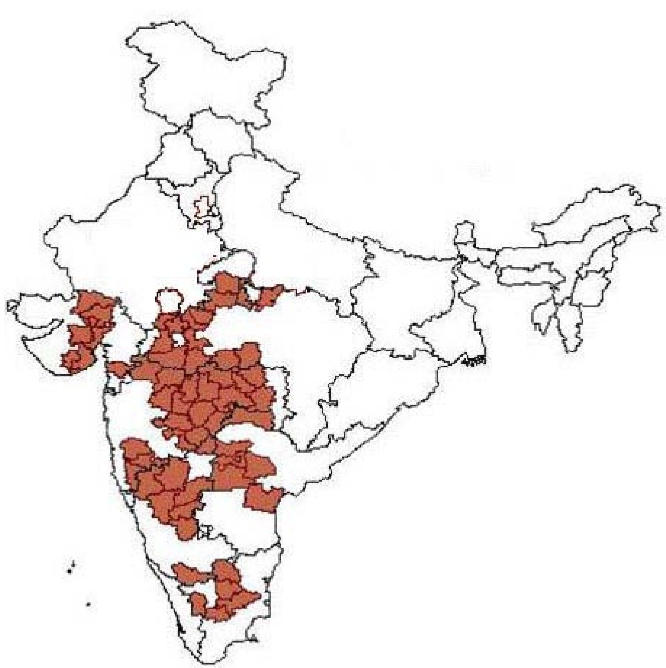

Fig. 2. Grain mold map of India

Karnataka Andhra Pradesh, Tamil Nadu, Madhya Pradesh, Rajasthan and Gujarat [4]. With the change in climate, cropping pattern, introduction of high yielding hybrids and varieties, the disease scenario has also changed and once minor disease has become major later. Grain mold of *kharif* sorghum is one such disease, which has changed its significance over time. Gradually, it has become number one disease of *kharif* sorghum for its devastating effects on yield and quality. Gravity of the situation was so that all the organizations involved in research and development of *kharif* sorghum in India allocated major share of their resources to tackle the problem. Regional station of Indian Agricultural Research Institute (IARI) at Hyderabad, which was subsequently developed to a full fledge institutes as National Research Center for Sorghum, the State Agricultural Universities (SAUs) in the state of Maharashtra, Karnataka, Andhra Pradesh, Tamil Nadu and Madhya Pradesh, and the International Crop Research Institute for Semi-arid Tropics (ICRISAT) Patancheru, were intensely involved on grain mold research and developed and released many grain mold tolerant high yielding hybrids and varieties over time. This book portrays the journey of grain mold research from its inception to the present time with emphasis on India.

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Traditional
Improved

2. HISTORY

The beginning

During earlier days, a panicle disease of grain sorghum having similarities with present day 'grain mold' was known as 'head mold'. One of the earliest available literatures using the term 'head mold' was an M Sc thesis by JF Smith, from Department of Botany and Plant Pathology, Oklahoma Agricultural and Mechanical College, USA [1]. Before the release of early maturing composite hybrids and varieties in India the Indian farmers were used to grow mostly traditional, long duration, tall, and photoperiod sensitive sorghum cultivars or land races. Because of their photosensitivity they would flower late and mature after monsoon is over. Grain mold was not recognized as a constraint on these traditional cultivars mainly because their grains were not caught in rain. Downy mildew, anthracnose and leaf blight were the major yield limiting diseases on *kharif* sorghum in those days. The scenario changed with the introduction of short duration, short height composite sorghum hybrids and varieties. With a view to increase food production, research efforts were directed towards development of high yielding hybrids in food crops especially that was started with the sorghum with the advent and use of male sterility technique. Major breeding efforts to avoid climatic vulnerability of rainfed sorghums had been to develop cultivars whose duration would match with the duration of the rainy season, as against the long duration (5–6 months) for traditional cultivars. Such hybrids and varieties would mature in 90–110 days and confer stability of production, even in years of low rainfall [2]. This led to development of many short duration hybrids and varieties and four-fold increase of yield in food grains and that ushered in green revolution in India. With this development the crop phenology was dramatically changed to photoperiod-insensitive, short- and medium-duration, high-yielding sorghum hybrids in India. These hybrids would mature early and often caught in occasional late rains in October leading to 'grain deterioration' and consequent low market price. That was the time the balancing act between earliness for yield stability and necessity of avoiding grain deterioration (grain mold) started [3].

Sixties

During 1960s there was hardly any mention of the word 'grain mold' of sorghum in Indian literature [4]. This is the period the Indian farmer mostly grew **photoperiod sensitive**, late maturing and tall Indian land races. Composite sorghum hybrids and varieties just started coming. The first composite sorghum hybrid (CSH 1) in India was released in 1964. Subsequently high-yielding hybrid CSH 2 (1965) and variety CSV 1 (1968) were released in India up to the end of 1969. There might be some sporadic infections on grain that time also, which is not reported. A few workers started investigations on the problem of head molds in India [5].

Seventies

This was the period when today's 'grain mold' was being termed in different names. Various terminologies were being used in papers and reports on the topic across the globe. The terms **head mold**, seed mold, grain mold, grain weathering, and grain deterioration were all encountered. Sorghum scientific community in many countries in Africa, Americas, and Asia, however, started using the phrase 'grain mold' for fungal deterioration of sorghum grain in the field. Towards the end of 1970s an international workshop on sorghum diseases (known as 'Sorghum diseases- A world review) was held at Hyderabad, India in December 1978, in which several sorghum researchers across the country participated [6]. Grain mold, downy mildew, ergot, smut, leaf diseases-each disease was discussed at length in separate sessions. The anomalies in naming grain mold were also discussed. This workshop probably helped researchers to use a common name 'grain mold' to describe the diseased appearance of sorghum grain resulting from the infection of the developing grain by one or more parasitic fungal species.

Till the end of 1979 India had released a total of 6 short duration (100-120 days), short-height (150-200 cm), high-yielding **composite sorghum hybrids** (CSH 1 to CSH 6) and 9 varieties (CSV 1 to CSV 6, GJ 9, CO 21, CO 22) for cultivation by the farmer. Fungal infection of sorghum grain become increasingly visible in the field on these early maturing hybrids and varieties, and the incidences drew attention of the researchers. However, the phrase 'grain mold' was not figured in Indian literature (except ICRISAT Patancheru) until 1980s. The All-India Coordinated Sorghum Improvement Project (AICSIP) that started functioning in 1970 at Hyderabad as a part of the program initiated by the Indian Council of Agricultural Research (ICAR), in which all sorghum-growing states participate through their agricultural universities, used the term 'grain deterioration' or 'head mold' to describe above fungal deterioration of sorghum grain in the field. That time pathological works in India were mainly revolved around determining the cause of the disease, and fungal genera involved [7]. Head mold was reported to be severe in Vidarbha, Marathwada of Maharashtra; parts of Andhra Pradesh; Coimbatore, North Arcot (a former district in Madras Presidency), and Salem districts of Tamil Nadu [8]. Various organic acids related compounds, propionic acid were tried for mold management and were found effective as a mold inhibitor at moisture contents of 18-24 percent [9]. The disease showed a high potential for damage, especially of short-duration, compact-headed strains whose grain-formation stage coincided with heavy rains. The process of grain deterioration received attention of the breeders. Selection criteria to breed for resistance were worked out. Tan plant type, low water absorption capacity when soaked for a period of 2 hr, and the breaking strength of grain (hardness) were the criteria used for selection of types resistant to grain deterioration [10]. Screening for resistance had identified the released variety CSV-4 as the best available for that time [2].

Eighties and beyond

During eighties grain mold had been recognized as the most important and economically damaging disease of *kharif* sorghum throughout the world. Use of the term '**grain mold'** had been stabilized and researchers across the globe started using this phrase without much deviation, though there was few reports that used different name for the disease. Plant breeders, pathologists, biochemists and other plant scientists grouped together to find solution of the problems so that quality grain of sorghum can be produced during rainy season. An international symposium on sorghum was held during November 1981 at ICRISAT, Patancheru, India, where grain mold was discussed at length by representative from different countries. The proceedings of the symposium were published as "Sorghum in Eighties" in 1982. After a gap of seven years the world sorghum community again met to particularly discuss sorghum diseases. Sorghum and Millets Diseases- A Second World Review was held at Harare, Zimbabwe, in Mar 1988. Progress made on sorghum diseases including grain mold since the first global workshop held in India in 1978 was reviewed to set research needs and goals for the next decade [11]. Few comprehensive reviews on the subject have been published during this period to address the problem [11, 12, 13, 14, 15].

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3. PATHOLOGICAL ADVANCEMENTS

What is grain mold

Grain mold is a complex fungal disease of sorghum grain. The disease starts with infection at the time of anthesis and reaches its peak at grain maturity. Its adverse effects do not end at grain maturity and continues even after harvest and in storage. If the discussion is restricted up to harvest, the disease can be divided into two parts for the sake of discussion. The first part is between start of infection at anthesis to physiological maturity of the grain, which is termed as grain mold. While the second part is from physiological maturity to harvest, this can be termed as **grain weathering**. Grain weathering is a post-physiological maturity problem when grains turn discolored and tissues are damaged by fungal colonization due to wet weather [1]. This term, in broad sense, is used to denote total deterioration of grains due to physical, biochemical and fungal infections. During this phase, damage is mostly caused by saprophytic mode of action of the fungi which colonize the exposed grain when active defense mechanisms in the host (grain) cease to operate. Many species of fungi are involved with the deterioration of the grain [2]. This division between grain mold and weathering is convenient to discuss the disease with respect to causal organisms, symptoms and the effects on the host- all of which are different in the above two stages. During second world review of sorghum diseases this topic along with terminology and definition was discussed at length [2].

Both the spelling 'mold' and 'mould' are frequently used by the researchers of grain mold. The various funguses that grow on organic matter are termed as 'mould'. This general terminology of mould, however, better fits with 'grain weathering', in which fungal growth is more of saprophytic in nature and external growth (bloom) is clearer than in 'grain mold'. Initial infection by mold pathogen occurs at the time of anthesis on the spikelet tissues. They colonize glum tissues and the pericarp of the developing caryopses during the next four weeks [3]. At physiological maturity, colonies of fungi can be observed in the starchy mesocarp and the cross and tube cells of the pericarp in all cultivars [4].

The first visible symptom is pigmentation of the spikelet tissues including sterile lemma, palea, lodicules and glumes (Plate 1a). Anthers and filaments can also be infected depending on severity of infection. Early infection results in loss of caryopsis formation [5], florets blasting (Plate 1b), poor seed setting and development of small and shriveled grains [3]. Fungal infection can be detected on immature grain as early as 8-10-day old grain [6]. Under humid conditions severely infected grains (particularly in susceptible lines) become fully covered by fungal growth even before PM and such grains disintegrate under slight pressure. Disintegration of molded grain before PM is termed as '**pre-mature kernel rot**' or simply 'kernel rot' or 'seed rot' (Plate 1c). Fungal growth first occurs at the hilar end of the grain, and subsequently extends on the pericarp surface. Often the infected grains are discolored and discoloration is more prominent on white-grain than in brown/red grain sorghum. Some apparently normal grain may not show external symptoms but produce fungal growth on incubation. Internal colonization of grain often leads to **sprouting** of grains in the field under wet conditions (Plate 1e). Such 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 happen as early as 15 days after pollination [7, 8].

Occasionally the fungus damages large areas of panicle including peduncle and rachis branches and spikelets, resulting in blighted panicle or **head blight** (Plate 2a). Moisture content in grains of such blighted panicles becomes significantly less (10.4%) than that of normal panicles (13.0%) at harvest [3]. The term '**head mold**' was mostly used during seventies and before to describe a condition where the spikelet tissues and grains become molded (Plate 2b). Mold growth varied on the heads from a light scattered spotting to complete coverage. In many cases the spikelets and kernels are held tightly together by this growth so that the head become a quite rigid. Additional damage to bagged sorghum heads might be caused by various insects, including the aphid (*Aphis maidis*), and earworm (*Heliothis armigera*) [9].

Plate 1. Grain mold symptoms: a) spikelet tissue discoloration, b) Floret blasting, c) Premature seed rotting, d) Grain molding, e) Sprouting (arrow), f) Grain weathering (color grain).

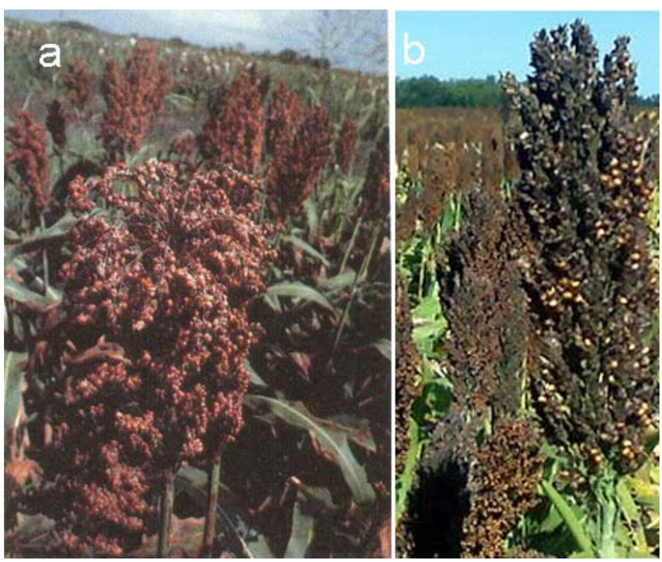

Plate 2. a) Head blight (Source: Frederiksen, 1986), b) Head mold.

Post maturity fungal colonization generally produces moldy appearance of grain maturing under humid environments [2]. The color of mold vary depending on the fungal species involved, for example, *Fusarium moniliforme* produces pinkish–white to orange-white powdery growth , while *Phoma* produces black discoloration, *Curvularia* shiny, velvety black, fluffy growth on the grain surface [10]. The grain mold infection often results in moldy growth known as "**blackening**" of the head [11, 12, 13].

Causal organisms

Sorghum grain mold is caused by fungi, which can be broadly grouped into two categories- **pathogens** (or parasites) and saprophytes. Fungi that can initiate infection on florets and subsequently to immature grains are the pathogens. Chiefly the species of *Fusarium, Curvularia* and *Alternaria* are responsible for this. Among *Fusarium* species *F. andiyazi*, *F. proliferatum* and *F. thapsinum* are proven pathogens of sorghum grain mold [5, 65, 68]. They cause severe infection in pericarp, mesocarp and endosperm. Heavy mycelia aggregation is found in the embryonal axis, hylar void, and scutellum and at the point of grain attachment. Infection is generally more at hylar region followed by middle and stylar region [14]. Growth of the mold pathogen can easily be detected at hilar region of immature grain on incubation (Plate 3). *Curvularia lunata* infects all parts of seed including pericarp, seed coat, aleurone layer, endosperm and embryo [15]. There is report of pathogenecity differences in *C. lunata* isolates from different parts of India [16]. Possibly there is some competition between the major pathogens (*Fusarium* & *Curvularia*), especially when severity of infection is towards higher side. This is revealed by the significant negative association between infection frequency of *Fusarium* and *Curvularia* when intensity of infection is greater than 25% (Fig. 1). Few other fungi namely *Alternaria* and *Bipolaris,* spp. are also detected in immature grain sporadically in low frequency.

It is observed that more the early (8-day-old grain) infection more is the mold severity at physiological maturity. Therefore, frequency of early infection plays significant role in deciding grain mold severity on maturity grain [18] (Fig. 2). Study conducted to quantify natural infection frequency of fungi in early or milk stage kernel (8-10 day old grain) on major sorghum locations in India reveals that *Fusarium* (16.1%) and *Curvularia* spp. (7.9%) are the predominant pathogens across locations [17].

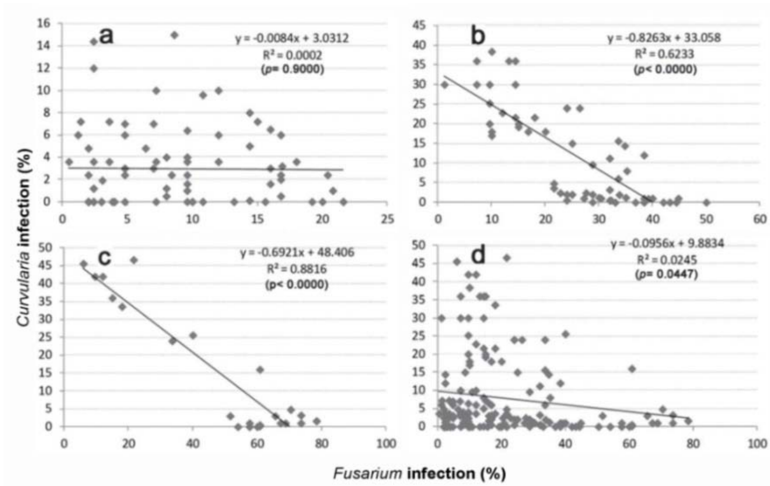

Fig. 1. Frequency of *Fusarium* and *Curvularia* infections on 8-day old immature grain. When combined infection frequency (a) less than 25%, (b) 26 to 50%, (c) more than 50%, and (d) overall (0 to 80%) (Source: Das et al., 2017).

Plate 3. Grain mold pathogen growth at hilar region of parental line 296B.

When grain is nearing maturity (or matured) many saprophytes colonize on its surface if weather conditions are warm and humid. More than 40 fungal genera have been found associated with mature or molded or weathered sorghum grain. A few of these fungi may be pathogens which might have infected spikelet tissues or immature grains and persisted thereon (as parasite) but majority are **saprophytes**. The most frequent one

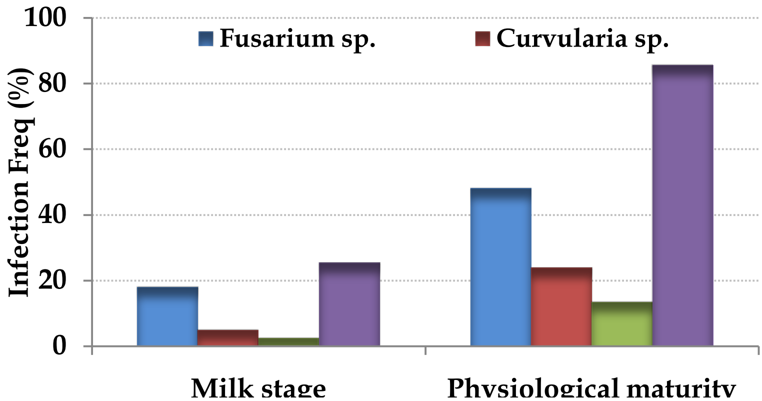

Fig. 2. Fungal infection frequency of sorghum seed at milk and physiological maturity stages.

is *Fusarium moniliforme* (presently divided into 14 species of these, *F. andiyazi*, *F. nygamai*, *F. proliferatum*, *F. thapsinum* and *F. verticillioides* are associated with sorghum grain) [19, 20]. Other *Fusaria* that are not *F. moniliforme* but have been frequently isolated from sorghum grain include *F. anthophilum*, *F. chlamydosporum*, *F. culmorum*, *F. equiseti*, *F. graminearum*, *F. oxysporum*, *F. pallidoroseum*, *F. sacchari*, *F. semitectum*, *F. solani* and *F. sporotrichioides*. They produce wide range of variations in growth, morphology, color, texture when grown on synthetic media (Plate 4). *Curvularia lunata* is the second most frequent fungi associated with sorghum grain mold. *F. moniliforme* and *C. lunata* are of significance worldwide [21, 22, 23] (Plate 5 & 6). Other prominent genera are *Phoma*, *Alternaria*, *Drechslera*, *Cladosporium*, *Olpitrichum*, *Exserohilum*, *Gonatobotrytis* and *Aspergillus* spp [21, 24]. More information on fungi associated with sorghum grain can be available in the pictorial guide published by ICRISAT [25].

Plate 3. Variations in *Fusarium* cultures associated with sorghum grain.

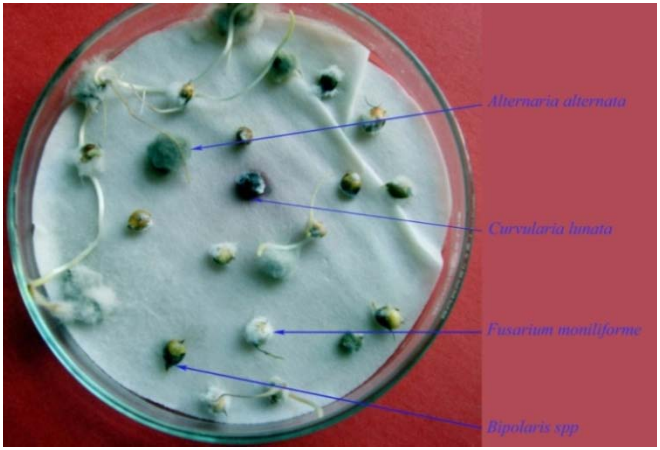

Plate 5. Infection of grain mold on sorghum seed.

Genetic diversity and several **vegetative compatibility** groups (VCGs) have been reported in the genus *Fusarium* [26]. 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. The genetic diversity among isolates of *Fusarium* and *Curvularia* was studied [27]. Studies on mating populations of Indian isolates of Fusarium primarily from mycotoxins point of view are rare.

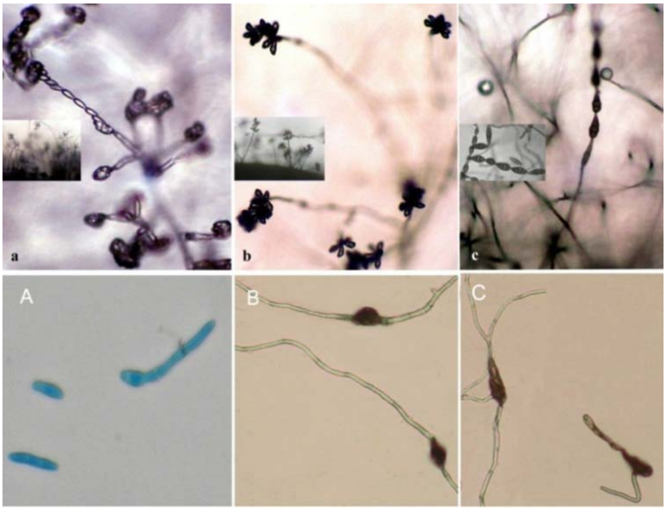

Plate 6. Microscopic view of reproductive structure and spore germination of major grain mold fungi: a) *Fusarium* spp., b) *Curvularia* spp., and c) *Alternaria* spp.

Sources of inoculum

Fungi causing grain mold of sorghum can be soil-borne, airborne, or carried in plant residue, and can be recovered from any part of an infected plant (root to the flower) [19, 28, 29]. **Primary sources** of inocula in the field are mostly plant debris containing fungal hyphae and conidia. Special fungal structures may not be essential for survival of *F. moniliforme*. The conidia and hyphae in sorghum stalks can survive two winters without any loss of viability or pathogenicity [30]. Its microconidia can survive up to 900 days at different levels of humidity and temperature under laboratory conditions [31]. The natural air-borne inocula present over sorghum field during rainy season are suggested to be sufficient for development of grain mold without any artificial inoculation [32]. Studies with volumetric **spore trap** (Plate 7) fitted over sorghum field showed that concentration of spores of mold causing fungi varied at different times within a day. Maximum spore count was between evening six to midnight [33] (Fig .3).

Plate 7. Burkard volumetric spore trap installed in a sorghum field.

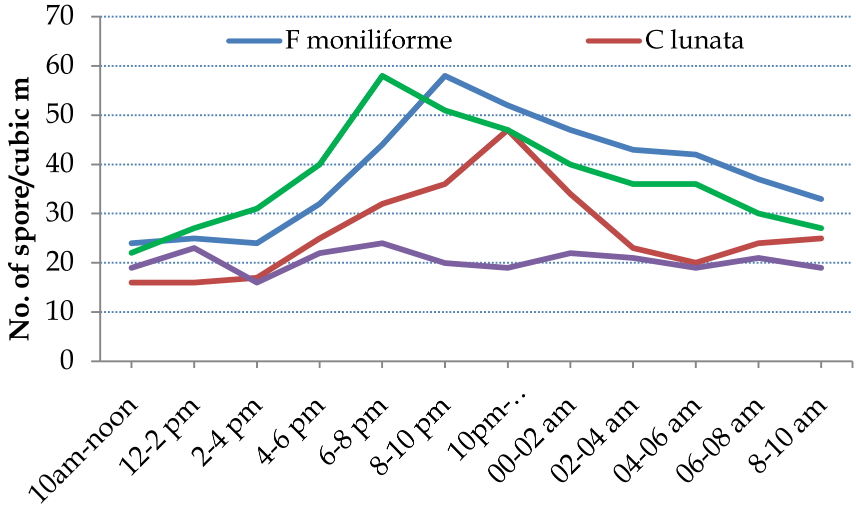

Fig. 3. Spore concentration of grain mold fungi in sorghum field (Source: Adapted from Indira and Muthusubramanian, 2004).

Favorable factors for disease

Among the three components of a disease triangle the weather and host factors are more crucial for grain mold development than the other. The third factor is the pathogen, which are multiple in numbers for grain mold and have frequent presence in air. It was observed that artificial inoculation of panicle with fungal spore or no inoculation did not make much difference in mold development in the field provided sufficient relative humidity was ensured with sprinkler irrigation [34]. Therefore, the roles of pathogen/ parasite/ saprophyte are less variable for this disease. However, there may be variation in infection frequency among fungi and grain development stages indicating that individual fungi might have different windows for maximum infection during the grain developmental stages [35]. Spores of *Fusarium, Curvularia* and *Alternaria* are observed in the field during post flowering to hard dough stages and their frequency increase after grain maturity [32].

Grain mold is possibly one of a few diseases of crop plants where weather factors, particularly relative humidity play dominant and determining role. Prevailing weather conditions from flowering to maturity particularly humidity and temperature are the most important factors for grain mold development. Warm and humid climate is conducive for development of the disease. Wet weather condition following flowering is necessary for grain mold development and longer the duration of wetness, more is the incidence of mold development [36, 37, 38]. With increase in wetness duration, there is corresponding increase in grain infection by *C. lunata, Cladosporium, Fusarium oxysporum, Bipolaris australiensis, F. moniliforme, F. Pallidoroseum* and *Phoma sorghina* [39]. Relative humidity, rainfall, number of rainy day and minimum temperature during 4-6 weeks after flowering shows significant correlation with mold incidence [40, 41]. Fungal sporulation and grain mold severity increases on most sorghum genotypes with increasing incubation temperature from 25–28 ^{o}C with an RH levels of 95–98% [42].

Sorghum lines (the host) is another important factors determining grain mold development. Wide variations are observed in grain mold resistance among the sorghum genotypes including germplasm, improved lines and cultivars, indicating definite role of host in grain mold. Short height (~150-200 cm) plant with compact panicles and less glum cover on seed generally favors mold development. One of the possible reasons of fewer molds on tall plant with loose panicle is the quick drying of the panicle after rain. Panicle structure and glum cover play significant role in premature seed rot and grain mold and these characters can be used in precise evaluation of the disease [6]. Many other plant or grain characters have strong relation with grain mold which will be discussed in mechanism section.

Assessment of mold severity

Visual scoring of disease severity is recorded either following a qualitative (immune, resistant, moderately resistant, susceptible and highly susceptible) or a quantitative scale (1–9 scale, 1–5 scale or percentage). The quantitative scores are useful for statistical analysis of resistance. They can also be converted into qualitative forms to describe disease reaction types. The grain mold severity is measured in two stages- first at physiological maturity or before harvest in standing crop (known as panicle grain mold score or **PGS**) and secondly on threshed grain after harvest (known as threshed grain mold score or TGS). PGS is a better indicator for genetic resistance than TGS. During the process of threshing and cleaning many soft, damaged and rotten grains are removed and remain unrepresented in the sample on which TGS is recorded. **TGS** indicates how much clean grain can be obtained after threshing for commercial use. For recording PGS randomly selected 10 panicles in a row are observed for percent grain infection and seed surface covered by mold growth and an average of 10 panicles is determined. Similarly, for TGS percent grain infection is determined by observing a seed sample drawn from the threshed grains. The percent infection is converted to a 1–5 scale, where 1= resistant, while 5= more than 75% of the grain is infected. Later on a refined 1–9 scale, where minor differences in resistance could be worked out, was adopted. Presently the 1–9 scale is followed for almost all the diseases internationally both for measuring PGS and TGS [1, 34, 43] (Table 1). There are two other less popular methods for measuring grain mold severity *viz*., colony forming units (CFU) and ergosterol method. CFU method measures the degree of fungal colonization per unit of kernel tissue. This is measured by serial dilution of dried, ground kernel tissue, plating on selection media, and counting of colonies. Infection frequencies can also be measured by plating and incubating the entire kernel on blotting paper or on a selective agar medium. In **ergosterol** method the concentration of

Table 1. Visual grain mold severity rating scale (1-9 scale)

Severity Grade	Percent grains infected in a panicle/ threshed sample	Disease Reaction
1	<1	Highly Resistant
2	1-5	Resistant
3	6-10	Resistant
4	11-20	Moderately resistant
5	21-30	Moderately resistant
6	31-40	Susceptible
7	41-50	Susceptible
8	51-75	Highly Susceptible
9	>75	Highly Susceptible

ergosterol (a sterol which is very much specific to fungi and a measure of quantities of total fungal mass present in the seed) is measured on grain sample [44]. Studies on ergosterol concentration in mold susceptible and mold resistant sorghum at different stages of grain development revealed that its concentration increased with increasing days after flowering in the mold susceptible lines and was 10 fold higher in grains collected at 50 days after flowering than in corresponding mold resistant lines [45]. Ergo sterol quantity was found highly correlated to visual grain mold score [46].

Screening techniques

In a simple term 'screening' is a strategy or technique to identify or filter out a desired individual from a population. Screening for disease resistance is, therefore, a technique to identify disease resistant sources. The basic requirement of a screening programme is availability of a large number of variable germplasm, in which a desired resistance can be searched. The plant materials can be screened either in a greenhouse or in the field, depending on the requirement and availability of the facility. Screening under field conditions can be performed using artificial inoculation or natural inoculation. Humidity can be created by sprinkler or mist irrigation (Plate 8). Unlike field conditions, in a greenhouse, weather

Plate 8. Creation of humidity by overhead mist irrigation in grain mold screening block.

parameters (humidity, temperature, light intensity) can be regulated and hence, the results are more repeatable and reliable. Sound knowledge on biology and epidemiology of the disease, culture or strain of the pathogen, resistant and susceptible lines, inoculation method, artificial disease development techniques and disease rating scales are sentential. The basic principle is to provide adequate pathogen inoculum at the most susceptible growth stage of the crop under optimal environmental conditions for infection and disease development [47]. The known resistant line (check) score is used for comparison with the scores of other lines and the susceptible line score helps in deciding whether adequate disease pressure was prevalent. Screening techniques for grain mold have been developed and subsequently refined over the years.

Study on comparative evaluation of various **field screening** techniques for grain mold *viz*., inoculating panicles with grain mold fungi, bagging of panicles and providing overhead sprinkler irrigation on rain free days showed that mold resistance screening without inoculation and bagging of panicles was feasible if overhead sprinkler irrigation is used from flowering to harvest (for 54 days from flowering) [34]. However, when ambient relative humidity is low, sprinkler irrigation may not be effective in maintaining sufficient humidity for adequate grain mold development. Inoculation or non inoculation had little distinctive effect on the severity of the disease if adequate humidity was available [48]. A **laboratory-screening** technique for screening sorghum lines for resistance to *F. moniliforme, F. semetecturm* and *C. lunata* was developed [49]. The technique involved inoculation of seeds with the spores of grain mold fungi, transferring inoculated seeds into pre-sterilized Petri plates and incubation at 28 ^{o}C for 5-6 days in humidity chambers. This method is advantageous to screen photoperiod sensitive materials, which cannot be reliably screened using field-screening method. Few recent literatures have described detailed methodology of screening for grain molds [1, 47].

Resistant sources

In sorghum, most of the disease resistant sources come from the **primary gene pool** represented by the five basic races (*bicolor, guinea, caudatum, kafir* and *durra*) and their ten intermediate combinations. Numerous sorghum germplasm have been screened worldwide over the years for identification of resistant to grain mold and many resistant sources have been identified. These sources are mostly resistant or moderately resistant type and represent different gene pool. Absolute resistance against grain mold is rare mainly because of complexity of causal fungi and involvement of multiple resistance mechanisms. **Zera-zera** germplasm form Sudan and Ethiopia were used in India to develop high yielding grain mold tolerant lines. Up to beginning of eighties grain mold screening were mainly carried out under natural field conditions [36, 38, 50, 51, 52, 53]. The lines IS Nos 452, 455, 472, 2327, 14332, 2261, 9225, Funks 814, E35-1, E35-1, TNS 25 and TNS 28 were reported to be resistant to grain mold. A total of 7132 photoperiod sensitive germplasm lines that represented many sorghum growing regions of the world were screened with artificial inoculation at ICRISAT [34]. About 156 colored-grain lines had high levels of mold resistance. Of these, 14 mold resistant lines lacked testa, suggesting that that grain mold resistance without testa is possible. Later on many mold resistant sources have been identified in white grain lines devoid of testa layer [54, 55, 56]. Apart from germplasm, grain mold resistance has also been identified in many parental lines, segregating materials and recombinant inbred lines [43, 57, 58, 59, 60]. Some valuable sources of grain mold resistance have been listed in Table 2. It can be noted that till now, high level of resistance in white grain bold seeded lines has been a challenge. White grain **guinea** sorghum has been used in many breeding programme for improvement of mold resistance. It is a West African race producing hard corneous grains on open pendulous panicles.

Table 2. Sources of grain mold resistance

Screening conditions	Resistant sources	Remarks	References
Field	SC No. 0630, 0297, 0566	Resistant to *Fusarium* & *Curvularia*	[3]
	SC 103-11E, SC 650-11E, SC 748-5	Resistant	[61]
	Indian land races E 12, E 5, EJ 15	Resistant to Moderately resistant	[62]
	DSR-GMN No. 41, 42, 46, 52, 58, 59	Resistant to Moderately resistant	[60]
Field with Sprinkler	Guinea lines IS No. 7173, 23773, 23783, 34219	Immune	[63]
	Guinea lines IS No. 7326, 4963, 5726, 4011, 5292, 27761	Resistant to Moderately resistant	[63]
	B-lines ICSB 382, 384, 400, 321, IVSB 401; R-lines PVK 801, GD 65028, ICSR 93034, NSS 254, RSSV 106 resistant; ICSR 89058, ICSR 91011, IS 41675, GD 65055, ICSV 96105	Resistant to Moderately resistant	[58, 59]
	Fifty mini-core collections consisting of 5 basic races (*bicolor*, *guinea*, *caudatum*, *kafir*, *durra*) and 10 intermediate races	Resistant	[56]
	Sorghum accessions from Sudan PI No. 570011, 570027, 569992, 569882,	Resistant	[64]

	571312, 570759		
Field with artificial inoculation	Sorghum accessions from Uganda PI534117, PI576395, SC719-11E	Resistant	[65]
	GM RIL No. 25, 92, 98, 124, 203, 170, 83, 169	Resistant to Moderately resistant	[43]
	RIL No. 4, 166, 92, 118, 161, 172, 30	Resistant to *Fusarium* induced seed rotting	[6]
	Sorghum accessions from Burkina Faso PI No. 586182, 586186, 647705, 647706, 647707, 647708, 647710, 647712	.	[66]
Artificial inoculation	IS No. 625, 2821, 2825, 2867, 3547, 8545, 8614, 8763, 8848, 9353, 9487, 9498, 10301, 10892, 11227, 14332, 14375, 14380, 14384, 14387, 14388, 17141, 18759, 20620, 21454	Resistant to Moderately resistant	[34]
Laboratory	Photoperiod sensitive guinea lines IS No. 7326, 4963, 5726, 4011, 5292, 27761	Resistant to Moderately resistant	[49]
Laboratory and field	Converted Zera-zera selections IS No. 18758C-618-2, 18758C-618-3, 18758C-710-4, 18758C-710-5	Resistant	[67]

Plate 9. Grain mold resistant sources. a) White grain, b) Color grain.

Apart from small, hard grain structure its grain mold resistance properties probably stems from their continuous selection from high rainfall areas in Malawi and Tanzania. Hard seeded guinea sorghum lines like B58586, IS14375 and IS14387 can be used as good sources of grain mold resistance [46]. The resistant lines have been used for development of grain mold resistant sources (Plate 9). The limitation of using guinea lines is that the resistance has been difficult to transfer in an agronomical superior background.

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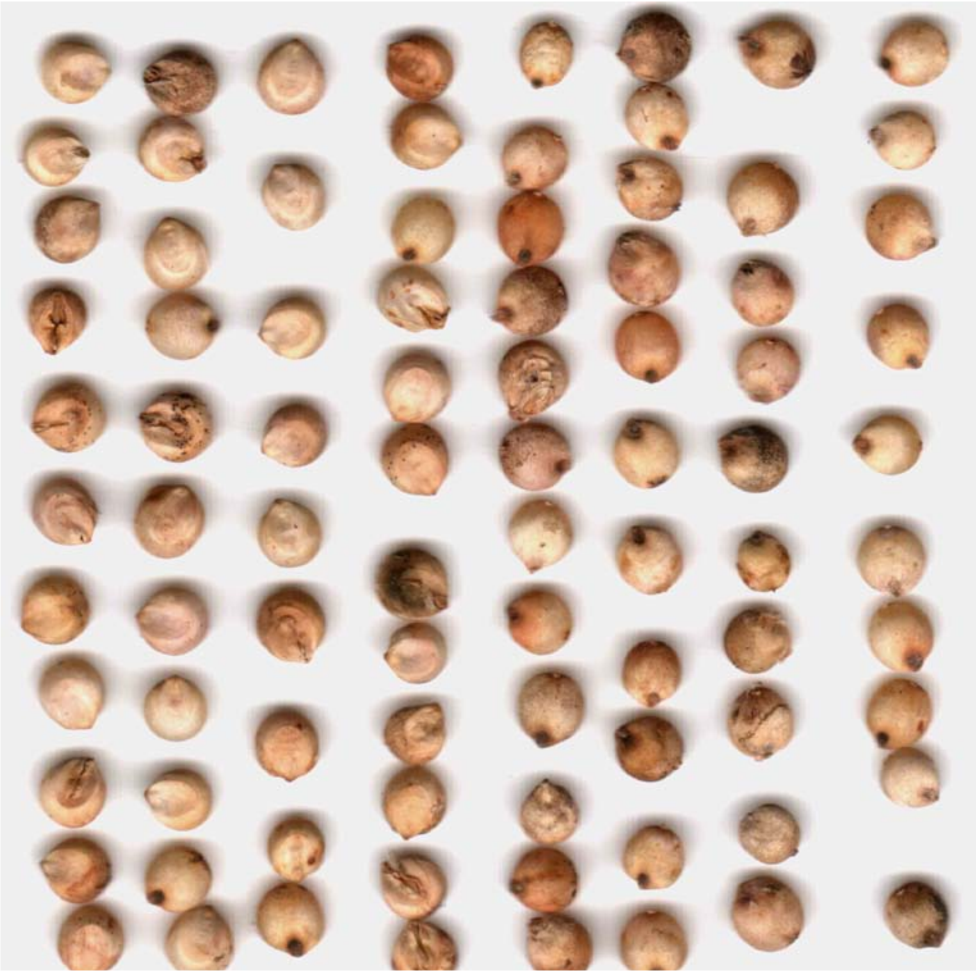

4. ECONOMIC SIGNIFICANCE

Grain mold is an immensely important disease of rainy season grain sorghum across the world causing both quantitative and qualitative losses [1]. The disease has severe effect on grain yield, quality, market value, seed-quality and ultimately on the grain-based end products [2]. It is difficult to estimate losses accurately since it involves steps starting from production to marketing and finally utilization of the grain or seed. A heavy rain at grain maturity stage affected 4 lakh ha of sorghum and caused a loss of US$ 46 million in Texas during 1976 [3]. The annual losses on a conservative scale were estimated to be US$ 130 million across the globe [4] and US$ 50–80 million in India [5].

Grain yield

Grain mold is one of the major constraints for sorghum cultivation. Production loss ranges from 30-100% depending on cultivar, and prevailing weather conditions during flowering to harvesting [6]. In case of highly susceptible varieties yield loss of up to 100% may be observed under favorable environmental conditions [7]. Early infection causes reduction in caryopsis formation, arrest of kernel development, decrease in grain mass and grain density [8]. All these are ultimately reflected in grain yield. Infection by the major grain mold fungi (*F. moniliforme* & *C. lunata*) is reported to interfere with carbohydrate translocation to developing kernels, and thus cause reduction in seed size and weight without visible mold development [9, 10]. Grain weight loss due to mold infection is often significant and varies from 40–70% [11, 12, 13].

Grain quality & marketability

Apart from yield gain quality is another important aspect, which is adversely affected due to grain mold. The disease reduces quality of grain and its acceptability in the market. During the process of post-maturity weathering, sorghum grains are colonized mostly by saprophytic fungi, causing discoloration and resultant reduction in market price [14, 15]. Molded grains with visible mold sign fetch in around 20% lower market price than normal grain [16]. Market price of grain was reported to reduce by 10 and 30% when it recorded grain mold score of 3 and 4, respectively (on a 1–5 scale) [17]. Moreover, grain mold fungi secrete enzymes that can degrade starch in endosperm and germ tissues [18]. *F. moniliforme* may stimulate synthesis of enzymes responsible for initiation of germination and the subsequent-breakdown of endosperm tissue. Regardless of the source, these enzymes reduce feed or food value of the grain. Other important qualities of sorghum grains like storage quality, food and feed processing quality, cooking quality, and nutritive value of food and feed are also affected in molded grains [19].

Seed quality

Seed quality is important for growing the crop. Various physiological and biochemical tests on seed give an insight about the biological quality of the grain and its value as seed. Fungus infected seeds often exhibits reduction in germination and emergence, which causes poor stands in fields. Seedlings coming out of molded seed may be killed after emergence, or their growth may be reduced [20]. Certain grain mold pathogens causes losses in seed mass [21, 22, 23], grain density [14], germination [24, 25] and seed viability [26]. Parameters determining seed quality decline with increasing temperature and relative humidity that support colonization and sporulation by the mold fungi [27]. *Fusarium* species have relatively more adverse effect on seed germination of sorghum than other fungi [28, 29]. **Path analysis** for seed germination reveals that premature seed rot (-0.43) and panicle mold score (-0.28) has maximum direct effect on seed germination, accompanied with less interference by other factors. Premature seed rot is the most important predicting parameter for determining seed germination in molded grains [30] (Fig. 1). There may be significant reduction of crude fat, starch and ash content in molded seeds [31]. Effects on seed quality vary among genotypes with white or colored grain. Decrease in test weight and seed germination in white cultivars is more than the red or brown ones [32]. However, mere discoloration of grain does not mean that it is always internally infected or there is loss in total seed quality. Involvement of *Fusarium* spp. is also suggested in pre-harvest sprouting of seed in the panicle, which leads to loss of seed weight, viability and chalkiness of grain [24, 33].

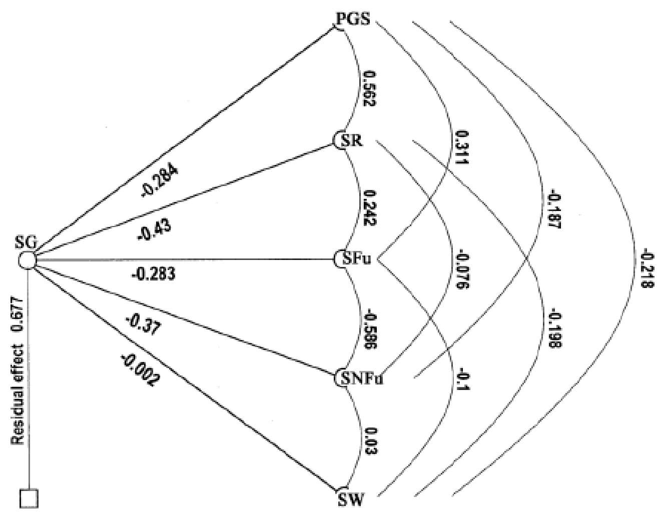

Fig. 1. Phenotypic path coefficient diagrams for seed germination (SG), panicle grain mold score (PGS), seed rot (SR), seed-borne *Fusarium* (SFu), seed-borne non-*Fusarium* (SNFu) and seed weight (SW) (Source: Das et al., 2012).

Mold and mycotoxins

Several fungi that grow on sorghum grain are able to elaborate toxins and other secondary metabolites, which makes sorghum grain unfit for human consumption as well as for cattle and poultry feed. Major grain mold associated fungi that can produce mycotoxins are the species of *Fusarium* and *Aspergillus*. *Fusarium* infection may not be always visible from outside. Apparently healthy looking grain often grows *Fusarium* colony on incubation (or in storage under humid conditions) (Plate 1). *F. moniliforme* and *Fusarium proliferatum* can produce a family of mycotoxins such as **Fumonisins** B_1, B_2, and B_3. Other *Fusarium* species belonging to Liseola section of the genus *Fusarium* are also reported to produce mycotoxins such as fumonisins, moniliformin, fusaproliferin, fusaric acid, fusarins, beauvericin, and gibberellic acids [34]. *Fusarium* isolates from different sorghum locations in India showed great variations in fumonisin B_1 elaboration, on artificially inoculated grains (range: 0-82143 µg/kg) [35] (Fig. 2). Around 32% (of total 65) isolates were non-toxigenic and ~6% were highly toxigenic (FB_1>5000 µg/kg). Morphological characters of *Fusarium* isolates and their relation with fumonisin B_1 production revealed that isolates with powdery−cottony growth and pink to yellow pigmentation generally were toxigenic (Plate 2). While isolates exhibiting dense growth and without pigmentation were non-toxigenic.

Plate 1. Healthy looking sorghum grain produce Fusarium colony on incubation.

Fumonisin B_1 contamination in field samples ranged from 5-1398 µg/kg grain and varied over locations and genotypes. Few genotypes showed toxins below safety limits. The extent of fumonisin contamination in sorghum samples collected from markets and households in sorghum-growing regions of Andhra Pradesh was reported to be higher in rain affected and moldy samples compared to that in normal samples [36]. The outbreak of fumonisin toxicity in the human population during October 1995 in parts of the Deccan Plateau in India was associated with unseasonal rains. Most of the crops that were harvested during that period became visibly moldy. Most of the mycotoxins are thermo-labile and they are not destroyed during industrial processes. Analysis of Lager beer samples for the presence of deoxynivalenol, fumonisin B1, zearalenone and aflatoxin B1 revealed that about 66–100% of the samples were contaminated with one or other mycotoxins [37].

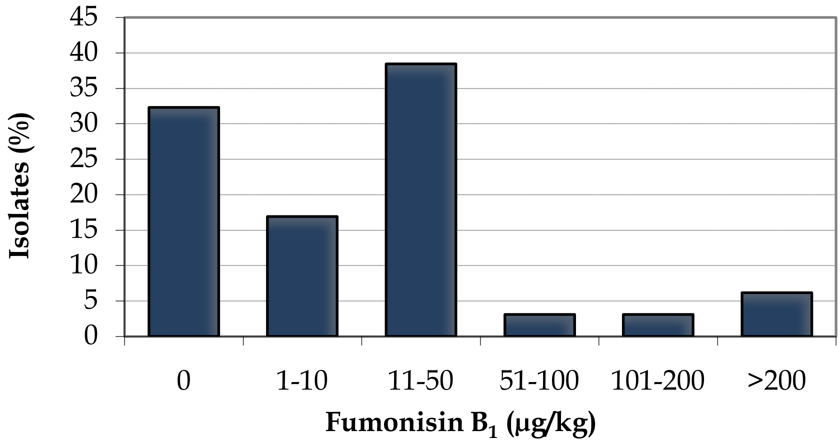

Fig. 2. Frequency of toxigenic and non-toxigenic isolates of *Fusarium* spp. in sorghum grain samples (Source: Das et al., 2010).

Plate 2. Fusarium associated with sorghum. A) Single spore colony, B) Growth on artificially inoculated sorghum grain, C) Colony characters of toxigenic *Fusarium* spp., and D) *Growth* on sorghum in field (Source: Das et al., 2010).

Apergillus contamination of sorghum grain is common in storage (Plate 3). *Aspergillus flavus* and *A. parasiticus* are two of the grain mold causing fungi that produce **aflatoxin**. They can extensively damage the starch granules in the endosperm (Plate 4). These fungi can be found virtually everywhere. Aflatoxin is a byproduct of mold growth in a wide range of commodities including sorghum. Aflatoxins (B_1, B_2, G_1, G_2), fumanosin B_1 and zearalenone are detected in sorghum based malt, wort and beer in Botswana [38, 39]. Recently a total of 1606 *kharif* sorghum grain samples collected during four years (2005–2008) from seven sorghum growing states were analyzed for aflatoxin B1 contamination. Around 73% of the

Plate 3 a) *Aspergillus* spp., associated with sorghum grain, b) Pure culture.

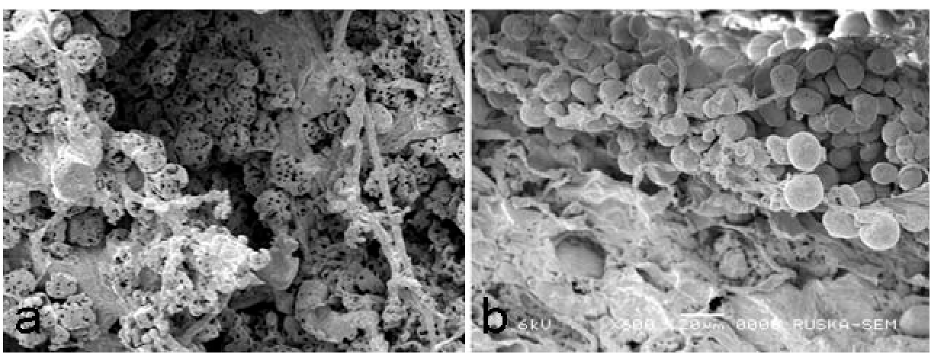

Plate 4. Scanning electron micrograph of sorghum grain showing (a) damaged, and (b) undamaged tissue and starch granules

samples were positive for the toxin and in ~3% samples level of toxins was above safety limit (20µg/kg) (Fig. 3) [40]. This suggests that mycotoxin contamination in *kharif* sorghum is low to medium and grain is mostly safe for consumption. Aflatoxin is considered to be the most potent, naturally occurring carcinogen. It has been linked to various health problems in both humans and animals. 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.

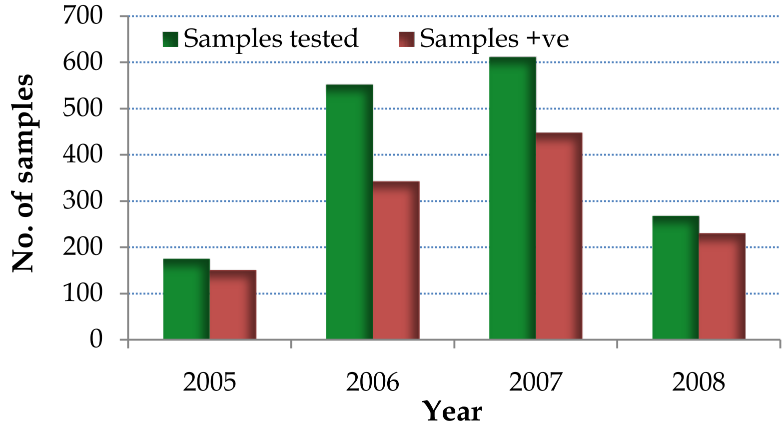

Fig. 3. Aflatoxin B_1 contamination of *Kharif* sorghum in India (Source: Adapted from Ratnavathi et al., 2012).

Impact on crop area

There is sharp decline in sorghum area in India since 1985 (Fig. 4) [41]. As area under winter (*rabi*) sorghum is more or less constant in India this decline is mainly due to reduction in *kharif* sorghum area [42]. Grain mold is a serious

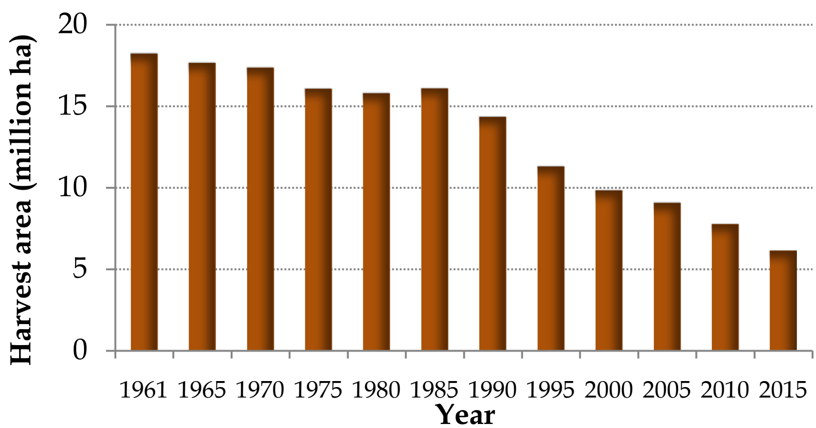

Fig. 4. Changes in sorghum area in India over time (Source: FAO STAT, 2017).

problem on *kharif* sorghum for which effective control measures under high disease pressure is not available. Therefore, the disease is considered to be one of the many reasons for decline in *kharif* sorghum area. Other important factor

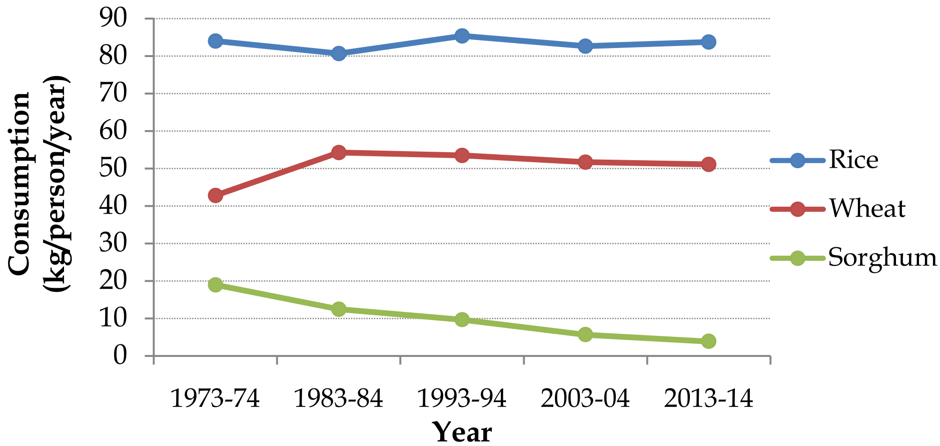

Fig. 5. Trends in direct consumption patterns of sorghum, wheat and rice in rural India during 1973 to 2014.

for reduction in *kharif* sorghum area is less demand for sorghum. Demand is the most vital driving force for the farmer to decide which crop to grow. Demand for sorghum has been declined over time due to a host of factors associated with reduced consumption (Fig 5). The major one is easy availability of fine cereals like rice and wheat at a cheaper rate under public distribution system. Other factors like tedious and time consuming preparation process, rapid urbanization, rising in capita income, change in food habit particularly preference to fast food by younger generations, and preference to cash payment by laborers over food grain in kind [43].

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5. MECHANISMS OF RESISTANCE

Knowledge of disease resistance mechanisms operating in a plant is essential for selection of useful traits in a disease resistance breeding programme. When the number of mechanism is less and each contributing significantly on resistance it becomes relatively easy for the breeder for selection of important traits and *vice-a-versa*. Unfortunately, for sorghum grain mold mechanisms governing resistance are quite high with each contributing meagerly. Mechanisms of grain mold resistance are somewhat different for pathogen induced mold (grain mold) and saprophyte induced mold (weathering). Accordingly, available information on mechanisms of resistance targeted either towards grain mold or grain weathering. Unlike other plant diseases, in which mechanisms of resistance operating toward prevention of infection or subsequent colonization inside the host are targeted, grain mold resistance mechanisms are chiefly concentrated the grain and it physical, chemical and biochemical properties along with plant, panicle and spikelet characters. Not much literature is available that targeted initial infection at the time of anthesis. Mechanisms of mold resistance against early infection are meager especially in white grain sorghum. Relative importance of the resistance mechanisms in relation to grain type and mold development stages are summarized in Table 1.

Table 1. Mechanisms of mold resistance in color and white grain sorghum.

Mechanisms of resistance	Brown & red grain		White grain		Related references[b]
	Early infection	Weather ing	Early infection	Weather ing	
Panicle compactness	nr	+[a]	nr	+[a]	[6, 7, 8, 9]
Glume cover	nr	+[a]	nr	+[a]	[6, 7, 8, 9]
Glume pigmentation	nr	+	nr	+	[9]
Grain hardness	nr	+++	nr	+++	[9, 10]
Polyphenols (tannins)	+	+++	nr	nr	[8, 11, 12]
Flavonoids (flavan-4-ols)	+	++	nr	nr	[13]
AFP (chitinases, glucanases, sormatin, PR-10, RIPs)	++	+	++	+	[1, 2, 3, 4, 5]

Importance: low (+), medium (++) and high (+++). [a] Conflicting reports regarding role in resistance. 'nr' indicates that the role is unknown or insignificant. [b] Includes references that give some indications or provide direct or indirect information for determining role. (Source: Das et al., 2012)

Plant, panicle and floral characters

Duration and plant height

Effects plant characters such as plant traits (height, duration), panicle structure (compactness), and floral traits (glum cover, length) on grain mold resistance have been explored quite extensively. Long duration photo period sensitive sorghum is known for mold resistance due to escape mechanism that enables such lines to avoid mold development. Traditional Indian cultivars that were cultivated before seventies are examples where such mechanisms were in operation. Little variation in days to flowering (or maturity duration), however, may not work for avoidance of mold as there will be chance of being caught in rain. That might be the reasons why many studies did not find a strong correlation between days to flowering and grain mold severity [8, 30]. Plant height may have some role in mold resistance. Compared to dwarf plant the taller one is more flexible, which allow quick movement and rapid drying of panicles in air and thus reduce duration of grain surface wetness. It is observed that taller plants generally develop fewer molds than shorter one under the same microclimate in the field. However, the difference may not be significant especially under high disease pressure (e.g. artificial inoculation, continuous rain or frequent sprinkler irrigation) [28, 29].

Panicle structure

Panicle structure (loose, semi-compact, compact) determines the microclimate around seed. It may not play role in determining early infection on the floret but can influence post-infection colonization. Loose panicle generally dries quicker after rain than a compact one and thus influence mold development. It was observed that in 36 recombinant inbred lines panicle compactness was significantly (p<0.001) associated with premature seed rot [29]. However, all cultivars with loose panicles are not resistant to mold (e.g, BulkY: loose panicle but mold susceptible) and *vice-a-versa* (e.g., SVD9601). This indicates that the reason for resistance/ susceptibility associated with panicle structure may be more related to the physical factors (microclimate) than genetic. There are conflicting reports on role of panicle compactness on mold resistance [6, 7, 8, 9].

Glume cover and color

Physical and chemical properties of glumes play role in providing resistance against grain mold. The glume appears to be the first defense against fungal invasion, later it is the chemical and physical properties of cgrain that appear to be more important in imparting resistance to grain molds [15]. Negative correlation of grain mold incidence with glume cover (r=-0.56), glume length and glume area was reported. Seeds completely enclosed in long papery glumes exhibited high level of resistance to grain molds. There are also conflicting reports about the role of glume cover on mold resistance [6, 7, 8, 9]. Glume color showed a strong association with mold resistance [25, 27]. Wide variations are available in glume color of white grain lines (Plate 1). Generally darker glume provides better mold resistance. It may be possible to enhance grain mold resistance in white-grained sorghum by incorporating colored-glume character.

Plate 1. Variations in glume color of white grain sorghum lines.

Grain characters

Antifungal proteins

Preformed plant **defense proteins** like chitinase, β-glucanase, sormatin and ribosome-inactivating proteins (RIPs) play role in defense against early infection by mold pathogen. The hydrolytic enzymes such as chitinase and β-glucanase have been shown to be 'upregulated' after treatment with fungal elicitor or when they were infected [1]. Sorghum proteins, sormatins are closely related to the thaumatin-like proteins which can turn-on incompatible (resistance) interactions [2]. They act by permeabilizing fungal membranes and may work in concert with the hydrolytic enzymes [3]. Levels of chitinases, β-1, 3-glucanases, sormatins, and PR-10 are reported to be more in resistant cultivar than in susceptible when challenged with mold pathogen [4, 5]. Their levels increase during seed development till physiological maturity and then reduced substantially [4]. With information from many literatures it can be said that a link between antifungal proteins and grain mold resistance is now established. It can be possible to select lines with high levels of these proteins in the developing, mature and infected grain by direct analysis or by using antibodies or molecular markers.

Pericarp properties

Pericarp plays important role in mold resistance. The sorghum kernel is composed of three parts, the pericarp (outer covering), the endosperm (storage tissue), and the embryo. The pericarp can be subdivided into the epicarp, mesocarp, and the innermost endocarp. **Waxy coating** outside the pericarp prevents fungi and the water to enter grain, thus provide an important barrier for mold resistant. **Free phenolic** compounds in the pericarp inhibits fungal invasion on immature grain and play vital role against mold. Their levels are significantly more in immature than mature grain in resistant cultivar. Resistant cultivars respond more quickly to fungal invasion than susceptible one via increased levels of phenolic compounds in glume tissues. Extensive deterioration of the grain does not occur before physiological maturity because the developing grain contains 3–10 times more specific phenolic compounds than the mature grain [15]. The phenolics, however, may not have role in grain weathering as mold susceptible cultivars were found to contain more of them than the resistant one in mature grains.

Color of the grain depends on the pigmentation in pericarp. Red pericarp contains secondary metabolites like **flavan-4-ols** that confers resistance to grain weathering to some extent. Concentration of flavan-4-ols in mold resistant sorghum was found to be at least 2-times higher than the susceptible [13]. However, red pericarp alone is not good enough for mold resistance and many lines with red pericarp are found susceptible. Co-presence of red pericarp (flavan-4-ols) and pigmented testa (tannin or flavan-3-ols) provide additive effects on mold resistance. The associations of flavan-4-ols and tannins with mold resistance have been demonstrated in cultivars with color pericarp and with pigmented testa [16].

The mesocarp (the middle layer of the pericarp) plays important role for grain mold resistance. This layer may be thick (contains more number of small starch granules) or thin (less number of small starch granules). Grains with thin mesocarp exhibit more resistant to weathering than thick one [17]. This may be related to initial water absorption rate (**water imbibitions rate**), which is relatively more when mesocarp is thick. Water enters the cross and tube cells of the pericarp, and rapidly moves around the grain. There is variation in rate of water uptake among sorghum genotypes, especially during initial two

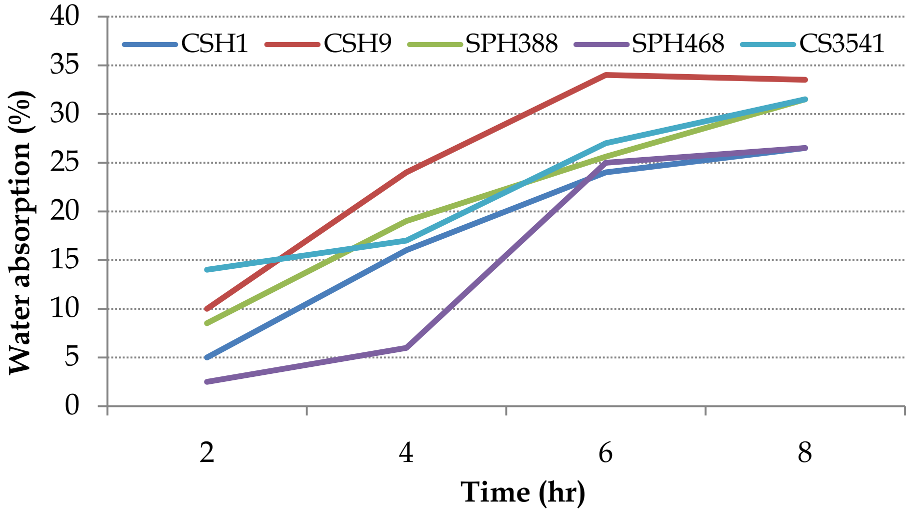

Fig. 1. Genotypic variation in water absorption rate at 30 °C, (Source: Adapted from Somani and Indira, 2000).

hours. The initial absorption rate is slower in mold resistant than susceptible genotypes (Fig 1). In fact, low water absorption capacity (when soaked for a period of 2 hours) was used as a criterion for selection of sorghum genotypes against grain deterioration [18]. Like initial rate, total water absorption (in 20 hr) is also less in mold resistant (0.80–0.99% of grain weight) than susceptible (1.61–3.08%) genotypes [19].

Testa layer

Some sorghum grains have a highly pigmented layer just beneath the pericarp, called testa (or subcoat). The pigmentation of testa is due to presence of high concentration of **polyphenols or tannins** (flavan-3-ols) [17]. Tannins possess anti-microbial properties. A pigmented testa containing condensed tannins is the most important trait that confers grain mold resistance [12] in some color sorghums. White grain sorghum that is cultivated in India and many African countries do not possess testa layer in grain. Therefore, this property is of no use for developing grain mold resistance in white-grain sorghum. Moreover, tannins are not good for health as they are known to reduce dry-matter digestibility by binding proteins and possibly making complex with digestive enzymes. The high-tannin sorghums are known for bird-resistance but have poor feed efficiency to livestock.

Endosperm structure

Sorghum **endosperm** consists of two distinct portions- the peripheral, extremely dense, corneous endosperm and beneath it the air-space filled floury portions. The corneous endosperm shows rather more resistance to weathering than floury because of the denser structure and organization in the former [17]. Water and fungi are not able to proceed as readily through a denser structure than a loose, floury endosperm. Corneous endosperm provides hardness to the grain. **Grain hardness** and density has direct relationship with mold resistance and harder the grain and more is the resistance [8, 9, 10, 20, 21, 22]. Genotypic variations are available for this character [19] that can be utilized for developing mold resistant cultivar (Fig. 2). Since grain mold develops under wet conditions, a key factor to consider is the ability to retain hardness by mold-resistant grain [23]. It has been suggested that for genotypes to be mold resistant, their grains must be hard, have high grain density with a corneous endosperm. Hard grains contain high amount of storage protein **kafirin**, which comes under a group of plant storage proteins 'prolamins'. Prolamin (name is because they content a high amount of the amino acid 'proline') is found in many cereals and the sorghum prolamin is called kafirin. Kafirin is one of the most hydrophobic proteins and has antifungal properties. In most circumstances, a high degree of hardness is not compatible with grain characters required for food quality [9]. An appropriate level of grain hardness may be required in grains without compromising on its food-making properties. Recently kafirin is being considered as viable alternate source of protein. Although, kafirin is noted for its slow digestibility it does not trigger any adverse response when consumed by celiacs. These properties make kafirin potentially valuable in both food and non-food applications, especially as a bioplastic and encapsulating agent [31]. Also, efforts are on to improve the functional and nutritional properties of kafirin as a functional food ingredient. For white grain types, grain hardness is the most durable resistance mechanism [24]. Resistance to pre-harvest sprouting is likely to be another factor that helps grains to retain hardness under wet conditions.

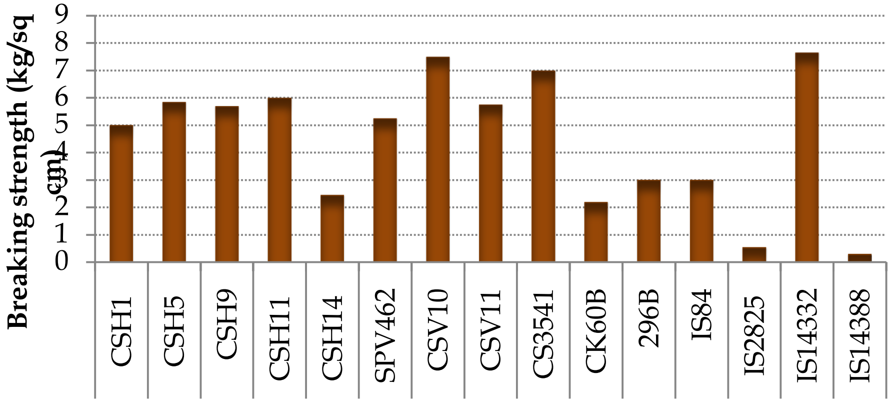

Fig. 2. Variations in grain hardness in sorghum hybrid, variety and parents.

It can be summarized that resistance to grain mold is conferred by a range of physical and chemical properties of plant and grains that include hard grain, loose panicle, increased glum coverage, glum color, pigmented testa, red pericarp, phenolic compound, high levels of condensed tannin, phenolic acids and flavan-4-ols. How they can be incorporated into a single mold resistant cultivar is real challenge.

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6. GENETICS OF RESISTANCE

Grain mold development is governed by many traits, few of which may be qualitatively inherited, but their individual contribution to total grain mold resistance generally account for only a small portion of the total variation. Therefore, many genes might be involved in grain mold resistance.

Genetics of mold resistance

Grain mold resistance is a complex problem as it is caused by many fungi and governed by many traits [1]. Various studies on grain mold resulted in identification of three different mechanisms of resistance governed by i) morphological characters (open panicle structure, seed hardness, corneous endosperm, pigmented testa, and red pericarp), ii) secondary metabolites and their distribution and quantity (flavan-4-ol content), and iii) antifungal proteins present in the seed endosperm and their type and quantity [2]. Grain mold resistant is polygenic in nature [3] and there may be involvement of a minimum of four to ten genes [4]. There are major and minor genes, additive and epistatic effects with significant G × E interactions [5, 6].

In **color grain** sorghum mold resistance is governed by dominance and epistatic interactions [6]. The genetic control of mold was studied in seven crosses of two resistant genotypes, where P_1, P_2, F_1, F_2, BC_1 and BC_2 families of each cross were evaluated under field conditions and subjected to generation mean analysis. The study showed that resistance was dominant character and was controlled by two or three major genes. Additive gene effects were found in all seasons. Significant dominance effects of similar magnitude of additive effects were observed in the crosses [6]. The generation means analysis of a cross between Sureno (a dual purpose mold resistant variety) and RTx430 (an inbred line, highly susceptible to mold) revealed significant differences in mold incidence between the generations in all the eight environments and involvement of a minimum of four to ten genes [4]. A diallele study involving crosses between resistant and susceptible lines under artificial inoculation indicated that additive gene action was predominant in the inheritance of resistance to *Fusarium* molds [7]. In *Curvularia* mold both additive and non-additive components of variance determined the expression of reaction [8]. Generation mean analysis of resistance to *Curvularia* and

Fusarium, indicated large dominance effects and significant epistatic effects. Additive and additive × additive effects were also present but only next in importance to the dominance effects [9, 10]. Studies with F_1, F_2, BC_1 and BC_2 crosses between susceptible and resistant lines revealed that mold inheritance was governed by four independently assorting genes- two with complementary interactions and the other two additive interactions [11]. Studies on genetics of caryopsis associated with mold resistance suggested that R-Y- pericarp color and I (intensifier) genes conferred dominant resistance individually and their effecter were additive when present together [12]. The complex genetics of mold resistance is due to the presence of different mechanisms of inheritance from various sources.

Grain mold resistance in the **white-grain** sorghum is controlled by polygenes with significant additive × additive gene interactions and G x E interactions [4, 13]. There were significant genotype x environment (linear) interaction and significant environmental effects for grain mold resistance at physiological maturity, while at harvest maturity only environmental effects were significant [14]. It was concluded that grain mold occurring before physiological maturity is influenced by genetics and to some extent by environment while that occurring after physiological maturity is influenced completely by environment. That may be the reasons why breeding for grain mold resistance by pedigree method for last three decade has not paid much dividend [15]. Due to variation in the casual pathogen from location to location, resistance to grain mold should be evaluated in target environments [13]. Evaluation of segregating population for resistance and selection for stable derivatives in advanced generations in different environments could be effective [16].

Genetics of associated characters

The expression of open glume character is dominant and that of closed glume is recessives [17]. Three pairs of genes were involved in gaping glumes, which was dominant over normal glumes [18]. In F_2 generation, the segregation for glume color was found to be 3:1 indicating a single gene difference, reddish purple glume being dominant over black purple [19, 20]. Digenic segregation with interaction was found when black glume line was crossed with straw glume line [21]. Trigenic segregation was found in crosses between varieties with deep and light purple glume. The F_2 ratio was 45:19, with deep purple color dominant over light purple color glumes [22]. Seed color was found governed by single dominant gene when red seed was crossed with white seed [23]. Two genes were involved in the inheritance of phenol content (catechin equivalents) [24]. Genes at loci 7 were responsible for different characters affecting caryopsis traits R, Y, I, Z, B1, B2 and S genes [25, 26]. A generation means analysis of 27B × B58586 was taken up for sorghum grain hardness, indicating polygenic nature of inheritance of grain hardness. This also showed additive gene effects and additive and additive gene interactions [27].

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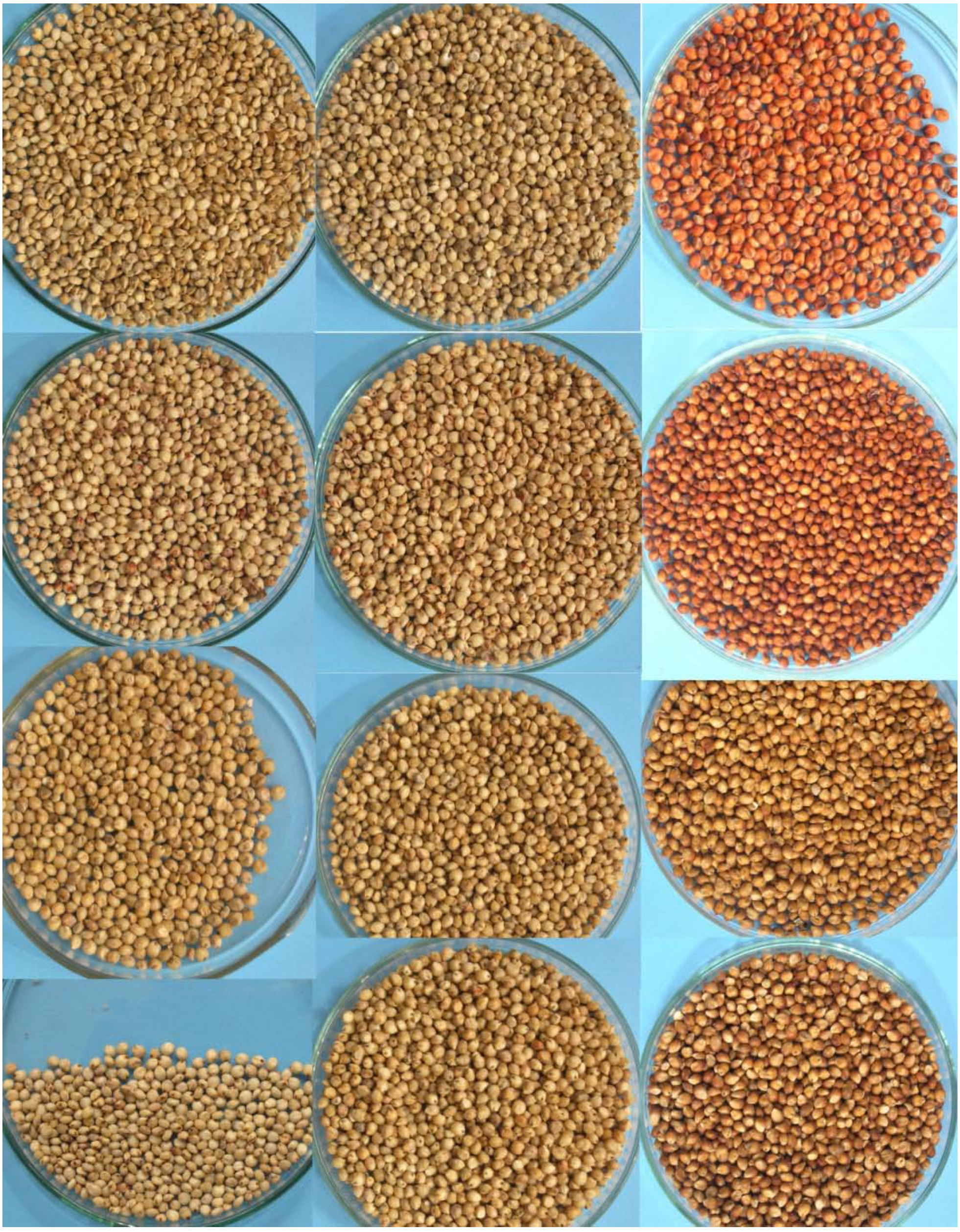

7. BREEDING FOR RESISTANCE

The infection caused by mold in *kharif* sorghum grain deteriorates the grain physically as well as chemically causing reduction in grain size, blackening and making them unfit for human consumption. Once the grain quality is improved the demand for *kharif* sorghum will increase for food, feed and starch industries. Hence, any improvement in grain quality cannot be separated from improvement for grain mold resistance which benefits the farmers with premium price. In view of the seriousness of the grain mold problem, it is necessary to develop new varieties and hybrids with mold resistance. This is the most effective means of managing the damages, as it does not involve extra input cost by the farmers.

Conventional breeding

Materials and strategies

Initially derivatives of **Zera-zera germplasm** form Sudan and Ethiopia were used extensively in breeding programmes in India to produce high yielding mold tolerant progenies. These progenies were better than high yielding elite lines at low disease pressure. A total of 370 parents including Zera-zera and elite lines were utilized to develop grain mold resistant lines [1]. The F_2 population was screened under natural infestation and F_3/F_4 under artificial inoculation to obtain lines that are less susceptible to *Curvularia* and *Fusarium*. It was further reported that increase of level of resistance could be achieved by selective inter-mating of resistant progenies in specific crosses or recurrent selection in random mating populations. However, under moderate to high mold pressure, these lines became susceptible [2]. A **random mating** population involving 24 experimental or partially converted restorer or maintainer lines chosen for their resistant to *Fusarium* head blight was screened at various locations and the sources of agronomically desirable B and R lines resistant to *F. moniliforme, F. semitectum* and *F. roseum* were developed. Efforts were made to develop grain mold resistant sorghum lines by improving B lines in white grain background, which can be incorporated in other agronomically superior entries [3, 4]. Breeding efforts were made at Indian Institute of Millets Research (IIMR) on developing white grain sorghum lines with high level of resistance to mold complex [5, 6]. Since A_1 based cytoplasmic lines are widely used, to increase genetic diversity, A_2 based cytoplasms as an alternate source of resistance has been advocated [7, 8]. This concept has also been utilized at IIMR, Hyderabad in the years 2004 and 2005.

Superior lines with high yield and resistance to grain mold were identified from crosses involving elite grain mold susceptible lines with resistance lines, and subsequent selfing generations [9]. **Hybrid breeding** is also encouraged for grain mold resistance in order to obtain high yielding, uniform and stable genotypes across environments [10]. Studies were carried out on 168 hybrids and their parents across four environments in India in order to identify the good combiners for a breeding program aimed at improving grain mold resistance [11]. Similarly, studies with ten divergent parents and their forty-five F1 hybrids were taken up with the aim to study the inheritance of some mold resistance traits and identify useful parents for an efficient breeding scheme. Studies with twenty-five sorghum hybrids in seven locations in Brazil helped to identify some hybrids combining adaptability and stability across locations [10]. Mold resistant hybrids (Partners NK6638, Asgrow A571, AN600 RN610 and Pioneer 83G19) were identified in Senegal, West Africa [12, 13]. High yielding hybrids with resistance to grain molds were identified by evaluating them over five years across four locations in the Central and South-East regions of Senegal [14]. They observed significant effects of year, genotype and their interaction on grain mold resistance and yield.

Different breeding strategies that are being following at present at IIMR to tackle the grain mold problem involves

- The *caudatum* and *kafir* races have been exploited well in all the Indian sorghum improvement programs. *Guinea* race germplasm which has genes for high yields (heavy panicles), grain quality and grain mold resistance could not be exploited so far because of their high photosensitive nature. Efforts are initiated to exploit the yield and grain mold genes from *guinea* germplasm by taking up rigorous crossing and back crossing programs.

- So far in all the Indian programs, research is towards the development of white grained sorghum. But keeping in view the potential of red sorghums in the feed industry and also their relative high level of resistance to grain molds, the *kharif* sorghum improvement program is targeting the development of high yield red sorghum cultivars.

- Grain mold problem is being handled using population improvement program also which was initiated during early 2000. Now a hand full of lines which are very early (maturing in <100 days) along with better level of resistance to grain molds are being evaluated in multilocation trials.

Approaches

Different conventional breeding approaches were followed to tackle the problem of grain molds. Mainly pedigree breeding was used making crosses involving resistant sources and selections in the segregating populations. However, major breeding efforts during the last three decades to develop grain mold resistance in high yielding genotypes have not paid many dividends. Success has been limited because of many mechanisms governing resistance, complex genetics and high environmental influence [15]. This hampers the progress and we expect only marginal increases in the levels of resistance through conventional breeding methods. The grain mold disease occurs in two phases: grain mold that occurs before physiological maturity (PM) caused by pathogenic fungi, and grain weathering that occurs after PM caused by pathogens and saprophytes. Pathogens infect and develop grain mold whenever environmental conditions are favorable. However, most of the infections take place at flowering. *Fusarium* and *Curvularia* are responsible for major damage (around 60%) in India [16]. Regarding mechanisms of resistance against saprophytic molds in white-grain cultivars that are used for food in India, present understanding is that grain hardness is the only indicator that contributes to resistance. Occurrence of saprophytic mold was entirely governed by environment whereas that of pathogenic mold was partly controlled by host resistance & partly by environment [15]. It is logical to believe that resistance against pathogenic fungi is more under genetic control than that against saprophytes. Expression of defense genes in sorghum increased rapidly following inoculation with mold pathogen. Real time PCR showed that mRNA encoding PR-10 was up-regulated in glumes & spikelets of resistant cultivars [17].

At IIMR, **population breeding** approach was initiated to tackle grain molds during 2000 and two populations were targeted, one for female parent (B line) development and another for male parent (R line) development. There is an improvement of grain mold incidence over 3 cycles of random mating (Fig. 1). Improvement to the tune of grain mold score 7 in cycle I and score of 6.4 in cycle II was realized in B lines from the base score of 9.0. Similarly improvement of 6.5 score in cycle I and 5.4 in cycle II was realized over the base score of 8 in case of R lines. The genotypes were stabilized and a trial on grain mold resistance was taken up at four centers with 165 genotypes derived from population breeding during *kharif* 17.

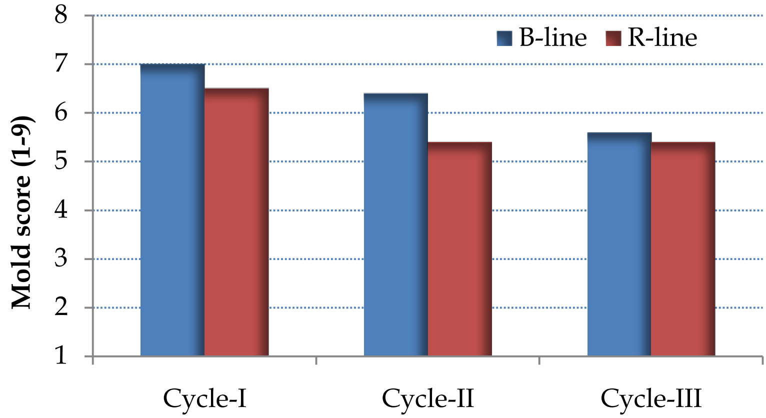

Fig. 1. Improvement of grain mold resistance over random mating cycles.

Out of these, 33 genotypes which performed well across locations were tested with resistant (B58586) and susceptible (296B) checks across four locations (Parbhani, Akola, Dharwad and Hyderabad). Ten genotypes were found to have either PGS or TGS less than 3.5 score. Panicle shape ranges from loose to semi-compact and the glume color also varies. Of these, GM 4-18, GM8-18 and GM 33-18 are more promising and on par with resistant check for grain mold score (Table 1).

Table 1. Promising derivatives of population breeding for grain mold resistance

Entry	TGS	DF	100 seed wt	Plant ht (cm)	Panicle shape	Glume cover	Glume color
GM 013	4.03	70	1.91	146	SC	1/4	Br
GM 017	4.47	66	2.77	250	SL	3/4	L red
GM 032	4.56	73	2.58	187	SC	1/4	Red
GM 036	5.23	68	2.98	261	L	>1/2	Red
GM 077	4.75	72	2.64	200	SC	1/4	Red
GM 078	4.75	78	2.52	299	L	1/2	Br
GM 094	3.85	71	2.36	244	SC	1/2	Br
GM 112	3.95	60	2.43	168	SL	1/2	Br
GM 146	4.65	69	2.29	154	SL	1/2	Br
296 B	8.48	72	2.58	134	SC	1/4	Straw
B 58586	4.42	73	1.91	299	Lax	Full	L br
CD (5%)	2.81	5	0.49	32	-	-	-
CV (%)	27.8	4.5	15.1	13.6			

Limitations

Conventional breeding has its own limitations. Progress and limitations of this method have been outlined in literature [4, 18]. This method mainly exploited grain hardness trait in white-grained sorghum for improvement of resistance. 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.

Molecular breeding

Since grain mold resistance is a very complex trait involving many resistance mechanisms with different genetic background, tackling this problem through conventional breeding approach is difficult. A trait which is associated with many unwanted characters, having complex genetics and environmental influence call for biotechnological intervention to resolve the problems. The advent of new biotechnological tools like quantitative trait loci (**QTL**) analysis and marker assisted selection (MAS) provide new opportunities for enhancing grain mold resistance [19]. Identification of QTL for such complex traits would improve the selection efficiency of the breeder. Development of molecular markers helps in selection of resistance and facilitates dissection of various aspects of resistance (e.g., 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). The QTL for grain mold incidence was dependent on environment, which is consistent with heritability estimates that show strong environmental and genotype–environment effects. Selection in specific environments is useful but it may not provide resistance across a wide range of environments [20]. Simple sequence repeat (**SSR**) markers linked to grain mold resistance QTLs provide a new approach for selection of resistant plants from segregating populations and provide a fast, cheap, and accurate method for tagging QTLs with agronomic importance in breeding programme.

Not much work has been done on identification of QTL for grain mold resistance. In a diversity study on 92 sorghum lines, comprising of 74 grain mold resistant and 18 susceptible lines, using 46 SSR markers it was reported that 12% of total observed variation was accounted for between grain mold resistant and susceptible types [21]. Secondary metabolites and plant defence proteins may play a greater role in plant defence against early infection. Levels of some antifungal proteins are more in resistant cultivar than in susceptible when challenged with pathogenic mold fungi [22, 23]. Grain mold incidence was observed to be influenced by five QTL, each accounting for the phenotypic variance between 10 and 23% [24]. The effects and relative positions of QTL for grain mold resistance were in accordance with the QTL distribution of several agronomic traits correlated with grain-mold incidence. Several genomic regions affected multiple traits including the one that affected grain mold incidence, plant height, panicle peduncle length, and grain-milling hardness, and others that influenced grain mold and plant height. Collectively, QTL detected in the population explained between 10% and 23% of the phenotypic variance. Grain mold QTL on LG7 near to Xtxp295 was consistently identified explaining 20% of phenotypic variation. In a recent study, two **SNP** loci linked to grain mold resistance have been identified using an association-mapping panel of 242 mini-core sorghum genotypes [25]. Among these, one contained a NB-ARCLRR class of R gene (Sb02g004900) that shares 37% identity and 57% similarity to the non-host resistance gene of maize, *Rxo1*. However, the map positions of the SNP markers did not overlap with the grain mold QTL detected by other researchers [24]. This could possibly be due to the differences in the pathogen among various environments causing differences in resistance expression [26]. Much of the identified QTLs were associated with traits that modulate resistance indirectly and major effect QTLs or genes with direct effect have not been identified. The complex nature of the fungal species involved, limitations in phenotyping methods, quantitative nature of genetic resistance, and signficant impact of environmental factors on the disease are among the major reasons for limited success in identification of major-effect grain mold resistant loci.

Study on genome wide association using 1425 diverse Ethiopian landraces of sorghum identified a major grain mold resistance locus containing tightly linked and sequence related MYB transcription factor genes [27]. The locus contains yellow seed1 (Y1), a likely non-functional pseudo gene (Y2), and yellow seed3 (Y3). SNPs and other sequence polymorphisms that alter the Y1 and Y3 genes correlated with susceptibility to grain mold and provided strong genetic evidence. They reported that the expression of both Y1 and Y3 genes in the developing grain and glumes of a widely known susceptible sorghum line, RTx430, were severely reduced but significantly increased in the resistant line, RTx2911. In addition, the expression of flavonoid biosynthesis genes such as Dihydroflavonol-4-reductase 3 (DFR3) was significantly induced in the resistant line in response to inoculation by a mixture of spores from different molding fungi while the susceptible line displayed reduced expression. The data suggest that the MYB genes and their grain and glume specific expressions may determine the differential regulation of the flavonoid biosynthesis pathway genes, the synthesis of 3-Deoxyanthocynidins and ultimately responses to molding fungi.

In an effort to identify quantitative loci (QTL) for grain mold resistance, IIMR has developed a RIL population of the cross between '296 B' (grain mold susceptible elite parent) and 'B 58586' (grain mold resistant parent). About 200 RILs along with the parents were characterized in six environments over three years (3 years x 2 locations) for grain mold resistance [15]. On an average the susceptible (296B) and resistant (B58586) parents recorded grain mold scores of 6.6 and 2.7 respectively at physiological maturity. The grain mold score in RILs varied from 2.6 to 6.6. Efforts are on to identify stable QTL for grain mold resistance and associated traits, which would help in breeding for grain mold resistant elite parental lines.

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8. MANAGEMENT

Available options for management of gain mold are reasonably limited. Benefit cost ratio is the determining and driving force for taking up any disease control measure in agriculture. For grain mold of sorghum this ratio is mostly in disadvantage due to low return from the crop. As the disease started becoming prominent during the seventies and turning to be the number one problem of *kharif* sorghum later on, research for its management intensified. Many technologies and strategies have been developed during last 40 years, which is discussed here.

Utilization of disease escape mechanism

Disease escape may be considered as conditions or situations that help crop to avoid being diseased because of separation, in space or time, of susceptible host, infective pathogen and favorable weather. This avoidance principle provides a powerful tool for management of disease. Long duration (150-180 days) traditional Indian cultivars that were being grown by the farmers before the introduction of short duration high yielding composite sorghum cultivars were escaping grain mold since time immemorial, because their flowering and grain filling were happening during rain free period. Present day cultivars take less time and mature in 100-120 days, a duration which can fit suitably in the cropping system and also does not expose the crop to terminal drought brought about by early recession of rain. Present window of sowing *kharif* sorghum in India (1st June to 30th July), is found to increase the incidence of molds, because flowering and grain filling take place during August-September, two high rainfall months in India. Two options can be there for disease escape to occur- either preponing or postponing the sowing time. Postponing sowing to August will delay the harvest and subsequent sowing of the rabi crop. Late planting also increases the incidence of shoot fly [1]. In case of preponing, sowing is to be done by mid-February (so that crop matures by mid-June, before onset of monsoon), which will be hardly possible as sowing cannot be taken up due to lack of rain (if irrigation is available farmers generally do not go for sorghum and prefer to use that water to grow cash crop). In fact, seed production of *kharif* sorghum is taken up with irrigation during February-May window in non-traditional *kharif* areas, which not only produce clean grain but also improve yield. However, how far this window can be practicable in traditional *kharif* sorghum belt remains another question. Manipulating sowing date based on input from highly precise advanced weather forecasting model may be thought of especially for high input intensive agriculture like in developed countries. However, such approach that needs support of the institutional infrastructure may not be successful in developing countries [2].

Harvesting at physiological maturity and drying

Grain mold development is a continuous process that starts at flowering and reached its peak just after physiological maturity of grain if weather conditions are favorable. Delayed harvesting often increases mold incidence due to prolong wet weather conditions [3]. Therefore, harvesting as soon as crop reaches physiological maturity (black layer formation at hilar end) is of utmost importance. However, such grain must be dried immediately to reduce moisture level from ~18 to ~12%. The infection in the field is carried over to storage and rapidly spoil the grains if moisture level in not maintained at desired level (~12%). To tackle the problem of grain weathering, the concept of community dryer was introduced, where grains can be artificially dried immediately after harvest at physiological maturity (Plate 1). Working of low-cost community driers was demonstrated under National Agricultural Innovation Project (NAIP) in some districts in Maharashtra (Parbhani and Akola) and Karnataka (Dharwad). The drying capacity is around 1.5 tons per hr, which is quick enough to restrict mold growth. This technology not only increased the quality of produce but also returned higher market price of grain. Profit was more (~50%) during a season of high (2001) and moderate (2003) rainfall compared to a dry (2002) season (Table 1) [4]. However, economic viability of this technology will be more realized if drying cost is less, the demand for sorghum increases and there is a premium price for clean grains.

Plate 1. Community sorghum dryer for artificial drying of sorghum

Table 1. Monetary gain when harvested at physiological maturity (PM) followed by artificial drying over normal maturity (NM) and sun drying

Treatment	Price of produce (Rs/ tonne)		
	2001	2002	2003
Harvesting at PM & artificial drying	5409	4637	5500
Harvesting at NM & sun drying	3490	4214	3783
Incremental returns	1919	423	1717
Monetary gain (%)	55.0	10.0	45.4

Source: Adapted from Audilakshmi et al., 2005.

Application of chemical, botanical and bioagents

Chemical, botanical and biocontrol agents for mold management are used for two purposes. Firstly, seed treatment for removing seed-born infection and improvement of initial plant vigor and stand. Secondly, spray application on standing crop for control of grain mold. Seed treatments is not cost intensive and therefore, returns better benefit than spray. Spraying with chemical, botanical or biocontrol agent is not popular in sorghum as they need to be applied on to standing crop and require money for labor and purchase of chemicals by the resource poor farmers. Many reports are available that claim significant efficacy of these fungicides in management of grain mold and few are summarized in Table 2. However, most of them are experimental studies lacking large scale demonstrations in actual farm situations. Major issue here is benefit cost ratio, which hardly favors the farmers. Sorghum being relatively tall cereal (especially varieties are tall as they are grown for dual purpose) spraying is difficult and application cost is also more.

However, spray applications may be beneficial for specific conditions. Commercial sorghum seed production is considered as a high value production system, where cost intensive control measures can be affordable. Use of fluorescent pseudomonas is advantageous as it increases germination, seedling vigor, reduces grain mold and improve seed quality [5] (Plate 2). Spraying of fungicides at 80% anthesis significantly lessens seed-borne infection at milk stage and grain mold severity at maturity. Propiconazole spray effectively reduces 65% of *Fusarium*, 89% of *Curvularia* and 67% of total fungal infection on milk stage grain over no spray [6]. Recently sorghum and millets are increasingly becoming popular and there is upward trend in sorghum prices. This gives an opportunity to relook the use of fungicides for mold management.

Table 2. List of some chemicals, botanicals and bio-agents reported effective for management of grain mold

Application	Treatment	Control	Reference
Seed treatment	Thiram/ Chloranil/ @ 1 oz/bushel; Agrosan GN 2 oz/ bushel	Reduction of moldy growth of sorghum seed	[7]
	Ferbam	Improvement of germination, reduce seedling blight	[8]
	Carbendazim	Increase plant height, grain yield	[9]
	Thiram (3g/ kg seed) and Bavistin (2.5g/ kg seed)	Reduce seed infection by *Fusarium* and *Curvularia*	[10]
Foliar spray	Mancozeb + Captan @ 0.2%. Three sprays (flowering, 15, 25daf)	Reduce grain mold, increase grain yield	[11]
	Captafol @0.2% or Carbendazim + triademefon	Significantly controlled *Fusarium, Curvularia* and *Phoma*	[12]
	Zimmu (onion & garlic extract) formulation 50 EC, 3 ml/L (v/v). Three sprays (60, 75, 90daf)	Significantly reduce grain mold and increased grain weight and grain hardness	[13]
	Talc formulation of Fluorescent Pseudomonad (0.2%). Two sprays (flowering, 20daf)	Significantly increase germination, seedling vigor and reduce grain mold	[5]
	Thiram+ Carbendazim @0.2%; Captan + Mancozeb @ 0.3%. Three sprays (flowering, 15, 25daf)	Significant control of grain molds in sorghum hybrid CSH14	[14]
	Spray of Propiconazole 25EC @ 0.1% at flowering	Significantly reduce seed-borne infection at milk stage	[6]

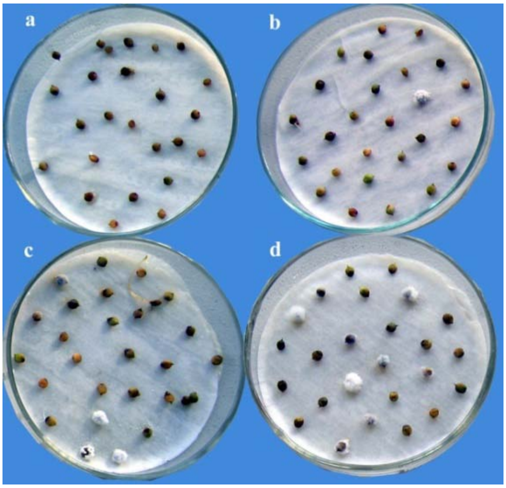

Plate 2. a) Propiconazole sprays at flowering reduce mold infection on immature grains of CSH16: a) treated, c) control and CSH23: b) treated, d) control.

Growing mold tolerant cultivar

Above discussed grain mold management methods such as avoidance or use of chemical, botanical or biological control measures are not always practically feasible or effective. Sorghum farmers hardly take any management practice for disease control on standing crop and they need technology intensive seed materials that will take care of all problems. Therefore, host plant resistance has been the most preferred method of sorghum grain mold management under integrated disease management strategies. And for long time major share of research activities on mold management has been on the aspect of improving host resistance. Efforts to produce sorghum cultivars with tolerance to grain mold by conventional breeding methods have yielded partially successful results, where tolerance is effective under moderate disease pressure (~moderate rainfall). A high degree of resistance that can be effective under high rainfall conditions could not be built-in. The main reasons behind this include the facts that the resistance is complex, involves many fungi, governed by many genes, with significant genotype by environment interactions [15, 16, 17, 18]. Available cultivars in India can withstand moderate disease pressure.

There have been considerable improvements in grain mold resistance levels of sorghum hybrids and varieties during last few decades in India. An analysis of initial and advanced hybrids and varieties that were tested under All India Coordinated Sorghum Improvement Project from 1990 to 2013 indicated that there was decreasing trends in panicle mold score (PGS), which is an indicator of mold resistance. As per trend exhibited, the PGS was reduced from 4.6 to 3.9 for hybrids and 4.3 to 3.7 for varieties during this period (Fig. 1 & 2). Absolute scores, however, are different and they fluctuate the over year depending on weather conditions. This is because mold severity in cultivar is highly weather dependant. A year of high rainfall increases the severity to great extent as absolute resistance is not available for grain mold.

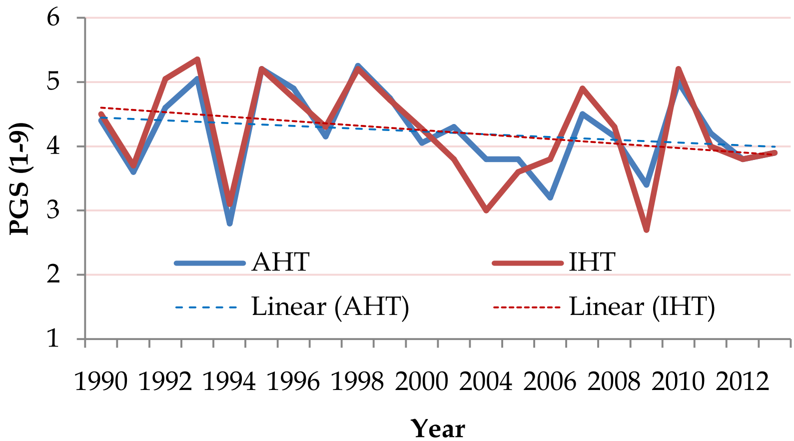

Fig. 1. Year-wise all India means for panicle mold score (PGS) for initial and advanced sorghum hybrids tested in AICSIP from 1990 to 2013.

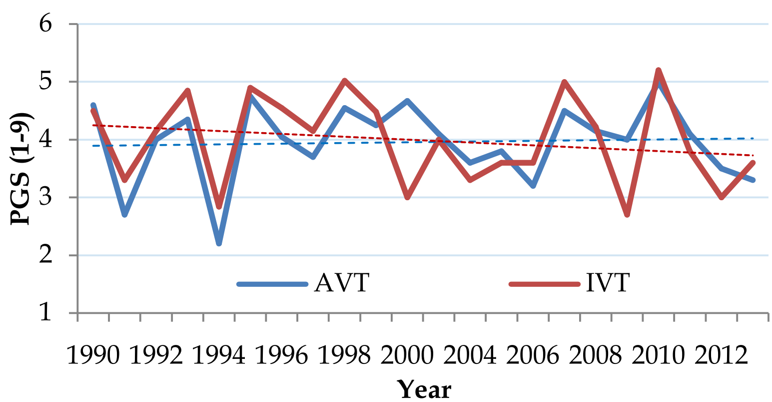

Fig. 2. Year-wise all India means for panicle grade mold score (PGS) for initial and advanced sorghum varieties tested in AICSIP from 1990 to 2013.

Many grain mold tolerant lines (GMRP 4, GMRP9, GMRP13, GMRP25, GMRP28, GMRP 33) were developed at MAU Parbhani [19] and other research stations (ICSV91008, ICSV95001) [20]. Few superior mold tolerant cultivars that are widely cultivated in India are CSH16, SVD9601, PVK801, CSH9 and SPV462 (Plate 3).

Plate 3. Grain mold tolerant cultivars (a) hybrid CSH16, (b) variety SVD9601.

Other methods

Off the field methods of grain mold management especially against post-harvest grain deterioration were tried in various different ways. One such method was pearling of molded grain for improving overall look and grain quality. In this process a mechanical dehuller is used to rub off the outer pericarp, on which fungal growth is present in the grain superficially (Plate 4).

Plate 4. Demonstration of sorghum dehuller to farmers.

After removal of pericarp the appearance of moldy grain can be improved and grains appear almost as clean as non-moldy grains [21]. There was about 16% increase in market price by polishing the molded grain, with minor loss in grain weight (about 5%). Removal of moldy pericarp not only eliminates most of the fungus from the grain but also reduce the chances of mycotoxins contamination. However, large scale adoption of this technology and its economical feasibility has been in question. After harvesting the sorghum panicles farmers keep them in a heap for threshing later. In case of rain the wet grain deteriorates fast and does not fetch good price. Heat generated inside cause further deterioration. Treatment of such heap with 4% acetic acid 2 times a day for 3 days was found effective to reduce grain deterioration and improved the market price [22].

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9. ALTERNATE USE OF MOLDED GRAINS

Apart from normal uses of sorghum grain for food and feed purpose, molded grains have found alternative uses in industries. Grains of sorghum are used as poultry feed, animal feed and alcohol production in the distilleries and starch based products in starch industry. At present in India poultry feed sector is using approximately 1.30 million tons; animal feed sector about 0.45 million tones and alcohol distillers about 0.092 million tons annually.

Alcohol production

The mold grains are available in large quantities and prices are also cheap compared to that of clean grains. This persuades the alcohol industries to use these grains as cheap source of carbohydrate. Now technologies to utilize molded grain for alcohol production have been standardized by the private industries in India. This **potable alcohol** is used as liquor and in the pharmaceutical industry. In addition, the grain is processed in brewing industries to produce alcoholic beverages. The alcohol recovery from grain is ~400 L/ton and the byproduct can be used as cattle feed [1]. A study conducted by National Research Center for Sorghum, Hyderabad with Seagram R&D Institute, Nasik, Maharashtra revealed that CSH16 and CSH18 were the superior hybrids for alcohol production [2]. Alcohol made from grain sorghum can be a good alternative to produce ethanol for blended petrol. This has got further policy boost from the Government of India, who has given permission to blend this alcohol with petrol. Besides bio-ethanol produced from sweet sorghums, molded grains have also found to be a better alternative in the production of ethanol. Considerable amount of sorghum utilization is taking place in grain alcohol sector, mainly using molded grains [3]. Gradually non-food uses of sorghum grain are emerging as a major market for sorghum farmers [4]. Sixteen percent of total production of rainy season grain and forty percent of marketed sorghum are used for industrial utilization, mainly in the poultry and grain alcohol sectors [5].

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10. FUTURE RESEARCH AND STRATEGY

Future research on sorghum grain mold should be specific enough to cater the need of the farmers as well as the industries in the twenty-first century. Huge amount of works on the subject has already been done and there is hardly any common aspect, which has not been investigated to contain the disease during last 50 years. Limited success has been achieved but that is not adequate to fully manage or solve the problem. During this journey few crucial experiences have been learnt which indicate that total elimination of the problem as a disease on a naked grain is not easy. From many angels this disease is different from other plant diseases. Long coexistence of the causal agents with the host (~50% time of the total crop duration) warrants many host weapons to act together against the disease and so comes many mechanisms and necessity to incorporate them to a single cultivar. And this was never an easy target. Therefore, only host resistance will not hold good to tackle it. The situation needs new ideas adequately supported by new strategies. Some of the aspects, which may hold promise for future are discussed here, with particular emphasis to India.

Search for new escape mechanism

Disease escape is one of the best management methods to avoid disease to occur. The best example of this in India is *rabi* sorghum, which escape grain mold as it flowers and matures in dry weather. For kharif sorghum we need to escape favorable weather conditions for mold development *viz*., to avoid entire rainy season. As discussed in previous section the only available period for this is **February to May window** and sowing can be done with irrigation. Crop during this period not only expected to give significantly higher yield but also produce clean grain, thus fetching higher market price. How far this is practicable in reality is another question, as in case of water availability farmers prefer cash crop. Moreover, this window also may clash with *rabi* crops in the area. Alternately, we need to search for a new escaping mechanism that should work even in normal *kharif* season. Development of cultivar with **gooseneck panicle** may be a good strategy for this. In several occasions people have come across stray plants in the field with gooseneck panicle. Grains in such panicles are observed to be mold free in the same field where normal crop developed moldy grains. Mechanism of escape probably involves creation of unfavorable microclimatic conditions for fungal spore germination and subsequent infection. Spikelet tissues in such panicle act more or less like an umbrella to the grain. As glumes are downward facing, such spikelet does not hold water and water-ladened fungal spores and thus significantly reduce wetness duration of grain. This is a hypothesis based on natural observation, which needs to be proved or disproved with experimental evidence. However, before exploitation of this mechanism little basic ground works need to be undertaken. Firstly, it should be established that gooseneck trait is governed by plant genetics and not a random phenomenon. Secondly, gooseneck trait is mostly found in *rabi* type and the character needs to be incorporated in *kharif* type sorghum.

Focus on hard-bold grain

Among about two dozens of studied mechanisms of grain mold resistance in sorghum, **grain hardness** is the only one available in Indian white-grain sorghum that ensures mold resistance in field conditions even under high disease pressure. Harder the grain high is the mold resistance. Hardness in sorghum is imparted by corneous endosperm which contain high amount of storage protein kafirin. **Kafirin** being highly hydrophobic not only resist water movement inside grain but also possess antifungal properties. Presently there is less focus on hard grain in India as kafirin present in it is known to slows digestibility and reduce food making properties. However, fact is now-a-days kharif sorghum is hardly used for food making. It is the high quality *rabi* sorghum grains, which are mostly used for food purpose in India. Kharif grains mostly goes for poultry and animal feed and alcohol production. Gradually there should be definite planning for a total shift in use of *kharif* grain for industrial purpose, which many developed countries already doing. Promoting moldy *kharif* grain for these alternate uses also need some precautions as they may contain mycotoxins. Although they are being used for feed and alcohol production, harmful effects of mycotoxins always remain, as toxins are mostly thermo-labile and are not degraded under industrial processes [1]. In this context, mold free hard grain will be better option than moldy soft grains. Hard grains are mostly small in size and efforts should be made to maintain hardness in bigger grain size. There is indication that kafirin (sorghum storage protein) itself has potential future. Recently kafirin is being considered as viable alternate source of protein. On this, sorghum has advantage over wheat because kafirin does not trigger any adverse response when consumed by celiacs. These properties make kafirin potentially valuable in both food and non-food applications, especially as a bioplastic and encapsulating agent [2]. Also, efforts are on to improve the functional and nutritional properties of kafirin as a functional food ingredient.

Orientation towards color grain

Color sorghum with red pericarp contains flavonoids that confer better mold resistance than white sorghum. When grain with red pericarp also contains pigmented testa beneath the pericarp, provide additive effects on mold resistance. So far in India, research is towards the development of white grain sorghum. But keeping in view the potential of red sorghums in the **feed industry** and also their relatively resistance to grain molds, the *kharif* sorghum improvement program should also focus the development of high yield red sorghum cultivars. Developed countries like South and North Americas, Europe and Australia mostly grow color sorghum for animal feed. Red sorghum has export potential can also be developed so as to use for biscuit industries, where color is not an important criteria. Tannin may be an issue in color sorghum with testa. Though tannins have anti-nutritional properties varieties can be developed for low tannin content. Low tannin sorghum varieties are available in many countries. An vital property of tannin is that sorghum containing tannin is **bird resistant**, an important deciding parameter in present day agriculture [3].

Exploitation of molecular tools

Molecular biology tools have not been utilized much in sorghum grain mold research. One reason may be involvement of many mechanisms of resistance, many fungi and large environmental effects on the disease. In such condition a particular trait may account only a meager amount of the total observed variation [4]. Magnitude of variance being high repeatability of results in various studies carried out in different environment may be low [5]. Still identification and incorporation of resistance to grain mold is the most important aspect for a cultivar development programme of *kharif* sorghum. Molecular markers may be useful for identification of QTL for mold resistance and thus would improve the selection efficiency of the breeder.

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