ANALYSIS OF GENETIC VARIABILITY OF GRAIN MOULD RESISTANCE IN GRAIN SORGHUM

By

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DECLARATION

"I declare that the thesis hereby submitted by me for the degree of Philosophiae Doctor at the University of the Free State is my own independent work and has not previously been submitted by me at another University/Faculty. I further cede copyright of the thesis in favour of the University of the Free State"

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Leo Thokoza Mpofu

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PREFACE

This thesis consists of five chapters in an attempt to explain issues surrounding grain mould in sorghum. The first chapter is a literature review of work that has been done in the field of grain mould research. It focuses on grain mould definition, causes of grain mould, consequences of grain mould, how the disease develops and conditions that favour growth and development of the disease. It also describes current options for managing grain mould and limitations that have led to development of this research program.

Chapter 2 focuses on a green-house experiment that was carried out to assess the nature of host-plant-to-pathogen (G x P) genetic interactions. This experiment confirms that there are genes in sorghum plants that are responsible for resistance to individual grain mould fungi that have been previously bungled as the grain mould fungi complex. A biplot is used to explain the nature of the observed G x P interactions. It further recommends the use of markers to pyramid those genes so that broad adaptation of sorghum genotypes may be achieved.

Chapter 3 presents multi-environment testing, at Cedara-1, Cedara-2 and Potchefstroom, of 12 sorghum parental lines with their hybrids (27 hybrids) to evaluate gene action involved in genetic resistance to grain mould. This chapter also assesses levels of heritability that can be attained with this resistance. This chapter also has an in depth analysis into the best way of measuring grain mould severity i.e. visual scoring versus use of ergosterol concentration measurement.

Chapter 4 is a follow-up on Chapter 3. This Chapter emanates from the observed genotype by environment interaction. (G x E) interaction was highly significant after

ANOVA and could be one of the reasons why the experiment failed to detect differences among the 39 genotypes for variation in ergosterol concentration. A Biplot is therefore used here to assess the nature of the G x E.

Chapter 5 is an independent assessment into levels of mycotoxins that can be expected from the various environments where this research was conducted. Mycotoxins are dangerous substances and levels of their concentrations are controlled by legislations to protect consumers. This chapter determines levels of three selected mycotoxins namely aflatoxin, zearalenone and deoxynivalenol (DON) across the three locations used.

CHAPTER 1

Genetic variability for grain mould resistance in sorghum

LITERATURE REVIEW

Introduction

1.1. What is grain mould

Grain mould is one of the most serious biotic constraints in the production of grain sorghum (*Sorghum bicolor* (L.) Moench). Different names and definitions have been given for grain mould. Forbes *et al.* (1992) defined grain mould as a condition (deterioration of grain) resulting from all fungal associations with sorghum spikelet tissues occurring from anthesis to harvest. This definition has now been widely accepted. As a result, grain mould is of particular concern in areas where the period between anthesis and harvest coincides with high humidity and warm temperature. This is the case in areas where improved short and medium duration cultivars that mature before the end of the rains have been adopted (Stenhouse *et al.*, 1997). The sorghum panicle (unlike the maize cob) is exposed to insects, moulds and other environmental elements. This makes the grains vulnerable to attack by bugs leaving behind areas for fungal invasion (Ratnadass *et al.*, 2001).

1.2. Causal fungi and their variability

Fungi in more than 40 genera have been associated with sorghum grain mould (Williams and Rao, 1981). Mycoflora analysis of sorghum kernels over the years reveal that some of the most important species include *Fusarium graminearum* Schwabe, *Fusarium thapsinum* Klittich, Leslie, Nelson et Marasas sp. nov. 1996, *Curvularia lunata* (Wakker) Boedijn, *Phoma sorghina* (Sacc.) Boerma *et al.* and *Alternaria alternata* (Fr.) Keissl. because they are more frequently isolated from moulded grain (Williams and Rao, 1981; Bandyopadhyay *et al.*, 1991; Esele *et al.*, 1993; Erpelding and Prom, 2006).

Fusarium thapsinum and *Curvularia lunata* seem to be consistently associated with infection at early grain development stages across most climates and geographies (Bandyopadhyay and Chandrashekar, 2000; Singh and Bandyopadhyay, 2000; Prom *et al*., 2003**)**. The occurrence and frequency of isolation of the various grain mould fungal species varies between growing seasons and between sorghum lines indicating significant genotype by environment interactions (Erpelding and Prom, 2006). Other fungi that have been isolated include *Fusarium semitectum*, *Bipolaris* spp, *Dreschslera* spp, and *Colletotrichum graminicola* (Little, 2000; Singh and Bandyopadhyay, 2000). Most of these are facultative parasites or saprophytic fungi and are associated with grain weathering. Fungi such as *Aspergillus* spp, (especially *Aspergillus glaucus*) and *Penicillium* spp. contribute to post harvest deterioration and toxin contamination (Frederiksen, 1986).

Variation within the *Fusarium* species was widely reviewed by Leslie and Marasas, 2002. *Fusarium* species recovered from sorghum stalks and grain associated

with stalk rot and grain mould were previously named *Fusarium moniliforme sensu lato*. However, this name represented an unacceptably broad species concept (9 species) and was changed to *Fusarium verticillioides* and the name of the teliomorph is *Gibberella moniliformis* (Seifert *et al.*, 2003). Currently, the *Fusarium* from the *Liseola* section has been expanded to 10 taxa (Lesley *et al.*, 2005). Toxicity, pathogenicity, and genetic differentiation of five of the *Fusarium* species (formerly bungled as *Fusarium moniliforme*) were evaluated by Lesley *et al.*, 2005. The results indicated that these species differ sufficiently in terms of plant pathogenicity and toxin production profile. *Fusarium verticillioides* was shown to be a prolific fumonisin producer whereas *Fusarium thapsinum* produces moniliformin (secondary metabolites). Such observations bring about the need to therefore assess the degree of resistance and inheritance patterns available against each precise *Fusarium* strain in the present breeding material. This result concurred with the findings of Jardine and Leslie (1992, 1999). *Fusarium verticillioides* is associated with maize while *Fusarium thapsinum* is associated with sorghum (Leslie and Marasas, 2002). For this reason, fumonisin is found mainly in maize and moniliformin in sorghum.

Variability in *Curvularia lunata* in India was evaluated by Somani *et al.*, (1994). Four isolates from four locations were studied for their cultural characteristics and pathogenicity. They observed differences in levels of virulence between these isolates suggesting the need for further research within *Curvularia lunata* to determine the level of resistance in the sorghum germplasm against these strains.

Variability within grain mould causing fungi is further complicated by the effect of the environment. The predominant species vary with location, year and season.

Mansuetus *et al*. (1997) found that the fungi that attack sorghum grain in Tanzania are always constant but disease expression is a function of the environment and the host genotypes. They observed six *Fusarium* mating types occurring at one site and even on the same panicle. This observation implies that different types of grain mould fungal inoculum may be available in the surroundings at any location but the one that is favoured by the environment will dominate and cause disease.

1.3. Infection

1.3.1. Methods of infection

Researchers have distinguished between grain infection and grain colonization (Forbes *et al.*, 1992). Infection occurs at the base of the grain, near the pedicel and interferes with grain filling and may cause a premature formation of the black layer (Castor and Frederiksen, 1981). This condition leads to reduction in grain size (a cause of reduction in yield), a symptom often associated with grain mould. Colonization occurs primarily on the exposed part of the grain not covered by the glume and may be limited to that area (Forbes *et al.*, 1992). The process of colonization disrupts and degrades the internal structure of the kernel from the outside inwards, resulting in the reduction of grain quality and seed viability. Sporulation follows colonization and mould appears on the kernel surface. The color of the mould depends on the fungi involved. The cells of the developing kernels produce compounds (pigments) in response to fungal colonization resulting in grains that are pink, white or black (Castor and Frederiksen, 1982). Grain mould is most common and severe on white grained

sorghum and has thus restricted the adoption of improved varieties in Africa (Mukuru, 1992).

1.3.2. Relationship with weather

A hot and humid environment, during and after maturation, promotes extensive damage of the grain by mould fungi. Research conducted in West and Central Africa (Ratnadass *et al.*, 2001) in 1996 and 1997 on grain from 28 research stations in10 countries, indicates that highly significant correlations exist between mean maximum relative humidity (RHmax) and grain mould rating during early plant growth [5-40 days after anthesis (das)] and between end of flowering and harvest (65-125 das). Scatter diagrams of grain mould scores versus RHmax indicate that when RHmax <90%, the mean grain mould score for 21 genotypes was consistently low, but when RHmax ≥95%, the mean grain mould score increased. This indicates that grain mould incidence increases with increase in humidity. Other weather variables did not show any correlation with mould incidence. This observation is consistent with earlier research by Bandyopadhyay and Mughogho, 1988. This brings about the need to research further the effect of environmental factors on the epidemiology of the fungal species in order to be able to manipulate these weather variables (if possible) to achieve long term solutions to the problem of grain mould.

Mould damaged grain cannot be decorticated and the grain is no longer useful as food or feed. Grain mould incidence and severity increases if harvesting is delayed until after grain maturity and wet conditions persist (Forbes *et al.*, 1992; Bandyopadhyay *et al.*, 2002). However, harvesting at physiological

maturity when moisture levels are below 18% reduces grain mould damage (Christensen, 1970). Grain should be dried to 10-12% moisture after harvesting using grain drying technology or sun-drying to avoid moulding during storage and further processing (Bandyopadhyay *et al.*, 2002). Proprionic acid has been used as a mould inhibitor during the drying process to reduce the risk of mould development (Shetty *et al*., 1995).

1.3.3. Relationship with insects

Grain mould severity increases with insect damage (Sharma, 1993; Marley and Malgwi, 1999; Ratnadass *et al*., 2001). Sorghum head bugs, *Calocoris angustatus* Lethiery and *Eurystylus oldi* Poppius, cause major damage in India and West Africa respectively (Sharma, 1993). These head bugs, nymphs and adults, feed or oviposite on developing grain leaving punctures through which mould fungi can enter and colonize the grain. Insect damaged grain becomes shriveled, tanned and under severe infestation becomes completely invisible outside the glumes (Sharma *et al.*, 2000). Insect damage to grain renders most mould resistant genotypes susceptible by breaching their resistance. Host plant resistance to insect is the most effective way of controlling insects. Considerable genetic resistance and cytoplasmic male sterility (CMS) based resistance exists in sorghum (Steck *et al.,* 1989; Sharma and Lopez, 1992a, 1992b; Sharma *et al.,* 1994; Sharma *et al.,* 2000). Cytoplasmic male sterility based resistance was shown to have dominance and partial dominance type of gene action (Sharma *et al.,* 2000). Patterns of resistance to head bugs follow the same trends as traits

associated with grain mould resistance (Esele *et al.*, 1993; Sharma *et al.*, 2000) and include grain hardness, presence of a pigmented testa, and a red pericarp with an intensifier gene. Genotypes with all of the above traits show less susceptibility to both head bugs and grain moulds. Thus it should be possible to develop combined resistance to both grain mould and head bugs (Sharma *et al.*, 2000).

1.4. Damage caused by grain mould

1.4.1. Economics

Grain mould is a very important biological constraint to sorghum production globally. In highly susceptible cultivars, losses can reach 100% (Williams and Rao, 1981). Very little information is available in literature on the scale of the economic impact of grain mould on sorghum production and utilization especially after year 2000. In 1992, grain mould caused an estimated loss of US$130 million globally (ICRISAT, 1992). These losses can be accounted for by the effect of grain mould on the physical properties of grain. These losses affect both quality and quantity of sorghum grain and includes (1) mouldy and discolored pericarp, (2) a soft and chalky endosperm, (3) decreased grain filling and size, (4) sprouting (reduced germination of seed, (5) mycotoxin production, (6) decreased dry matter, density and test weight, and (7) altered composition of phenolic compounds (Waniska *et al.*, 1992).

The loss in quantity implies reduction in yield. Early infection of the spikelets occurs from the outside inwards. Infection of the grain itself occurs at the base near the pedicel and can interfere with grain filling and cause premature

formation of black layer (Castor, 1981). This leads to development of smaller seeds, which results in farmers getting reduced yields per plot of land.

The importance of sorghum in the livelihood of the African rural poor cannot be over-emphasized. Most rural societies in Africa are poor and live in hot semi-arid areas where sorghum is their main source of income, food and feed for their animals (Hall *et al*., 2000a). However, sorghum production is problematic (especially poor grain quality and availability of grain) to such an extent that it is being overlooked (in terms of research and development) in preference for maize production (Hall *et al*., 2000a; 2000b). Early infection and post maturity colonization of sorghum grain by mould fungi are the primary cause of quality reduction. There is therefore a need for relevant institutions to reform their rules, policies and norms to bring about institutional innovations that can be manipulated or leveraged to alleviate financial and food security losses caused by grain mould (Hall *et al*., 2000b). Although sorghum is primarily a subsistence crop, there are indications that this situation can be improved if proper research is done to find ways to improve this crop. Sorghum grain has a great potential for utilization on a commercial scale by the brewing and poultry industries to replace maize that has been projected to become very expensive in the near future (Seshaiah, 2000).

1.4.2. Effect on seed viability

Grain mould fungi cause abnormal growth of seedlings and decrease seed viability (Castor and Frederiksen, 1980; Garud *et al*., 2000; McLaren *et al*., 2002;

Prom *et al*., 2003). According to (McLaren *et al*., 2002) *Alternaria* spp. are the main cause of reduced germination in South Africa accounting for 88% of the reduction. These fungi cause the grain to germinate on the panicle (pre-harvest sprouting) after black layer formation if wet conditions persist (Bandyopadhyay *et al.*, 2000). Such pre-harvest sprouted grains become soft due to the digestion of parts of the endosperm by α-amylases. Fungal colonization of embryos leading to formation of smaller seeds and seed dormancy is the main cause of reduction in germination. At College Station in Texas USA, Prom *et al*. (2003) noted that *C. lunata* has the ability to infect and kill seeds without producing significant visible symptoms especially under hot and dry weather conditions. This means that visual assessment of grain is not sufficient as a measure of potential damage to germination caused by mould fungi. It is therefore important to conduct germination tests on seed to be used by farmers.

1.4.3. Mycotoxin production

A major concern associated with grain mould is the production of mycotoxins and secondary metabolites that are harmful to human and animal health and productivity. Apart from sorghum, mycotoxins can accumulate in maize, soybean, groundnuts and other food and feed crops in the field or during improper storage. Several fungi are capable of producing mycotoxins but the most important are *Aspergillus*, *Fusarium* and *Penicillium* spp. (Sweeney and Dobson, 1998). *Fusarium* spp. are field fungi whilst *Aspergillus* and *Penicillium* spp. are storage fungi. There are five common mycotoxins found in food and these are

aflatoxins, ochratoxin, fumonisins, deoxynivalenol (DON, vomitoxin), and zearalenone (ZEA) (Bandyopadhyay *et al.*, 2000).

1.4.3.1. Aflatoxins

Aflatoxins are considered the most important mycotoxins. Major producers of aflatoxins are *Aspergillus flavus* Link, and *A. parasiticus* Speare (Bandyopadhyay *et al.*, 2000). These fungi are found virtually everywhere growing in soils and decaying plant material and cause stored grains to heat and decay. They produce aflatoxins as a byproduct of growth on many commodities, including sorghum, before and after harvest. Aflatoxins are differentiated into B (B1 and B2), M (M1 and M2), and G (G1 and G2) sub-types based on structure, chromatographic and fluorescent characteristics. Aflatoxin B1 is the most potent (Husein and Brasel, 2001). It binds to DNA, disrupting the genetic code, thereby promoting generation of cancerous tumors. It is also responsible for poor performance in livestock and poultry. Legislation regulates maximum allowable contamination levels at 5 ppb in Europe, 10 ppb in USA and commodities must be tested to ensure that levels are below this value for human and animal consumption. The LD50 of aflatoxins for most species ranges from 0.5 – 10 mg/kg body weight (Rustom, 1997). However, aflatoxins in sorghum are not as serious as in maize, groundnut and other oil rich seeds because sorghum is a relatively poor substrate for *Aspergillus* spp. (Bandyopadhyay *et al.*, 2000).

1.4.3.2. Ochratoxin

Ochratoxin is produced by *Aspergillus ochraceus* Wilhelm and *Penicillium viridicatum* Westling (Bandyopadhyay *et al.*, 2000). Van der Merwe *et al.* (1965) described the first occurrence of ochratoxin in maize in South Africa. This toxin can also be found in sorghum and barley together with aflatoxins (Bandyopadhyay *et al.*, 2000). Ochratoxin has been reported to cause endemic nephropathy in humans and porcine nephropathy in a number of mammalian species (Lowe and Arendt, 2004). Turkeys and poultry suffer lower productivity, and other animals suffer kidney malfunction due to ochratoxicoses (Bandyopadhyay *et al.*, 2000). Maximum allowable levels of contamination are 10-20 ppb.

1.4.3.3. Fumonisins

Fumonisins are categorized into three groups: B1, B2, and B3. They are produced by *Fusarium verticillioides (moniliforme)* and *Fusarium proliferatum* (Matsushima) Nirenberg (Bandyopadhyay *et al.*, 2000). *Fusarium verticillioides* (mating population A) is a soil borne plant pathogen found mainly in maize that produces large quantities of fumonisins. Fumonisins have been found in a wide range of products including rice, yams, sorghum, hazelnut, pecans, and cheeses (Doko *et al.*, 1995) emphasizing the importance of testing. In sorghum, mating population F is predominant and produces less fumonisins (Leslie and Mansuetus, 1995). Fumonisins found in maize have been associated with esophageal cancer in the Transkei region of South Africa (Sydenham *et al.*, 1990).

1.4.3.4. Deoxyvalenol

Deoxynivalenol (DON or vomitoxin) is produced by *Fusarium graminearum* (Bandyopadhyay *et al.*, 2000). This toxin has been found in wheat, barley, sorghum and maize (Bilgrami and Choudhary, 1998). Deoxynivalenol is classified under the largest group of *Fusarium* mycotoxins called trichothecenes that are divided into types A, B, C, and D according to their molecular structures (Miller *et al*., 1991). Deoxynivalenol is classified as type B. This toxin can cause vomiting, feed refusal, immune suppression, diarrhea, and weight loss in animals (Bandyopadhyay *et al.*, 2000).

1.4.3.5. Zearalenone

Zearalenone (ZEA) is also produced by *Fusarium graminearum* together with deoxynivalenol (Bandyopadhyay *et al.*, 2000). This toxin is found in maize and sorghum and has been detected in beer and sour porridge prepared from contaminated maize and sorghum (Sibanda *et al.*, 1997). ZEA is a macrocyclic lactone with high binding affinity to oestrogen receptors and low acute toxicity (Diekman and Green, 1992). It causes a wide range of reproductive problems to livestock which include, infertility, vulva oedema, vaginal prolapse, mammary hypertrophy in females and feminization in males, pseudo pregnancy and abortion with pigs being the most affected species (Bandyopadhyay *et al.*, 2000). Zearalenone toxicity can lead to losses to farmers by lowering productivity and reproductive ability of livestock.

It is clear that grain mould is a very important cereal disease that scientists have to take seriously since cereals are a carbohydrate source for many people the world over. If left unattended, this disease will cause a reduction in production (plant and animal productivity), nutritional value, and market value of most cereals. The best protection against mycotoxins is to monitor their presence in feeds and foods by continuously testing grains from harvest to processed products. Since prevention is better than cure, managing grain moulds in the field helps to reduce the overall amount of damage.

1.4.4. Effect on seed sorghum

Commercial production of hybrid seed in sorghum started with the discovery of cytoplasmic-nuclear male-sterility (CMS) system designated as A1 (milo) (Stephens and Holland, 1954). Additional CMS systems (A2, A3, and A4) were later identified (Moran and Rooney, 2003). Almost all commercial sorghum hybrids have been produced using A1 cytoplasm for the past 45 years (Moran and Rooney, 2003). However, there is higher risk with respect to stability of production and vulnerability to disease when a single CMS system with narrow nuclear genetic diversity of both male sterile (A-) lines and restorer (R-) lines is used. This is evident from the outbreak of southern corn leaf blight on maize hybrids based on a Texas cytoplasm in 1970 (Tatum, 1971). In sorghum, type of cytoplasm (A1 or A2) does not affect grain mould severity and *Fusarium* head blight incidence (Stack and Pedersen, 2003) but has been shown to increase sorghum susceptibility to rust (*Puccinia purpurea* Cooke), zonate leaf spot

(*Gloeocercospora sorghi* Bain & Edgerton ex Deighton), and leaf blight (*Exserohilum turcicum* (Pass) K. J. Leonard & E. G. Suggs) (Rodriguez *et al*., 1994).

1.5. Measurement of grain mould

1.5.1. Visual scoring

Measurement of grain mould severity is important as it ties in with other areas of research, including epidemiology and host resistance. Visual scoring has been the most popular means of quantifying grain mould. This method can be used to estimate severity (degree of colonization of a uniform sample indicated by signs or discoloration), incidence (proportion of grain affected), or damage (reduction in grain size), (Bandyopadhyay *et al.*, 2000). Visual scoring uses a common scale of well defined units such as percent of grain surface affected (Forbes, 1986; Bandyopadhyay and Mughogho, 1988). This method is easy to use and a large number of samples can be screened in a short time. Even though visual scoring has been shown to have bias against light-colored grains and is subject to individual judgments, it has been shown to significantly correlate with more reliable methods like measurement of ergosterol concentration (Seitz *et al*., 1983; Forbes, 1986).

1.5.2. Ergosterol concentration

Since visual scoring evaluates grain mould severity superficially, there is a need to assess internal colonization. Most attempts have measured the proportion of infected grains using selective media (Castor, 1981) or chemical treatment of grain (Gopinath and Shetty, 1985) to remove bias towards the more competitive (fast growing) component of the mycoflora on the seed surface (Bandyopadhyay *et al.*, 2000). These methods estimate the amount of viable fungal tissue (propagules per gram of seed tissue).

Measurement of ergosterol concentration is a more sensitive method of estimating total (viable and non viable) fungal biomass which considers all fungal growth events that have taken place (Seitz *et al.*, 1977). Ergosterol is the predominant sterol component of all fungi (Weete, 1974) and it differs significantly from sterols of higher plants. It is therefore not a native constituent of grains. The primary role of sterols in nature is as architectural components of membranes (Nes, 1974). Ergosterol concentration procedure has been used to distinguish levels of grain mould resistance (Jambunathan *et al*., 1991). This procedure provides an indication for the extent of internal mould colonization which is not externally visible. Therefore, a combination of assessment of severity of different fungi (visually or on agar) in a grain sample in conjunction with ergosterol measurement indicates the identity of the fungi and their quantity.

1.6.Management of grain mould

1.6.1. Avoidance and chemical control

Traditional landrace sorghum varieties have been grown in most parts of the world over many years. These varieties often escape grain moulds because they are photoperiod sensitive and late maturing such that they flower and mature after rains have stopped and the risk of grain mould is very low i.e. avoidance mechanism (Curtis, 1968). However, these varieties have low adaptation outside their natural habitat, tend to be bulky, are susceptible to many diseases and have low yields. In some areas farmers grow high-tannin, brown sorghums because these have been shown to resist both grain mould and bird damage (Mukuru, 1992; Waniska *et al.*, 1992). However, high-tannin sorghum is unacceptable to most end-users because tannins are responsible for lower protein digestibility, dark colors and astringency (Hagerman *et al.*, 1998; Waniska, 2000; Rooney, 2005). Chemical control of grain mould has been shown to be very effective (Gopinath and Shetty, 1987) but is not economical for most subsistence sorghum growers (Mukuru, 1992). In most cases, avoidance and chemical control is impractical hence, use of resistant cultivars offers the most sustainable and effective means of control.

1.6.2. Resistance breeding and defense mechanisms

Since sorghum is grown in the semi-arid regions with very little and unpredictable rainfall, there is a need for farmers to maximize yield on the limited moisture received during the rainy season. In Africa, most research programs are

introducing white-grained, short-duration and short-statured cultivars that mature before the end of the rainy season (Stenhouse *et al.*, 1997). This has led to an increase in the incidence of grain mould as has been noted in India and parts of China, the US and Latin America (Stenhouse *et al.*, 1997).

The fact that some true breeding cultivars vary in resistance to grain mould indicates that there are genes responsible for resistance. However, this resistance is complex and involves several mechanisms (Forbes *et al.*, 1992; Waniska *et al.*, 2001; Little and Magill, 2004). These mechanisms and the genes involved work synergistically to realize resistance. Most of the time (not always), resistance genes in a cultivar are race-specific and are inherited in a simple Mendelian way (Collinge and Slusarenko, 1987). This is supported by the concept of gene-for-gene interaction that hypothesized that for each gene for resistance in a host, there is a matching gene for virulence in the pathogen (Flor, 1971). There is need to identify these genes and pyramid them in order to have a broader and more stable resistance mechanism.

Sources of grain mould resistance cut across a wide morphological variability and diversity in taxonomic races and geographic origin (Bandyopadhyay *et al.*, 1988). Many years of research have shown that there are three primary sources of sorghum grain mould resistance. These include physical or structural characteristics and biochemical traits of the seed, glume and panicle (Chandrashekar *et al.*, 2000; Reddy *et al.*, 2000; Waniska, 2000). Defense mechanisms can be classified as either passive (constitutive or pre-existing features) or active (inducible or switched on after infection by pathogen). Passive

sorghum grain mould defense mechanisms include (but not limited to) grain hardness, panicle compactness and shape, presence or absence of a testa layer, photoperiod sensitivity and glume coverage.

1.6.2.1. Grain hardness

Grain hardness has been shown to be key in conferring grain mould resistance by several independent researchers (Menkir *et al.*, 1996; Ghorade and Shekar, 1997; Audilakshmi *et al.*, 1999). Grain hardness acts as a physical barrier to penetration by fungal hyphae. Jambunathan *et al.* (1992) reported a collection of landrace sorghums from Orissa in India that where of white grain type without a testa layer but were resistant to grain mould. These landraces where reported to have extremely hard endosperms. However, populations from the different regions of the world have their own preference regarding hardness depending on the end use. In India, Sudan and Ethiopia, soft grains are preferred for making *chapatti*, *kisra* and *injera*, respectively. In West Africa, on the other hand, more corneous endosperms are preferred for making *tô* (Audilakshmi *et al.*, 1999). This has implications when it comes to utilizing grain hardness to manage grain mould in these areas.

1.6.2.2. Phenols

Plants synthesize a vast array of metabolites that are toxic to potential pests and pathogens. These metabolites include antibiotic phenols that occur constitutively in plant cells and function as pre-formed inhibitors associated with

passive non-host resistance (Stoessl, 1983). All sorghums contain phenols. Phenols are divided into three major groups: phenolic acids, flavonoids and tannins (Chung *et al.*, 1998). Sorghum containing tannins is referred to as tannin or brown sorghum even though the pericarp color may be white, yellow or red (Waniska, 2000). Sorghums have been divided into three groups according to the levels of tannins (Price and Butler, 1977) and genotype of the grain (Rooney and Miller, 1982). Group I sorghums do not have a pigmented testa, contain low phenols and no tannins (food types). Group II has a testa with tannins but do not have a spreader trait (B_1-B_2-ss). Group III has a testa with tannins that spread to the pericarp due to the presence of a spreader gene (B_1-B_2-S-).

Harris and Burns (1973) reported that sorghum tannin content was significantly and negatively correlated with pre-harvest seed-moulding scores. Jambunathan *et al.* (1986) and Jambunathan *et al.* (1990) reported that the concentration of flavan-4-ols in grain sorghum was responsible for resistance to fungal invasion. Condensed tannins, athocyanidins, some phenolic acids, and flavan-4-ols are some of the secondary metabolites that have been associated with sorghum grain mould resistance (Butler, 1988; Nicholson and Hammerschmidt, 1992).

Choice of grain color varies according to preference and end-use (Audilakshmi *et al.*, 1999). In India, for example, white grained varieties with straw glumes are preferred as they do not want glume color to taint the food products. In most parts of Africa, some farmers can tolerate high tannin brown sorghums as they prefer them for their bird resistance and better taste in beer

brewing. Waniska (2000) described food-type sorghums as ‘sorghums with a tan secondary-plant-color, straw colored glumes, white or clear pericarp, no pigmented testa, intermediate to hard endosperm texture, and increased resistance to grain weathering that mill into products with bland flavor, white color, and no off-colors’. Some Group I sorghums that are white colored and without a pigmented testa were shown to be resistant to grain mould (Rodriguez-Herrera *et al.*, 1999). They proved to co-express high levels of all four antifungal proteins as a resistance mechanism. Use of high phenol content can therefore be used selectively, just like grain hardness, by breeders in their selection criteria for grain mould resistance.

Some fungi have developed sophisticated ways of overcoming plants’ physical defenses. Plants therefore have evolved active biochemical defense mechanisms that act synergistically with passive resistance mechanisms to express resistance. When a cell wall or cell membrane is breached by a pathogen, the host reaction differs between resistant and susceptible hosts (Forbes *et al.*, 1992). Susceptible sorghums allow the fungi to grow and ramify throughout the placental sac and into the endosperm and even the embryo without much change in pericarp color (indicating a compatible interaction). In resistant sorghums, pigmentation occurrs rapidly at the infection site (indicating an incompatible interaction) (Forbes *et al.*, 1992). This pigmentation is the first visible symptom of host response and is caused by the rapid accumulation of phenols, formation of lignin and accumulation of cell-wall appositions such as papillae. Other

alterations may include esterification, polymerization and crosslinking of phenols and esters in the cell walls of infected cells.

These physiological activities result in discoloration, auto-fluorescence and rapid (hypersensitive) cell death of host tissue around the pathogen. The sum total of these changes is the creation of an inhibitory environment which restricts further growth of the pathogen either by starvation or poisoning or physically restricting it or a combination of the three (Collinge and Slusarenko, 1987; Graham and Graham, 1991; Nicholson and Hammerschmidt, 1992; Little, 2000). These physiological activities in the cells of resistant cultivars are a result of transcriptional elicitation of defense genes (Little, 2000). The defense genes are activated when pathogen-derived products interact with host-derived receptors encoded by resistance genes i.e. the recognition mechanism (Daly, 1984; Day, 1984).

1.6.2.3. Secondary metabolites

Another active defense mechanism is the formation of secondary metabolites. This involves the production of phytoalexins from the phenylpropanoid and flavonoid biosynthesis pathways (Collinge and Slusarenko, 1987; Little, 2000). The amino acid phenylalanine is a precursor for the synthesis of phenolic compounds. In healthy tissues, this amino acid is usually incorporated into proteins. Following infection, however, this amino acid is converted to trans-cinnamic acid and enters the pathway for biosynthesis of phenylpropanoid compounds. Phenylpropanoid and flavonoid metabolisms are common in many

plant cells especially during flowering (van der Meer *et al*., 1993). Induction of phytoalexin biosynthesis is associated with accelerated *de novo* synthesis of phytoalexin biosynthetic enzymes phenylalanine ammonia-lyase (PAL) and chalcone synthase (CHS). Metabolism of phenylpropanoid by the enzyme PAL results in the accumulation of antifungal furanocoumarins (phytoalexin). PAL activity responds to several stimuli including fungal infection (Orczyk *et al.*, 1996). Cui *et al*. (1996) demonstrated an increase in PAL activity within a few hours of infection, in seedlings of the sorghum lines BTx635 and RTx7078 when inoculated with *Bipolaris maydis* (Nisikado & Miyake) Shoem. The phenylpropanoid pathway is switched to the flavonoid biosynthesis pathway by CHS (Little, 2000). Cui *et al*. (1996) also demonstrated an increase in CHS activity, within few hours of infection, in seedlings of the sorghum lines BTx635 and RTx7078 when inoculated with *Bipolaris maydis.* An increase in PAL and CHS activity comes as a defense mechanism in response to infection since *Bipolaris maydis* is a non-pathogen of sorghum. There is need therefore for breeders to select or screen germplasm for ability to produce PAL and CHS in grain mould hotspots during the breeding process.

1.6.2.4. Antifungal proteins

An important factor in screening for grain mould resistance is the profile of antifungal proteins (AFPs) also known as storage proteins or pathogenesis-related (PR) proteins. These proteins are enzymes and are produced in response to biotic and abiotic stress. They have been identified in many plants including beans,

peas, pearl millet, maize (Abeles *et al.*, 1970; Mauch *et al.*, 1984) and sorghum (Kumari and Chandrashekar, 1994; Seetharaman *et al.*, 1997; Rodriguez-Herrera *et al.*, 1999; Bueso *et al.*, 2000; Bejosano *et al.*, 2003). They have been shown to inhibit fungal growth *in vivo* and *in vitro* (Seetharaman *et al.*, 1996; Seetharaman *et al.*, 1997).

Sormatin, chitinase, β-1,3-glucanase and ribosome-inactivating proteins (RIP) are all AFPs and have been identified in sorghum (Seetharaman *et al.*, 1996). β-1,3-glucanases have been isolated in many plants (Boller, 1985) including sorghum (Darnetty *et al.*, 1993) and act by hydrolyzing β-1,3-glucans found in the cell walls of many fungi (Bartnicki-Garcia, 1968). They range in molecular mass from 21 to 31 kDa (Boller, 1985) and are important plant defense proteins in that they are induced by fungal infection and or damage (Krishnaveni et al., 1999) and inhibit growth of most pathogenic fungi.

Chitinases are enzymes of 25 – 35 kDa molecular mass that hydrolyze chitin. Three isozymes of chitinase from sorghum seeds were purified and their biochemical and antifungal properties determined (Krishnaveni *et al.*, 1999). These isozymes were induced upon colonisation by both insects and fungi. There are however reports of instances where high levels of chitinases have been reported in susceptible plants (Krishnaveni *et al.*, 1999).

Sormatin is a smaller protein with a molecular mass of ~22 kDa (Seetharaman *et al.*, 1997). It acts by causing permeabilization of fungal membranes (Vigers *et al.*, 1991). High concentrations of this protein have been reported in sorghum and maize seed extracts (Darnetty *et al.*, 1993). Sormatin is a homolog of the maize

AFP zeamatin (Vigers *et al.*, 1991). Sormatin production in sorghum has been shown to be induced by fungal attack (Rooney, 1998) indicating that it is one of the plant's defense mechanisms. It however acts later than other AFPs as it has been reported to peak at 30 days after anthesis (DAA).

Ribosome inactivating proteins are found in seeds, roots, leaves and sap of many plants and they range in size from 28 to 31 kDa (Darnetty *et al.*, 1993). They inhibit protein synthesis in pathogenic fungal cells by RNA N-glucosidase modification of 28 s-RNA (Logemann *et al.*, 1992). A 30 kDa RIP was reported in sorghum (Seetharaman *et al.*, 1996). Rodriguez-Herrera *et al.*, (1999) reported that grain mould resistant lines constitutively expressed higher RIP levels under both grain mould free environments and under grain mould environments compared to grain mould susceptible lines.

Rodriguez-Herrera *et al.*, (1999) found that a combination of AFPs in sorghum inhibits fungal growth more efficiently than does either enzyme alone. This co-expression of AFPs is responsible for grain mould resistance in food type sorghums of Group I. Joch *et al.* (1995) demonstrated that hydrolytic activity of chitinase or β-1,3-glucanase on cell walls in transgenic tobacco could result in an increased uptake of RIPs into fungal cells. Therefore ability to co-express AFPs is essential for grain mould resistance.

Bejosano *et al.* (2003) revealed that the environment significantly affects the levels of AFPs in resistant and susceptible sorghums. This research follows on the findings of Seetharaman *et al.*, (1996) that AFPs are mobile and leach from the karyopsis due to rain and changes in atmospheric temperature. As a result, the

ability of sorghums to resist fungal invasion was directly and positively influenced by their retention/production of AFPs (Bejosano *et al.*, 2003). Susceptible cultivars lost or failed to retain their AFPs from 30 to 50 DAA. Therefore breeders should use ability to co-express and retain AFP's as a screening mechanism when selecting for grain mould resistance in their nurseries.

1.6.2.5. Number of genes

As indicated above, sorghum grain mould resistance has been shown to be determined by several qualitative loci that include grain hardness, panicle compactness and shape, presence or absence of a pigmented testa layer, photoperiod sensitivity, glume coverage, production of phenols, antifungal proteins and other secondary metabolites. However, these loci do not account for all the variation observed for grain mould resistance in sorghum (Rooney and Klein, 2000). Rodriguez-Herrera *et al.* (2000) estimated 4 to 10 genes to contribute to grain mould resistance in sorghum. However, this number is an under-estimation of the actual number of genes involved as indicated in the discussion above. This could be because some genes that influence grain mould infection were not segregating in the material used and or because of genetic linkage. Generation mean analysis of a cross between 'Sureño' and 'RTx430' indicated that sorghum grain mould resistance has additive, dominace and epistatic effects (Rodriguez-Herrera *et al.*, 2000). This resistance is also moderate to highly heritable (Dabholkar and Baghel, 1983; Rodriguez-Herrera, 2000) even

though it has to be selected for specific environments due to high genotype by environment interactions.

1.6.2.6. MAS and QTL analysis

The availability of advanced biotechnological developments recently has opened new avenues that breeders can utilize more efficiently to breed for grain mould resistance. Quantitative trait loci (QTL) analysis and marker assisted selection (MAS) are some of the tools that provide hope for such developments (Rooney and Klein, 2000; Rodriguez-Herrera *et al.*, 2000). Attempts to identify molecular markers for genes involved in sorghum grain mould resistance were reviewed by (Rooney and Klein, 2000; Rodriguez-Herrera *et al.*, 2000; Klein et al., 2001). They found five QTLs each accounting for between 10 to 23% of the observed grain mould incidence variance. However, some of these QTLs affected multiple traits and hence could be confounding if not used properly in MAS.

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CHAPTER 2

Variability in genotype x pathogen interaction for grain mould resistance in sorghum

ABSTRACT

Sorghum panicles were inoculated with five fungi frequently isolated from sorghum grain to determine the relationship between fungal pathogenicity and host genetic resistance. A collection of 11 sorghum genotypes sourced from Southern Africa regional trials supported by the International Sorghum and Millets Collaborative Research Support Program (INTSORMIL CRSP) was used. Panicles of selected genotypes were inoculated at anthesis with *Fusarium graminearum, Fusarium thapsinum, Curvularia lunata, Phoma sorghina* and *Alternaria alternata* spores. Panicles were sampled at 50 days after anthesis and visually scored for grain mould severity and evaluated for ergosterol concentration. There were highly significant differences in the levels of fungal pathogenicity on the different sorghum genotypes. These differences accounted for 58.4% of observed variation in ergosterol concentration. Genotype by pathogen (G x P) interactions accounted for 33.5% of the observed ergosterol concentration variation. The implication is that different genotypes reacted differently to different fungi. The genotypic reactions of the hosts accounted for 8.1% of the observed ergosterol concentration variation. Overall, fungal pathogenicity is the most important factor to consider in the evaluation of germplasm for grain mould resistance. However, fungal pathogenicity also depends on host genetics. Individual host genes associated with resistance to individual grain mould

fungi need to be identified and manipulated into sorghum hybrids and cultivars. Possible sources of resistance could be identified by use of biplot analysis of G x P interactions. Visual scoring for grain mould is insufficient without identifying causal fungi. A multiple regression model involving all the fungal species accounted for 67% of the variation in the final visual grain mould damage rating. *Alternaria alternata* accounted for 52% of the final visual grain mould damage rating. However, *Fusarium thapsinum* and *Phoma sorghina* were the most abundant fungi across all genotypes. Thus, it should be possible to identify individual host resistance genes and pyramid them in order to get a broad sense resistance mechanism that will hold against all important fungi across environments and seasons.

INTRODUCTION

Grain mould is a significant constraint to sorghum (*Sorghum bicolor* (L.) Moench) production and utilization. It is a particular problem in areas where the period between anthesis and harvest coincides with high humidity and warm temperatures. Most of the fungi isolated from moulded grain are facultative parasites and the predominant species differ across locations and seasons. The significance of sorghum grain mould has been highlighted in Africa, Asia and the Americas (Fredericksen *et al*., 1982: ICRISAT, 1987). Grain mould reduces yield, nutritional quality, seed viability, kernel weight and market value (Forbes *et al.,* 1992). Grain mould fungi are also responsible for the production of potent mycotoxins and secondary metabolites that are harmful to human and animal health and productivity (Castor and Frederiksen, 1980). Fungi in more than 40 genera have been associated with sorghum grain mould (Williams and Rao, 1981). Mycoflora analysis of sorghum kernels over the years reveal that some of the most important species include *Fusarium graminearum* Schwabe, *Fusarium thapsinum* Klittich, Leslie, Nelson et Marasas sp. nov. 1996, *Curvularia lunata* (Wakker) Boedijn, *Phoma sorghina* (Sacc.) Boerma *et al*. and *Alternaria alternata* (Fr.) Keissl. because they are more frequently isolated from moulded grain (Williams and Rao, 1981; Bandyopadhyay *et al.,* 1991; Esele *et al.,* 1993; Erpelding and Prom, 2006).

Grain mould is a result of a complex fungus-host interaction. This interaction needs to be fully investigated and understood before a durable solution to grain mould damage is found. Many researchers have referred to grain mould as a disease caused by many fungi collectively and have therefore sought to find a resistance mechanism that would restrict all grain mould fungi simultaneously. Grain characteristics that have been

associated with mould resistance include grain hardness, a thin pericarp, a thick surface wax layer, a pigmented testa, a red pericarp, high concentration of tannins and flavan-4-ols, antifungal proteins, grain density and grain integrity, open panicles with long glumes, and plant height (Glueck and Rooney 1980; Mukuru, 1992; Esele *et al*., 1993; Rodriguez-Herrera *et al*., 1999; Waniska *et al.*, 2001). Most of these traits are qualitative traits. Overall, grain mould resistance is believed to be multigenic with recent estimates indicating a minimum of 4 to 10 genes controlling resistance in white-grained sorghums (Rodriguez-Herrera *et al*., 2000).

Most research into grain mould resistance has acknowledged the significant effect of the environment on the expression of resistance mechanisms. However, the environment is the effect of the climate (weather conditions) and location whereas very little attention has been given to the pathogen itself. As a result, most recommendations on grain mould resistance emphasize the need to select for each environment. This approach however is limiting as environment changes with seasons even within the same location.

Before resistance mechanisms can be investigated, it is essential to understand fungal pathogenicity. Somani *et al.* (1994) reported the presence of strains of *Curvularia lunata* that poses different levels of pathogenicity to sorghum in India. On the other hand Jardine and Leslie (1992) found no differences in pathogenicity among two mating populations of *Fusarium* spp. Infection and colonization patterns were found to differ for *Fusarium* spp. and *Curvularia lunata* (Castor, 1981). These differences may partially explain why resistance to the two pathogens also differs and why the expression of resistance under changing conditions varies. After evaluating several thousand

germplasm lines in the International Grain Mould Resistance Screening Program (Indira *et al.,* 1991) noted that no genotype was immune to grain mould. This shows that no single genotype has all the necessary resistance genes. However, recombination breeding (crosses between lines with some resistance and susceptible ones) improved varietal resistance to grain mould. This improvement could be attributed to chance recombination among crosses. Informed crosses could have achieved more genetic gain toward achieving immunity. The little gain observed was achieved using visual grain mould rating during the screening process under natural infection.

This research seeks to identify sorghum genotypes with specific resistance and to quantify the effect of different grain mould fungi on host response. This will enable researchers to make their crosses more accurately and target specific pathogens with specific resistance genes for all environments.

MATERIALS AND METHODS

1. Genetic material and fungal cultures.

In August 2006, a diverse array of 11 sorghum genotypes with different reactions to mould was carefully selected for this program. The 11 genotypes were sourced from a Southern Africa sorghum regional breeding nursery supported by the International Sorghum and Millets Collaborative Research Support Program (INTSORMIL CRSP) of the USAID.

The experiment was conducted in a green-house at the University of the Free State in Bloemfontein South Africa. The selected genotypes where classified into three groups R (resistant), I (intermediate), and S (susceptible) (Table 1) based on field grade score (FGS) data from a number of years of prior field evaluations. This data was obtained using a rating system described by (Bandyopadhyay and Mughogho, 1988). Ratings where based on a 1 to 5 scale in which 1 = no visible mould; 2 = 1 to 10%; 3 = 11 to 25%; 4 = 26 to 50%; 5 = more than 50% of kernels in the panicle moulded. Group (R), are resistant genotypes and have a score (x) such that $x \leq 2$. Group (I), are intermediate resistant and $2 < x \leq 3$. Group (S), are susceptible genotypes and $3 < x \leq 5$.

Plants were maintained at 25 – 30 °C with regular irrigation and fertilization. Seed from each line was sown into a steam sterilized soil:peat mixture (3:1) mix in 25-cm diameter pots and thinned to two plants per pot after emergence. There was one pot per replication. A split-plot design with three replications was used with fungus as the main treatment (whole-plot) and genotypes as the sub-plots within treatment. The design was completely random with genotypes and fungi taken to be

fixed in the analysis model. Panicles were visually scored for grain mould severity and harvested 50 days after anthesis and were evaluated for ergosterol content.

2. Sources of isolates.

Isolates of *F. graminearum, and F. thapsinum* were obtained from grain collected at Potchefstroom during 2005-2006 season and maintained in the culture collection at the University of the Free State.

Isolates of *Curvularia lunata, Phoma sorghina* and *Alternaria alternata* were obtained from bulk sorghum grain sampled from a local brewing company. Grain was surface sterilized in 1% sodium hypochlorite solution for 3 minutes followed by three rinses in sterile distilled water. The grain was dried on sterile blotting paper and from 50 to 100 seeds were plated onto malt extract agar (MEA) medium (Biolab Diagnostics SA (Pty) Ltd) to which streptomycin (Caps Pharmaceuticals SA (Pty) Ltd / (Edms) Bpk was added at a rate of 0.3 ml per liter of medium. Individual fungal colonies from grains were transferred to half-strength potato dextrose agar (PDA) (Biolab) to assess colony morphology. Single spore isolates obtained following serial dilution of spores collected from colonies growing on ½ PDA were cultured on full strength PDA to increase inoculum.

3. Inoculum production.

Conidial suspensions of *F. graminearum, F. thapsinum, Curvularia lunata, Phoma sorghina* and *Alternaria alternata* were prepared by plating single colony agar plugs onto a culture plate and harvesting conidia by scraping the colonized agar plate

with a flame sterilized bacterial spreader. The solution was filtered through autoclaved cheese cloth to remove mycelial fragments. Conidial suspensions were calibrated to 1 x 10^6 conidia per ml with a haemocytometer.

4. **Plant inoculation**.

Individual genotypes were inoculated with spores of the five fungi and the treatments were replicated three times. Heads sprayed with sterile water served as a control. Panicles were inoculated at grain milk dough stage. Inoculum was sprayed at all angles on to the panicles until runoff. Panicles were immediately covered with a plastic bag for seven days to maintain high relative humidity and promote initial infection and colonization. The incidence of grain mould and its damage was scored visually as described above at maturity. The identity of grain mould fungi in the greenhouse was confirmed by confirmatory re-isolations in the lab.

5. **Ergosterol extraction and determination**.

At maturity, grain was harvested from each treatment replicate and ergosterol was determined according to the method of Seitz *et al.* (1977) as modified by Jambunathan *et al.* (1991). A 10 g sorghum grain sample from each panicle was ground using a laboratory mill and sieved through a 0.4 mm screen. Ergosterol was then extracted from the sample with 50 ml of methanol (MeOH) by vigorously mixing with a magnetic stirrer in a 100 ml beaker for 30 minutes. The mixture was allowed to settle and 25 ml of clean extract was decanted and added to a screw capped test tube containing 3 g of potassium hydroxide (KOH). The mixture was

thoroughly agitated on a vortex mixer to dissolve KOH. *n*-Hexane (10 ml) was added and the mixture was incubated at 75 °C in a water bath for 30 minutes and allowed to cool to room temperature. Distilled water (5 ml) was added, and after mixing thoroughly, the solution was allowed to cool to room temperature. The upper hexane layer was removed with a syringe and transferred to a test tube. Hexane (10 ml) was added to the remaining aliquot in the screw capped test tube and mixed thoroughly and the upper hexane layer was again removed and pooled with the earlier aliquot. The procedure was repeated one more time. The three pooled hexane extracts in the test tube were evaporated to dryness in a hot water bath maintained at 75 °C. The residue was re-dissolved in 5 ml of methanol (HPLC grade) and filtered through a 0.45 μm filter (Millex-HV, Millipore Corp., Bedford, MA). A 2 ml aliquot of the filtrate was maintained in a -80 °C freezer for ergosterol determination.

Ergosterol content in the filtrate was determined using a Shimadzu DGU-20A5 Prominence Degasser high performance liquid chromatograph (HPLC) with auto injector SIL-20A. The extract was loaded onto a silica based reverse-phase column (C18 110Å 5 μm particle size, 150 x 4.6 mm). The mobile phase was methanol-water (96:4 v/v) at a flow rate of 1.2 ml/min. The column temperature was maintained at 50 °C and the absorbance of eluted ergosterol was detected with a SPD-M20A prominence diode array detector at 282 nm. The standard ergosterol (Sigma) had a retention time of 8.2 minutes. The area under the graph for all chromatograms was converted to ergosterol concentration in μg/g using the following best fit formula:

$$Y = 0.1008e^{7E\text{-}08AREA}$$

Where Y = ergosterol concentration in μg/g of grain.

AREA = area under the chromatogram graph of micro-absorbance units (mAU) versus time (minutes).

6. **Statistical analysis.** All analyses were done using SAS (SAS Enterprise Guide, 2005) and Microsoft Excel. Kolmogorov-Smirnov (K-S) test and Bartlett's test were conducted prior to data analysis to test for homogeneity of error and normality respectively (Steel and Torrie, 1980). Data were subjected to analysis of variance, and student's t test and least significance differences at the 5% significance level were used to compare means. A multiple regression analysis was computed with mean final FGS (measure of kernel discoloration) as a dependent variable and mean ergosterol concentration of each fungus as the independent variable. The maximum R-square improvement method of SAS was used to determine the relative importance of the different fungal species in kernel discoloration.

   Biplot analysis was used to explain the significant genotype by pathogen (G x P) interaction observed after ANOVA. The biplot was obtained using Microsoft Excel add-in Biplot software. Biplot analysis is a multivariate method for graphing row and column elements (genotype and pathogens in this case) in principal component analysis of two-way contingency tables and to detect interaction in two-way analysis on variance tables (Gower and Hand, 1996). Two principal components (PCs 1 and 2), obtained by singular value decomposition using principal component analysis after transforming the data with "Columns Centered and Standardized" transformation of the two way contingency table, where calculated and used to

graphically explain G x P interaction. Interpretation of the Biplot was done according to the methods reported by Yan and Hunt (2002).

RESULTS AND DISCUSSION

There were significant differences in the responses of genotypes to inoculation by grain mould fungi as well as between pathogens when ergosterol concentration was used as an assessment criterion to measure grain mould severity as indicated by the ANOVA (Table 2). Fungi accounted for most of the variation observed (58.4%) in ergosterol concentration. This means that there are major differences in pathogenicity among the selected fungal species. However, host x pathogen interaction was limited, with the exception of *Alternaria alternata* and *Fusarium thapsinum* which showed most variation (Figure 2). This result implies that sorghum genotypes reacted differently when inoculated with *Alternaria alternata* and *Fusarium thapsinum*. It also indicates presence or absence of host resistance genes in different sorghum genotypes against *Alternaria alternata* and *Fusarium thapsinum.* Observed differences in pathogenicity may be due to different modes and sites of action of the pathogens as observed with grain diseases of other cereals caused by *Fusarium* spp. (Trail, 2000).

This observation concurs with previous studies of grain mould resistance that indicated the involvement of a few major genes (Kataria *et al.*, 1990; Esele *et al.*, 1993; Shivana *et al.*, 1994; Sharma *et al.*, 2000). Advanced molecular techniques have been used to determine grain mould resistant germplasm and genomic regions important in reaction to the disease appear to have been identified (Klein *et al*., 2001).

Esele (1995) noted the importance of use of molecular markers and recombinant DNA technology to isolate resistance genes and incorporate them into acceptable varieties (gene pyramiding).

Curvularia lunata, *Fusarium graminearum* and *Phoma sorghina* showed very little variation in pathogenicity across genotypes. This result implies that sorghum genotypes reacted similarly to these three pathogens. Similarity in fungal pathogenicity may be due to similarity in the host genetic constitution that is either effective or ineffective in limiting infection and subsequent colonization.

The significant genotype x pathogen interaction which accounted for 33.5% of observed variation (Table 2) could be attributed mostly to the variation in pathogenicity in *Alternaria alternata* and *Fusarium thapsinum*. Biplot analysis as displayed in Figure 3 explains the G x P interaction. The model used in this analysis explained 87% of the observed variation (PC1 55% and PC2 32%). The biplot was divided into 5 sectors with fungi as the vertex entries and are referred to as sector AOB, sector BOC, sector COD, sector DOE, and sector EOA. The 11 genotypes were scattered across the biplot. A genotype falling within a sector implies that it had a compatible reaction (disease/grain mould developed in that genotype) with the nearest fungus in that sector. Genotype 1 is therefore susceptible to *Fusarium thapsinum* and *Alternaria alternata* because it is closest to the two fungi in sector BOC but it is resistant to *Fusarium graminearum* in the opposite side in sector DOE. Similarly, genotypes 8 and 11 are susceptible to *Fusarium graminearum* but resistant to *Fusarium thapsinum* and *Alternaria alternata.* The resistance genes in these genotypes could be isolated and moved between genotypes.

Over the years preceding this study, genotype 1 had a mean FGS of 1.25 (Table 1) and had been classified as resistant (McLaren, unpublished). This study has indicated that genotype 1 is susceptible to *Fusarium thapsinum* and *Alternaria alternata.* This analysis illustrates that visual scoring for resistance to unnamed grain mould fungi can be misleading in the sense that in areas or seasons when there is no *Fusarium thapsinum* and or *Alternaria alternata,* genotype 1 will appear unaffected with a FGS score of less than 2 and be classified as resistant. When the season is favourable to *Fusarium thapsinum* and or *Alternaria alternata,* even in the same area, or when genotype 1 is planted in another area where *Fusarium thapsinum* and or *Alternaria alternata* are prevalent, genotype 1 could succumb to grain mould. Inter-specific variation has been shown to have greater epidemiological significance than intra-specific variation in sorghum grain mould (Jardine and Leslie, 1992; Mansuetus *et al.,* 1997; Singh and Bandyopadhyay, 2000). There is need to identify causal species in the area before assessing grain mould severity.

There are no genotypes in sectors COD and EOA but only *Phoma sorghina, Curvularia lunata* and the control. This means there were no genotypic responses to the two fungi and the control i.e. all host plants are either resistant or susceptible to *Phoma sorghina* and *Curvularia lunata*. This is also confirmed by the almost horizontal graphs for *Phoma sorghina, Curvularia lunata* and the control in Figure 2. Sector AOB only has genotype 4 in it. This means genotype 4 reacted in the same way to all fungi i.e. either has the resistance gene to all five fungi or it does not. Borderline genotypes like genotype 3 and genotype 6 are susceptible to fungi in the two sectors surrounding them.

The significance of genotype by environment interaction in addition to the complications of resistance breeding for grain mould has long been highlighted (Indira *et al.*, 1991). This is further complicated by the wide use of visual grain scoring for discoloration as a measure of grain mould severity. Analysis during this research showed no correlation between ergosterol concentration and FGS (Pearson correlation coefficient 0.05^{ns}). Table 3 shows genotype 1 which is "resistant" according to previous visual field scoring as having the highest mean ergosterol concentration over all pathogens. Genotype 10 which is supposedly susceptible under field conditions has the lowest ergosterol concentration. This discrepancy could be explained by the variation in distribution of fungal biomass in the kernels of resistant and susceptible genotypes. It could be assumed that resistant genotypes can accumulate more fungal biomass than susceptible genotypes as long as that fungal biomass is limited to the periphery/pericarp of the kernel and fails to reproduce i.e. the kernel still looks clean.

Table 4 shows that *Fusarium thapsinum* and *Phoma sorghina* were the most prolific grain colonizers across all genotypes. Resistance to these two fungi should therefore be prioritized. On the other hand, *Alternaria alternata* is responsible for most of the variation observed in kernel discoloration (Table 5). *Alternaria alternata* contributed 52% of the observed variation in kernel discoloration and very little additional variation was observed when other fungi where added to the model. Table 6 shows that, on average, most resistant genotypes had lower FGS ratings and most susceptible genotypes had higher FGS ratings.

FGS only indicates the severity of grain mould in the area but does not indicate the causal pathogen. Future use of FGS should be accompanied by identification of causal fungi in the specific areas so that breeders can look for sources of genes for specific environments. This outcome has serious implications to efforts to breed for grain mould resistance. All breeding programs consider grain mould as a disease caused by a complex of fungi without giving due emphasis to the identity of the predominant causal fungal species in each environment. This research has shown clearly that different fungi have different levels of pathogenicity depending on the genotype of the host. Identifying and stacking or pyramiding resistance genes to the various predominant fungi, may lead to broad sense resistance mechanism in all commercial sorghum hybrids and even open pollinated improved cultivars with special traits that are also resistant to grain mould (Esele *et al.,* 1993). It becomes important therefore that breeders locate and identify host genes that are responsible for the resistance that was observed above. Resistance to *Fusarium graminearum* for example could be moved from genotype 1 to either genotype 8 or genotype 11 by conventional means or marker assisted selection (when markers have been identified), or *vice versa* with resistance to *Fusarium thapsinum*. This can be extended to other fungal species by using a broader range of genotypes and more fungal species as deemed necessary from fungal isolations from infected kernels.

Several efforts have already gone a long way to identify possible resistance mechanisms to grain mould (Rooney and Klein, 2000; Esele *et al.,* 1993; Bandyopadhyay *et al.,* 1988). Five QTLs associated with grain mould resistance were identified by Klein *et al*., (2001). Rodriguez-Herrera *et al.* (2000) estimated 4 to 10

genes to contribute to grain mould resistance in sorghum. More effort is needed to link each resistance mechanism to each particular fungus associated with grain mould.

CONCLUSION

Fungal pathogenicity for grain mould varies from one species to another. This variation depends on host genotype in terms of presence or absence specific resistance genes. It is important to identify and isolate host resistance genes in order to pyramid them in desired varieties.

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Table 1. Pedigree, field grade score (FGS), and level of resistance of 11 sorghum genotypes planted in the greenhouse in 2006 for evaluation for genetic response to grain mould.

Genotype #	Pedigree	FGS	Resistance level[a]
1	(87EO366 * WSV387)-HF14	1.25	R
2	(ISCV 1089BF * MACIA)-HF2-CA2-AE	1.5	R
3	(MACIA * DORADO)-HD2----CA3	1.75	R
4	(MACIA * TAMU428)-LL9	3	I
5	(Segaolane*WM#322)-CG1-BGBK-CCBK	2.5	I
6	(SV1*Sima/IS23250)-LG15-CG1-BG2-BGBK	2.5	I
7	(90EO328*CE151)-LA37	1.25	R
8	Kuyuma	4.25	S
9	R.9645_(RTx430*Sureno)-B12	4.5	S
10	R.9732_(ADN55*Tx430)-B10	4	S
11	SRN39_Striga Res.	3	I

[a] Level: R = resistant ($x \leq 2$); I = intermediate resistant ($2 < x \leq 3$); S = susceptible to grain mould $3 < x \leq 5$

Table 2. Analysis of variance for ergosterol concentration for 11 genotypes treated with *F. graminearum, F. thapsinum, Curvularia lunata, Phoma sorghina* and *Alternaria alternata* spores in the greenhouse at Bloemfontein South Africa in 2006.

SOURCE	DF	SS	MS	F	% of (G + P + GP)
Model	66	3746.51[a]	56.77	110.40**	-
GENOTYPE	10	20.06	2.01	3.90**	8.1
PATHOGEN	5	144.22	28.84	56.10**	58.4
GEN x PATH	50	82.84	1.66	3.22**	33.5
Error	110	56.56	0.51		
Total	176	3803.08			

[a] R Squared = 0.985 (Adjusted R Squared = 0.976)
** = Significant at P = 0.01.
Dependent Variable: ERGOSTEROL CONCENTRATION

Table 3. Mean ergosterol concentration (μg/g) across eleven sorghum genotypes inoculated with *Fusarium graminearum, Fusarium thapsinum, Curvularia lunata, Phoma sorghina* and *Alternaria alternata*.

Genotype	Resistance level	N	Mean	Std Dev
1	R	16	5.30[a]	2.27
3	R	16	5.10[ab]	1.60
4	I	16	4.70[abc]	1.16
2	R	16	4.50[abc]	1.30
5	I	16	4.48[abc]	0.78
7	R	16	4.30[bc]	1.07
8	S	16	4.30[bc]	1.07
9	S	16	4.27[bc]	1.49
6	I	16	4.03[c]	1.07
11	I	16	4.02[c]	0.97
10	S	16	3.98[c]	0.99
LSD (0.05)	-	-	0.9152	-
CV (%)	-	-	29.44	-

Means followed by the same letter(s) are not significantly different at 5% level of significance by Fisher's LSD method.

Table 4. Mean ergosterol concentration (μg/g) associated with five fungi and the control inoculated onto 11 sorghum genotypes in the greenhouse.

Fungus	N	Mean	Std Dev
Fusarium thapsinum	33	5.38[a]	2.20
Phoma sorghina	33	5.34[a]	0.11
Curvularia lunata	33	4.79[b]	0.002
Fusarium graminearum	33	4.07[c]	0.002
Alternaria alternata	33	3.27[d]	0.67
Control	11	2.68[d]	0.0009
LSD[1] (0.05)	-	0.4852	-
LSD[2] (0.05)	-	0.8404	-
CV (%)	-	22.42	-

1. [1] = Least Significance Difference for mean comparisons excluding the control.
2. [2] = Least Significance Difference for mean comparisons against the control only.
3. Means followed by the same letter(s) are not significantly different at 5% level of significance by Fisher's LSD method.

Table 5. Regression models that best described relationships between final visual grain mould rating associated with five fungal pathogens and ergosterol concentrations in 11 sorghum genotypes in the greenhouse in 2006, using maximum *R*-square improvement technique.

Fungi	R^2	**F**
Alternaria alternata (ALT)	0.52	39.27**
Curvularia lunata (CUR)	0.04	0.41^{ns}
Fusarium graminearum (GRA)	0.09	0.87^{ns}
Fusarium thapsinum (THA)	0.15	1.63^{ns}
Phoma sorghina (PHO)	0.04	0.39^{ns}
Control (CON)	0.04	0.37^{ns}
ALT CON	0.54	4.66*
ALT CON CUR	0.54	2.73^{ns}
ALT CON CUR GRA	0.63	2.60^{ns}
ALT CON CUR GRA PHO	0.64	1.78^{ns}
ALT THA CUR GRA PHO CON	0.67	1.36^{ns}

** = Significant at P = 0.01.
* = Significant at P = 0.05.
^{ns} = not significant.

Table 6. Breakdown of the distribution of genotypes by class (R, I, and S) based on visual field grade scores for the 11 genotypes at maturity after inoculation in the greenhouse with five fungal pathogens.

	Level of grain mould resistance		
Mould rating[a]	**Resistant**	**Intermediate**	**Susceptible**
1	35.9	28.1	6.3
2	35.9	25.0	14.6
3	10.9	21.8	22.9
4	7.8	7.8	14.6
5	7.8	17.2	41.6

[a] Ratings: 1 = no visible mould; 2 = 1 to 10%; 3 = 11 to 25%; 4 = 26 to 50%; 5 = more than 50% of kernels in the panicle moulded.

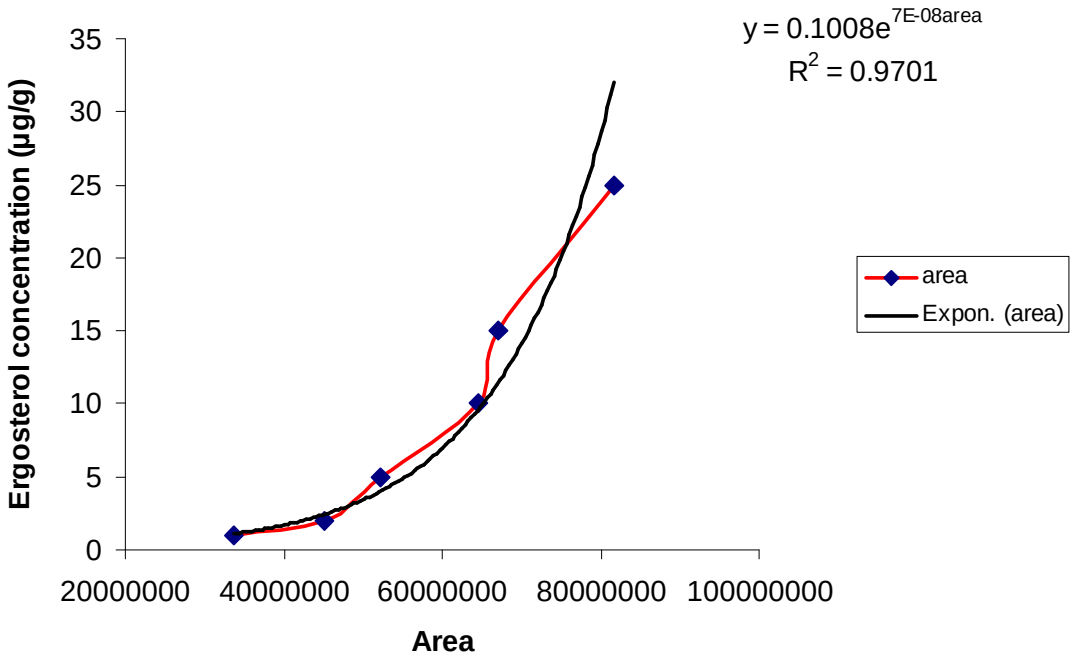

Figure 1. Standard-ergosterol concentration (µg/g) versus area under the chromatogram curve for the observed ergosterol concentration and the predicted ergosterol concentration using the exponential transformation as the best fit.

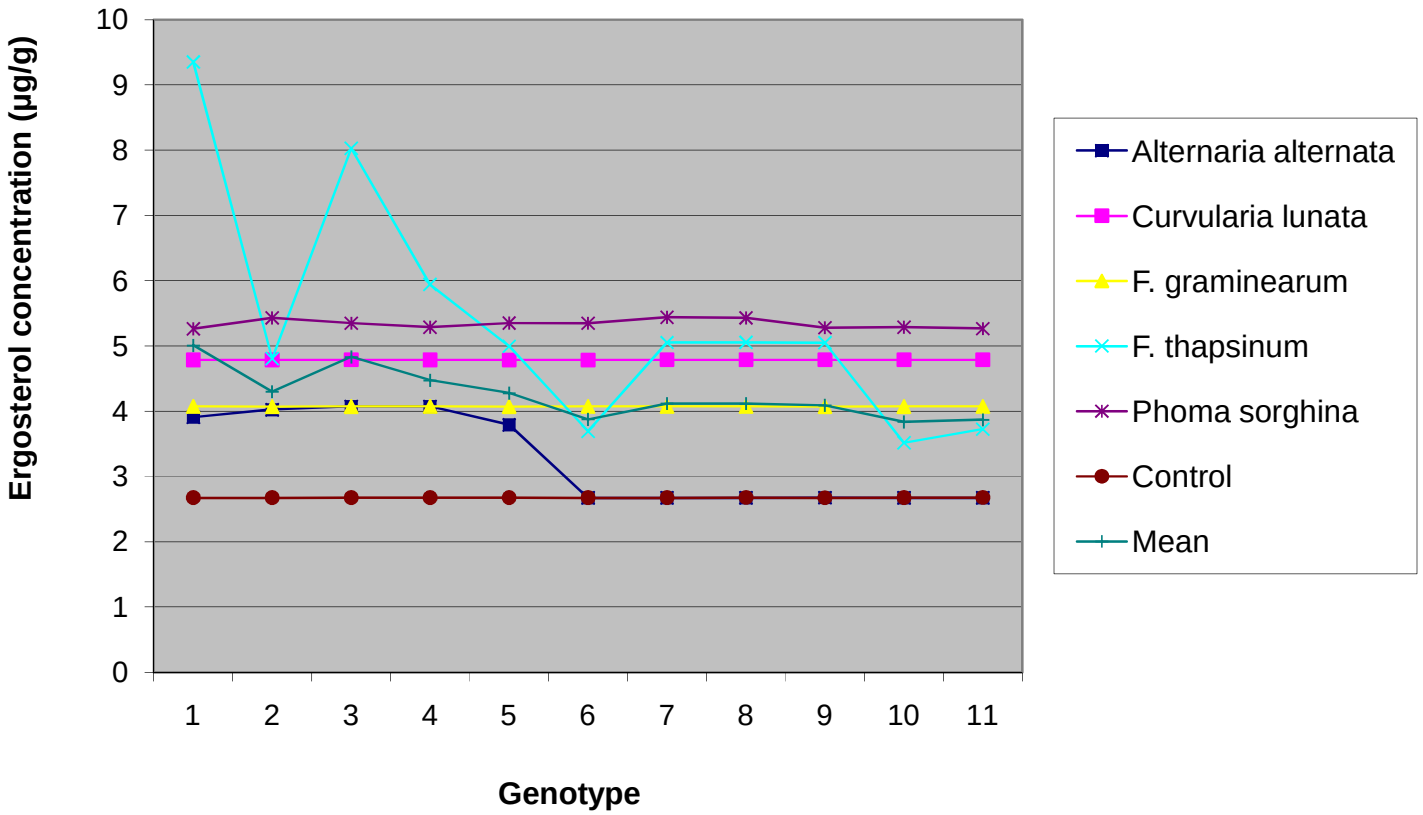

Figure 2. Mean ergosterol concentration across 11 genotypes inoculated with *Fusarium graminearum*, *Fusarium thapsinum*, *Curvularia lunata*, *Phoma sorghina* and *Alternaria alternata* in the greenhouse at Bloemfontein South Africa in 2006.

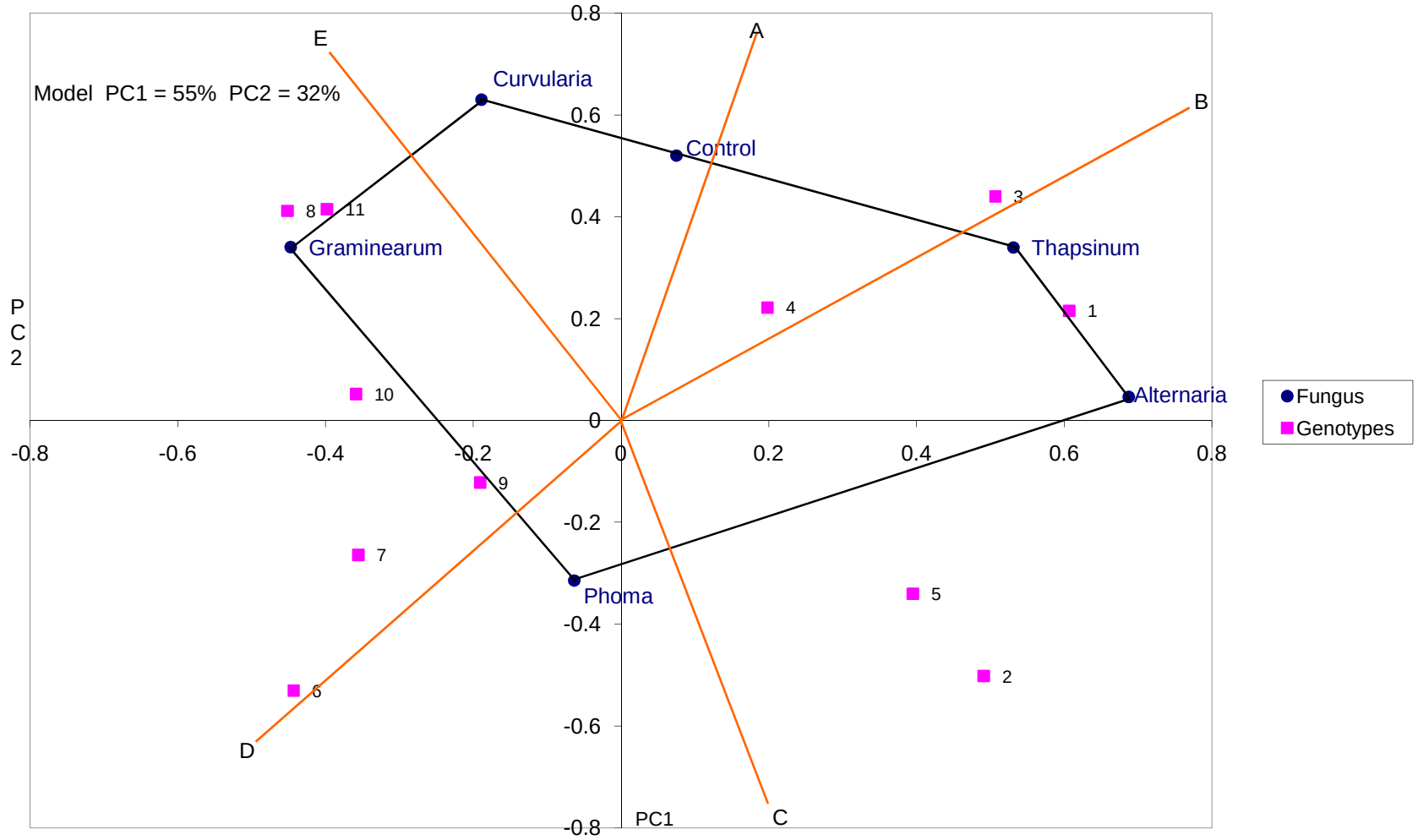

Figure 3. Biplot analysis of the nature of interaction between the 11 sorghum genotypes and 5 fungal species commonly found in the sorghum grain mould complex.

CHAPTER 3

Evaluation of heritability and gene action controlling grain mould resistance in sorghum.

ABSTRACT

Gene action and heritability for grain mould resistance in sorghum [*Sorghum bicolor* (L.) Moench] were investigated using a selection of specific parental lines. A North Carolina Experiment II mating design was used to test the combining ability of 9 random pollen parents (LM101, LM103, LM104, LM108, LM109, LM114, LM116, LM117, and LM120) with varying levels of grain mould resistance to a different set of three random seed parents (LM122, LM124 and LM130). The 27 resulting progenies and the parental lines were evaluated in a randomized complete-block design at Cedara-1, Cedara-2 and Potchefstroom during the 2007-2008 season. Differences in ergosterol concentration were used as a measure of level of grain mould resistance among all genotypes. Use of ergosterol concentration as a measure of grain mould severity did not correlate with visual field scoring. Other traits measured include plant yield, plant height, kernel hardness, field grade score, days to flowering, glume color and seed color. The combined analysis of variance showed no genotypic variance for grain mould resistance. The expression of grain mould resistance was also not stable with significant genotype x location interaction. The analysis from Potchefstroom showed significant differences among genotypes whereas Cedara-1 and 2 showed no differences. Additive genetic

variance was greater than dominance variance for all traits except grain mould resistance. A significant heterosis of -20.15% was observed for grain mould resistance indicating the importance of use of hybrid seed. LM124 (white seeded female) and LM130 (brown seeded female) produced resistant hybrids. Due to very high environmental variance, grain mould heritability could not be detected. Yield indicated the highest heritability of 0.41 at Cedara-1, plant height 0.39 at Cedara-2 and kernel hardness 0.38 at Potchefstroom.

INTRODUCTION

Grain sorghum [*Sorghum bicolor* (L.) Moench] is an important cereal crop with 57 924 metric tonnes produced in the world in 2004 (FAOSTAT data, 2004). For some of the world's most food insecure people sorghum remains the only viable food grain. Average grain yield for sorghum in most parts of Africa remains below 1 ton/ha (Chisi, 1996). The lower average grain yield is primarily because sorghum cultivation in Africa is still mainly characterized by traditional farming practices with low inputs (no inorganic fertilizer or pesticides) and use of traditional varieties or landraces. Biotic and abiotic stresses like high temperature, low and erratic rainfall, poor soils, pests and diseases are predominant in sorghum producing areas of Africa. Sorghum originates in Africa hence the largest diversity in cultivated and wild sorghum is in Africa (Doggett, 1970: De Wet and Harlan, 1971). Despite its positive adaptation to the African climate, sorghum continues to be dogged by many pests and diseases that reduce its productivity.

Grain mould has been traditionally described as a condition (deterioration of grain) resulting from all fungal associations with sorghum spikelet tissues occurring from anthesis to harvest (Forbes *et al.*, 1992). Fungi in more than 40 genera have been associated with sorghum grain mould (Williams and Rao, 1981). Mycoflora analysis of sorghum kernels over the years reveal that some of the most important species include *Fusarium graminearum* Schwabe, *Fusarium thapsinum* Klittich, Leslie, Nelson et Marasas sp. nov. 1996, *Curvularia lunata* (Wakker) Boedijn, *Phoma sorghina* (Sacc.) Boerma *et al.* and *Alternaria alternata* (Fr.) Keissl. because they are more frequently isolated from moulded grain (Williams and Rao, 1981; Bandyopadhyay *et al.*, 1991; Esele *et al.*, 1993; Erpelding and Prom, 2006).

A hot and humid environment, during and after maturation, promotes extensive damage of the grain by mould fungi. As a result, grain mould is of particular concern in areas where the period between anthesis and harvest coincides with high humidity and warm temperature.

Several sources of resistance against grain mould and their methods of inheritance have been identified (Bandyopadhyay *et al.*, 1988: Esele *et al.*, 1993: Menkir *et al.*, 1996). Both qualitative and quantitative loci having dominant, additive and epistatic effects have been shown to influence grain mould resistance. Endosperm texture, presence and color of glumes, pericarp traits such as color and presence of a pigmented testa layer are some of the traits associated with resistance. Rodriguez-Herera *et al.*, (2000) estimated that 4 to 10 genes contributed to grain mould resistance in sorghum. Both pigmented and non-pigmented sorghum lines with varying levels of resistance have been identified and used in crosses to develop new improved grain mould resistant lines and hybrids with limited success (Rooney and Klein, 2000). The lack of success could be attributed to an incomplete understanding of the genetics of interaction of sorghum grain mould resistance genes with fungal pathogenesis genes. Whilst there is lots of literature on the nature of the resistance and the genes associated with it, there is very little information on how these resistance genes interact with fungal genes to cause disease or prevent it. The resistant lines that have been identified in literature, e.g. Sureño, have not been genetically characterized to identify their resistance genes. Identification and characterization of such resistance genes in Sureño and other mentioned lines in literature could help sorghum geneticists investigate ways of moving those genes to where they could be useful. The detection of epistatic effects in grain mould resistance may indicate

the need to investigate gene combinations that enhance resistance. But it all begins with identifying and characterizing respective sources of resistance that already exist.

The role of the environment in this interaction has not been thoroughly or properly researched or articulated since environment is known to play a major role in all such cases. Currently, it has been generally accepted that selection in specific environments is a useful tool in enhancing grain mould resistance build up. But this means sorghum geneticists have to keep making crosses and selections for every environment each time the pathogen mutates or migrates to new environments. This research looks into gene action and heritability of grain mould resistance genes by investigating the performance of a selection of sorghum lines that vary from resistant to intermediate to susceptible across three environments. This information should help geneticists in making a choice of parental lines to use in hybrid crosses and improve the speed and efficiency of increasing genetic gain for grain mould resistance.

MATERIALS AND METHODS

1. Genetic material.

During the 2006-2007 season, nine pollinator lines (LM101, LM103, LM104, LM108, LM109, LM114, LM116, LM117, AND LM120), with varying levels of resistance to grain mould, were crossed to three A1 cytoplasmic-genetic male-sterile sorghum seed lines (LM122, LM124 AND LM130) without reciprocals at Potchefstroom (Table 1) using a North Carolina Experiment II mating design (Comstock and Robinson, 1948). The 9 male lines were sourced from a southern Africa sorghum regional breeding nursery sponsored by the International Sorghum and Millets Collaborative Research Support Program (INTSORMIL CRSP) of USA. The 3 female lines were sourced from Texas A&M University sorghum breeding program. The male lines were selected to represent different levels of resistance to grain mould i.e. resistant, intermediate and susceptible. These classifications were based on visual scoring data collected over several years of field evaluation within the region.

All male lines had white grains but varied for glume color. Presence of glumes and their color is one of the traits known to contribute towards resistance to grain mould. There were five panicle shapes coded as described in the descriptors for sorghum hand book (IBPGR and ICRISAT, 1993). 1 – Very lax panicle (typical of wild sorghum), 6 – Semi-loose erect primary branches, 7 – Semi-loose drooping primary branches, 8 – Semi-compact elliptic, and 9 – Compact elliptic. Panicle shape also contributes to grain mould resistance. One female line (LM122) had tan glumes and the other two (LM124 and LM130) had brown glumes. Two females (LM122 and LM124) had white grains and one

(LM130) had brown grain. This variation was used in an effort to capture maternal effects.

2. Experimental design

During the 2007-2008 season, seeds of the 27 single crosses plus the 12 parental lines as well as the three maintainer lines (male fertile) of the A1 testers used in the experiment were sown in a randomized complete block design (RCBD) with three replications in single 5 m row plots 0.75 m apart. The experiments were rain fed and standard agricultural practices were the same for all plots. The experiment at Cedara was planted on two dates. The first one was planted on the 27th of November and the second one on the 12th of December 2007 and the two planting dates were treated as different environments. The third experiment was planted at Potchefstroom on the 6th of December 2007. At Cedara, 10 plants per plot were bagged with transparent aerated bags to avoid bird damage. At Potchefstroom, the whole experiment was planted under a large bird cage net. Assessment of grain mould was done under natural infestation. 10 representative plants/panicles were harvested from each plot for further processing.

3. Characters measured

3.1. **Ergosterol content (ergo).** Ergosterol was determined according to the method of Seitz *et al.* (1977) as modified by Jambunathan *et al.* (1991). A 10 g sorghum grain sample from each panicle was ground using a laboratory mill and sieved through a 0.4 mm screen. Ergosterol was then extracted from the sample with 50 ml of methanol (MeOH) by vigorously mixing with a magnetic stirrer in

a 100 ml beaker for 30 minutes. The mixture was allowed to settle and 25 ml of clean extract was decanted and added into a screw capped test tube containing 3 g of potassium hydroxide (KOH). The mixture was thoroughly agitated on a vortex mixer to dissolve KOH. 10 ml of *n*-Hexane was added and the mixture was incubated at 75 °C in a water bath for 30 minutes and then allowed to cool to room temperature. 5ml of distilled water was then added, and after mixing thoroughly, the solution was allowed to cool down to room temperature. The top hexane layer was removed with a syringe and transferred to a test tube. To the remaining aliquot in the screw capped test tube, 10 ml of hexane was added and mixed thoroughly and the top hexane layer was removed and pooled with the earlier aliquot. The procedure was repeated one more time. The three pooled hexane extracts in the test tube were evaporated to dryness in a hot water bath. The residue was re-dissolved in 5 ml of methanol (HPLC grade) and filtered through a 0.45 μm filter (Millex-HV, Millipore Corp., Bedford, MA), and 2 ml of the filtrate was kept in a -80 °C freezer before ergosterol determination. Ergosterol content in the filtrate was determined using a Shimadzu DGU-20A5 Prominence Degasser high performance liquid chromatograph (HPLC) machine with auto injector SIL-20A. The extract was loaded on a silica based reverse-phase column (C18 110Å 5 μm particle size, 150 x 4.6 mm with two Securiguard cartridges). The mobile phase was methanol-water (96:4 v/v) at a flow rate of 1.2 ml min^{-1}. The column temperature was maintained at 50 °C and the absorbance of eluted ergosterol was detected with an SPD-M20A prominence diode array detector at 282 nm. The standard ergosterol (Sigma) had a retention time of 8.2

minutes. The standard ergosterol was loaded in 1.0, 2.5, 5.0, 7.5, 10.0, 15.0, and 25.0 μg ml^{-1} to standardize measurement of samples and also to determine the correct peak position on the chromatograph. The area under the graph for all sample chromatograms was converted to ergosterol concentration in μg/g of grain using the following best fit formula:

$$Y = 0.1008e^{7E\text{-}08AREA}$$

Where Y = ergosterol concentration in μg/g of grain.

AREA = area under the chromatogram graph of micro-absorbance units (mAU) versus time (minutes).

3.2. **Plant yield (yield).** Plant yield was recorded in grams as an average of 10 representative plants per plot.

3.3. **Plant height (height).** Plant height was measured in cm as an average of 10 representative plants from the ground to the top of the panicle.

3.4. **Kernel hardness (hardness).** A Tangential Abrasive Dehulling Device (TADD) was used for abrasive decortication milling of samples as described by (Reichert *et al.*, 1982). The device consists of eight sample cups (diameter = 5cm; depth = 5 cm) set at a distance of 1 mm above the abrasive surface. The abrasive surface consists of a horizontally rotating disk (1725 rpm) on which sand paper (P60, 3M, 454HL) had been glued. Each sample was abraded for 1, 2, and 3 minutes respectively. The weight of the abraded sample, at the mentioned time intervals, was measured and the percent abraded material recorded. A

regression of % abraded material versus time was done. The Abrasive Hardness Index (AHI) was calculated by multiplying the inverse of the slope of the regression line by 60 seconds. AHI was expressed as the time, in seconds, required to remove 1% of the grain mass by abrasion. The higher the AHI, the harder the grain.

3.5. **Field grade score (fgs).** The 10 selected panicles from each replication of each genotype were scored visually for grain mould severity at harvest using a 1 to 5 scale as described by (Bandyopadhyay and Mughogho, 1988) where R = resistant ($fgs \leq 2$); I = intermediate resistant ($2 < fgs \leq 3$); S = susceptible to grain mould $3 < fgs \leq 5$.

3.6. **Number of days to flowering (dma).** Number of days from germination to the day when 50% of the plants within a plot were flowering was noted. This data was not collected from Potchefstroom for logistical reasons.

3.7. **Glume color (glgcl).** Visual determination of either tan or brown was used. Tan grouped all pale shades and brown grouped all dark shades i.e. red and purple though they are genetically different.

3.8. **Seed color (sdcl).** Visual determination of either white or brown was used. White grouped all shades of white and brown grouped all colored grains (red, lemon yellow and other).

4. Statistical analysis

4.1. Factorial analysis

Analysis of variance and combining ability were performed per established methods (Hallauer and Miranda, 1988) with the PROC GLM procedure of SAS

(SAS Institute, 2003). Replications and environments and corresponding interactions were considered random effects and genotypes were considered fixed effects. Genotypes were fixed so that inferences, whether different or similar, could be made on later analysis with similar experiments at the same locations. Tests of significance for genotype, parent, female parent, male parent, hybrid and male by female effects for all traits were made by testing these mean squares against their respective environmental interactions (Table 2). Each environmental interaction term was tested against the pooled error mean square. Bartlett's test for homogeneity of error variances was used to test the validity of combining data from individual environments. Results indicated that the error variances were homogeneous and data from each environment was normally distributed. Therefore, combined analysis was conducted to make comparisons across environments. ANOVA for individual environments was done as indicated in (Table 3). All terms were tested against pooled error mean square. Mean comparisons were conducted using Fisher's least significant difference (LSD) procedure, with a probability level of 0.05.

A factorial analysis with a mathematical model underlying the analysis of variance was as follows:

$$Y_{ijke} = \mu + F_i + M_j + (FM)_{ij} + R(E)_{ke} + (ME)_{je} + (FE)_{ie} + (FME)_{ije} + \varepsilon_{ijke}$$

where,

Y_{ijke} = the observed trait value on the hybrid between the i^{th} female and the j^{th} male in the k^{th} replication at environment e of the experiment.

μ = mean effect.

F_i= the effect of the ith female parent, i = 1 to 4. Attributable to differences in general combining ability among female parents.

M_j = the effect of the jth male parent, j = 1 to 10. Attributable to differences in general combining ability among male parents.

$(FM)_{ij}$ = the interaction effect of the ith female and the jth male. Attributable to differences in specific combining ability.

$R(E)_{ke}$ = the effect of the kth replication within environment e, k and e = 1 to 3.

$(ME)_{je}$ = the interaction effect of the jth male and the eth environment.

$(FE)_{ie}$ = the interaction effect of the ith female and the eth environment.

ε_{ijke} = the effect of the subplot which had the hybrid between the ith female and the jth male in the kth replication of the eth environment of the experiment (experimental error).

4.2. Calculation of variance components and heritabilities (Becker, 1975)

Variance components were calculated using the following formulae:

$$\sigma_e^2 = MS_e$$

$$\sigma_{mf}^2 = \frac{MS_{mf} - MS_e}{r}$$

$$\sigma_m^2 = \frac{MS_m - MS_{mf}}{fr}$$

$$\sigma_f^2 = \frac{MS_f - MS_{mf}}{mr}$$

Heritabilities were calculated using the following formulae:

$$\sigma_A^2 = 4\sigma_f^2 = 4\sigma_m^2 \qquad \text{and} \qquad \sigma_D^2 = 4\sigma_{mf}^2$$

$V_P = 4\sigma_m^2 + 4\sigma_f^2 + 4\sigma_{mf}^2 + \sigma_e^2$ - Where V_P is total phenotypic variance

$$h_m^2 = \frac{4\sigma_m^2}{V_P}$$

$$h_f^2 = \frac{4\sigma_f^2}{V_P}$$

$$h_{m+f}^2 = \frac{2\left(\sigma_m^2 + \sigma_f^2\right)}{V_P}$$

The standard errors for variance components and heritabilities were calculated using the following formulae:

$S.E.\left(\sigma^2\right) \approx \sqrt{\frac{2}{k^2}\sum_g \left(\frac{MS_g^2}{f_g + 2}\right)}$ - Same for all variance components

Where k = coefficient of variance denominator component, MS_g = g^{th} mean square, f_g = degrees of freedom of the g^{th} mean square.

$S.E.\left(h_m^2\right) \approx \frac{4\left\{S.E.\left(\sigma_m^2\right)\right\}}{V_P}$ - Same for all heritabilities

4.3. Calculation of high parent heterosis

$$\text{High-parent heterosis} = \left[\frac{F_1 - HP}{HP}\right] * 100$$

Where F_1 = Mean of the F_1 offspring/hybrid

HP = Mean of highest performing parent or best parent

High parent heterosis is of more practical and applicable use to make fast significant gains in the trait of interest.

4.4 Estimates of the general combining ability (GCA)

GCA of each parental line was calculated as the difference between the grand mean and the marginal hybrid mean of each line. Significance of the GCA effect for each line was tested using a two-tailed *t*-test procedure in SAS. Standard errors for GCA effects for both males and females were calculated following procedures described by Cox and Frey, (1984). Specific combining ability was obtained as deviation of individual crosses from the performance expected from the average of the parents. The formulas are as follows:

GCA = A* - Y**

SCA = AB – (A*+B*)/2

where

A* = mean performance of line A.

Y** = general mean.

AB = mean performance of the hybrid between lines A and B.

$SE_{GCA} = \{MS_{ml}[(m-1)/mflyr]\}^{1/2}$ or $SE_{GCA} = \{MS_{fl}[(f-1)/mflyr]\}^{1/2}$

RESULTS AND DISCUSSION

1. ANOVA

1.1. Combined ANOVA

Growth environment significantly affected all four traits between experiments (Table 4). This may be because of differences in weather conditions among test environments. All the four traits are significantly affected by variations in total rainfall received and its distribution, humidity, and variations in temperature and soil types. The R-squares for all traits were above 0.50 hence acceptable.

For ergosterol concentration, there were significant differences between the parental lines and hybrid groups as shown by the significance of the Parent vs. Hybrid term. This indicates expression of heterosis for grain mould resistance. Use of hybrids should therefore be recommended hence choice of parents becomes even more critical. However, in this experiment, no significant differences were observed among all genotypes i.e. no significant differences between parental lines used and between hybrids generated. The reason for failure to detect genotypic variation in this case could be poor selection of parental lines used in making the crosses. Since parent lines used in this experiment were not significantly different genetically for grain mould resistance (Genotypes not significant) according to ergosterol concentration measure, it becomes almost impossible for the F1 to express resistance as expected.

Correlation analysis in this research shows that there is no correlation between visual field scoring and ergosterol concentration (-0.01^{ns}) (Table 16). The genetic material used for this research was based on selections made after visual scoring for grain mould severity hence it is possible that the classification into the three categories of resistant, intermediate and susceptible could be wrong. Previous research has proven that ergosterol assay is the most sensitive, rapid and convenient method of measuring fungal growth (Seitz *et al.*, 1977). This discrepancy could lead to failure for the F1 to exhibit expected genotypic/phenotypic ratios according to wrongly presumed parental genotypes hence failure to detect genotypic variation.

Most of the variation observed for ergosterol concentration was due to environmental effects. Gen*Env was highly significant and this was mainly from the female parents' variable response to environmental changes. This means that the significant differences observed among female lines were largely due to environmental effects. There is need therefore to select female lines based on their performance in each environment. This finding concurs with previous research (Indira *et al.*, 1991; Rodriguez-Herrera *et al*., 2000) which indicated that grain mould resistance is highly sensitive to environmental effects. This could be because grain mould resistance is multigenic with at least 4 to 10 genes estimated to be involved (Rodriguez-Herrera *et al*., 2000) with additive, dominance and higher order epistatic interactions. As a result, it has been generally accepted that selection in specific environments is a useful tool in enhancing grain mould resistance build up.

However, (Chapter 2) indicates presence of significant pathogen*host genotypic interaction in the nature of grain mould resistance. This interaction has not been thoroughly investigated in most previous research efforts. A detailed investigation in the genetic nature of the host resistance genes versus pathogen virulence genes could stop confounding genotypic variance into environmental variance or unexplained variance. The current failure to detect genotypic variance could then be due to similar host response for all genotypes previously classified wrongfully as resistant (R), intermediate (I) or susceptible (S) to the predominant fungal pathogenesis in each of the three environments. For example, all three classes (R, I and S) could actually have resistance genes against *C. lunata* hence react similarly in an environment where *C. lunata* is predominant. In an environment where *Phoma* is predominant the same group of genotypes may fall into different categories according to whether they have resistance genes against *Phoma*. This explanation once again brings about the need to categorize host genotypes according to their response to specific fungi (using ergosterol concentration analysis as an indicator of mould severity) instead of their visual scoring response to a complex of fungi as it has been the norm in most previous research.

One way of improving the ability to detect genotypic variation for resistance is the use of artificial inoculation of the trials in the field with a concoction of various conidia of selected important fungi combined with the use of overhead sprinkler irrigation during dry periods in order to increase disease pressure and reduce the effect of erroneous or uneven occurrence of natural

inoculum under natural infestation i.e. reduce environmental effects (Bandyopadhyay and Mughogho, 1988). However, this method was not followed here for logistical reasons.

The ANOVA for yield indicates existence of significant heterosis i.e. significant Parent vs. Hybrid effect. This also means use of hybrids over inbreds should be recommended. Also there were significant differences between genotypes. This difference is observed between parental lines and between hybrids. Within parental lines both male and female effects were significant and there were significant differences between male and female group effects. Within hybrids male GCA effects accounted for most of the hybrid effects. This indicates that yield is regulated by additive gene action from the male parental lines and no dominance effects as seen by the non-significance of the M x F (sca) term. This means when breeding for yield one should use a lot of male tester lines with a few good female lines. Gen x Env interaction was very significant and was expressed at both parental and hybrid effect levels. In the parental effect only males were significant. This means that male parental lines performed differently at each location. Within hybrids both the male and female effects had significant interaction with the environment. (Maman *et. al.*, 2004) indicated how location, year, and water regime influences number of panicles per square meter, kernel weight, potential kernel and kernels per panicle in determining grain yield in sorghum.

The ANOVA for plant height indicates presence of heterosis. Table 16 indicates a correlation of 0.55** between plant yield and plant height. This

means that taller plants tend to have more yield than shorter plants. There were significant differences between genotypes and these can be seen among both parental lines and hybrids. In parental lines, these differences are more significant among male lines only. However, differences between male and female line groups were not significant. Differences among hybrid lines were due to Male GCA, Female GCA and M x F SCA effects indicating the presence of male and female additive gene effects and dominance effects. Growth environment did not have any significant effect on plant height except for significant effect on the male effect within hybrids.

All observed variation for grain hardness was mainly genetic. There were no environmental effects except for the female effects within hybrids. This impresses the importance of choosing female lines based on their performance within an environment. The significance of genotypes means that there were significant genotypic differences among the genotypes across all environments. Genotypic differences were significant for both parental lines and hybrids. In parental lines, these differences were significant for both male and female lines and there were significant differences between male and female lines. These parental differences were passed on to the F1 generation in the form of additive male and female gene effects (Male GCA and Female GCA). There were no dominance gene effects for grain hardness. Female genetic effects were higher than male genetic effects within both parents and hybrids. This could be due to maternal effects that come about due to the *3n* nature of the kernel endosperm with *2n* coming from the mother plant.

1.2. Cedara-1 ANOVA

There were no significant differences among genotypes for ergosterol concentration (Table 5). This translated to no differences between parental lines and within hybrids. All of these effects had smaller mean squares than error mean square (MS_e). This is an indication of very high environmental effects since MS_e represents environmental variance in this case. The weather in Cedara tended to be highly humid with higher average rainfall but average daily temperature was low during the grain filling period. However, some heterosis was observed in the form of the significant Parent vs. Hybrid term.

The ANOVA for plant yield indicates that there were significant differences between genotypes. These differences were observed among both parental lines and hybrids. Among parental lines, the males were responsible for the observed differences and the females did not have a significant effect. Among the hybrids, both male and female additive gene actions were significant (Mgca and Fgca respectively) whereas dominance gene action (M x F sca) was not significant. However, heterosis was very significant and was observed in the form of the significant Parent vs. Hybrid term.

The ANOVA for plant height indicates that there were significant differences between genotypes. These differences were observed among both parental lines and hybrids. Among parental lines, the males were responsible for the observed differences and the females did not have a significant effect. There were no significant differences between male and female lines among parental lines. Among the hybrids, only male additive gene action was significant (Mgca)

whereas female additive gene action (Fgca) and dominance gene action (M x F sca) were not significant. Heterosis was significant indicating that hybrids were significantly taller than their parental lines at this location.

The ANOVA for kernel hardness indicates that there were significant differences between genotypes. These differences were observed among parental lines and not among hybrids. Among parental lines, both males and females were responsible for the observed differences with females contributing significantly higher variance than males as seen by the significance of the Male vs. Female term. Among the hybrids, there were no significant differences but significant male additive gene action (Mgca) was observed. Heterosis was not significant indicating that hybrids were not significantly harder than their parental lines at this location.

1.3. Cedara-2 ANOVA

The non-significance of males, females and their interaction for ergosterol concentration was once again due to high environmental effects (MS_e) (Table 6). This location was a difficult one with lots of non-significant terms. Replications were not effective in reducing experimental error for ergosterol concentration, yield and plant height. The only significant difference observed for ergosterol concentration was between male and female parental lines. However, this difference was not passed onto the hybrids. This non-significance of terms could also be attributed to lower average temperatures that were experienced during grain filling period for the late planted experiment. The lower temperatures caused a reduction of grain mould severity hence mean ergosterol concentration for both Cedara-1 and Cedara-2 are lower than for Potchefstroom (Tables 13, 14 and 15).

The ANOVA for plant yield indicates that there were significant differences between genotypes. These differences were due to both male and female parental effects. Male parental lines also performed significantly higher than female parental lines. Hybrids performed significantly better than the parental lines as indicated by the significance of the Parent vs. Hybrid term. The significance of the hybrid term was due to Male GCA effects only. No dominance gene action was detected.

There were significant differences among genotypes for plant height. These differences were due to significant hybrid and parental line differences. Among the parental lines only male lines were significant. Among hybrids only

the males once again were significant. There was significant heterosis for height as seen by the significant Parent vs. Hybrid term.

The significance of genotypic differences for grain hardness was due to the effect of parental lines only. There were no significant differences among hybrid lines. There were no heterotic effects either even though male and female parental lines were significantly different.

1.4. Potchefstroom ANOVA

The CV for ergosterol concentration at this location was very high (64.49%) as indicated in Table 7. This location had the higher ergosterol concentration than the other two (18.62 μg/g compared to 5.31 μg/g and 4.75 μg/g for Cedara-1 and Cedara-2 respectively). This could be because Potchefstroom had warmer temperatures during the grain filling period compared to Cedara-1 and Cedara-2. Therefore genotypic differences among genotypes could be detected at this location however parents and hybrids were not significant. Some level of heterosis was also present at this location.

The ANOVA for grain yield indicates that significant genotypic differences among all genotypes existed and were among both parental lines and hybrids. However among parental lines the male and female groups were not significant even though the two groups were significantly different. Among hybrids male additive gene action (Mgca) and dominance gene action (M x F sca) were significant. There was also significant heterosis for yield.

There were significant genotypic differences among genotypes for plant height and these were observed among both parental lines and hybrids. The male lines effect within parental lines was solely responsible for all variation observed among parental lines. Among hybrids both male and female GCAs were significant indicating an additive gene action effect without dominance since M x F sca was not significant. Heterosis for height was very significant indicating that hybrids were generally taller than their parental lines.

The ANOVA for kernel hardness indicates that there were significant differences among genotypes and these were among both parental lines and hybrids. Among parental lines the males had a significant effect over the females. Among the hybrids both male and female additive effects (Mgca and Fgca) were significant though the female GCA was stronger than the male GCA indicating once again maternal effects on this trait. There were no dominance effects (M x F sca) for kernel hardness. Heterosis was not significant at this location just like at the other two locations.

2. Means

2.1. Combined means

Even though there were no significant differences among genotypes for ergosterol concentration analysis in the ANOVA (Table 4), some inference can be made about some observed trends. Only three mean groupings were observed with group (ab) having 36 out of 39 genotypes (Table 8). Individual entry observations ranged from a minimum of 1.32 μg/g to a maximum of 71.38 μg/g. This led to a very high CV of 93.20%. However average ergosterol levels across locations ranged from the highest of 17.25 μg/g (LM109) to the minimum of 3.52 μg/g (LM122). From this range, any ergosterol concentration above 12 μg/g is considered susceptible. A concentration between 12 μg/g and 8 μg/g is considered intermediate and any concentration below 8 μg/g is considered resistant.

LM109 was the most susceptible genotype with the highest mean ergosterol concentration of 17.25 μg/g. LM109 was initially classified as intermediate resistant (Table 1) with brown glumes, white seeds and with a semi-loose panicle shape according to visual scoring. All its traits point towards a genotype with good grain mould resistance traits but ergosterol concentration analysis classifies it as the most susceptible genotype across locations. This highlights the need to rely on ergosterol concentration analysis as a means of scoring for level of resistance.

LM122 was the most resistant genotype with the least mean ergosterol concentration of 3.52 μg/g. LM122 is a tan plant with tan glumes, a semi-loose

with erect primary branches panicle shape, female line with resistance to grain mould. It can therefore be considered a source of grain mould resistance in non-pigmented sorghums. Its ability though to combine favorably with selected male lines is tested as indicated below.

LM122 hybrids had the highest positive average heterosis of 159.98% (Table 9) for ergosterol concentration analysis among the three female lines used. This means that despite LM122 being the most resistant line among both parental and hybrid lines, this line produced hybrids that are more susceptible than itself i.e. failed to pass the resistance to its offspring. This is evident from LM122 hybrids having a mean of 9.11 μg/g compared to 8.30 μg/g and 8.60 μg/g for LM124 and LM130 hybrids respectively (Table 8). (Table 10) indicates that LM122 had a positive GCA value of 0.466 and most of the positive GCA for this line was observed at Potchefstroom (Table 11). This observation can be explained by the fact that LM122 hybrids also had softer endosperms compared to their parental lines as indicated by their mean heterosis and GCA at -11.91% and -1.065 respectively for kernel hardness. LM122 also had no interesting SCA combinations with any of the male lines used (Table 12).

(Table 9) also indicates an average heterosis of -20% for ergosterol concentration meaning that use of hybrid seed could lead to a significant 20% gain in grain mould resistance. This level of heterosis was observed despite the fact that genotypes were not significantly different for ergosterol concentration analysis (ANOVA Table 4). This means that if a different set of parental lines with a different set of properly identified resistance genes, in both male and

female lines were used, then more gain and or differentiation among the F1 generation would have been observed. This finding further proves the need to identify good parental lines i.e. parental lines with gene combinations that lead to an increase in genetic resistance to grain mould. (Chapter 2) provides evidence of the need to play around with host genotype to counter pathological virulence. There is need therefore to use advanced technological approaches like marker assisted selection (MAS) and quantitative trait loci (QTL) analysis to identify useful genetic markers and quantitative loci on chromosomes associated with specific resistance genes in order to select good parental lines. There were very small gains from heterosis for plant height and kernel hardness (11.39% and 6.6% respectively). Plant yield had the highest level of heterosis (57.45%) emphasizing the already proven advantages of using hybrid seed over open pollinated varieties.

LM130 was the best female line for grain mould resistance. Despite the fact that LM130 was the most susceptible female line with a mean of 13.24 μg/g for ergosterol concentration, this line is the only female line that produced hybrids with a mean lower than the best parent at -12.89% mean high parent heterosis (Table 9). This also shows involvement of over-dominance effects or gene action for grain mould resistance. This line also had a negative GCA value of -0.083 (Table 10). Some of LM130 hybrids were susceptible like the cross to LM108 (Table 8). The top four most resistant hybrids came from crosses between LM130 to LM103, LM109, LM114 and LM101 respectively (Table 11). These are crosses between the least resistant female and the least resistant male

lines as indicated in Table 8. This observation implies that best combiners for grain mould resistance are not always the best performing individual lines. It is safe to assume that good grain mould resistance genes exist in both maternal and paternal lines even though they may not be expressed for some reason but when combined in good combinations, they get expressed. Therefore selection of parental lines for use in crosses for grain mould resistance research should be based on testing for combining ability based on observation of line performance in hybrid crosses.

LM130 has brown glumes and brown seeds with a semi-compact elliptic panicle shape (Table 1). All its hybrids had brown glumes and brown seeds i.e. had a high content of phenols, and their panicle shapes ranged from semi-compact elliptic to semi-loose drooping primary branches panicle shapes (7 and 8 classes only). Pigmented plants with pigmented kernels have been shown to have more grain mould resistance than their non-pigmented counterparts. Phenol content especially tannin and flavan-4-ol content have been associated with grain mould resistance (Bandyopadhyay *et al.,* 1988: Audilakshmi *et al.,* 1999). Phenol content therefore could have a significant role in the observed LM130 effect.

However on the other hand, LM124 (white seeded female) showed a high level of resistance with an ergosterol concentration of 6.7 μg/g (Table 8). This line passed its resistance to its offspring and had several crosses that showed good resistance. LM124 had the best GCA value of -0.383 (Table 10) indicating that its hybrids were the most resistant in general. Crosses with LM109 and LM114 performed better than parental lines with SCA values of -5.03 and -4

(Table 11). This shows that not only seed color (phenol content) accounts for the resistance. Since LM122 had white kernels like LM124 but failed to pass resistance traits to the offspring, it therefore means white seeded genotypes possess resistance genes that are more complex and need more investigation.

LM117 was the most stable male line with a GCA value of -1.266 and its pedigree is R.9645_(RTx430*Sureño)-B12. This line has Sureño genes in it and Sureño is a popular dual-purpose grain and forage variety with good resistance to grain mould (Meckenstock *et al*., 1993). Even though LM117 had a high concentration of ergosterol, its hybrids were more resistant especially the cross to LM130 (Table 12).

Grain yield ranged from a mean of 831.44 g/plot (hybrid 7) to 137.11 g/plot (LM122). Means for grain yield indicate that LM101 and LM109 were the best performing parent lines with a mean yield of 510.7 g/plot and 501.9 g/plot (Table 8) respectively. However in hybrid performance, LM101 had a positive GCA whereas LM109 had a negative GCA (Table 10). Among female parents LM130 had the highest yield but in hybrid performance LM122 had the highest and most significant GCA and heterosis (Tables 8, 9 and 10). Therefore LM122 may not be useful in transferring grain mould resistance but it can be useful when selecting for yield. Among male lines LM116 was the most stable with a significant GCA value of 178.73. The cross between LM122 and LM116 produced the highest heterosis at 122.72 %. A significant 57.45% heterosis for yield was observed (Table 9) indicating the importance of using hybrid seed.

Individual plant height ranged from 120 cm to 205 cm above ground. A significant heterosis of 11.39 % was observed (Table 9). According to the range for plant height, a height above 180cm is tall, 150 – 180 cm is intermediate and anything less than 150 cm is dwarf. All parental lines were in the intermediate height range. Among female parents only LM122 hybrids had a few tall hybrids with the highest mean heterosis of 11.46 % and the only significant positive GCA of 2.84 (Tables 9 and 10). LM130 had the least average heterosis of 8.35 % indicating that its hybrids did not grow too tall (Table 9). Plant height is not significantly correlated to ergosterol concentration hence grain mould resistance (Table 19). In this case plant height would be important for management purposes like weeding, spraying and hand versus combine harvesting. Preferable height would therefore vary from one place to another but intermediate height would suit most situations.

Individual hardness index values ranged from a maximum of 20.91 sec/g to a minimum of 1.88 sec/g. According to the observed range any timing above 14 sec/g is hard, 7 – 14 sec/g is intermediate and any timing below 7 sec/g is soft. All parental lines were therefore in the intermediate range except for LM122 which was soft at 4 sec/g (Table 8). None of the genotypes could be classified as hard across all locations. LM122 hybrids were softer than an average hybrid with an average heterosis of -11.91% and a significant GCA value of -1.065 (Tables 9 and 10). LM124 had the best heterosis among female lines at 5.06 % (Table 9) and the best significant GCA value at 0.698 (Table 10). LM130 had a positive heterosis and a significant GCA value at 2.01 % and 0.366 respectively coming

second to LM124 for use in selection for kernel hardness. Among male lines, LM109 and LM104 were the most stable in producing harder endosperm hybrids with significant GCA values of 1.525 and 0.745 respectively (Table 10).

The ratio of GCA/SCA is the same as the ratio of additive to dominance variance or gene action. A ratio greater than 1 indicates additive gene action prevalence whereas a ratio less than 1 indicates dominance gene action. As indicated in (Table 13), grain mould resistance exhibits mainly dominance whereas yield, height and kernel hardness exhibit additive variance or gene action. The dominance action for grain mould resistance is equally from both male and female parents. The dominance observed here could be complete, partial and over-dominance since high parent heterosis was calculated. Plant yield and plant height exhibited additive variance mainly due to the male parent effect whereas for grain hardness this variance was due to the female parent effect. This observation for grain hardness is to be expected since hardness is dominated by maternal effects due to the 3*n* nature of the endosperm with 2*n* coming from the female parent.

2.2. Cedara 1 means

Since genotypes were not significant for ergosterol concentration analysis, all means were in one group (Table 14). However, mean performance of hybrids was significantly different from that of the parent lines (Table 5) and was calculated to be 24 % heterosis. This means hybrids were more susceptible than their parental lines and this applies to all three female lines at this location. GCA values were not significant for any of the lines (Table 11). Individual plot observations ranged from a minimum of 1.5 μg/g to a maximum of 11.08 μg/g. Mean ergosterol concentration for the location was low at 5.05 μg/g and ranged from 3.35 μg/g for hybrid 15 to 7.06 μg/g for hybrid 17. This could have been caused by low temperatures experienced during the grain filling period hence grain mould severity was low leading to failure to detect genotypic differences.

Significant differences were detected among genotypes for plant yield (Table 5). The significant heterosis was calculated to be 59%. Hybrid 2 was the highest yielding genotype at 1054.7 g/plot and LM120 was the lowest at 208.7 g/plot (Table 14). LM122 was the lowest yielding female line but its hybrids had the highest mean yield at 752.1 g/plot. This line is therefore good for use in selection for plant yield at this location.

Significant differences were detected among genotypes for plant height (Table 5). The significant heterosis was calculated to be 14%. Hybrid 2 was the tallest genotype at 191.7 cm and LM117 was the shortest at 140 cm (Table 14). LM130 hybrids had the desirable intermediate height at 176.5 cm and the other two female lines produced tall hybrids.

Significant differences were detected among genotypes for kernel hardness (Table 5). The heterosis was calculated to be 11% but it was not significant. LM109 was the hardest genotype at 10.1 sec/g and LM122 was the softest at 3.6 sec/g (Table 14). LM130 hybrids were the hardest with a mean hardness of intermediate 7.7 sec/g.

2.3. Cedara-2 means

Since genotypes were not significant for ergosterol concentration (Table 6) all means were in one group except for hybrid 22 which had the highest concentration at 23.08 μg/g (Table 15). This location had the lowest mean ergosterol concentration compared to other locations at 4.41 μg/g. This could also be due to the fact that mean temperatures were low during the grain filling period. Grain mould severity was significantly reduced to the extent that genotypic responses could not be detected. A very high CV of 129.48% and small R-square of 0.33 were also observed.

Analysis for plant yield revealed significant differences among genotypes (Table 6). The significant heterosis was calculated to be 97.8%. Individual plot observations reveal that Hybrid 7 was the best performing entry at 851 g/plot and LM122 was the lowest at 10 g/plot (Table 15). LM122 once again had the lowest yield but its hybrids performed the best. This line continues to outperform the other two female lines in hybrid performance.

There were significant differences among genotypes for plant height (Table 6). The observed significant heterosis was calculated to be 11.3%. LM117 was the shortest at 138.3 cm and hybrid 3 was the tallest at 188.3 cm (Table 15). Only LM122 had a couple of tall hybrids whereas LM130 continues to have good height in its hybrids.

There were significant differences among genotypes for kernel hardness (Table 6). Heterosis was not significant at this location. Hybrid 9 was the softest genotype at 3.9 sec/g and hybrid 27 was the hardest at 10.7 sec/g (Table 15).

LM122 resulted in the highest number of hybrids with soft endosperms whilst LM130 hybrids were the hardest with a mean of 7.9 sec/g.

2.4. Potchefstroom means

This was the only location where there were significant differences among genotypes for ergosterol concentration analysis (Table 7). This could be because mean temperatures during the grain filling period at this location were higher than at the other two locations. Grain mould pressure was therefore higher here as indicated by a high mean ergosterol concentration of 18.61 μg/g (Table 16), bringing out significant genotypic responses that could be detected. The observed heterosis was calculated at -27.2 % indicating that hybrids were more resistant than the parental lines. LM109 was the most susceptible with a mean concentration of 42.81 μg/g and LM122 was the most resistant at 5.29 μg/g. However, LM122 resistance was not passed onto its hybrids since LM122 hybrids had the highest concentration at 18.34 μg/g compared to the other two female lines. On the other hand LM130 was the most susceptible female line at 33.77 μg/g but its hybrids had the lowest concentration compared to the other female lines at 14.37 μg/g. LM130 is therefore more useful at this location as a good parent for grain mould resistance.

There were significant differences among genotypes for plant yield (Table 7). The significant heterosis was calculated to be 57 %. Individual plot observations reveal that Hybrid 14 was the best performing entry at 705 g/plot and LM122 was the lowest at 167 g/plot (Table 16). LM122 once again had the lowest yield but its hybrids performed the best. This line continues to outperform the other two female lines in hybrid performance.

There were significant differences among genotypes for plant height (Table 7). The observed significant heterosis was calculated to be 10%. LM117 was the shortest at 145 cm and hybrid 6 was the tallest at 186.7 cm (Table 16). Only LM122 had a couple of tall hybrids whereas LM130 continues to have good height in its hybrids.

There were significant differences among genotypes for kernel hardness (Table 7). Heterosis was not significant at this location. LM122 was the softest genotype at 4.5 sec/g and hybrid 14 was the hardest at 16.6 sec/g (Table 16). LM122 resulted in the highest number of hybrids with soft endosperms whilst LM124 hybrids were the hardest with a mean of 11.7 sec/g.

3. Variance components and heritability

3.1. Cedara-1

For ergosterol concentration analysis, only environmental variance was observed (Tables 5 and 17). This therefore led to a lack of estimates for heritability (Table 18). The total phenotypic variance of 2.86 in Table 18 is all due to environmental effects. As explained earlier, low mean temperatures during the grain filling period led to low grain mould severity hence failure to bring out genotypic responses to grain mould. Spraying of trials with a concoction of conidia of important grain mould fungi could have increased grain mould severity and brought about more significant genotypic responses that could have been detected.

For plant yield analysis, only the male variance component was significant implying that male lines were responsible for most of the variation observed for plant yield at this location (Tables 5 and 17). Average heritability for plant yield (h^2_{m+f}) across all three locations was highest at this location at 0.41 and it was the highest heritability across all four measured heritabilities even though all heritabilities were less than 50%. This can also be confirmed from the fact that Cedara-1 had the highest mean yield across all three locations at 585.21 g/plot compared to 443.62 g/plot and 463.62 g/plot for Cedara-2 and Potchefstroom respectively (Tables 14, 15, and 16). This also implies that Cedara-1 is the best environment where all genotypes performed at their best for plant yield hence

will be best suited for selection for plant yield trials. Use of more male lines crossed to a few good female lines will be the best approach.

Analysis for plant height indicates a significant male variance component at 44.97 (Table 17). This indicates that male lines once again had more effect on this trait than the female lines. Average heritability of this trait was at 0.37 (Table 18).

Analysis for kernel hardness indicates that the male variance component was higher than the female one even though it was not significant (Table 17). Average heritability at this location was at 0.22 (table 18)

3.2. Cedara-2

Similarly to Cedara-1 ergosterol concentration analysis at this locality, only environmental variance was observed (Tables 6 and 17). This therefore led to a lack of estimates for heritability (Table 18). The total phenotypic variance, the lowest across all environments, of 0.58 in Table 18 is all due to environmental effects. This environment was the most unfavorable for grain mould development. Once again low mean temperatures during the grain filling period led to low grain mould severity hence failure to bring out genotypic responses to grain mould. Spraying of trials with a concoction of conidia of important grain mould fungi could have increased grain mould severity and brought about more significant genotypic responses that could have been detected.

For plant yield analysis, only the male variance component was significant implying that male lines were responsible for most of the variation observed for plant yield at this location (Tables 6 and 17). Average heritability for plant yield (h^2_{m+f}) across all three location was 0.37.

Analysis for plant height indicates a significant male variance component at 55.48 (Table 17). This indicates that male lines once again had more effect on this trait than the female lines. Average heritability of this trait was at 0.39 (Table 18).

Analysis for kernel hardness indicates only the female variance component at 0.12 even though it was not significant (Table 17). The male component was negative. Only female heritability at this location was recorded at a very low level of 0.07 (Table 18) implying that this location is not good for use to select for kernel hardness.

3.3. Potchefstroom

Unlike Cedara-1 and Cedara-2 for ergosterol concentration analysis at this environment some genotypic variance was observed (Tables 7 and 17). This variance was in the M x F SCA effect at 5.14 (Table 17) causing the significant heterosis observed in the ANOVA (Table 7). This location had the highest total phenotypic variance at 126.96 compared to the other two environments (Table 18). This environment was the most favorable for grain mould development. Higher mean temperatures during the grain filling period led to a significant

increase in grain mould severity hence ability to bring out genotypic responses to grain mould.

For plant yield analysis, the male variance component was significant implying that male lines were responsible for most of the variation observed for plant yield at this location (Table 17). The significance of the M x F SCA effect means there was some dominance effects on yield at this location. Average heritability for plant yield (h^2_{m+f}) across all three locations could not be measured due to negative female components. This location had the lowest total phenotypic variance compared to the other two environments.

Analysis for plant height indicates presence of all three variance components (Table 17). The male effect was more dominant and this indicates that male lines once again had more effect on this trait than the female lines. Average heritability of this trait was at 0.38 (Table 18). This location had the lowest total phenotypic variance for this trait.

Analysis for kernel hardness indicates both male and female variance components at 1.52 and 3.53 respectively (Table 17). The larger female component implies that the female lines had more role to play in this trait. Total phenotypic variance and average heritability for hardness were highest at this location at 26.49 and 0.38 respectively (Table 18) implying that this location is the best for use to select for kernel hardness.

4. Correlations

A lack of correlation (-0.01ns) between visual field grade scoring and ergosterol concentration analysis as a measure of grain mould severity has important implications (Table 19). Simply put, individual observation of what is happening on the outside of the kernel does not necessarily reflect what is happening inside the kernel. Due to some resistance mechanisms possessed by the host, some genotypes seem to be able to prevent deep penetration and colonization of the endosperm by restricting colonization to the periphery of the kernel hence leading to high visual field scores whilst total ergosterol concentration analysis indicates low levels of ergosterol. Ergosterol concentration analysis should therefore be recommended especially at advanced levels of selection in the breeding process since this analysis tends to take more time and money to carry out.

Plant yield is significantly correlated to plant height. This implies that taller plants tend to give more grain than shorter ones. However, plant height preferences are dependent on management mechanisms and other personal preferences hence they vary from place to place. Other correlation values that are significant are lower than 0.5 implying that more than half the time they do not hold even if they are significant.

CONCLUSION

It is evident therefore that use of visual scoring as a measure of grain mould severity can be misleading as observed from the wrongful classification of some of the parental lines into the three classes mentioned above. This wrongful classification can lead to selecting wrong parental lines to use in hybrid crosses and this will in turn lead to very little or no genetic gain in overall grain mould resistance levels as has been the case over the years. A very important observation here is the fact that the most resistant lines observed are not always the best parental lines to use in hybrid combination. The best parental lines should be selected based on performance in test crosses to selected good combiners. The best parental lines must be able to pass the resistance to their offspring and they achieve that if they are crossed to lines with complementary gene combinations. Dominance effects seem to be more prevalent for grain mould resistance further indicating the need to identify the responsible genes in order to be able to pyramid them in genotypes with good agronomic traits.

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Genotype number	Sex	Code	Pedigree	Panicle shape†	Glume Color	Seed Color	FGS	Mould reaction‡
1	Male 1	LM101	(87EO366 * WSV387)-HF14	8	tan	white	1.25	R
2	Male 2	LM103	(ISCV 1089BF * MACIA)-HF2-CA2-AE	9	brown	white	1.50	R
3	Male 3	LM104	(MACIA * DORADO)-HD2----CA3	7	brown	white	1.75	R
4	Male 4	LM108	(Segaolane*WM#322)-CG1-BGBK-CCBK	7	brown	white	2.50	I
5	Male 5	LM109	(SV1*Sima/IS23250)-LG15-CG1-BG2-BGBK	7	brown	white	2.50	I
6	Male 6	LM114	(90EO328 * CE151)-LA37	1	tan	white	1.25	R
7	Male 7	LM116	Kuyuma	8	tan	white	4.25	S
8	Male 8	LM117	R.9645_(RTx430*Sureno)-B12	9	tan	white	4.50	S
9	Male 9	LM120	SRN39_Striga Res.	9	tan	white	3.00	I
10	Female 1	LM122	ATx631	6	tan	white	-	-
11	Female 2	LM124	ATx623	8	brown	white	-	-
12	Female 3	LM130	A02059	8	brown	brown	-	-

† Panicle shape codes as described in the descriptors for sorghum hand book IBPGR and ICRISAT, 1993.
‡ Mould reaction as described by (Bandyopadhyay and Mughogho, 1988) R = resistant (FGS $\leq$ 2); I = intermediate resistant (2 < FGS $\leq$ 3); S = susceptible to grain mould 3 < FGS $\leq$ 5

Table 1. Pedigrees, codes, plant and grain characteristics of male and female parental lines used to generate hybrids in a design II mating scheme planted at Cedara and Potchefstroom during the 2007 – 2008 season.

Table 2. Analysis of variance model used across the three environments for all hybrids and parents.

Source	F Test	Expected Mean Square (EMS)
Env	MS_E / MSe	$\sigma_e^2 + grphfm\sigma_E^2$
Rep(Env)	$MS_{R(E)} / MSe$	$\sigma_e^2 + gphmf\sigma_{R(E)}^2$
Genotype	MS_G / MS_{GxE}	$\sigma_e^2 + rphmf\sigma_{GxE}^2 + rphmfe\sigma_G^2$
Parent	MS_P / MS_{PxE}	$\sigma_e^2 + grhfm\sigma_{PxE}^2 + grhfme\sigma_P^2$
Male	MS_M / MS_{MxE}	$\sigma_e^2 + grphf\sigma_{MxE}^2 + grphfe\sigma_M^2$
Female	MS_F / MS_{FxE}	$\sigma_e^2 + grphm\sigma_{FxE}^2 + grphme\sigma_F^2$
Male vs. Female		
Parent vs. Hybrid		
Hybrid	MS_H / MS_{HxE}	$\sigma_e^2 + grpfm\sigma_{HxE}^2 + grpfme\sigma_H^2$
Male (Mgca)	$MS_{M(H)} / MS_{MxE}$	$\sigma_e^2 + grphf\sigma_{MxE}^2 + grphfe\sigma_M^2$
Female (Fgca)	$MS_{F(H)} / MS_{FxE}$	$\sigma_e^2 + grphm\sigma_{FxE}^2 + grphme\sigma_F^2$
M x F (sca)	MS_{FxM} / MS_e	$\sigma_e^2 + grphe\sigma_{FxM}^2$
Gen x Env	MS_{GxE} / MS_e	$\sigma_e^2 + rphmf\sigma_{GxE}^2$
Parent x Env	MS_{PxE} / MS_e	$\sigma_e^2 + grhfm\sigma_{PxE}^2$
Male x Env	MS_{MxE} / MS_e	$\sigma_e^2 + grphf\sigma_{MxE}^2$
Female x Env	MS_{FxE} / MS_e	$\sigma_e^2 + grphm\sigma_{FxE}^2$
Hybrid x Env	MS_{HxE} / MS_e	$\sigma_e^2 + grpfm\sigma_{HxE}^2$
Male x Env	MS_{MxE} / MS_e	$\sigma_e^2 + grphf\sigma_{MxE}^2$
Female x Env	MS_{FxE} / MS_e	$\sigma_e^2 + grphm\sigma_{FxE}^2$
Male x Fem x Env	MS_{MxFxE} / MS_e	$\sigma_e^2 + grph\sigma_{MxFxE}^2$
Error		σ_e^2

Table 3. Analysis of variance model used for single environments for all hybrids.

Source	F Test	Expected Mean Squares (EMS)
Rep	MS_R / MS_e	$\sigma_e^2 + ghpmf\sigma_R^2$
Genotype	MS_G / MS_e	$\sigma_e^2 + rpmfh\sigma_G^2$
Parent	MS_P / MS_e	$\sigma_e^2 + grhmf\sigma_P^2$
Male	MS_M / MS_e	$\sigma_e^2 + grphf\sigma_M^2$
Female	MS_F / MS_e	$\sigma_e^2 + grphf\sigma_F^2$
Male vs. Female		
Parent vs. Hybrid		
Hybrid	MS_H / MS_e	$\sigma_e^2 + grpmf\sigma_H^2$
Male (Mgca)	MS_M / MS_e	$\sigma_e^2 + grphf\sigma_M^2$
Female (Fgca)	MS_F / MS_e	$\sigma_e^2 + grphf\sigma_F^2$
M x F (sca)	MS_{FxM} / MS_e	$\sigma_e^2 + grph\sigma_{MxF}^2$
Error		σ_e^2

Table 4. Mean squares from the analysis of variance for ergosterol concentration (µg/g), plant yield (g), plant height (cm), and seed hardness (Sec/g) across the three environments during the 2007-2008 season.

		Mean Squares			
Source	**DF**	**Ergosterol**	**Yield**	**Height**	**Hardness**
Env	2	7533.28**	689285.39**	1613.75**	232.91**
Rep(Env)	6	2041.90**	67045.76^{ns}	125.43^{ns}	20.21**
Gen	38	88.95^{ns}	238661.81**	1065.76**	19.85**
Parent	11	163.39^{ns}	131304.02**	432.24**	32.73**
Male	8	127.52^{ns}	113599.34**	581.10**	25.74**
Female	2	220.96^{ns}	183268.59**	8.33^{ns}	28.64**
Male vs. Female	1	335.13^{ns}	169012.35**	89.20^{ns}	96.91**
Parent vs. Hybrid	1	357.85*	3124414.41**	23877.56**	20.29*
Hybrid	26	47.12^{ns}	173091.93**	456.41**	14.38**
Male (Mgca)	8	19.27^{ns}	465876.92**	1156.94**	18.36**
Female (Fgca)	2	15.03^{ns}	143604.31^{ns}	527.16**	71.37**
M x F (sca)	16	65.05^{ns}	30385.38^{ns}	97.30*	5.26^{ns}
Gen x Env	76	102.12**	41591.16**	53.00^{ns}	4.68^{ns}
Parent x Env	22	164.57*	24319.80**	50.23^{ns}	1.98^{ns}
M x Env	16	145.21^{ns}	31884.60**	61.07*	2.05^{ns}
F x Env	4	228.52*	3949.70^{ns}	15.28^{ns}	1.01^{ns}
Hybrid x Env	52	50.79^{ns}	49498.32**	48.36^{ns}	5.95^{ns}
M x Env	16	44.89^{ns}	83420.70**	68.29^{ns}	5.32^{ns}
F x Env	4	69.79^{ns}	105770.21**	48.92^{ns}	22.09**
M x F x E	32	51.37^{ns}	25503.15^{ns}	38.33^{ns}	4.25^{ns}
Error	342	77.51	50958.95	160.55	5.87
R-Square	-	0.57	0.56	0.76	0.52
Mean	-	9.36	497.30	169.50	8.07

*, ** Indicates significance at $P = 0.05$ and $P = 0.01$ respectively.
^{ns} Indicates non- significance at $P \leq 0.05$.

Table 5. Mean squares from the analysis of variance for ergosterol concentration (µg/g), plant yield (g), plant height (cm) and seed hardness (Sec/g) for Cedara-1 during the 2007-2008 season.

		Mean Squares			
Source	**DF**	**Ergosterol**	**Yield**	**Height**	**Hardness**
Rep	2	217.33**	44246.83^{ns}	163.03^{ns}	20.73**
Gen	38	2.27^{ns}	157889.25**	481.22**	5.76**
Parent	11	1.70^{ns}	65689.26**	166.67**	10.40**
Male	8	1.48^{ns}	69508.58**	216.67*	8.46*
Female	2	1.39^{ns}	64552.44^{ns}	33.33^{ns}	10.20*
Male vs. Female	1	4.12^{ns}	37408.33^{ns}	33.33^{ns}	26.30**
Parent vs. Hybrid	1	17.31*	1242901.55**	11172.74**	5.72^{ns}
Hybrid	26	1.93^{ns}	155165.70**	203.09**	3.96^{ns}
Male (Mgca)	8	1.85^{ns}	392866.00**	473.92**	6.26*
Female (Fgca)	2	0.31^{ns}	280148.04*	191.05^{ns}	5.76^{ns}
M x F (sca)	16	2.18^{ns}	20692.76^{ns}	69.17^{ns}	2.59^{ns}
Error	114	2.75	75081.09	205.94	3.73
R-Square	-	0.70	0.70	0.78	0.56
Mean	-	5.05	585.21	172.99	7.31

*, ** Indicates significance at $P = 0.05$ and $P = 0.01$ respectively.

^{ns} Indicates non- significance at $P \leq 0.05$.

Table 6. Mean squares from the analysis of variance for ergosterol concentration (µg/g), plant yield (g), plant height (cm), and seed hardness (sec/g) for Cedara-2 during the 2007-2008 season.

		Mean Squares			
Source	**DF**	**Ergosterol**	**Yield**	**Height**	**Hardness**
Rep	2	18.07^{ns}	92229.31^{ns}	154.06^{ns}	19.00*
Gen	38	31.53^{ns}	111154.89**	400.65**	6.30*
Parent	11	4.47^{ns}	93847.66**	246.15**	8.04**
Male	8	3.98^{ns}	94664.58**	322.45**	7.39**
Female	2	3.74^{ns}	87908.78*	2.78^{ns}	5.86**
Male vs. Female	1	9.86*	99190.08*	122.45^{ns}	17.60**
Parent vs. Hybrid	1	31.39^{ns}	1237307.81**	6631.34**	12.17^{ns}
Hybrid	26	42.99^{ns}	75163.60**	226.38**	5.34^{ns}
Male (Mgca)	8	42.75^{ns}	185482.73**	560.03**	3.69^{ns}
Female (Fgca)	2	37.78^{ns}	58576.94^{ns}	216.98^{ns}	8.94^{ns}
M x F (sca)	16	43.76^{ns}	22077.37^{ns}	60.73^{ns}	5.71^{ns}
Error	114	32.21	52685.40	162.28	4.60
R-Square	-	0.33	0.71	0.83	0.49
Mean	-	4.41	443.62	165.60	7.20

*, ** Indicates significance at $P = 0.05$ and $P = 0.01$ respectively.

^{ns} Indicates non- significance at $P \leq 0.05$.

Table 7. Mean squares from the analysis of variance for ergosterol concentration (µg/g), plant yield (g), plant height (cm), and seed hardness (sec/g) for Potchefstroom during the 2007-2008 season.

		Mean Squares			
Source	**DF**	**Ergosterol**	**Yield**	**Height**	**Hardness**
Rep	2	5890.30**	64661.14^{ns}	59.19^{ns}	20.91^{ns}
Gen	38	259.38*	52799.99**	289.89**	17.16**
Parent	11	486.35^{ns}	20406.69*	119.89**	18.25**
Male	8	412.49^{ns}	13195.37^{ns}	164.12**	13.99**
Female	2	672.88^{ns}	38706.78^{ns}	2.78^{ns}	14.59^{ns}
Male vs. Female	1	704.21^{ns}	41497.12*	0.23^{ns}	59.70**
Parent vs. Hybrid	1	1808.57*	696184.90**	6481.63**	9.54^{ns}
Hybrid	26	103.77^{ns}	41759.27**	123.67**	16.98**
Male (Mgca)	8	64.45^{ns}	54369.59**	259.57**	19.06*
Female (Fgca)	2	116.54^{ns}	16419.75^{ns}	216.98*	100.84**
M x F (sca)	16	121.84^{ns}	38621.54**	44.06^{ns}	5.46^{ns}
Error	114	197.56	25110.38	113.43	9.24
R-Square	-	0.63	0.71	0.85	0.63
Mean	-	18.62	463.07	169.91	9.71

*, ** Indicates significance at $P = 0.05$ and $P = 0.01$ respectively.

^{ns} Indicates non- significance at $P \leq 0.05$.

Table 8. Genotype means for ergosterol concentration (µg/g), plant yield (g), plant height (cm), and seed hardness (sec/g) across the three locations during the 2007-2008 season.

entry	code	genotype	ergo	yield	height	hardness
Male 1	LM101	1	13.66^{ab}	510.7^{defghij}	166.7^{hi}	9.3^{abcdef}
Male 2	LM103	2	13.08^{ab}	440.6^{ghijklm}	156.1^{jk}	8.7^{abcdefghi}
Male 3	LM104	3	8.45^{ab}	463.0^{fghijkl}	159.4^{jk}	8.4^{bcdefghi}
Male 4	LM108	4	6.86^{ab}	355.2^{jklm}	159.4^{jk}	8.7^{bcdefghi}
Male 5	LM109	5	17.25^{a}	501.9^{efghijk}	168.9^{gh}	10.6^{ab}
Male 6	LM114	6	16.00^{a}	310.1^{lmn}	158.3^{jk}	9.2^{abcdef}
Male 7	LM116	7	6.88^{ab}	373.3^{jklm}	156.1^{jk}	7.0^{ghi}
Male 8	LM117	8	12.87^{ab}	280.1^{mno}	141.1^{l}	7.9^{cdefghi}
Male 9	LM120	9	11.97^{ab}	172.7^{no}	152.8^{k}	4.6^{jk}
Male means			11.9	378.6	157.6	8.3
Female 1	LM122	10	3.52^{b}	137.1^{o}	156.1^{jk}	4.0^{k}
Female 2	LM124	11	6.70^{ab}	303.6^{lmno}	154.4^{k}	7.3^{efghi}
Female 3	LM130	12	13.24^{ab}	421.1^{hijklm}	156.1^{jk}	7.0^{ghi}
Female means			7.8	287.3	155.5	6.1
Hybrid 1	LM122xLM101	13	7.32^{ab}	702.9^{abc}	174.4^{cdefg}	7.5^{defghi}
Hybrid 2	LM122xLM103	14	13.30^{ab}	691.3^{abc}	183.3^{ab}	8.7^{bcdefghi}
Hybrid 3	LM122xLM104	15	12.92^{ab}	625.3^{bcdef}	184.4^{a}	7.4^{defghi}
Hybrid 4	LM122xLM108	16	6.81^{ab}	566.6^{bcdefgh}	180.0^{abcd}	7.1^{fghi}
Hybrid 5	LM122xLM109	17	9.23^{ab}	556.8^{bcdefgh}	175.6^{cdefg}	8.3^{cdefghi}
Hybrid 6	LM122xLM114	18	10.94^{ab}	632.9^{bcde}	179.4^{abcd}	6.5^{ij}
Hybrid 7	LM122xLM116	19	10.11^{ab}	831.4^{a}	181.1^{abc}	6.9^{hi}
Hybrid 8	LM122xLM117	20	5.62^{ab}	464.0^{fghijkl}	158.9^{jk}	7.5^{defghi}
Hybrid 9	LM122xLM120	21	6.11^{ab}	341.7^{klm}	183.3^{ab}	4.6^{jk}
LM122 hybrid means			9.11	601.4	177.8	7.2
Hybrid 10	LM124xLM101	22	11.17^{ab}	543.0^{bcdefghi}	178.9^{abcde}	8.8^{abcdefghi}
Hybrid 11	LM124xLM103	23	10.74^{ab}	710.1^{ab}	175.0^{cdefg}	9.2^{abcdefg}
Hybrid 12	LM124xLM104	24	8.24^{ab}	633.0^{bcde}	178.9^{abcde}	10.0^{abc}
Hybrid 13	LM124xLM108	25	5.84^{ab}	657.9^{bcde}	176.1^{cdef}	8.5^{bcdefghi}
Hybrid 14	LM124xLM109	26	6.95^{ab}	583.3^{bcdefgh}	176.1^{cdef}	11.0^{a}
Hybrid 15	LM124xLM114	27	7.35^{ab}	514.3^{defghij}	176.1^{cdef}	8.1^{cdefghi}
Hybrid 16	LM124xLM116	28	8.73^{ab}	709.3^{ab}	176.7^{bcdef}	9.4^{abcde}
Hybrid 17	LM124xLM117	29	7.85^{ab}	326.9^{lmn}	153.9^{k}	7.9^{cdefghi}
Hybrid 18	LM124xLM120	30	7.84^{ab}	379.1^{ijklm}	176.7^{bcdef}	7.6^{defghi}
LM124 hybrid means			8.30	561.9	174.3	8.9
Hybrid 19	LM130xLM101	31	7.15^{ab}	540.9^{cdefghi}	175.6^{cdefg}	8.7^{bcdefghi}
Hybrid 20	LM130xLM103	32	6.18^{ab}	637.8^{bcde}	179.4^{abcd}	7.9^{cdefghi}
Hybrid 21	LM130xLM104	33	6.97^{ab}	668.4^{abcde}	177.8^{abcdef}	9.6^{abcd}
Hybrid 22	LM130xLM108	34	14.27^{ab}	607.2^{bcdefg}	171.7^{fgh}	8.8^{abcdefghi}
Hybrid 23	LM130xLM109	35	8.50^{ab}	430.3^{hijklm}	173.9^{defg}	10.0^{abc}
Hybrid 24	LM130xLM114	36	8.15^{ab}	429.3^{hijklm}	172.2^{efgh}	7.9^{cdefghi}
Hybrid 25	LM130xLM116	37	8.55^{ab}	676.0^{abcd}	175.0^{cdefg}	8.4^{bcdefghi}
Hybrid 26	LM130xLM117	38	8.78^{ab}	359.1^{jklm}	161.7^{ij}	7.3^{efghi}
Hybrid 27	LM130xLM120	39	8.86^{ab}	306.3^{lmn}	168.9^{gh}	8.8^{abcdefgh}
LM130 hybrid means			8.60	517.3	172.9	8.6
Overall Mean	-	-	9.36	497.30	169.50	8.07
CV (%)	-	-	93.20	33.34	4.06	25.15
LSD (0.05)	-	-	11.79	167.21	7.12	2.26

Means followed by the same letters are not significantly different according to Fisher's LSD method.

Table 9. High parent heterosis for ergosterol concentration (μg/g), plant yield (g), plant height (cm), and seed hardness (sec/g) across the three locations during the 2007-2008 season.

Entry	Code	ergo %	yield %	height %	hardness %
Hybrid 1	LM122xLM101	107.95	37.63	4.62	-19.35
Hybrid 2	LM122xLM103	277.84	56.90	17.42	0.00
Hybrid 3	LM122xLM104	267.05	35.05	15.68	-11.90
Hybrid 4	LM122xLM108	93.47	59.52	12.92	-18.39
Hybrid 5	LM122xLM109	162.22	10.94	3.97	-21.70
Hybrid 6	LM122xLM114	210.80	104.10	13.33	-29.35
Hybrid 7	LM122xLM116	187.22	122.72	16.02	-1.43
Hybrid 8	LM122xLM117	59.66	65.66	1.79	-5.06
Hybrid 9	LM122xLM120	73.58	97.86	17.42	0.00
LM122 mean heterosis		159.98	65.60	11.46	-11.91
Hybrid 10	LM124xLM101	66.72	6.32	7.32	-5.38
Hybrid 11	LM124xLM103	60.30	61.17	9.79	9.52
Hybrid 12	LM124xLM104	22.99	36.72	12.23	19.05
Hybrid 13	LM124xLM108	-12.84	85.22	10.48	-2.30
Hybrid 14	LM124xLM109	3.73	16.22	4.26	3.77
Hybrid 15	LM124xLM114	9.70	65.85	11.24	-11.96
Hybrid 16	LM124xLM116	30.30	90.01	13.20	28.77
Hybrid 17	LM124xLM117	17.16	7.67	-0.32	0.00
Hybrid 18	LM124xLM120	17.01	24.87	14.44	4.11
LM124 mean heterosis		23.90	43.78	9.18	5.06
Hybrid 19	LM130xLM101	-46.00	5.91	5.34	-6.45
Hybrid 20	LM130xLM103	-52.75	51.46	14.93	-9.20
Hybrid 21	LM130xLM104	-17.51	44.36	11.54	14.29
Hybrid 22	LM130xLM108	108.02	44.19	7.72	1.15
Hybrid 23	LM130xLM109	-35.80	-14.27	2.96	-5.66
Hybrid 24	LM130xLM114	-38.44	1.95	8.78	-14.13
Hybrid 25	LM130xLM116	24.27	60.53	12.11	20.00
Hybrid 26	LM130xLM117	-31.78	-14.72	3.59	-7.59
Hybrid 27	LM130xLM120	-25.98	-27.26	8.20	25.71
LM130 mean heterosis		-12.89	16.91	8.35	2.01
Mean of Parents	-	10.87	355.78	157.12	7.73
Mean of Hybrids	-	8.68	560.19	175.00	8.24
Heterosis (Parents vs Hybrids)	-	-20.15	57.45	11.39	6.60

Table 10. General combining ability estimates for ergosterol concentration (μg/g), plant yield (g), plant height (cm), and seed hardness (sec/g) across the three locations during the 2007-2008 season.

Entry	Code	ergo	yield	height	hardness
Male Parent	LM101	-0.139	35.395	1.296	0.093
	LM103	1.389	119.543**	4.259**	0.341
	LM104	0.691	82.062**	5.370**	0.745**
	LM108	0.289	50.358	0.926	-0.122
	LM109	-0.457	-36.716	0.185	1.525**
	LM114	0.129	-34.679	0.926	-0.733**
	LM116	0.446	178.728**	2.593	0.014
	LM117	-1.266	-176.864**	-16.852**	-0.647**
	LM120	-1.083	-217.827**	1.296	-1.216**
	SE	2.19	32.40	1.42	0.26
Female Parent	LM122	0.466	41.235**	2.840**	-1.065**
	LM124	-0.383	1.691	-0.741	0.698**
	LM130	-0.083	-42.926**	-2.099**	0.366**
	SE	1.37	5.70	0.35	0.09
Hybrid means	-	8.69	560.20	175.00	8.23

*, ** Significant GCA effects determined by two-tailed *t*-test ($P = 0.05$ and $P = 0.01$ respectively).

Table 11. Estimates of general combining ability for ergosterol concentration in Cedara-1, Cedara-2 and Potchefstroom during the 2007-2008 season.

		Ergosterol concentration		
Entry	**Code**	**Cedara-1**	**Cedara-2**	**Potch**
Male Parent	LM101	0.497	-0.984	0.069
	LM103	-0.101	-0.344	4.613
	LM104	0.025	0.061	1.986
	LM108	-0.818	5.595	-3.909
	LM109	-0.285	-0.002	-1.083
	LM114	-0.322	-1.157	1.867
	LM116	0.620	-0.401	1.118
	LM117	0.377	-1.749	-2.426
	LM120	0.006	-1.018	-2.236
	SE	0.45	2.18	2.68
Female Parent	LM122	-0.116	-0.825	2.340
	LM124	0.096	-0.530	-0.715
	LM130	0.020	1.355	-1.625
	SE	0.09	1.02	1.80
Hybrid means	-	5.31	4.75	16.00

Table 12. Specific combining ability for ergosterol concentration (μg/g) across the three locations during the 2007-2008 season.

Entry	**LM122**	**LM124**	**LM130**	**Avg SCA**
LM101	-1.27	0.99	-6.30	-2.19
LM103	5.00	0.85	-6.98	-0.38
LM104	6.94	0.67	-3.88	1.24
LM108	1.62	-0.94	4.22	1.63
LM109	-1.16	-5.03	-6.75	-4.31
LM114	1.18	-4.00	-6.47	-3.10
LM116	4.91	1.94	-1.51	1.78
LM117	-2.58	-1.94	-4.28	-2.93
LM120	-1.64	-1.50	-3.75	-2.29
Avg SCA	1.45	-0.99	-3.96	-1.17

Table 13. Ratio of general combining ability (GCA) for male and female effects to specific combining ability (SCA) (GCA/SCA) for agronomic traits among hybrids across the three locations during the 2007-2008 season.

Character	GCA_M	GCA_F	SCA	GCA_M/SCA	GCA_F/SCA
Ergosterol	19.27	15.03	65.05	0.30	0.23
Yield	465876.92	143604.31	30385.38	15.33	4.73
Height	1156.94	527.16	97.30	11.89	5.42
Hardness	18.36	71.37	5.26	3.49	13.57

Table 14. Genotype means for ergosterol concentration (µg/g), plant yield (g), plant height (cm), and seed hardness (sec/g) at Cedara-1 during the 2007-2008 season.

entry	code	genotype	ergo	yield	height	hardness
Male 1	LM101	1	3.48	664.7^{cdefghi}	170.0^{defghi}	8.5^{abc}
Male 2	LM103	2	4.71	603.3^{cdefghijkl}	161.7^{ghij}	8.2^{abcd}
Male 3	LM104	3	4.81	420.3^{hijklmno}	158.3^{ij}	8.7^{abc}
Male 4	LM108	4	4.12	341.3^{klmno}	158.3^{ij}	7.8^{abcd}
Male 5	LM109	5	5.46	537.3^{defghijklm}	165.0^{efghij}	10.1^{a}
Male 6	LM114	6	4.57	445.3^{ghijklmno}	163.3^{fghij}	8.7^{abc}
Male 7	LM116	7	5.37	541.7^{defghijklm}	153.3^{jk}	5.9^{cdefg}
Male 8	LM117	8	4.03	280.3^{mno}	140.0^{k}	6.1^{cdefg}
Male 9	LM120	9	5.49	208.7^{o}	160.0^{hij}	4.9^{efg}
Male means			4.67	449.2	158.9	7.6
Female 1	LM122	10	3.58	234.3^{no}	156.7^{ij}	3.6^{g}
Female 2	LM124	11	4.67	363.0^{jklmno}	153.3^{jk}	7.3^{abcde}
Female 3	LM130	12	3.41	527.0^{efghijklmn}	160.0^{hij}	6.0^{cdefg}
Female means			3.89	374.8	156.7	5.7
Hybrid 1	LM122xLM101	13	5.82	748.0^{bcdef}	173.3^{cdefgh}	7.2^{abcde}
Hybrid 2	LM122xLM103	14	4.90	1054.7^{a}	191.7^{a}	7.9^{abcd}
Hybrid 3	LM122xLM104	15	5.44	795.3^{abcde}	188.3^{ab}	7.5^{abcde}
Hybrid 4	LM122xLM108	16	4.50	780.3^{abcdef}	188.3^{ab}	7.3^{abcde}
Hybrid 5	LM122xLM109	17	5.38	629.0^{cdefghijkl}	178.3^{abcde}	7.0^{bcde}
Hybrid 6	LM122xLM114	18	5.32	869.3^{abc}	176.7^{bcdef}	7.6^{abcde}
Hybrid 7	LM122xLM116	19	5.38	1037.7^{ab}	186.7^{abc}	7.0^{bcde}
Hybrid 8	LM122xLM117	20	4.38	488.3^{fghijklmno}	163.3^{fghij}	6.5^{bcdef}
Hybrid 9	LM122xLM120	21	5.61	366.7^{ijklmno}	186.7^{abc}	3.8^{fg}
LM122 hybrids means			5.19	752.1	181.5	6.9
Hybrid 10	LM124xLM101	22	5.85	635.0^{cdefghijk}	186.7^{abc}	9.3^{ab}
Hybrid 11	LM124xLM103	23	5.47	883.3^{abc}	181.7^{abcd}	6.9^{bcde}
Hybrid 12	LM124xLM104	24	4.90	656.0^{cdefghij}	183.3^{abcd}	8.7^{abc}
Hybrid 13	LM124xLM108	25	4.86	835.7^{abcd}	183.3^{abcd}	6.1^{cdefg}
Hybrid 14	LM124xLM109	26	5.58	718.0^{cdefgh}	183.3^{abcd}	7.7^{abcde}
Hybrid 15	LM124xLM114	27	3.35	601.0^{cdefghijkl}	180.0^{abcd}	7.6^{abcde}
Hybrid 16	LM124xLM116	28	6.83	890.7^{abc}	183.3^{abcd}	8.6^{abc}
Hybrid 17	LM124xLM117	29	7.06	382.0^{ijklmno}	158.3^{ij}	7.7^{abcde}
Hybrid 18	LM124xLM120	30	4.75	346.0^{klmno}	185.0^{abc}	5.3^{defg}
LM124 hybrid means			5.40	660.9	180.6	7.6
Hybrid 19	LM130xLM101	31	5.75	611.3^{cdefghijkl}	176.7^{bcdef}	8.4^{abc}
Hybrid 20	LM130xLM103	32	5.26	818.7^{abcde}	185.0^{abc}	6.6^{bcdef}
Hybrid 21	LM130xLM104	33	5.66	773.0^{abcdef}	185.0^{abc}	8.3^{abc}
Hybrid 22	LM130xLM108	34	4.11	649.3^{cdefghij}	175.0^{bcdefg}	7.9^{abcd}
Hybrid 23	LM130xLM109	35	4.11	330.3^{lmno}	178.3^{abcde}	9.2^{ab}
Hybrid 24	LM130xLM114	36	6.29	536.7^{defghijklm}	176.7^{bcdef}	7.1^{bcde}
Hybrid 25	LM130xLM116	37	5.58	744.0^{bcdefg}	178.3^{abcde}	7.9^{abcd}
Hybrid 26	LM130xLM117	38	5.62	264.3^{mno}	163.3^{fghij}	6.7^{bcdef}
Hybrid 27	LM130xLM120	39	5.58	211.3^{o}	170.0^{defghi}	7.6^{abcde}
LM130 hybrid means			5.33	548.8	176.5	7.7
Mean	-	-	5.05	585.21	172.99	7.31
CV (%)	-	-	34.27	31.36	4.78	22.51
LSD (0.05)	-	-	4.74	299.50	13.67	2.895

Means followed by the same letters are not significantly different according to Fisher's LSD method.

Table 15. Genotype means for ergosterol concentration (µg/g), plant yield (g), plant height (cm), and seed hardness (sec/g) at Cedara-2 during the 2007-2008 season.

entry	code	genotype	ergo	yield	height	hardness
Male 1	LM101	1	4.02^{a}	529.0^{bcdefghi}	166.7^{defgh}	8.1^{abcd}
Male 2	LM103	2	4.29^{a}	391.0^{fghijklm}	153.3^{ijk}	7.6^{abcd}
Male 3	LM104	3	4.95^{a}	528.0^{bcdefghi}	161.7^{fghi}	7.5^{abcd}
Male 4	LM108	4	3.05^{a}	282.3^{ijklmn}	158.3^{ghij}	7.9^{abcd}
Male 5	LM109	5	3.47^{a}	501.0^{cdefghij}	170.0^{cdef}	9.0^{abc}
Male 6	LM114	6	2.73^{a}	147.0^{mno}	150.0^{jk}	8.3^{abcd}
Male 7	LM116	7	4.43^{a}	248.0^{jklmno}	156.7^{hij}	5.5^{def}
Male 8	LM117	8	2.41^{a}	213.0^{lmno}	138.3^{l}	6.3^{bcdef}
Male 9	LM120	9	6.04^{a}	37.7^{no}	143.3^{kl}	4.0^{ef}
Male means			3.93	319.7	155.4	7.1
Female 1	LM122	10	1.71^{a}	10.0^{o}	151.7^{ijk}	3.9^{ef}
Female 2	LM124	11	3.92^{a}	241.0^{klmno}	151.7^{ijk}	6.2^{bcdef}
Female 3	LM130	12	2.54^{a}	344.3^{ghijklm}	150.0^{jk}	6.4^{bcdef}
Female means			2.72	198.4	151.1	5.5
Hybrid 1	LM122xLM101	13	3.13^{a}	686.7^{abcde}	171.7^{bcdef}	6.6^{bcdef}
Hybrid 2	LM122xLM103	14	3.84^{a}	438.3^{efghijkl}	173.3^{bcde}	7.6^{abcd}
Hybrid 3	LM122xLM104	15	5.21^{a}	763.3^{ab}	188.3^{a}	7.2^{bcde}
Hybrid 4	LM122xLM108	16	5.34^{a}	518.3^{bcdefghi}	176.7^{bcd}	6.9^{bcdef}
Hybrid 5	LM122xLM109	17	5.36^{a}	568.7^{bcdefgh}	170.0^{cdef}	8.2^{abcd}
Hybrid 6	LM122xLM114	18	3.00^{a}	515.7^{bcdefghi}	175.0^{bcde}	6.5^{bcdef}
Hybrid 7	LM122xLM116	19	2.76^{a}	851.0^{a}	178.3^{abc}	6.0^{cdef}
Hybrid 8	LM122xLM117	20	2.61^{a}	316.3^{hijklm}	150.0^{jk}	8.2^{abcd}
Hybrid 9	LM122xLM120	21	4.10^{a}	291.7^{ijklmn}	181.7^{ab}	3.9^{f}
LM122 hybrid means			3.93	550.0	173.9	6.8
Hybrid 10	LM124xLM101	22	4.92^{a}	617.7^{abcdef}	176.7^{bcd}	8.3^{abcd}
Hybrid 11	LM124xLM103	23	4.72^{a}	619.7^{abcdef}	170.0^{cdef}	8.1^{abcd}
Hybrid 12	LM124xLM104	24	4.02^{a}	728.3^{abc}	175.0^{bcde}	8.4^{abcd}
Hybrid 13	LM124xLM108	25	2.62^{a}	593.3^{abcdefg}	171.7^{bcdef}	8.2^{abcd}
Hybrid 14	LM124xLM109	26	5.22^{a}	327.0^{hijklm}	170.0^{cdef}	8.6^{abcd}
Hybrid 15	LM124xLM114	27	4.47^{a}	461.0^{defghijkl}	171.7^{bcdef}	6.3^{bcdef}
Hybrid 16	LM124xLM116	28	4.84^{a}	708.7^{abcd}	170.0^{cdef}	8.2^{abcd}
Hybrid 17	LM124xLM117	29	3.06^{a}	306.0^{ijklm}	145.0^{kl}	5.9^{cdef}
Hybrid 18	LM124xLM120	30	4.13^{a}	375.7^{fghijklm}	171.7^{bcdef}	6.1^{bcdef}
LM124 hybrid means			4.22	526.4	169.1	7.6
Hybrid 19	LM130xLM101	31	3.26^{a}	465.3^{defghijkl}	173.3^{bcde}	8.3^{abcd}
Hybrid 20	LM130xLM103	32	4.66^{a}	480.7^{cdefghijk}	175.0^{bcde}	8.1^{abcd}
Hybrid 21	LM130xLM104	33	5.21^{a}	620.3^{abcdef}	173.3^{bcde}	9.4^{ab}
Hybrid 22	LM130xLM108	34	23.08^{b}	522.3^{bcdefghi}	166.7^{defgh}	6.8^{bcdef}
Hybrid 23	LM130xLM109	35	3.67^{a}	444.0^{efghijkl}	165.0^{efgh}	7.7^{abcd}
Hybrid 24	LM130xLM114	36	3.32^{a}	274.3^{ijklmn}	166.7^{defgh}	7.1^{bcdef}
Hybrid 25	LM130xLM116	37	5.45^{a}	608.7^{abcdef}	168.3^{cdefg}	7.2^{bcdef}
Hybrid 26	LM130xLM117	38	3.34^{a}	314.7^{hijklm}	158.3^{ghij}	6.0^{cdef}
Hybrid 27	LM130xLM120	39	2.97^{a}	411.0^{fghijkl}	173.3^{bcde}	10.7^{a}
LM130 hybrid means			6.11	460.1	168.9	7.9
Mean	-	-	4.41	443.62	165.60	7.20
CV (%)	-	-	129.48	34.52	3.96	26.89
LSD (0.05)	-	-	9.22	258.12	11.02	3.31

Means followed by the same letters are not significantly different according to Fisher's LSD method.

Table 16. Genotype means for ergosterol concentration (µg/g), plant yield (g), plant height (cm), and seed hardness (sec/g) at Potchefstroom during the 2007-2008 season.

entry	code	genotype	ergo	yield	height	hardness
Male 1	LM101	1	33.48^{abc}	338.3^{ijklmno}	163.3^{e}	11.2^{bcdefgh}
Male 2	LM103	2	30.24^{abc}	327.3^{jklmno}	153.3^{g}	10.4^{bcdefghi}
Male 3	LM104	3	15.59^{abc}	440.7^{defghijklmn}	158.3^{efg}	9.1^{cdefghijk}
Male 4	LM108	4	13.40^{bc}	442.0^{defghijklmn}	161.7^{ef}	10.3^{bcdefghi}
Male 5	LM109	5	42.81^{a}	467.3^{cdefghijklm}	171.7^{d}	12.7^{bcd}
Male 6	LM114	6	40.70^{ab}	338.0^{ijklmno}	161.7^{ef}	10.7^{bcdefghi}
Male 7	LM116	7	10.84^{c}	330.3^{jklmno}	158.3^{efg}	9.5^{bcdefghij}
Male 8	LM117	8	32.17^{abc}	347.0^{hijklmno}	145.0^{h}	11.3^{bcdefgh}
Male 9	LM120	9	24.37^{abc}	271.7^{no}	155.0^{fg}	5.0^{lm}
Male means			27.07	366.9	158.7	10.0
Female 1	LM122	10	5.29^{c}	167.0^{o}	160.0^{ef}	4.5^{m}
Female 2	LM124	11	11.50^{c}	306.7^{klmno}	158.3^{efg}	8.2^{fghijklm}
Female 3	LM130	12	33.77^{abc}	392.0^{ghijklmn}	158.3^{efg}	8.4^{fghijkl}
Female means			16.85	288.6	158.9	7.1
Hybrid 1	LM122xLM101	13	13.02^{bc}	674.0^{ab}	178.3^{bcd}	8.8^{defghijkl}
Hybrid 2	LM122xLM103	14	31.16^{abc}	581.0^{abcdef}	185.0^{ab}	10.4^{bcdefghi}
Hybrid 3	LM122xLM104	15	28.11^{abc}	317.3^{jklmno}	176.7^{cd}	7.5^{hijklm}
Hybrid 4	LM122xLM108	16	10.58^{c}	401.0^{fghijklmn}	175.0^{cd}	7.1^{ijklm}
Hybrid 5	LM122xLM109	17	16.96^{abc}	472.7^{cdefghijklm}	178.3^{bcd}	9.6^{bcdefghij}
Hybrid 6	LM122xLM114	18	24.52^{abc}	513.7^{bcdefghi}	186.7^{a}	5.5^{klm}
Hybrid 7	LM122xLM116	19	22.18^{abc}	605.7^{abcd}	178.3^{bcd}	7.7^{ghijklm}
Hybrid 8	LM122xLM117	20	9.89^{c}	587.3^{abcde}	163.3^{e}	7.8^{fghijklm}
Hybrid 9	LM122xLM120	21	8.62^{c}	366.7^{ghijklmn}	181.7^{abc}	6.2^{jklm}
LM 122 hybrid means			18.34	502.1	178.1	7.9
Hybrid 10	LM124xLM101	22	22.75^{abc}	376.3^{ghijklmn}	173.3^{d}	8.7^{efghijkl}
Hybrid 11	LM124xLM103	23	22.04^{abc}	627.3^{abc}	173.3^{d}	12.5^{bcde}
Hybrid 12	LM124xLM104	24	15.79^{abc}	514.7^{bcdefghi}	178.3^{bcd}	12.8^{abc}
Hybrid 13	LM124xLM108	25	10.05^{c}	544.7^{abcdefg}	173.3^{d}	11.1^{bcdefgh}
Hybrid 14	LM124xLM109	26	10.05^{c}	705.0^{a}	175.0^{cd}	16.6^{a}
Hybrid 15	LM124xLM114	27	14.24^{abc}	481.0^{cdefghijk}	176.7^{cd}	10.2^{bcdefghi}
Hybrid 16	LM124xLM116	28	14.53^{abc}	528.7^{abcdefgh}	176.7^{cd}	11.5^{bcdefg}
Hybrid 17	LM124xLM117	29	13.44^{bc}	292.7^{mno}	158.3^{efg}	10.2^{bcdefghi}
Hybrid 18	LM124xLM120	30	14.65^{abc}	415.7^{efghijklmn}	173.3^{d}	11.6^{bcdefg}
LM124 hybrid means			15.28	498.4	173.1	11.7
Hybrid 19	LM130xLM101	31	12.43^{bc}	546.0^{abcdefg}	176.7^{cd}	9.4^{bcdefghijk}
Hybrid 20	LM130xLM103	32	8.63^{c}	614.0^{abcd}	178.3^{bcd}	9.0^{cdefghijk}
Hybrid 21	LM130xLM104	33	10.05^{c}	612.0^{abcd}	175.0^{cd}	11.1^{bcdefgh}
Hybrid 22	LM130xLM108	34	15.63^{abc}	650.0^{abc}	173.3^{d}	11.6^{bcdef}
Hybrid 23	LM130xLM109	35	17.73^{abc}	516.7^{bcdfghi}	178.3^{bcd}	13.2^{ab}
Hybrid 24	LM130xLM114	36	14.83^{abc}	477.0^{cdefghijkl}	173.3^{d}	9.6^{bcdefghij}
Hybrid 25	LM130xLM116	37	14.63^{abc}	675.3^{ab}	178.3^{bcd}	10.2^{bcdefghi}
Hybrid 26	LM130xLM117	38	17.38^{abc}	498.3^{bcdefghij}	163.3^{e}	9.1^{cdefghijk}
Hybrid 27	LM130xLM120	39	18.01^{abc}	296.7^{lmno}	163.3^{e}	8.1^{fghijklm}
LM130 hybrid means			14.37	542.9	173.3	10.1
Mean	-	-	18.61	463.06	169.91	9.71
CV (%)	-	-	69.34	22.92	2.95	23.84
LSD (0.05)	-	-	28.78	182.72	8.30	3.895

Means followed by the same letters are not significantly different according to Fisher's LSD method.

Table 17. Estimates of variance components for ergosterol concentration (ergo), plant yield (yield), plant height (height), and seed hardness (hardness) across the three locations during the 2007-2008 season.

	Characters			
Variance components	**ergo**	**yield**	**height**	**hardness**
Cedara-1				
σ_e^2	2.86**	45098.86**	68.65**	2.73**
	(0.39)‡	(6137.18)	(9.34)	(0.37)
σ_{mf}^2	-†	-	0.17	-
			(8.86)	
σ_m^2	-	41352.58*	44.97*	0.41
		(19536.71)	(23.69)	(0.33)
σ_f^2	-	9609.45	4.51	0.12
		(7341.28)	(5.08)	(0.15)
Cedara-2				
σ_e^2	0.58**	27286.89**	46.68**	4.87**
	(0.08)	(3713.28)	(6.35)	(0.66)
σ_{mf}^2	-	-	4.68	0.28
			(7.38)	(0.71)
σ_m^2	-	18156.15*	55.48*	-
		(9252.91)	(27.92)	
σ_f^2	-	1351.84	5.79	0.12
		(1558.10)	(5.73)	(0.25)
Potchefstroom				
σ_e^2	106.40**	12232.61**	31.75**	6.29**
	(14.48)	(1664.65)	(4.32)	(0.86)
σ_{mf}^2	5.14	8796.31*	4.10	-
	(15.16)	(4362.44)	(5.30)	
σ_m^2	-	1749.78	23.95	1.52
		(1018.99)	(13.00)	(0.97)
σ_f^2	-	-	6.40	3.53
			(5.71)	(2.64)

†Negative variance components were zeroed and are shown as -.
‡Numbers in parentheses are standard errors.
*,**Indicates significance at P = 0.05 and 0.01 levels, respectively.

Table 18. Heritability estimates for ergosterol concentration (ergo), plant yield (yield), plant height (height), and seed hardness (hardness) across the three locations during the 2007-2008 season.

	Characters			
Heritabilities	**ergo**	**yield**	**height**	**hardness**
Cedara-1				
h_m^2	-	0.66**	0.67	0.34
		(0.31)‡	(0.35)	(0.27)
h_f^2	-	0.15	0.07	0.098
		(0.12)	(0.08)	(0.12)
h_{m+f}^2	-	0.41	0.37	0.22
V_P	2.86	248947.00	267.25	4.85
Cedara-2				
h_m^2	-	0.69*	0.71*	-
		(0.35)	(0.36)	
h_f^2	-	0.05	0.07	0.07
		(0.06)	(0.07)	(0.15)
h_{m+f}^2	-	0.37	0.39	-
V_P	0.58	105318.90	310.48	6.47
Potchefstroom				
h_m^2	-	0.13*	0.57	0.23
		(0.07)	(0.31)	(0.15)
h_f^2	-	-	0.15	0.53
			(0.13)	(0.40)
h_{m+f}^2	-	-	0.36	0.38
V_P	126.96	54416.97	169.55	26.49

‡Numbers in parentheses are standard errors.
*,**Indicates significance at P = 0.05 and 0.01 levels, respectively.

Table 19. Pearson correlation coefficients for seven selected traits measured across the three locations during the 2007-2008 season.

	ergo	field score	panshape	height	yield	hardness
field score	-0.01ns					
panshape	-0.03ns	-0.06ns				
height	-0.03ns	-0.38**	-0.14**			
yield	-0.11*	-0.44**	-0.01ns	0.55**		
hardness	0.22**	-0.19**	-0.17**	0.11*	0.25**	
genotype	-0.08ns	-0.42**	-0.03ns	0.34**	0.16**	0.11*

*-Significant at P≤0.05.
**-Significant at P≤0.01.
ns -Not significant at P≤0.05.

CHAPTER 4

Genotype by environment interactions for grain mould resistance

ABSTRACT

Variation in genotype performance for grain mould resistance in sorghum [*Sorghum bicolor* (L.) Moench] was assessed over three environments. The objective of this study was to assess the significance and nature of genotype by environmental interactions in the expression of grain mould resistance. A North Carolina Experiment II mating design was used to test the combining ability of 9 random pollen parents with varying levels of grain mould resistance to a different set of three random seed parents. The 27 resulting progenies and the parental lines were evaluated for grain mould development in a randomized complete-block design at Cedara-1 (early planting), Cedara-2 (late planting) and Potchefstroom during the 2007-2008 season. Differences in ergosterol concentration in mature grain were evaluated and used as the primary measure of the level of grain colonization in genotypes. Significant G x E interaction effects on ergosterol were detected after analysis of variance across all three locations. Single site analysis was conducted to better explain the nature of the G x E interaction. Potchefstroom was the only location that showed significant genotypic responses to grain mould infection with a range of 5 to 43 μg/g in ergosterol concentration in parent lines and 8 to 31 μg/g in hybrids. Low grain mould pressure at Cedara-1 and Cedara-2 resulted in very low genotypic responses. A biplot was used to indicate all genotypic performances across the

three locations in a graphical design. Fungal species infecting sorghum kernels were isolated, counted and identified. Low genotypic responses at the Cedara locations seems to have been caused by prevalence of a less aggressive fungal species, *Mucor* spp. Weather variables did not correlate significantly with ergosterol concentration.

INTRODUCTION

Grain mould is a significant constraint to sorghum [*Sorghum bicolor* (L.) Moench] production and improvement in many parts of the world. Grain mould has been shown to reduce yield and quality of sorghum grains ranging from cosmetic deterioration of the pericarp to substantial deterioration of the endosperm and embryo (Rooney and Serna-Saldivar, 1991). Fungal damage may also involve reduction in seed viability, nutritional quality, kernel weight and market value of the grain. Moulded grain may also contain mycotoxins and present health hazards to both humans and animals (Castor and Frederiksen, 1980). This disease is more severe when grain development coincides with wet and warm weather conditions (Forbes *et al.*, 1992). Grain mould is caused by a number of fungal species belonging to different genera (Castor and Frederiksen, 1980; Williams and Rao, 1981; Forbes *et al.*, 1992). Some of the most important species include *Fusarium graminearum* Schwabe, *Fusarium thapsinum* Klittich, Leslie, Nelson et Marasas sp. nov. 1996, *Curvularia lunata* (Wakker) Boedijn, *Phoma sorghina* (Sacc.) Boerma *et al.* and *Alternaria alternata* (Fr.) Keissl. because they are more frequently isolated from moulded grain (Williams and Rao, 1981; Bandyopadhyay *et al.*, 1991; Esele *et al.*, 1993; Erpelding and Prom, 2006).

Chemical control of grain mould and avoidance mechanisms are not economically feasible for most resource poor sorghum farmers in the semi-arid regions where sorghum is widely grown (Bandyopadhyay *et al.,* 1991). Biological control has not been successful. The use of genetic resistance is the only long term and stable means of controlling grain mould. However, breeding for grain mould resistance has had limited success to date. Numerous genetic factors with complex underlying factors have been reported to influence grain mould resistance. Plant traits like panicle shape, plant height, and glume structure have been associated with grain mould resistance. Caryopsis traits such as grain hardness, a pigmented testa layer, antifungal proteins and pericarp color are also related with grain mould resistance (Esele *et al.*, 1993). However none of these factors solely explain the variation in grain mould resistance found in sorghum. Some of the unexplained variation can be attributed to the effect of the environment.

The role of environment in disease development and severity has been studied on numerous occasions but very little substantial information has been gathered. It would appear that genotype x environment interaction is high and thus influences the conclusions regarding the role of environment in the epidemiology of the disease complex. An understanding of how sorghum genotypes respond to certain environmental factors like weather variables and composition of causal fungi in the environment will go a long way in assisting sorghum breeders to choose test environments that elicit genotypic responses during the screening process. The objectives of this research were to (i) analyze and explain the nature of the observed genotype by environment interaction (ii) choose the best location for screening sorghum genotypes for grain mould resistance.

MATERIALS AND METHODS

1. Genetic material.

During the 2006-2007 season, nine pollinator lines (designated LM101, LM103, LM104, LM108, LM109, LM114, LM116, LM117, AND LM120, pedigrees indicated in Table 1) with varying levels of resistance to grain mould, were crossed to three A1 cytoplasmic-genetic male-sterile sorghum seed lines designated LM122, LM124 and LM130 pedigrees indicated in Table 1) without reciprocals at Potchefstroom using a North Carolina Experiment II mating design (Comstock and Robinson, 1948). The nine male lines were sourced from a Southern Africa sorghum regional breeding nursery sponsored by the International Sorghum and Millets Collaborative Research Support Program (INTSORMIL CRSP) of USA. The 3 female lines were sourced from Texas A&M University sorghum breeding program. The male lines were selected to represent different levels of resistance to grain mould i.e. resistant, intermediate and susceptible. These classifications were based on visual scoring data collected over several years of field evaluation within the region.

All male lines had white grains but varied in glume color. Presence of glumes and their color is one of the traits known to contribute towards resistance to grain mould (Esele *et al.* 1993). There were five panicle shapes coded as described in the descriptors for sorghum hand book (IBPGR and ICRISAT, 1993). Where 1 – very lax panicle (typical of wild sorghum), 6 – semi-loose erect primary branches, 7 – semi-loose drooping primary branches, 8 – semi-compact elliptic, and 9 – compact elliptic. Panicle shape also contributes to grain mould resistance (Esele *et al.* 1993). One female line

(LM122) had tan glumes and the other two (LM124 and LM130) had brown glumes. Two females (LM122 and LM124) had white grains and one (LM130) had brown grain. This variation was used in an effort to capture maternal effects.

2. Experimental design

During the 2007-2008 season, seeds of the 27 single crosses, 12 parental lines and the three maintainer lines (male fertile) of the A1 testers used in the experiment were planted in a randomized complete block design (RCBD) with three replications in single 5 m row plots with a 0.75 m inter-row spacing. The experiments were rain fed and standard agricultural practices were the same for all plots. The experiment at Cedara was planted on two dates, the first planted on the 27th November and the second on the 12th December 2007. The two planting dates were treated as different environments. The third experiment was planted at Potchefstroom on the 6th December 2007. At Cedara, 10 plants per plot were bagged with transparent aerated bags to avoid bird damage. At Potchefstroom, the whole experiment was planted under a large bird cage net. Grain mould development was dependent on natural infection. Ten representative plants/panicles were harvested from each plot at maturity for further processing.

3. Fungal isolation and colony counts

Grain was threshed from panicles of 10 randomly selected plants within each plot. One hundred grains from each treatment replicate were surface-sterilized in 1% sodium hypochlorite for three minutes followed by three rinses in sterile water. Grains were aseptically plated onto Petri dishes (5 kernels per dish) containing 1% Malt Extract Agar (MEA) medium amended with 0.3 ml streptomycin sulphate (Caps Pharmaceuticals SA

(Pty) Ltd) to prevent bacterial contamination. Individual fungal colonies growing from seed onto the MEA medium were counted and morphologically identified to genus level. Peptone PCNB Agar (PPA) or Nash-Snyder Medium [15 g peptone, 20 g agar, 1 g KH_2PO_4, 0.5 g $MgSO_4$, 750 mg PCNB (pentachloronitrobenzene) per liter of water] was used for the isolation of *Fusarium* spp. The *Fusarium* spp. from the PPA plates were transferred to carnation leaf agar (CLA) plates and incubated at 25 °C under fluorescent light for 12 h/day for 7 days to promote sporulation. The CLA plates were then used for single spore production and subsequent identification (Leslie *et al.*, 2006).

4. Statistical analysis

Analyses were done using PROC GLM procedure of SAS (SAS Institute, 2003) and Microsoft Excel. Data was subjected to analysis of variance. The combined ANOVA was performed by considering replications and environments and corresponding interactions as random effects while genotypes were considered as fixed effects. Biplot analysis was used to explain the nature of the significant genotype by environment (G x E) interaction observed after ANOVA. The biplot was obtained using Microsoft Excel add-in Biplot software. Biplot analysis is a multivariate method for graphing row and column elements (genotype and pathogens) in a principal component analysis of two-way contingency tables and to detect interaction in two-way analysis on variance tables (Gower and Hand, 1996). Two principal components (PCs 1 and 2), obtained by singular value decomposition using principal component analysis after transforming the data with "Columns Centered and Standardized" transformation of the two way contingency table, where calculated and used to graphically explain G x E interaction. Interpretation of the Biplot was done according to the methods reported by Yan and Hunt (2002).

RESULTS AND DISCUSSION

After confirming constant variance and normality of data, a combined ANOVA was carried out to determine the significance of environments and genotype x environment interaction effects on the expression of grain mould in parent and hybrid genotypes. The combined analysis indicates that environments were highly significant (Table 1). This means that all three environments were significantly different from each other in terms of their effect on grain mould development. Essential environmental factors include location, year, genotype of the predominant fungal species in the area and prevailing weather conditions (Bandyopadyay and Mughogho, 1988; Maloy, 1993; McGee, 1995). Generally, wet weather immediately after flowering is necessary for grain mould development and the longer the wet period the greater the mould development (Koteswara and Poornachandrudu, 1971; Rao and Williams, 1977; Menkir *et al.,* 1996; Hall *et al.,* 2000). This is the most critical period for infection by most grain mould fungi. Weather variables like high relative humidity, warm temperature and high rainfall significantly increase infection, sporulation and dispersal of grain mould fungi (Bandyopadyay *et al.,* 1991). Previous research has indicated that differences in environments lead to differences in grain mould pressure (Bandyopadyay *et al.,* 1991).

Table 2 indicates mean squares for ANOVA of individual environments. No significant differences among genotypes were observed at Cedara-1 and Cedara-2 but at Potchefstroom there were significant differences. Potchefstroom yielded an average ergosterol concentration of 18.62 μg/g compared to 5.05 μg/g and 4.41 μg/g for Cedara-1 and Cedara-2 respectively (Table 2). It is apparent that there was very low grain mould pressure at Cedara-1 and Cedara-2 leading to the inability for genotypic differences

among sorghum genotypes to be observed. The late planting at Cedara-2 was the most ineffective in bringing out those genotypic responses among the sorghum genotypes.

Analysis of individual environments indicated that, minimum relative humidity (Min r.h.) was higher at Potchefstroom than at Cedara during the first few weeks post anthesis (Figures 2 and Figure 3). This means that the Potchefstroom environment was more moist than the Cedara environment during this critical period. Since moisture promotes fungal infection, higher grain mould pressure was thus observed at Potchefstroom. Figure 4 indicates that at Potchefstroom there was more rainfall during the same critical period than at Cedara. Higher relative humidity and rainfall are therefore assumed to be responsible for the observed higher grain mould pressure at Potchefstroom.

The significance of the G x E interaction term in the ANOVA (Table 1) implies that differences in ergosterol concentration or levels of resistance to grain mould were influenced by environment or location. This observation has been confirmed by other independent researchers (Indira *et al.*, 1991; Singh and Agarwal, 1993; Mansuetus *et al.*, 1997; Tarekegn *et al.*, 2006). In this case, the significant G x E was mainly from the P x E interaction as seen from the significance of the F x E effect. This means that the female parental lines are more sensitive to changes in environment than the male parental lines and the hybrids.

More than 40 fungal genera are associated with grain mould (Williams and Rao, 1981) but only a few are regarded as important pathogens (Singh and Bandyopadyay, 2000). Most fungi are either facultative parasites or saprophytes (Esele *et al.*, 1993). Efforts to isolate, count and identify fungi from grain harvested from all three locations

indicated that *Mucor* spp. was the dominant fungus at Cedara-1 and Cedara-2. There were low frequencies of important grain mould fungal species like *Phoma* spp., *F. graminearum* and *Curvularia lunata*. *Mucor* spp. has been isolated from mouldy grain but is not considered as an important grain mould fungus (Williams and Rao, 1981). The predominance of *Mucor* spp. at Cedara-1 and Cedara-2 could be the reason for low grain mould pressure. At Potchefstroom on the other hand there was a high frequency of *Alternaria* spp., with some *Fusarium sacchari*, *Aspergillus niger* and *Phoma* spp. and other minor species. The wider variety of fungi at Potchefstroom could be responsible for higher levels of ergosterol concentration observed.

The biplot in Figure 1 explains how the various genotypes performed in the different environments. The model used in this analysis explained 74% of the observed variation (PC1 35% and PC2 39%). The three environments fall into different parts of the plot area indicating that the three environments were totally different from each other in terms of genotype performance. The biplot was divided into 3 sectors with environments as the vertex entries and are referred to as sector AOB, sector BOC, and sector COA. A genotype falling close to a particular environment indicates that the genotype had high ergosterol concentration in that particular environment i.e. was most susceptible in that environment. Genotype 34 is the only genotype closest to Cedara-2 and referring to Table 3, genotype 34 had a grain ergosterol concentration of 23.08 μg/g at Cedara-2. This was the highest concentration for this genotype across all three locations and was the highest within the Cedara-2 trial. The 39 genotypes are spread in all three sectors but most of them are grouped towards the centre implying that their performance was not totally biased towards any of the environments. However some genotypes i.e. 1, 12 8, 6 and 5

are located towards Potchefstroom indicating that they were more susceptible to grain mould at Potchefstroom. Genotypes 28 and 29 tend towards Cedara-1 indicating that they were more susceptible at Cedara-1.

CONCLUSION

Environmental variables play an important role in the expression of genotypic characters in living organisms. Grain mould pressure in a particular environment is a function of fungal species composition combined with weather variables (Tarekegn *et al*. 2006). Environmental conditions that promote the growth and development of more aggressive fungal species, as was the case at Potchefstroom, will result in higher grain mould severity making those environments grain mould hot spots. Prevalence of less aggressive fungal species like *Mucor* spp. will results in low grain mould severity since such species may fail to penetrate deeper into the endosperms of some genotypes resulting in low levels of ergosterol concentration as was the case at Cedara-1 and Cedara-2. Potchefstroom would be an ideal location to screen sorghum germplasm for grain mould resistance in South Africa since genotypic responses could be detected more easily at that location than at Cedara.

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Table1. Mean squares from the combined analysis of variance for ergosterol concentration in inbred lines and their hybrids under conditions of natural infection at three locations during the 2007-2008 season.

Sources	DF	Ergosterol
Env	2	7533.28**
Rep(Env)	6	2041.90**
Gen	38	88.95ns
Parent	11	163.39ns
Male	8	127.52ns
Female	2	220.96ns
Male vs. Female	1	335.13ns
Parent vs. Hybrid	1	357.85*
Hybrid	26	47.12ns
Male (Mgca)	8	19.27ns
Female (Fgca)	2	15.03ns
M x F (sca)	16	65.05ns
Gen x Env	76	102.12**
Parent x Env	22	164.57*
M x Env	16	145.21ns
F x Env	4	228.52*
Hybrid x Env	52	50.79ns
M x Env	16	44.89ns
F x Env	4	69.79ns
M x F x E	32	51.37ns
Error	342	77.51
R-Square	-	0.57
Mean	-	9.36

*, ** Indicates significance at $P = 0.05$ and $P = 0.01$ respectively.
ns Indicates non- significance at $P \leq 0.05$.

Table 2. Mean squares from the analysis of variance for ergosterol concentration in inbred lines and their hybrids under conditions of natural infection at Cedara-1, Cedara-2 and Potchefstroom during the 2007-2008 season.

		Ergosterol concentration		
Sources	**DF**	**Cedara-1**	**Cedara-2**	**Potch**
Rep	2	217.33**	18.07^{ns}	5890.30**
Gen	38	2.27^{ns}	31.53^{ns}	259.38*
Parent	11	1.70^{ns}	4.47^{ns}	486.35^{ns}
Male	8	1.48^{ns}	3.98^{ns}	412.49^{ns}
Female	2	1.39^{ns}	3.74^{ns}	672.88^{ns}
Male vs. Female	1	4.12^{ns}	9.86*	704.21^{ns}
Parent vs. Hybrid	1	17.31*	31.39^{ns}	1808.57*
Hybrid	26	1.93^{ns}	42.99^{ns}	103.77^{ns}
Male (Mgca)	8	1.85^{ns}	42.75^{ns}	64.45^{ns}
Female (Fgca)	2	0.31^{ns}	37.78^{ns}	116.54^{ns}
M x F (sca)	16	2.18^{ns}	43.76^{ns}	121.84^{ns}
Error	114	2.75	32.21	197.56
R-Square	-	0.70	0.33	0.63
Mean	-	5.05	4.41	18.62

*, ** Indicates significance at $P = 0.05$ and $P = 0.01$ respectively.

^{ns} Indicates non- significance at $P \leq 0.05$.

Table 3. Genotype means for ergosterol concentration (μg/g) at Cedara-1, Cedara-2 and Potchefstroom during the 2007-2008 season.

entry	code	genotype	Cedara-1	Cedara-2	Potch	Mean
Male 1	LM101	1	3.48	4.02[a]	33.48[abc]	13.66[ab]
Male 2	LM103	2	4.71	4.29[a]	30.24[abc]	13.08[ab]
Male 3	LM104	3	4.81	4.95[a]	15.59[abc]	8.45[ab]
Male 4	LM108	4	4.12	3.05[a]	13.40[bc]	6.86[ab]
Male 5	LM109	5	5.46	3.47[a]	42.81[a]	17.25[a]
Male 6	LM114	6	4.57	2.73[a]	40.70[ab]	16.00[a]
Male 7	LM116	7	5.37	4.43[a]	10.84[c]	6.88[ab]
Male 8	LM117	8	4.03	2.41[a]	32.17[abc]	12.87[ab]
Male 9	LM120	9	5.49	6.04[a]	24.37[abc]	11.97[ab]
Male means			4.67	3.93	27.07	11.9
Female 1	LM122	10	3.58	1.71[a]	5.29[c]	3.52[b]
Female 2	LM124	11	4.67	3.92[a]	11.50[c]	6.70[ab]
Female 3	LM130	12	3.41	2.54[a]	33.77[abc]	13.24[ab]
Female means			3.89	2.72	16.85	7.8
Hybrid 1	LM122xLM101	13	5.82	3.13[a]	13.02[bc]	7.32[ab]
Hybrid 2	LM122xLM103	14	4.90	3.84[a]	31.16[abc]	13.30[ab]
Hybrid 3	LM122xLM104	15	5.44	5.21[a]	28.11[abc]	12.92[ab]
Hybrid 4	LM122xLM108	16	4.50	5.34[a]	10.58[c]	6.81[ab]
Hybrid 5	LM122xLM109	17	5.38	5.36[a]	16.96[abc]	9.23[ab]
Hybrid 6	LM122xLM114	18	5.32	3.00[a]	24.52[abc]	10.94[ab]
Hybrid 7	LM122xLM116	19	5.38	2.76[a]	22.18[abc]	10.11[ab]
Hybrid 8	LM122xLM117	20	4.38	2.61[a]	9.89[c]	5.62[ab]
Hybrid 9	LM122xLM120	21	5.61	4.10[a]	8.62[c]	6.11[ab]
LM122 hybrid means			5.19	3.93	18.34	9.11
Hybrid 10	LM124xLM101	22	5.85	4.92[a]	22.75[abc]	11.17[ab]
Hybrid 11	LM124xLM103	23	5.47	4.72[a]	22.04[abc]	10.74[ab]
Hybrid 12	LM124xLM104	24	4.90	4.02[a]	15.79[abc]	8.24[ab]
Hybrid 13	LM124xLM108	25	4.86	2.62[a]	10.05[c]	5.84[ab]
Hybrid 14	LM124xLM109	26	5.58	5.22[a]	10.05[c]	6.95[ab]
Hybrid 15	LM124xLM114	27	3.35	4.47[a]	14.24[abc]	7.35[ab]
Hybrid 16	LM124xLM116	28	6.83	4.84[a]	14.53[abc]	8.73[ab]
Hybrid 17	LM124xLM117	29	7.06	3.06[a]	13.44[bc]	7.85[ab]
Hybrid 18	LM124xLM120	30	4.75	4.13[a]	14.65[abc]	7.84[ab]
LM124 hybrid means			5.40	4.22	15.28	8.30
Hybrid 19	LM130xLM101	31	5.75	3.26[a]	12.43[bc]	7.15[ab]
Hybrid 20	LM130xLM103	32	5.26	4.66[a]	8.63[c]	6.18[ab]
Hybrid 21	LM130xLM104	33	5.66	5.21[a]	10.05[c]	6.97[ab]
Hybrid 22	LM130xLM108	34	4.11	23.08[b]	15.63[abc]	14.27[ab]
Hybrid 23	LM130xLM109	35	4.11	3.67[a]	17.73[abc]	8.50[ab]
Hybrid 24	LM130xLM114	36	6.29	3.32[a]	14.83[abc]	8.15[ab]
Hybrid 25	LM130xLM116	37	5.58	5.45[a]	14.63[abc]	8.55[ab]
Hybrid 26	LM130xLM117	38	5.62	3.34[a]	17.38[abc]	8.78[ab]
Hybrid 27	LM130xLM120	39	5.58	2.97[a]	18.01[abc]	8.86[ab]
LM130 hybrid means			5.33	6.11	14.37	8.60
Overall Mean	-	-	5.05	4.41	18.61	9.36
CV (%)	-	-	34.27	129.48	69.34	93.20
LSD (0.05)	-	-	4.74	9.22	28.78	11.79

Means followed by the same letters are not significantly different according to Fisher's LSD method.

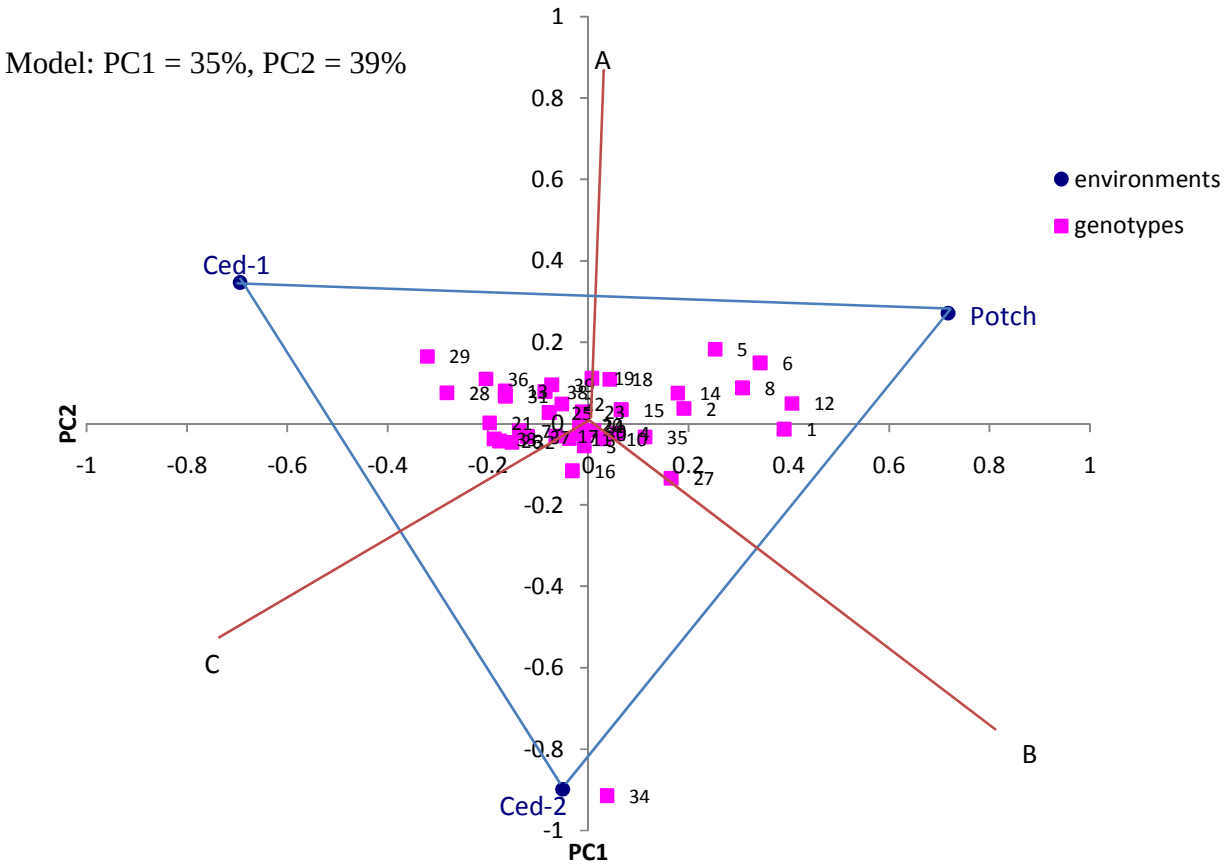

Figure 1. Biplot analysis depicting the nature of the G x E interaction observed across Cedara early planting (Ced-1), Cedara late planting (Ced-2) and Potchefstroom (Potch) during the 2007-2008 season.

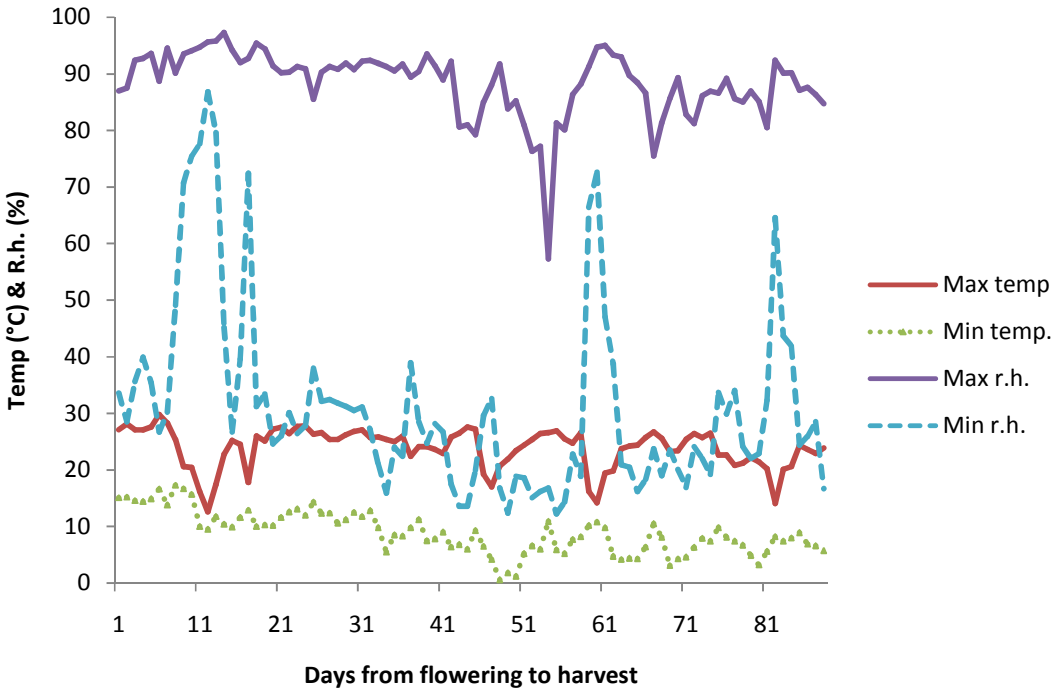

Figure 2. Temperature and relative humidity (r.h.) from the first day of flowering to harvest time at Potchefstroom during the 2007-2008 season.

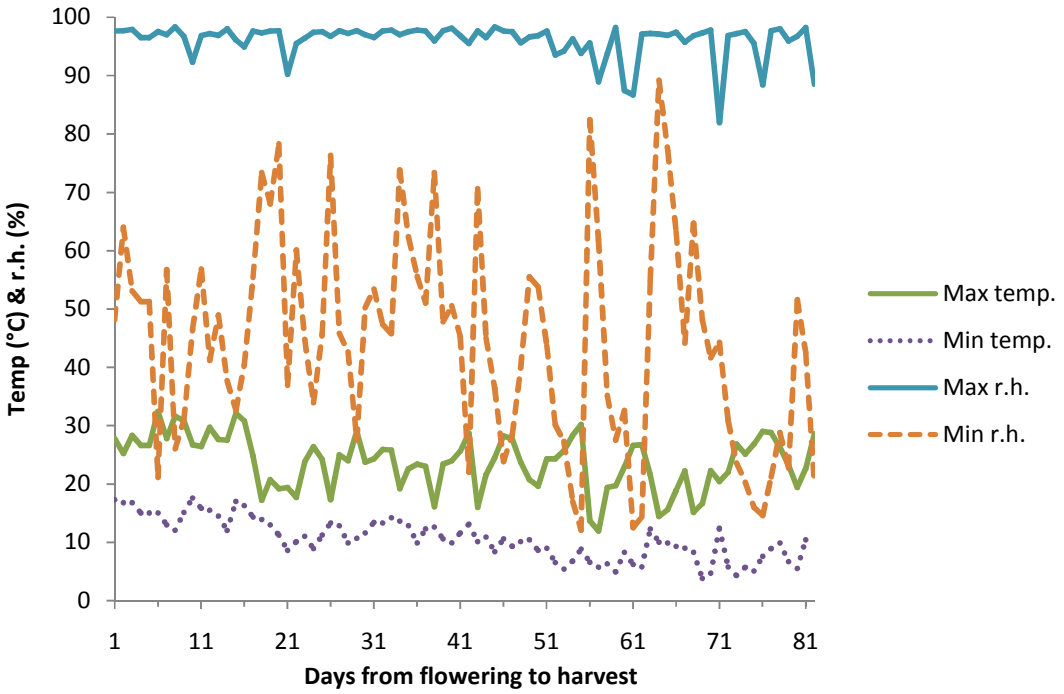

Figure 3. Temperature and relative humidity (r.h.) from the first day of flowering to harvest time at Cedara during the 2007-2008 season.

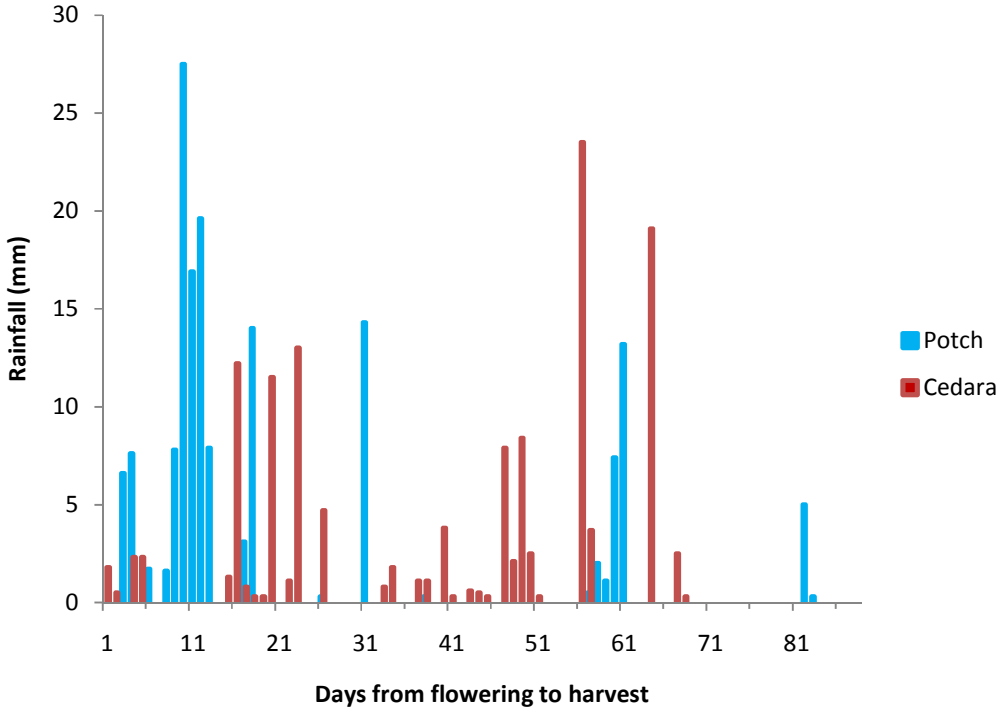

Figure 4. Rainfall (mm) distribution pattern from the first day of flowering to harvest time at Cedara and Potchefstroom during the 2007-2008 season.

CHAPTER 5

Mycotoxins and fungal biomass as they apply to sorghum grain across several genotypes in South Africa.

ABSTRACT

Mycotoxins have been detected in many foods, feeds and commodities as a result of growth of mould on crops and food. Mycotoxins are pathogenic to animals and humans as they may cause damage to critical body parts like the liver, central nervous system or the reproductive system. The objective of this study was to quantify the levels of three mycotoxins namely aflatoxin, deoxyvalenol (DON), and zearalenone across 39 sorghum genotypes grown at three diverse environments and to determine the extent to which G x E interaction affects toxin production. A North Carolina Experiment II mating design was used to test the combining ability of 9 random pollen parents with varying levels of grain mould resistance to a different set of three random seed parents. The 27 resulting progenies and the parental lines were evaluated for grain mould resistance levels in a randomized complete-block design at Cedara-1, Cedara-2 and Potchefstroom during the 2007-2008 season. The same genetic material was used to determine concentration levels of the three mycotoxins and ergosterol in grain harvested at maturity. Varying levels of ergosterol and mycotoxins were observed across the three locations. There was no correlation between ergosterol concentration and any of the mycotoxins evaluated. This indicates that mycotoxin concentrations are not related to total fungal biomass. The high concentration of mycotoxins at some localities suggests that environment plays a major role in toxin levels. Farmers at Potchefstroom should be aware of aflatoxins and

zearalenone while farmers at Cedara should choose varieties that tend to resist the development of DON and aflatoxins, while not ignoring zearalenone.

INTRODUCTION

Mycotoxins are natural, secondary metabolites produced by fungi on agricultural commodities in the field and during storage under a wide range of climatic conditions. About 200 different filamentous fungi species that produce toxins have been identified although the principal toxin producing fungi tend to fall within the genera *Aspergillus*, *Penicillium* and *Fusarium* (Husein and Brasel, 2001). Several hundred different mycotoxins have been discovered to date, exhibiting great structural diversity, which results in different chemical and physicochemical properties (Rustom, 1997). Mycotoxins are potent toxins and have a wide range of actions on animals and humans, including cyto-, nephro- and neurotoxic, carcinogenic, mutagenic, immunosuppressive and estrogenic effects (Bandyopadhyay *et al.*, 2000).

Because mycotoxins are unavoidable, naturally occurring compounds, regulations provide an important means to control the quality of the food and feed in which they occur and prevent them from proceeding through the food and feed chain to end users. Due to prevailing global international trade there is owing pressure for international legislation on mycotoxins in food and feed in order to avoid trade barriers and to protect the health of the consumer (Berg, 2003). The joint FAO/WHO Food Standards Programme and the Codex Alimentarius Commission (CAC) elaborate international food standards and codes of practice related to food (Berg, 2003). Each country has its own

means of enforcing implementation of those standards using their own internal structures. In most countries in Africa either the scientific basis for mycotoxin regulation is non-existent or the science has not been fully utilized (van Egmond, 1993). Three mycotoxins were reviewed in this research because they are considered to be important on sorghum in South Africa. These are aflatoxin, zearalenone and deoxynivalenol (DON).

Aflatoxins are considered to be the most important mycotoxins. Aflatoxicosis is poisoning that result from ingestion of aflatoxins in contaminated food or feed. Major producers of aflatoxins are *Aspergillus flavus* Link, and *A. parasiticus* Speare (Bandyopadhyay *et al.*, 2000). These fungi are found virtually everywhere growing in soils, decaying plant material and cause stored grains to heat and decay. They produce aflatoxins as a byproduct of growth on many commodities including sorghum before and after harvest. Aflatoxins are differentiated into B (B1 and B2), M (M1 and M2), G (G1 and G2) sub-types based on structure, chromatographic and fluorescent characteristics (Husein and Brasel, 2001). Aflatoxin B1 is the most potent (Husein and Brasel, 2001) as it binds to DNA, disrupting the genetic code thereby promoting generation of cancerous tumors. It is also responsible for poor performance in livestock and poultry. Legislation regulates maximum allowable contamination levels at 20 ppb and commodities must be tested to ensure that levels are below this value for human and animal consumption. The LD_{50} of aflatoxins for most species ranges from 0.5 – 10 mg/kg body weight (Rustom, 1997). However, aflatoxins in sorghum are not as serious as in maize, groundnut and other oil rich seeds because sorghum is not considered a good substrate by the *Aspergillus* fungi (Bandyopadhyay *et al.*, 2000).

Deoxynivalenol (DON or vomitoxin) is produced by *Fusarium graminearum* (Bandyopadhyay *et al.*, 2000). This toxin has been found in wheat, barley, sorghum and maize (Bilgrami and Choudhary, 1998). Deoxynivalenol is classified under the largest group of *Fusarium* mycotoxins named trichothecenes that are divided into types A, B, C, and D according to their molecular structures (Miller *et al*., 1991). Deoxynivalenol is classified as type B. This toxin can cause vomiting, feed refusal, immune suppression, diarrhea, and weight loss in animals (Bandyopadhyay *et al.*, 2000).

Zearalenone (ZEA) is also produced by *Fusarium graminearum* together with deoxynivalenol (Bandyopadhyay *et al.*, 2000). This toxin is found in maize and sorghum hence has been detected in beer and sour porridge prepared from contaminated maize and sorghum (Sibanda *et al.,* 1997). ZEA is a macrocyclic lactone with high binding affinity to oestrogen receptors and low acute toxicity (Diekman and Green, 1992). It causes a wide range of reproductive problems to livestock. These include infertility, vulva oedema, vaginal prolapse, mammary hypertrophy in females and feminization in males, pseudo pregnancy and abortion with pigs being the most affected species (Bandyopadhyay *et al.*, 2000). Zearalenone toxicity can lead to huge losses to farmers.

The purpose of the present study was to quantify genetic and environmental effects on mycotoxins production using 39 genotypes at 3 locations.

MATERIALS AND METHODS

1. Genetic material

During the 2006-2007 season, nine pollinator lines designated LM101, LM103, LM104, LM108, LM109, LM114, LM116, LM117, and LM120 (pedigrees indicated in Table 1), with varying levels of resistance to grain mould, were crossed to three A1 cytoplasmic-genetic male-sterile sorghum seed lines designated LM122, LM124 and LM130 (pedigrees indicated in Table 1) without reciprocals at Potchefstroom using a North Carolina Experiment II mating design (Comstock and Robinson, 1948). The nine male lines were sourced from a Southern Africa sorghum regional breeding nursery sponsored by the International Sorghum and Millets Collaborative Research Support Program (INTSORMIL CRSP) of USA. The 3 female lines were sourced from the Texas A&M University sorghum breeding program. The male lines were selected to represent different levels of resistance to grain mould i.e. resistant, intermediate and susceptible. These classifications were based on visual scoring data collected over several years of field evaluation within the Southern Africa region.

All male lines had white grains but varied for glume color. Presence of glumes and their color is one of the traits known to contribute towards resistance to grain mould (Glueck *et al.,* 1977; Mansuetus *et al.,* 1990). The structure of the sorghum panicle influences the micro-climate inside the head thereby affecting grain mould development. Compact heads favour development of grain mould as their closely packed panicles retain moisture that creates a suitable environment for grain mould development (Reddy and Nasrath, 1985). There were five panicle shapes coded as described in the descriptors for sorghum hand book (IBPGR and ICRISAT, 1993). 1 – Very lax panicle (typical of wild

sorghum), 6 – Semi-loose erect primary branches, 7 – Semi-loose drooping primary branches, 8 – Semi-compact elliptic, and 9 – Compact elliptic. One female line (LM122) had tan glumes and the other two (LM124 and LM130) had brown glumes. Two females (LM122 and LM124) had white grains and one (LM130) had brown grain. This variation was used in an effort to capture maternal effects.

2. Experimental design

During the 2007-2008 season, seeds of the 27 single crosses and the 12 parental lines as well as the three maintainer lines (male fertile) of the A1 testers used in the experiment were sown at Cedara and Potchefstroom in a randomized complete block design (RCBD) with three replications in 5 m row plots with a 0.75 m inter-row spacing. The experiment at Cedara was planted on two dates. The first was planted on the 27th November and the second on the 12th December 2007 and the two planting dates were treated as different environments. The third experiment was planted at Potchefstroom on the 6th December 2007. The experiments were rain fed and standard agricultural practices were the same for all plots. At Cedara, 10 plants per plot were bagged with transparent aerated bags to avoid bird damage. At Potchefstroom, the whole experiment was planted under a large bird cage net. Grain mould was dependent on natural infection. Ten representative plants/panicles were harvested from each plot at maturity (at 13% moisture content) for further processing.

3. Ergosterol measurement

Ergosterol concentration with grain samples was determined according to the method of Seitz *et al.* (1977) as modified by Jambunathan *et al.* (1991). A 10 g sorghum

grain sample from each panicle was ground using a laboratory mill and sieved through a 0.4 mm screen. Ergosterol was then extracted with 50 ml of methanol (MeOH) by vigorously mixing using a magnetic stirrer in a 100 ml beaker for 30 minutes. The mixture was allowed to settle and 25 ml of clear extract was decanted and added to a screw capped test tube containing 3 g of potassium hydroxide (KOH). The mixture was thoroughly agitated on a vortex mixer to dissolve the KOH. Ten ml of n-Hexane was added and the mixture was incubated at 75 °C in a water bath for 30 minutes and allowed to cool to room temperature. Five ml of distilled water was added, and after mixing thoroughly, the solution was allowed to cool to room temperature. The upper hexane layer was removed with a syringe and transferred to a test tube. To the remaining aliquot in the screw capped test tube, 10 ml of hexane was added and mixed thoroughly and the upper hexane layer was again removed and pooled with the earlier aliquot. The procedure was repeated one more time. The three pooled hexane extracts in the test tube were evaporated to dryness in a hot water bath. The residue was re-dissolved in 5 ml of methanol (HPLC grade) and filtered through a 0.45 μm filter (Millex-HV, Millipore Corp., Bedford, MA), and 2 ml of the filtrate was stored in a -80 °C freezer before ergosterol determination.

Ergosterol content in the filtrate was determined using a Shimadzu DGU-20A5 Prominence Degasser high performance liquid chromatograph (HPLC) machine with auto injector SIL-20A. The extract was loaded on a silica based reverse-phase column (C18 110Å 5 μm particle size, 150 x 4.6 mm with two Securiguard cartridges). The mobile phase was methanol-water (96:4 v/v) at a flow rate of 1.2 ml min^{-1}. The column temperature was maintained at 50 °C and the absorbance of eluted ergosterol was

detected with an SPD-M20A prominence diode array detector at 282 nm. The standard ergosterol (Sigma) had a retention time of 8.2 minutes. The standard ergosterol was loaded at 1.0, 2.5, 5.0, 7.5, 10.0, 15.0, and 25.0 μg ml-1 to standardize measurement of samples and also to determine the correct peak position on the chromatograph. The area under the graph for all sample chromatograms was converted to ergosterol concentration in μg/g of grain using the following best fit formula:

$$Y = 0.1008e7E\text{-}08AREA$$

Where Y = ergosterol concentration in μg/g of grain.

AREA = area under the chromatogram graph of micro-absorbance units (mAU) versus time (minutes).

4. Aflatoxin analysis

A 5 g sample was obtained from each sorghum genotype after grinding and sieving through a 20 mesh sieve. The sample was vigorously shaken in 25 ml of 70% methanol for 3 minutes using a mechanical shaker. The extract was filtered through a Whatman #1 filter and the filtrate was collected for analysis. The samples were analyzed using a Veratox Quantitative Aflatoxin Test Kit purchased from Neogen Corporation 630 Lesher Place, Lansing, MI 48912. A BioTek ELx808 microwell reader was used to read the concentration of aflatoxin per sample in parts per billion (ppb).

5. Deoxynivalenol (DON) analysis

A 10 g sample was obtained from each sorghum genotype after grinding and sieving through a 20 mesh sieve. The sample was vigorously shaken in 100 ml of distilled water

for 3 minutes using a mechanical shaker. The extract was filtered through a Whatman #1 filter and the filtrate was collected for analysis. The samples were analyzed using a Veratox 5/5 Quantitative DON Test Kit purchased from Neogen Corporation 630 Lesher Place, Lansing, MI 48912. A BioTek ELx808 microwell reader was used to read the concentration of DON per sample in parts per million (ppm).

6. Zearalenone analysis

A 5 g sample was obtained from each sorghum genotype after grinding and sieving through a 20 mesh sieve. The sample was vigorously shaken in 25 ml of 70% methanol for 3 minutes using a mechanical shaker. The extract was filtered through a Whatman #1 filter and 5 ml of the filtrate was collected. 1 ml of that extract was mixed with 4 ml of distilled water to give a 1:5 extract to water dilution. The diluted sample was then analyzed using a Veratox Quantitative Zearalenone Test Kit purchased from Neogen Corporation 630 Lesher Place, Lansing, MI 48912. A BioTek ELx808 microwell reader was used to read the concentration of zearalenone per sample in parts per billion (ppb).

RESULTS AND DISCUSSION

Mean ergosterol concentration determination indicated that grain from Potchefstroom had the highest fungal biomass concentration at 18.61 μg/g followed by Cedara-1 with 5.05 μg/g and finally Cedara-2 with 4.41 μg/g. Efforts to isolate, count and identify fungi from grain harvested from all three locations indicated a high frequency of *Mucor* spp. (>50%) at Cedara-1 and Cedara-2. *Mucor* spp inoculum is high in most environments and the fungus is often found as saprobes in soils, dead plant material (such as hay), horse dung, and fruits (Mucor mould website 24/08/09). *Mucor* spp. has been isolated from mouldy grain but is not considered as an important grain mould fungus (Williams and Rao, 1981). The prevalence of *Mucor* spp. at Cedara may account for the low levels of ergosterol observed at the two Cedara locations. Some *Phoma* spp., *F. graminearum* and *Curvularia lunata* were isolated at Cedara though the latter were at low frequencies. Potchefstroom on the other hand had a high frequency of *Alternaria* spp., with some *Fusarium sacchari, Fusarium graminearum, Aspergillus niger* and *Phoma* spp and other species at low frequency. A wider variety of aggressive fungi at Potchefstroom may be responsible for the observed higher levels of ergosterol concentration.

Differences in weather conditions between Cedara and Potchefstroom (Chapter 4) may account for the difference in fungal species composition and frequency observed. The high relative humidity and high rainfall immediately after anthesis observed at Potchefstroom, promoted development of grain mould fungi, hence increased grain mould pressure.

1. Aflatoxin analysis

The mean aflatoxin concentration across all three locations ranged from 0 ppb in LM122xLM108 to 15.88 ppb for LM130xLM108 (Table 1) indicating significant genotypic effects. The female line LM130 produced hybrids with a high average aflatoxin concentration of 12.99 ppb. This female line thus has limited value in crosses in areas where aflatoxin causing fungi are predominant. Figure 1 indicates that Potchefstroom had the highest mean aflatoxin levels and farmers in that environment should be wary of the risk of high levels of aflatoxin concentration in their sorghum grains. The high level of aflatoxin at Potchefstroom was to be expected because it is the only location where aflatoxin producing *Aspergillus* spp. were isolated. Inadequate post-harvest storage conditions of high moisture content and warm temperature increase aflatoxin levels even in grains with low frequencies of *Aspergillus* spp. (Blaney and Williams, 1991). Forbes *et al.,* (1992) reported that high moisture content during the season can lead to high aflatoxin levels (10 - 80 μg/g).

The joint FAO/WHO Food Standards Programme and the Codex Alimentarius Commission (CAC) has different regional maximum aflatoxin concentration limits. In South Africa the maximum allowable regulatory level in all food stuffs is 5 ppb (codex alimentarius) and the three locations produced some grain beyond this limit. Correlation between aflatoxin and ergosterol concentration across all three locations was highly significant but very low at 0.40** (Table 2). This is not a reliable correlation because it does not hold most of the time.

2. Deoxynivalenol (DON) analysis

The mean DON concentration across all three locations ranged from 0 ppm to 0.9 ppm for LM103 (Table 1). The male lines had the highest average concentration at 0.49 ppm. Maximum allowable regulatory DON concentration in commodities for human and animal consumption is 1 ppm (codex alimentarius). Figure 2 indicates that Cedara-1 had the highest mean for DON with some entries above the limit, hence farmers in that environment who plant their crops early should be wary of the risk of high levels of DON concentration in their sorghum grains. Cedara-2 also had some entries with high levels hence they must be very careful with the choice of variety that they use. On the other hand farmers in Potchefstroom do not seem to have a significant risk of high levels of DON concentration. Correlation between DON and ergosterol concentration across all three locations was highly significant but very low at -0.37** (Table 2). This is not a reliable correlation because it does not hold most of the time.

3. Zearalenone analysis

Zearalenone contamination is prevalent in many agricultural commodities especially in maize (Logreico *et al.,* 2003). Half of sorghum malt grain tested in South Africa was positive for zearalenone (Odhav and Naicker, 2002). Zearalenone has also been detected in maize porridge and sorghum beer from Swaziland and Lesotho (Sibanda *et al.,* 1997). Zearalenone has been detected in sorghum grains with no visible mould (Bowman and Hagler, 1991). The mean zearalenone concentration across all three locations ranged from 134 ppb for LM130xLM120 to 436 ppb for LM104 (Table 1). The

male lines had the highest average concentration at 305 ppb. Figure 3 indicates that Cedara-1 had the highest mean levels of zearalenone, hence farmers in that environment who plant their crops early should be wary of getting high levels of zearalenone concentration in their sorghum grains. Cedara-2 and Potch also had some entries with high levels hence they must be very careful with the choice of variety that they use. Correlation between zearalenone and ergosterol concentration across all three locations was highly significant but very low at -0.27** (Table 2). This is not a reliable correlation because it does not hold most of the time.

The non-significance of the correlations between ergosterol concentration and any of the three mycotoxins implies that the concentration of the toxins is not dependent on the amount of fungal biomass in the grain. This is to be expected because the total amount of fungal biomass in the grain while in the field is usually composed of several different fungi. The most abundant fungus in the locality may not necessarily be the one producing the toxin. If ergosterol concentration analysis was done on the mycotoxin producing fungus in isolation, then the results would have been different.

CONCLUSION

Mycotoxins are very harmful substances that must not be consumed by people and animals as they have detrimental effects. Mycotoxin concentration in sorghum varies from one location to another. It is not related to the total amount of fungal biomass in the grain. It is related to fungal species composition of the environment in which the crop is grown. It is essential to know the fungal species composition of the environment in which one is growing their sorghum crop. This would help in deciding which mycotoxins to screen for in the grain after harvesting.

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Table 1. Mean ergosterol (μg/g), zearalenone (ppb), deoxynivalenol (ppm) and aflatoxin (ppb) concentration across 39 sorghum genotypes at three locations during the 2007-2008 season.

entry	genotype	ergo	zea	don	afla
LM101	1	13.66	158.00	0.00	7.54
LM103	2	13.08	285.00	0.90	6.98
LM104	3	8.45	436.00	0.54	8.28
LM108	4	6.86	272.67	0.47	4.42
LM109	5	17.25	246.67	0.00	3.27
LM114	6	16.00	206.67	0.46	5.81
LM116	7	6.88	297.00	0.85	3.79
LM117	8	12.87	429.33	0.61	4.71
LM120	9	11.97	419.00	0.55	6.10
Male means	-	*11.89*	*305.59*	*0.49*	*5.66*
LM122	10	3.52	222.33	0.58	4.32
LM124	11	6.70	269.00	0.41	4.75
LM130	12	13.24	210.33	0.07	5.44
Female means	-	*7.82*	*233.89*	*0.35*	*4.84*
LM122xLM101	13	7.32	244.67	0.37	3.91
LM122xLM103	14	13.30	350.67	0.63	4.17
LM122xLM104	15	12.92	341.00	0.36	4.25
LM122xLM108	16	6.81	196.67	0.01	0.00
LM122xLM109	17	9.23	178.00	0.07	3.07
LM122xLM114	18	10.94	289.33	0.38	0.25
LM122xLM116	19	10.11	306.67	0.24	1.79
LM122xLM117	20	5.62	302.00	0.37	1.33
LM122xLM120	21	6.11	276.33	0.05	4.55
LM122 means	-	*9.15*	*276.15*	*0.28*	*2.59*
LM124xLM101	22	11.17	287.33	0.00	1.17
LM124xLM103	23	10.74	281.67	0.35	4.25
LM124xLM104	24	8.24	395.00	0.12	3.36
LM124xLM108	25	5.84	310.33	0.01	4.66
LM124xLM109	26	6.95	303.67	0.04	3.50
LM124xLM114	27	7.35	355.33	0.05	8.24
LM124xLM116	28	8.73	364.33	0.29	6.20
LM124xLM117	29	7.85	409.67	0.84	10.67
LM124xLM120	30	7.84	223.67	0.75	14.74
LM124 means	-	*8.30*	*325.67*	*0.27*	*6.31*
LM130xLM101	31	7.15	299.00	0.12	11.80
LM130xLM103	32	6.18	371.00	0.61	12.55
LM130xLM104	33	6.97	369.00	0.38	13.74
LM130xLM108	34	14.27	241.00	0.30	15.88
LM130xLM109	35	8.50	381.00	0.45	14.63
LM130xLM114	36	8.15	313.33	0.42	7.26
LM130xLM116	37	8.55	273.67	0.05	13.98
LM130xLM117	38	8.78	239.67	0.59	13.07
LM130xLM120	39	8.85	134.00	0.15	14.01
LM130 means	-	*8.60*	*291.30*	*0.34*	*12.99*
Grand mean	-	9.36	294.62	0.34	6.73

Table 2. Pearson's correlation coefficients for correlation analysis between ergosterol concentration and three mycotoxins analysed in sorghum grain in 2008.

	Ergosterol
Zearalenone	-0.27**
Deoxynivalenol	-0.37**
Aflatoxin	0.40**

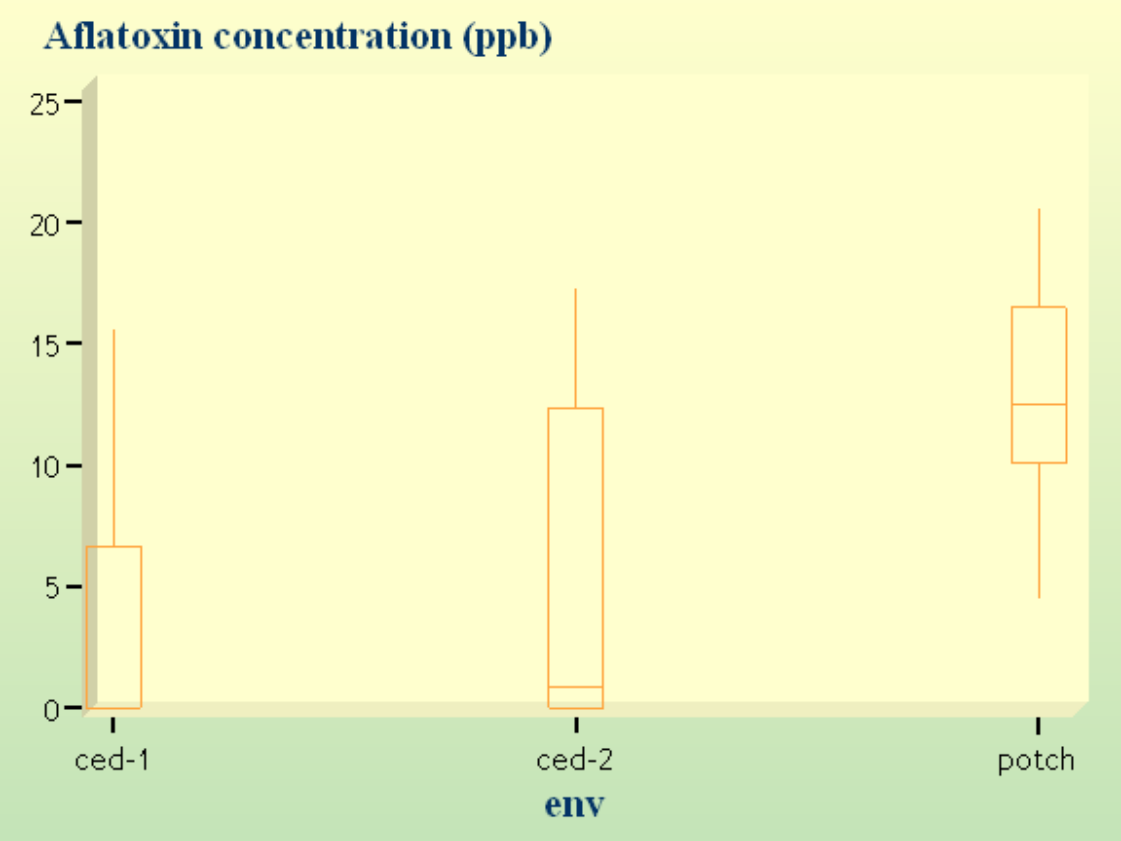

Figure 1. Levels of aflatoxin recorded in sorghum grain at Cedara-1, Cedara-2 and Potchefstroom during the 2007-2008 season.

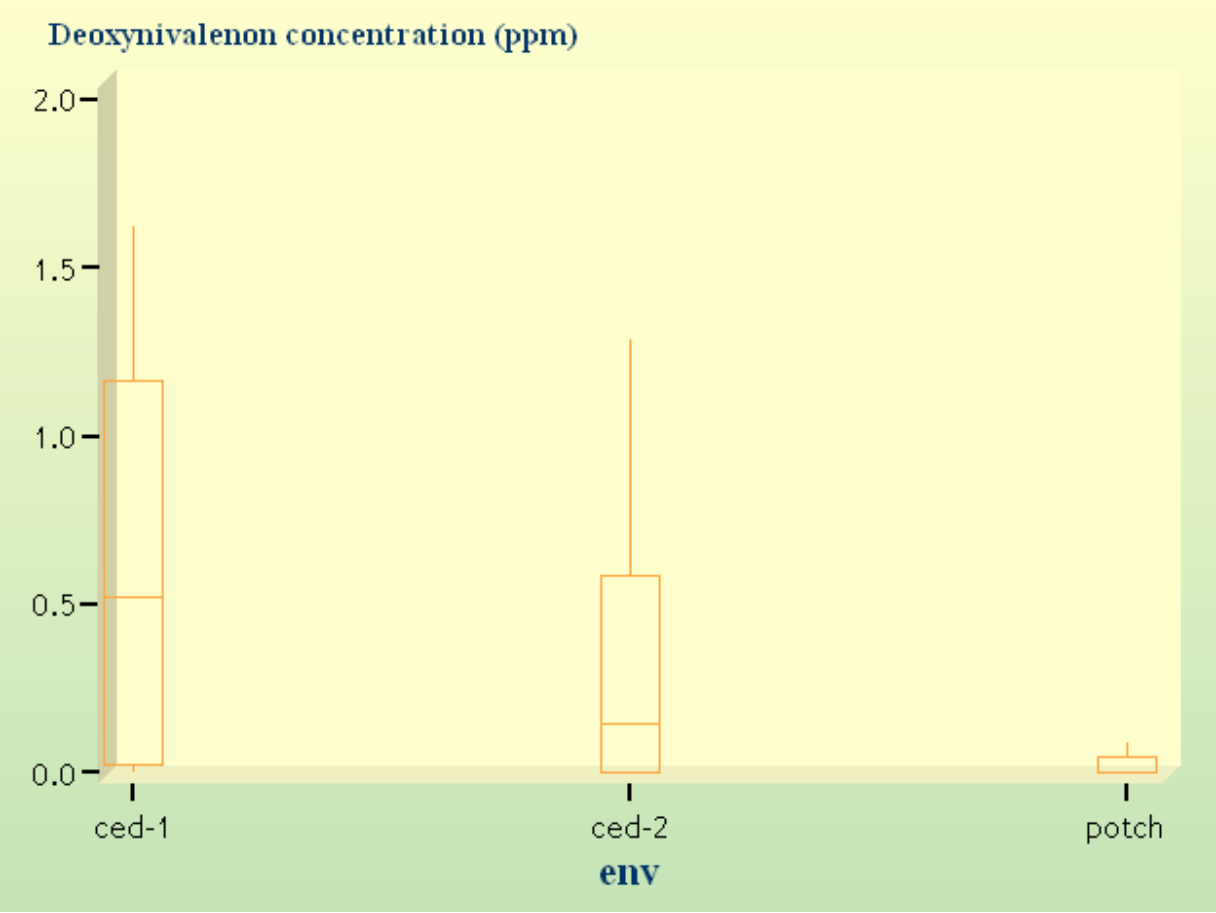

Figure 2. Levels of deoxynivalenol recorded in sorghum grain at Cedara-1, Cedara-2 and Potchefstroom during the 2007-2008 season.

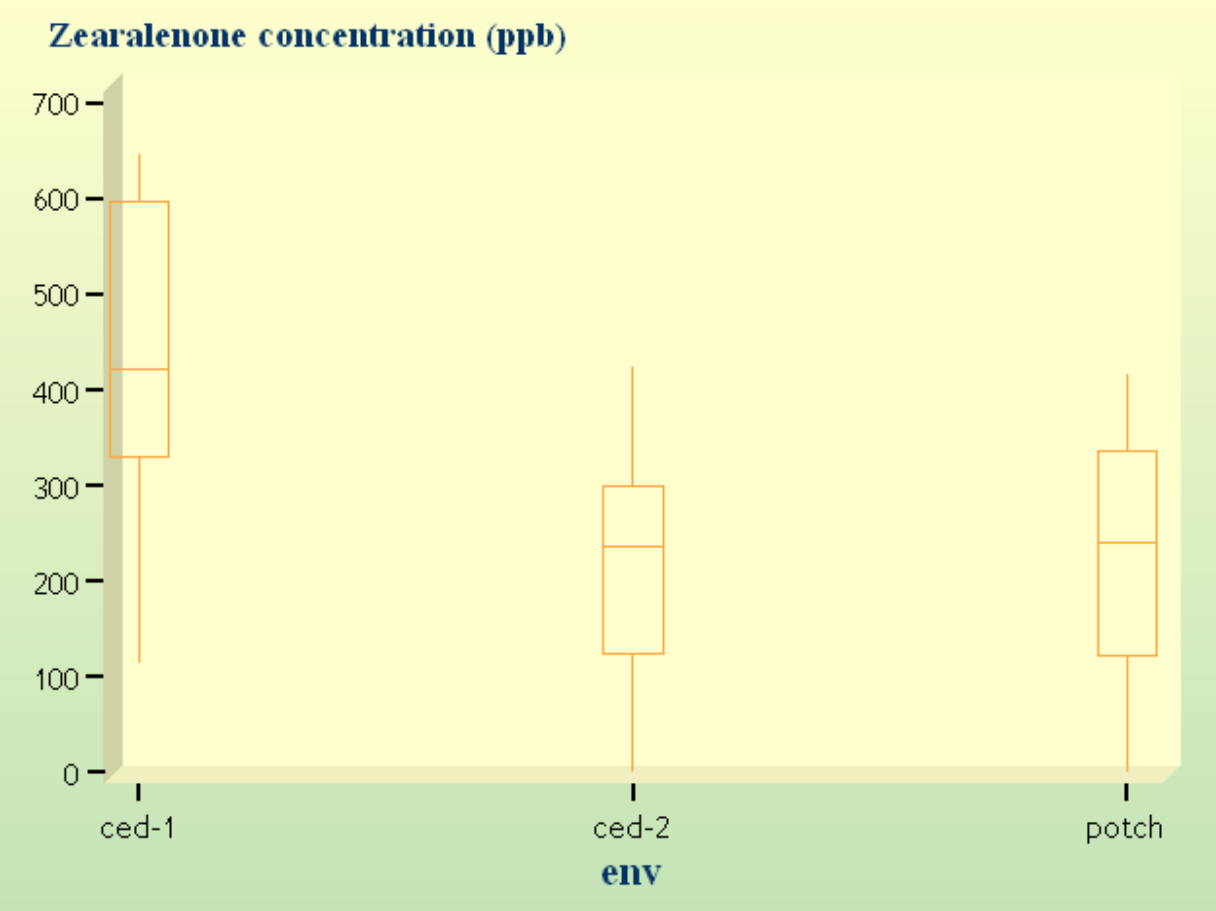

Figure 3. Levels of zearalenone recorded in sorghum grain at Cedara-1, Cedara-2 and Potchefstroom during the 2007-2008 season.

SUMMARY

In an effort to characterize the relationship between fungal pathogenicity and host genetic resistance, sorghum panicles of 11 genotypes were inoculated with five fungi frequently isolated from sorghum grain. Panicles were inoculated at anthesis with *Fusarium graminearum, Fusarium thapsinum, Curvularia lunata, Phoma sorghina* and *Alternaria alternata* spores. There were highly significant differences in the levels of fungal pathogenicity on different sorghum genotypes. These differences accounted for 58.4% of observed variation in ergosterol concentration. Genotype by pathogen (G x P) interactions accounted for 33.5% of the observed ergosterol concentration variation. The implication is that different genotypes reacted differently to different fungi. The genotypic reactions of the hosts accounted for 8.1% of the observed ergosterol concentration variation. Overall, fungal pathogenicity is the most important factor to consider in the evaluation of germplasm for grain mould resistance. Possible sources of resistance could be identified by use of biplot analysis of G x P interactions. Visual scoring for grain mould has limited value without identifying causal fungi. *Fusarium thapsinum* and *Phoma sorghina* were the most abundant fungi across all genotypes.

Gene action and heritability for grain mould resistance in sorghum were investigated using a selection of 9 random pollen parents with varying levels of grain mould resistance to a different set of three random seed parents. Differences in ergosterol concentration were used as a measure of level of grain mould resistance among all genotypes. Use of ergosterol concentration as a measure of grain mould severity did not correlate with visual field scoring. Other traits measured include plant yield, plant height, kernel hardness, field grade score, days to flowering, glume color and seed color. The

combined analysis of variance showed no genotypic variance for grain mould resistance. The expression of grain mould resistance was also not stable with significant genotype x location interaction. The analysis from Potchefstroom showed significant differences among genotypes whereas Cedara-1 and 2 showed no differences. Additive genetic variance was greater than dominance variance for all traits except grain mould resistance. A significant heterosis of -20.15% was observed for grain mould resistance indicating the importance of use of hybrid seed. LM124 (white seeded female) and LM130 (brown seeded female) produced resistant hybrids. Due to very high environmental variance, grain mould heritability could not be detected. Yield indicated the highest heritability of 0.41 at Cedara-1, plant height 0.39 at Cedara-2 and kernel hardness 0.38 at Potchefstroom.

The significance of genotype by environment interaction was assessed over three environments. Differences in ergosterol concentration were used as a measure of level of grain mould resistance among all genotypes. Significant G x E interaction was detected after analysis of variance across all three locations. Single site analysis was then done to better explain the nature of the G x E interaction. Potchefstroom is the only location that showed significant genotypic responses to grain mould infection. Low grain mould pressure at Cedara-1 and Cedara-2 caused very low genotypic responses. A biplot was then used to indicate all genotypic performances across the three locations in a graphical design. Fungal species infecting sorghum kernels were isolated, counted and identified. Low genotypic responses at the Cedara locations seems to have been caused by prevalence of a less aggressive fungal species, *Mucor* spp. Weather variables did not correlate significantly with ergosterol concentration.

Mycotoxin levels of aflatoxin, deoxyvalenol (DON), and zearalenone together with ergosterol concentration levels across 39 sorghum genotypes were measured. Varying levels of ergosterol and mycotoxins were observed across the three locations. There was no correlation between ergosterol concentration and any of the mycotoxins which indicates that mycotoxins concentration is not related to total fungal biomass. Mycotoxin concentration must therefore be related only to the biomass of the fungal species that is producing the mycotoxin among all fungal species infesting the grain. Farmers in Potchefstroom should be wary of aflatoxins and zearalenone while farmers in Cedara should choose varieties that tend to be low in DON and aflatoxins while not ignoring zearalenone.

OPSOMMING

‘n Poging is aangewend om die verhouding tussen fungus patogenisiteit en gasher genetiese weestand te bepaal en sorghum are van 11 genotipes is dus geinokuleer met vyf fungi wat gereeld vanaf sorghumgraan geisoler is. Are is geinokuleer by struifmeel stort met *Fusarium graminearum, Fusarium thapsinum, Curvularia lunata, Phoma sorghina* en *Alternaria alternata* spore. Hoogs betekenisvolle verskille in fungus patogenisiteit is op die verskillende sorghum genotipes waargeneem. Die verskille is vir 58.4% van die variasie in ergosterol konsentrasie verantwoordelik. Genotipe x patogeen (G x P) interaksie was vir 33.5% van die waargenome ergosterol konsentrasie verantwoordelik. Dit impliseer dat genotipes verskillend reageer teen verksillende fungi. Die genotipiese reaksies van die gashere was vir 8.1% van die waargenome variasie en konsentrasie verantwoordelik. Algeheel is fungus patogenisiteit die belangrikste faktor wat oorweeg moet word by die evaluering van kiemplasma vir graanskimmel weerstand. Moontlike bronne van weerskand kon identifiseer word deur biplot analise van G x P interaksies. Visuele meting van graanskimmel het beperkte waarde sonder om fungi te identifiseer en fungus biomassa in aanmerking te neem. *Fusarium thapsinum* en *Phoma sorghina* het die meeste voorgekom by alle genotipes.

Geen aksie en oorerfbaarheid van graanskimmel by sorghum weersand is bestudeer deur gebruik te maak van nege stuifmeel ouers met verskeie vlakke van graanskimmel weestand en ‘n ander stel van drie saadouers. Verskille in ergosterol konsentrasie is gebruik as maatstaf van die vlak van graanskimmel weerstand oor alle genotipes. Ergosterol konsentrasie as maatstaf van graanskimmel strafheid het nie met visuele veldmetigs gekorreleer nie. Ander eienskappe wat gemeet is sluit in plant

opbrengs, plant hoogte, graan hardheid, veldgraadmeting, dae tot blom, kaffie kleur en saad kleur. Die gekombineerde variasie analise het geen genotipiese variansie vir graanskimmel weerstand aangedui nie. Die uitdrukking van graanskimmel weerstand was nie stabiel met betekenisvolle genotipe x lokaliteit interaksies nie. Die analises vanaf Potchefstroom het betekenisvolle verksille tussen geotipes getoon terwyl Cedara-1 en 2 geen verkille getoon het nie. Additatiewe genetiese variansie was groter as dominante variansie vir alle eienskappe behalwe graanskimmel weerstand. 'n Betekenisvolle heterose van -20.15% is waargeneem vir graanskimmel weerstand wat die belangrikheid van bastersaad aandui. LM124 (witsaad wyfie) en LM130 (Bruinsaad wyfie) het weerstandbiedende basters geproduseer. Weens die hoë omgewings variasie kon graanskimmel oorerfbaarheid nie bepaal word nie. Opbrengs het die hoogste oorerfbaarheid van 0.41 getoon by Cedara-1, plant hoogter 0.39 by Cedara-2 en graanhardheid 0.38 by Potchefstroom.

Die betekenisvolle genotipe x omgewing interaksie is ontleed oor drie omgewings. Verskille in ergosteril konsentrasie is gebruik as maatstaf van graanskimmel weerstand tussen genotipes. Betekenisvolle G x E interaksies is waargeneem na 'n variansie analise oor al drie die lokaliteite. Enkel lokaliteit analise is uitgevoer om die aard van die G x E interaksie beter te verduidelik. Potchefstroom was die enigste lokaliteit wat 'n betekenisvolle genotipiese respons to gevolg gehad het. 'n Biplot is gebruik om alle genotipiese uitdrukkings voor te stel oor die drie lokaliteite in 'n grafiese ontwerp. Fungus spesies wat sorghumgrane geinfekteer het is geisoleer, getel en geidentifiseer. Lae genotipe respons by die Cedara lokaliteite is blykbaar veroorsaak deur die voorkoms van minder aggresiewe fungus spesies, bv. *Mucor* spp.

Weerveranderlikes het nie betekenisvol gekorreleer met die ergosterol konsentrasie nie. Mikotoksien vlakke van aflatoksien, deoxyvalenol (DON) en zearalenone saam met ergosterol konsentrasie vlakke oor 39 sorghum genotipes is bepaal. Verskeie vlakke van ergosterol en mikotoksiene is waargeneem oor die drie lokaliteite. Daar was geen korrelasie tussen ergosterol konsentrasie en enige van die mikotoksiene nie. Dit dui aan dat mikotoksiene nie verband hou met totale fungus biomassa nie. Mikotoksien konsentrasie moet dus in verband gebring word teenoor biomassa van fungus spesies wat mikotoksiene produseer in die omgewing waar die gewas gekweek word. Boere op Potchefstroom moet bedag wees vir die risiko van aflatoksiene en zearelenone. Boere op Cedara moet varieteite kies wat lae DON en aflatoksien to gevolg het maar moet bewus by van zearalenone.