Mycotoxigenic fungi associated with ear-rots in Zimbabwe: Identification and inheritance of resistance in southern and West African maize inbred lines

By

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Declaration

I declare that the thesis hereby submitted by me for the degree 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.

Dedication

To my wife (Lovejoy), my daughters (Makomborero and Tinomutenda), my late father (Patrick), my mother (Agness) and my late brother (Michael).

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Abbreviations and symbols

%	Percent
∑	Summation
°C	Degrees Celcius
µg	Microgram
µL	Microlitre
µm	Micrometre
M	1 mole
A1	Small scale resettled sector
A2	Large scale resettled sector
AFLP	Amplified fragment length polymorphism
AGRITEX	Agricultural Technical and Extension
ANOVA	Analysis of variance
ASI	Anthesis to silking interval
B1	Fumonisin B1 analogue
B2	Fumonisin B2 analogue
B3	Fumonisin B3 analogue
BCP_1S_1	S1 of a backcross with parent 1 as recurrent parent
Bt	*Bucillus thuriengensis*
CA	Communal area
CHT	Cob height
CIMMYT	International Maize and Wheat Improvement Centre
cm	Centimetre (s)
CML	CIMMYT maize line
DMP	Days to mid pollen shedding
DMS	Days to mid silking
DNA	Deoxyribonucleic acid
DON	Deoxynivalenol
E	Environment
E x Y	Environment by year interaction
EASP	Ear aspect

EC	Ears counted
EDTA	Ethylenediaminetetra acetate
EPO	Ear position
EPP	Ear per plant
ER	Ear rot
et al.	And others
F1	First filial generation
FAM	6-carboxyfluorescein
FAM	Fluorophores 6-carboxyfluorescein
FAO	Food and Agricultural Organisation
FB1	Fumonisin B1 analogue
FB2	Fumonisin B2 analogue
FB3	Fumonisin B3 analogue
FCSC	*Fusarium clamydosporum* species complex
FDA	Food and Drug Administration
FFSC	*Fusarium fujikori* species complex
FGSC	*Fusarium graminearum* species complex
FRET	Fluorescence resonance energy transfer
FUM	Total fumonisin content
g	Gram (s)
G	Genotype
G x E	Genotype by environment interaction
G x E x Y	Genotype by environment by year interaction
G x Y	Genotype by year interaction
GCA	General combining ability
GCA_f	General combining ability attributable to females
GCA_m	General combining ability attributable to males
GCPSR	Genealogical concordance phylogenetic species recognition
GD	Genetic distance
GDS	Grain disease score
GGE	Genotype and genotype by environment interaction

GLS	Grey leaf spot
GMB	Grain Marketing Board
GY	Grain yield (t ha^{-1})
H	Pride of Saline heterotic group
h^2	Heritability
H_2O	Water
ha	Hectare (s)
HC	Husk cover
HP	High-parent
HPH	High-parent heterosis
HPLC	High-performance liquid chromatography
HT	*Helminthosporium turcicum*
ID	Identity
IITA	International Institute for Tropical Agriculture
ITS	Internal transcribed spacer
KAPA	Kapa Biosystems
KASP	Kompetitive Allele Specific PCR
KASPar	KBioscience competitive allele-specific polymerase chain reaction
kg	Kilogram
kPa	Kilo pascal
KRC	Kadoma Reserch Centre
Ktaq	Klen Thermus aquaticus
l	Litre
LC	Liquid chromatography
LGC	LGC Limited
LSCFA	Large scale commercial farmers
LSD	Least significant difference
m	Metre (s)
M	Molar (s)
MABC	Marker-assisted back-crossing
MARS	Marker-assisted recurrent selection

masl	Metre (s) above sea level
Max	Maximum
MC	Moisture content
$MgCl_2$	Magnesium chloride
Min	Minimum
min	Minute
ml	Millilitre
ML	Maximum likelihood
MLST	Multilocus sequence typing
mm	Millimetre (s)
MP	Mid-parent
MPH	Mid-parent heterosis
MRD	Modified Rogers' distance
MSA	Malt salt agar
MSV	Maize streak virus
MT	Metric tonne
mtDNA	Mitochondria deoxyrebose nucleac aci
MTL	Maximum tolerable level
N3	Salisbury White
NaCl	Sodium chloride
NaOCl	Sodium hypochlorite
NaOH	Sodium hydroxide
NCDII	North Carolina Design II
ng	Nanogram (s)
NIV	Nivalenol
nm	Nanometre
NN	N3 heterotic group and another N3 heterotic group
NO	N3 heterotic group and an unknown heterotic group
NR	Natural region
nr	Not recorded
ns	Not significant

NTC	No template controls
OCO	Unknown heterotic group and another unknown heterotic group
OH	Unknown heterotic group and heterotic group H
OO	Unknown heterotic group and another unknown heterotic group
OPA	O-phthaldialdehyde
OPV	Open pollinated variety
OR	Old resettlement
P	F-probability
P	Natal Potchefstroom Pearl heterotic group
PCNB	Pentachloronitrobenzene
PCR	Polymerase chain reaction
PDA	Potato dexterose agar
pH	Measure of acidity/alkalinity
PH	P heterotic group and H heterotic group
PHT	Plant height
PI	P heterotic group and Iodine heterotic group
PIC	Polymorphic information content
PLS	*Phaeosphaeria* Leaf Spot
PMPH	Pelmitic mid parent heterosis
PMTDI	Provisional maximum tolerable daily intake
PO	P heterotic group and unknown heterotic group
PP	*Puccinia polysora*
ppm	Parts per million
Prob-T	Probability for t-test
PS	*Puccinia sorghi*
QPM	Quality protein maize
QTL	Quantitative trait loci
r	Pearson correlation coefficient
R2	10-14 days after silking
RAPD	Random amplified polymorphic DNA
RARS	Rattray Arnold Reserch Station

rDNA	Ribosomal deoxyrebose nucleac acid
RFLP	Restriction fragment length polymorphism
RL	Root lodging
ROX	6-Carboxyl-X-Rhodamine, succinimdyl ester
rpm	Revolutions per minute
rRNA	Ribosomal ribonucleic acid
SC	Southern Cross
SCA	Specific combining ability
SD	Standard deviation
SE	Standard error
sec	Second (s)
Sh_2	Shrunken gene
SL	Stalk lodging
SNP	Single nucleotide polymorphism
SPE	solid-phase extraction
spp	Species
SRC	Stapleford Reserch Centre
SS	SC heterotic group and another SC heterotic group
SSCA	Smal scale commercial areas
SSR	Simple sequence repeat
STB	Stalk-borer
t ha^{-1}	Ton per hectare
TEF	Translocation elongation factor
TEXT	Grain texture
TL	Total lodging
UK	United Kingdom
USA	United States of America
USAD	United States Agricultural Department
UV	Ultra violet
v/v	Percent volume by volume
VIC	2´chloro-7´-phenyl-1,4-dichloro-6-carboxyfluorescein

WACE	Weeks after crop emergence
WARC	West African Research Centre
WHO	World Health Organisation
xg	Centrifuge speed
XR	X-ray
Y	Year
ZEA	Zearalenone
µl	Microlitre
σ^2_e	Error variance
σ^2_g	Genotypic variance
σ^2_p	Phenotypic variance

Chapter 1

General introduction

1.1 Maize production in Africa

1.1.1 Importance

Almost every meal that is taken by the majority of people in sub-Saharan Africa, particularly in eastern, central and southern Africa, contains maize (*Zea mays* L.) as a sole or major component. Other countries in some regions of Africa such as central and West Africa have other sources of food besides maize which include yam, cassava, plantain and rice. Despite that, maize remains important in some regions of such countries with total estimated production surpassing the total production from those countries that regard maize as a staple crop. Nigeria, for instance was expected to produce 7.5 million metric tonnes (MT) of maize in 2014, a slight drop from the 2011 production of 9.25 million MT (USDA, 2014). Maize also constitutes the main component of animal feeds that man depends upon for sustenance. According to the USDA (2014), a total of 33.7 million MT of maize was estimated to be produced in 2014 in Africa. Due to production simplicity involved in maize such as no need to scare birds as is the practice with sorghum, and availability of cultivars adapted to traditionally non-maize environments, maize seems to be encroaching into such areas at a rapid rate. Poor production would constitute a national disaster in some countries with huge effects on the economy as importation becomes inevitable, hence successful production plays a large role in ensuring global food security (Edmeades *et al*., 2000).

1.1.2 Constraints in Africa

Despite the availability of high yield potential of maize cultivars on the market, there are several factors that affect its availability in sufficient magnitude as food. These include abiotic constraints such as recurrent drought (Kassie *et al*., 2013), inherent poor soil fertility, poor nutrients in the maize grain, poor agronomic practices and poor agricultural policies. Global warming further exacerbates the situation (Lobell *et al*., 2011) since maize has been demonstrated to be susceptible to drought and heat stresses (Cairns *et al*., 2012). African farmers face challenges that affect recommended practices

for the best yields to be obtained. These include late planting, poor weed control and where available, delayed fertilizer application. The poor nutritive quality of soil include low nitrogen and low pH, that have been identified as contributing to the low production of maize in Africa. Policies that allow access to finance have been recommended as a tool to increase productivity of maize (Abu *et al*., 2011) as lack of financial resources prevents attainment of good yields in maize production. Among the biotic factors that constrain maize production are insect pests, diseases (Kassie *et al*., 2013) and parasitic weeds caused by Striga species.

1.1.3 Quality

Malnutrition is prevalent in Africa, caused by both inadequate quantity of food and poor nutritive value of the maize that is grown and consumed. Through plant breeding programmes agronomically superior varieties are available in Africa, particularly in southern Africa, but they have poor nutritional value. Efforts have been made to ameliorate the nutritive value by breeding for high lysine and bio-fortified maize. Breeding for high lysine has faced some pleitropic challenges such as the *opaque-2* gene which has been closely linked with undesirable agronomic traits such as yet another biotic constraint, ear rots (Pixley and Bjarnason, 1992) which have been associated with mycotoxin production. Several biotic constraints exist which breeding programmes have endeavoured to overcome with great success. Among these are the complex fungi that cause ear rots which have been discovered to exude some hazardous metabolites called mycotoxins. Such biotic factors affect both the quality and quantity of maize as a source of food. These include fungal, bacterial, and viral diseases that affect the foliage, stalk and grain of maize. Among the fungal diseases that cause ear rotting, some can be sources of mycotoxins that affect the health of people and animals that depend on maize. The ear rot causing fungi include the *Sternocarpella (Diplodia), Aspergillus* and *Fusarium* species. Humans can contract secondary infections when they consume products from animals fed on contaminated products (Oyeru and Oyefolu, 2010). Such infections include acute toxicosis, liver cancer, morbidity in children suffering from kwashiorkor and esophageal cancer (Rheeder *et al*., 1992; Miller, 1996; Widstrom, 1996; Oyeru and Oyefolu, 2010). It is not only in Africa where higher levels of cob rots have been observed, but also in Europe, north and South

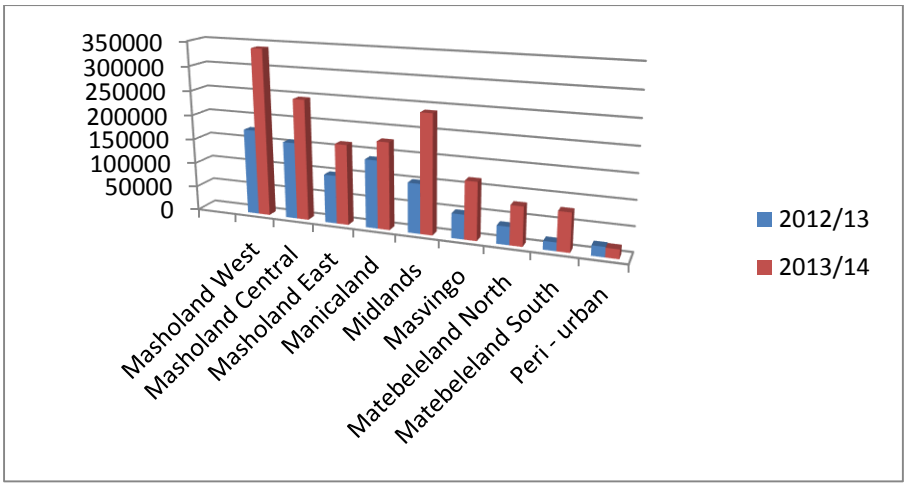
America and Asia (MacDonald and Chapman, 1997; Vigier *et al*., 1997; Logrieco *et al*., 2002).

In an effort to ameliorate the availability of food, breeding in Zimbabwe and most southern African countries has centred mainly on the development of hybrids that offer higher yields. Among these hybrids are those from which heterotic groups such as N3, K64R and SC have been developed which constitute most of the available maize hybrids in the region. Of these, the N3 heterotic group has been associated with high incidences of ear rots caused by the Fusarium, Aspergillus and Sternocarpella complexes. Despite its known susceptibility, it is widely used because of its good combining ability for yield. A similar situation prevails in the USA Corn Belt where derivatives of B73 that are very susceptible to aflatoxins caused by *Aspergillus flavus,* are widely used because of their superior yield potential.

1.2 Maize production in Zimbabwe

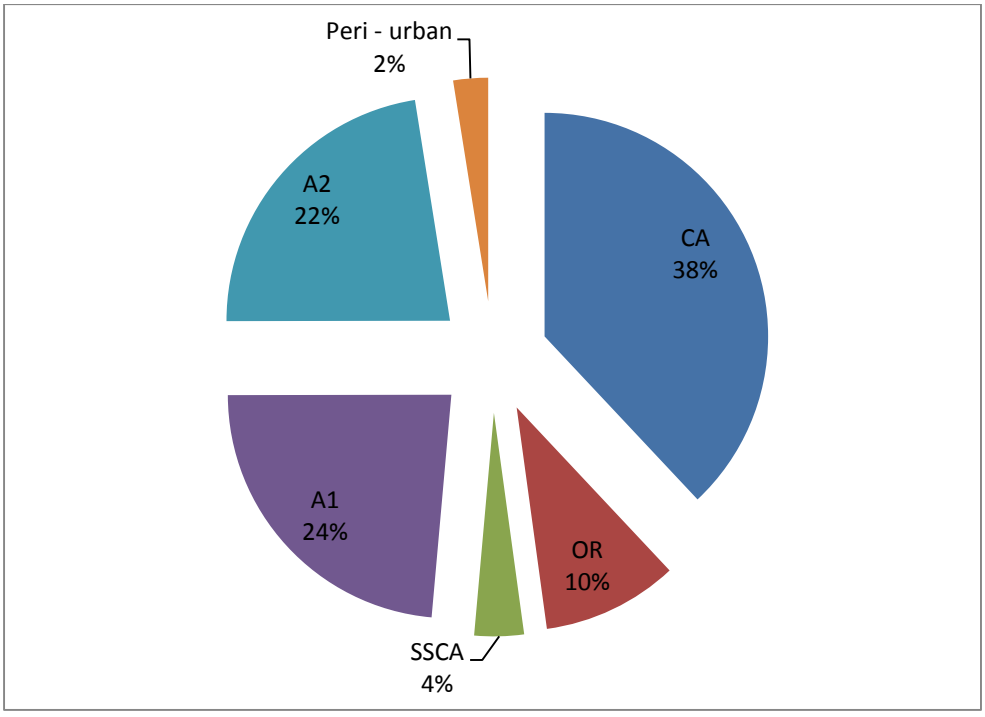
1.2.1 Importance

Zimbabwe views maize as synonymous with food as it is part of most of the meals taken by the majority of the people. Although it is seldom taken on its own since it basically consists of carbohydrates, the other dietary components such proteins and vitamins can easily be obtained from various other sources. The target production figure on an annual basis has been 2.1 million MT of which 1.8 million MT is for human, livestock and other industrial use while 300 000 MT goes towards the strategic grain reserve. Traditionally, the highest maize production is in Mashonaland West and Mashonaland East which are characterised by high rainfall (Figure 1.1). The highest production in terms of volumes come from the small holder communal farmers whose aggregated contribution supersedes other sectors due to number of farmers in that sector (Figure 1.2) despite having the lowest yield per unit area of about 0.5 MT ha^{-1} in the 2013/14 season as compared with an average of 2.5 MT ha^{-1} obtained from the commercial A2 sector (AGRITEX, 2014). More than 70% of the country's population is in the rural areas where farming, particularly maize production, is a way of life. The government recognised the role played by farmers and intervened in several ways to ensure availability of maize in the country.



Source: second round crop and livestock assessment report 2013/14 season

Figure 1.1 Maize production (MT) by province



A1=small scale resettled sector; A2=large scale resettled sector; CA=communal area; SSCA=small scale commercial area; OR=old resettlement

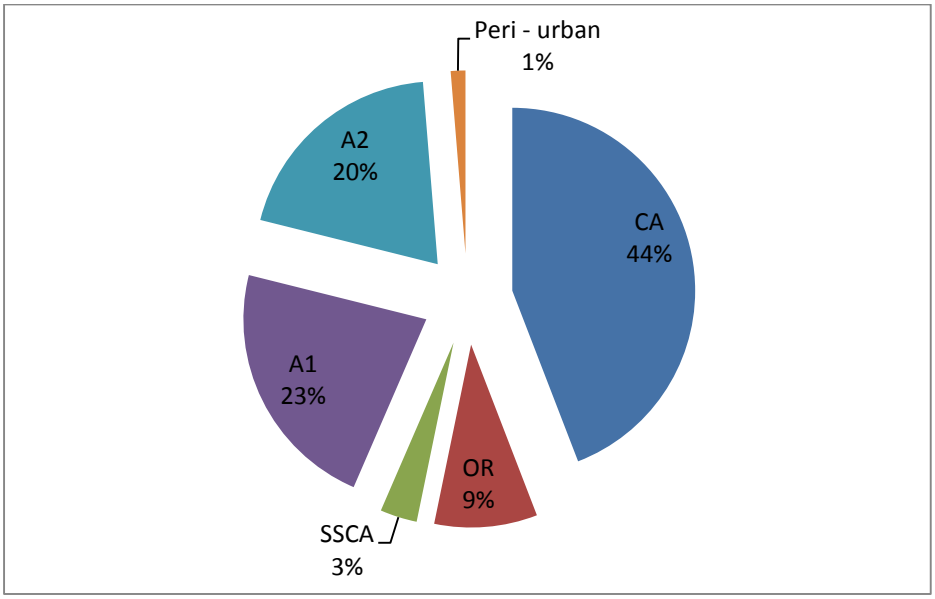
Source: second round crop and livestock assessment report 2013/14 season

Figure 1.2 Contribution by various sectors in the production of maize in Zimbabwe in the 2012/13 season

It has been supplying free inputs to small holder communal and A1 farmers to enable them to produce at least enough for household food security. It has been importing maize in years where production has been inadequate to meet local demand.

1.2.2 Constraints in maize production in Zimbabwe

Maize production has been fluctuating (Figure 1.4) due to various factors that include recurrent droughts, particularly the traditional mid-season drought that of late seems to be prolonged, late start of the growing season and unavailability of inputs, despite availability of high yielding hybrids. During the past decade when the country suffered the worst economic crisis, maize production was not spared. This is evidenced by low productivity as demonstrated in Figure 1.4. Production started to pick up with restoration of economic stability in 2010 but further declined in 2012, 2013, and 2014 as a result of drought, which remains the main limiting factor.



A1=small scale resettled sector; A2=large scale resettled sector; CA=communal area; SSCA=small scale commercial area; OR=old resettlement

Source: second round crop and livestock assessment report 2013/14 season

Figure 1.3 Contribution by various sectors in the production of maize in Zimbabwe in the 2013/14 season

The worst affected regions are the low lying areas that characterise most of Masvingo, Matebeleland North and South, and some parts of the Midlands, Mashonaland East and Central and Manicaland (AGRITEX Crop and Livestock Assessment Report, 2014). Production has been affected by high input costs as related to the price offered by the main purchaser, the Grain Marketing Board (GMB), a parastatal responsible for purchasing, storage and distribution of grain to various end users. Despite the opening up of the markets to private buyers, maize has remained unattractive as the GMB does not pay on time while private buyers offer even lower prices. This is in contrast to the alternative crops such as tobacco which has drawn more attention and has taken over some land that would otherwise be dedicated to maize. Such a shift has resulted in the decline in the farmers' contribution towards the strategic grain reserves, while keeping a certain hectarage for household consumption. Even urban dwellers have intensified maize production in open spaces within urban centres basically for household food security in what is referred to as peri-urban farming.

Besides these abiotic and socio-economic constraints, biotic factors have contributed towards a remarkable reduction of maize. Chief among these is the outbreaks of army worm (*Spodoptera exempta*). The pest attacks the crop at an early stage of development with damage that becomes difficult to correct as replanting will be too late for the crop to successfully give good yield.

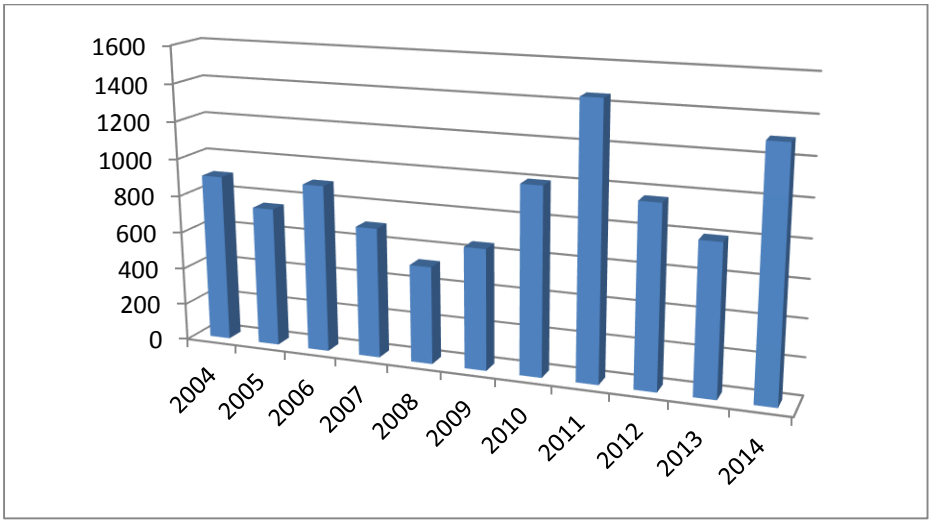


Figure 1.4 Maize production in Zimbabwe in the last 10 years in 1000 metric tonnes (USDA, 2014)

Leaf damaging diseases that include maize streak virus (MSV), leaf blight (*Exserohilum turcicum*) and grey leaf spot (*Cercospora zea-maydis*) cause severe reduction in yield. Their occurrence is sporadic and tends to occur in specific agro-ecological regions, particularly grey leaf spot (GLS). MSV is a country wide viral disease more prevalent where there is continuous cropping that allows the insect vector *Cicadulina mbila* to thrive throughout the year.

Some other diseases of major concern are those that affect the cobs and the grain itself. The most significant are *Sternocarpella maydis* and *Fusarium verticillioides* ear rots. The GMB used to grade maize delivered to its depots on the basis of, among other traits, infection with ear rots. With successive years of inadequate production levels, maize is being accepted under the same grade irrespective of its quality. While there is no loss to the farmer, the risk to the general population of consuming infected maize cannot be over emphasised. Fungal infection of grain results in production of metabolites such as fumonisins that are emitted by *Fusarium verticillioides,* aflatoxins from *Aspergillus flavus,* and zearalenone from *F. graminearum*. Fumonisins have been reported in Zimbabwe (Marasas, 1995; 2001; Gamanya and Sibanda, 2001). Mycotoxins zearalenone, moniliformin and fumonisin B_1 were detected in some samples collected from some GMB storage facilities in Zimbabwe (Mubatanhema *et al*., 1999). Aflatoxins as well as diplosporin and *Diplodia mycospora* were detected in some maize samples collected from the GMB that were visibly infected by some ear rot causing fungi (McFaden, 1985). In Zimbabwe, aflatoxins have been associated with groundnuts where a substantial amount is often observed. In some 56 groundnut samples collected and analysed by the government laboratory in 2013/14 season, aflatoxins were detected in 30 samples. However, no aflatoxins were detected in 24 maize grain samples analysed by the same government laboratory in the 2013/14 season (Nziramasanga, 2014). Out of the 47 samples of stock feeds, four samples had at least six parts per billion. This is not surprising as infected maize is normally put aside for livestock feed.

1.3 Mitigatory measures to address the above constraints

Besides addressing the socio-economic constraints, management can play an important role in addressing most of the problems affecting maize production.

In addition to implementation of good agronomic practices recommended after extensive research, one of the tools that have been implemented since the early 1930s has been breeding for superior germplasm that culminated into the release of SR52 in 1960 (Doswell *et al*., 1996). Such success has been attributed to the use of exotic germplasm that formed the basis of the current heterotic groups used in southern and eastern Africa, including in Zimbabwe (Ndhlela, 2012). Such heterotic groups have been maintained up to date. Such a success story has faced challenges of changing conditions with outbreaks of diseases that never occurred when various populations were made within these heterotic groups.

This necessitated further use of exotic germplasm, which, because of close association with international institutions such as the International Institute for Tropical Agriculture (IITA) and International Maize and Wheat improvement Centre (CIMMYT) that have breeding programmes in the region, made it easy to incorporate their germplasm into national and private breeding programmes. Such initiatives have succeeded to assist local private and public programmes with resistance to diseases such as GLS that was first observed in the USA in 1924, hence the germplasm from the Corn Belt inherently has resistance to this fungus (Ward *et al*., 1999). Besides that, introgression of exotic germplasm plays an important role in widening the genetic diversity as a decline in diversity in maize breeding programmes has been observed in several studies (Duvick *et al*. 2004). Use of exotic germplasm has been observed as one of the good strategies to increase diversity (Liu *et al*. 2003) thus reducing vulnerability associated with germplasm with a narrow genetic base.

The early breeding programmes managed to increase combining ability for yield, particularly for the high yielding potential areas with the best management practices including high fertilizer application, early planting, high precipitation as the target area which in Zimbabwe, for instance, was 1000-1800 m above sea level (MASL) characterised by high rainfall (Ndhlela, 2012). With the advent of global warming and changed socio-economic situation, such conditions no longer prevail, hence such germplasm does not perform as expected. New sources mainly from IITA and CIMMYT are being incorporated, which requires a better understanding on how such exotic material can be used in conjunction with existing germplasm that is adaptable to

the local conditions. Molecular tools become handy to address such issues with a possibility to predict heterosis.

With recurrent droughts, maize availability has been constrained such that every grain produced has been finding its way into the strategic reserves. This has led to acceptance of diseased kernels into the storage facilities which increase the chances of increased mycotoxin levels (McFaden, 1985). Most research on mycotoxins in Africa has centred on surveys to determine the extent of its prevalence and its effect on human and livestock (MacDonald and Chapman, 1997; Viljoen, 2003; USDA, 2006; Oyero and Oyefuro, 2010; Mukanga *et al*., 2010). The work done in Zimbabwe has predominately been on surveys (McFaden, 1985; Mubatanhema *et al*., 1999; Gamanya and Sibanda, 2001). The observations have mainly been based on visual morphological identification of such fungi before the advent of molecular sequencing technology that has the capacity to identify the gene sequence level that is not affected by the environment, which significantly compliments the morphological effort in distinguishing fungi. The level of resistance to mycotoxin within the existing varieties in southern Africa has not been quantified, although there is a limited level of various mycotoxins that may be allowed. The magnitude of contribution by these varieties in the accumulation of mycotoxins has not been quantified either.

Since a lot of work on maize improvement has been done within this region, it is important to understand how best the sources for the mycotoxin resistance can be utilised in maize breeding programmes. Understanding the resistance to mycotoxin inducing complexes of ear rots causing fungi, will contribute to the development of improved and healthier varieties that can improve the livelihood and health of the people in the region. The work done elsewhere on the type of gene action related to yield, and the inheritance of aflatoxins and the type of gene action were true for the material that were used. Since the results obtained from one geographical area mostly differ when the same trial is conducted elsewhere or when different genetic materials are used, it is appropriate to test the germplasm to be used within the local context (Falconer and Mackay, 1996).

The need to develop varieties that are agronomically superior, offer a higher nutritional value while safe-guarding the health of the consumers and their animals, becomes of paramount importance.

The work done in this study centred mainly on, a) evaluation of the magnitude of ear rots both under storage and field conditions and the likely effects that these ear rot causing fungi may cause to the consumers in the form of mycotoxins, b) usability of central and West African tropical lowland inbred lines in combination with southern African mid altitude inbred lines in both the lowland and the mid-altitude areas, c) inheritance of resistance to both ear rot causing fungi as well as the mycotoxin fumonisins which culminates in breeding for resistance to both, achieved through determination of combining ability for their scores as well as yield and other agronomic scores, d) molecular characterisation of the lines from both regions, including correlations between the genetic distance and heterosis.

It is with this background that this study on the gene action and heritability of resistance to the most commonly occurring ear rot causing fungi with a potential to produce mycotoxins, has been undertaken. Besides giving an in depth understanding on the type of gene action, this effort concurrently could assist in the development of varieties that can alleviate the health hazards associated with mycotoxins within the region.

1.4 Overall objective

The main objective of this study was to identify the most frequently occurring fungi in storage grain and to determine strategies of breeding towards its resistance and the metabolites that it exudes.

1.5 Specific objectives

1. To study the strains of fungi causing ear rotting and subsequently producing mycotoxins in Zimbabwe.
2. To conduct a phylogenetic study on the Zimbabwe *Fusarium verticillioides* isolates.
3. To determine combining ability and type of gene action controlling resistance to the most abundant mycotoxin producing fungi.

4. To determine the heterotic patterns of maize inbred lines from southern, central and western Africa.

5. To assess stability of agronomic performance of hybrids formed from lines developed in southern, central and western Africa.

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Chapter 2

Literature review

2.1 Mycotoxins

Mycotoxins are secondary metabolites produced by fungi on grain. Eight *Fusarium* spp. have been associated with fumonisin production but the most fumonisin producing species are *Fusarium verticillioides* (17 900 ug g^{-1}) and *F. proliferatum* (31 000 ug g^{-1}) of fumonisin analogue B_1 (Rheeder *et al.,* 2002). Among several mycotoxins produced by fungi, deoxynivalenol/nivalenol (DON), zearalenone, ochratoxin, aflatoxins and fumonisins have been the most reported and extensively studied (Pittet, 1998; Pitt, 2000). The gravity of the problems associated with mycotoxins has been expressed by the FAO (2004) with estimates that 25% to 50% of maize produced globally contain mycotoxins.The effect of fungi on crops such as maize is not only confined to yield reduction but also to build up of mycotoxins, such as fumonisins and aflatoxins. Fungal effects on ear rots have not been largely associated with yield loss (Mesterhazy *et al*., 2012) although Vigier *et al.* (1997) reported occasional high yield losses. The non-acceptance of grain that contains mycotoxins that exceed a certain limit has been an indirect yield loss associated with ear rots. The FAO singled out mycotoxin contamination caused by *Fusarium* spp as contributing 25% of the world food crops loss (Fareid, 2011). It has also been reported by Iheshiulor *et al*. (2011) that of the maize samples from the Philippines, Thailand and Indonesia, more than 50% contain FB_1 and FB_2, two of the 28 different analogues of fumonisins identified and found to be common in maize together with FB_3 (Rheeder *et al.,* 2002). The health of the human population in these and other countries is thus exposed to the hazards, especially the fumonisins, caused by often abundant infections from *F. verticillioides* that are found in maize, a staple food of the people mainly in sub-Saharan Africa (Gamanya and Sibanda, 2001; Fandohan *et al.,* 2003). *F. verticillioides* has been found to be the main mycotoxin causing fungi in South Africa, unlike *Aspergillus flavus* that is the main fungus associated with the problem of aflatoxin contamination in the Americas (Viljoen, 2003; Warburton *et al*., 2009). Mycotoxins may exist in the whole maize plant with variable distribution within different parts of the plant according to Schollenberger *et al*. (2012) who observed the occurrence of both A and B type trichothenecenes, some of which were siginificantly ($P<0.05$) distributed while some were not significantly ($P>0.05$) distributed.

2.2 Causal organisms for mycotoxins

Mycotoxins are caused by several ear rot causing organisms that include *Diplodia maydis* (Berk.) Sacc. [=*Stenocarpella maydis* (Berk.) Sutton], *F. verticillioides* that was recently renamed by Seifert *et al.* (2004) from [= *F. moniliforme* J. Sheld. (sexual stage: *G. moniliformis* Wineland)], and *F. subglutinans* (Wollenw and Reinking) Nelson *et al*. (1983) with *F. graminearum* Schwabe [teleomorph: *Gibberella zeae* (Schw.) Petch and *D. macrospora* Earle [= *S. macrospora* (Earle) Sutton] in maize found in southern Africa (Rheeder *et al*., 1994). Apart from these common genuses, mycotoxins are also caused by organisms in other genera that include the Aspergillus, Alternaria, and Penicillium.

2.2.1 Fusarium and fumonisins

2.2.1.1 *Fusarium* species

The mycotoxin complex has been associated with higher incidences of ear-rots, although ears without ear rot symptoms have also been found to sustain a substantial contamination by mycotoxins (Fandohan *et al.*, 2003; Morales-Rodriguez *et al*., 2007; Mukanga *et al*., 2010a). *Fusarium* spp. are regarded as field fungi since they have been reported to infect 50% of the maize kernels before harvesting (Fandohan *et al*., 2003). *F. verticillioides* exist in latent form inside the seed until the environmental conditions are favourable for development and growth. In the early 20th century, a possible cause of diseases affecting cattle, horses, pigs and chicken fed on mouldy maize in the USA was described as *F. moniliforme* which later became known as *F. verticillioides* (Kriek *et al*., 1981; Seifert *et al*., 2004). *F. verticillioides* has been described as an endophyte fungus with a tendency of having low visibility of symptoms on the kernel with a systemic tendency on the plant (Munkvold *et al.*, 1997a; b).

While there are several species of Fusarium that cause ear rots, their distribution may vary from one region to the other. In France, 12 species were identified by Folcher *et al.* (2009) with *F. verticillioides* and *F. proliferatum* being more prevalent in the south while in the north, *F. gramimearum* and *F. culmorum* were the most frequently occurring species. Similar results were observed in Hungary and the USA where, in the case of Hungary, the distribution is the same in drier years but differs in the wetter seasons (Mesterhazy *et al.*, 2012) suggesting that certain species occur under certain environmental conditions which tend to differ during the wetter season. Due to multiple

species occurrence, it is not surprising to observe different species of Fusarium on a single ear (Logrieco *et al*., 2002). However, the findings in other crops such as wheat (Snijders and van Eeuwijk, 1991; Mesterhazy, 1995) that the same QTLs were important for all the *Fusarium* species found, is consistent with almost the same QTLs being important in the resistance to various lines tested in different environments, also suggesting non-specificity in maize.

The ear rot causing fungi *F. verticillioides* is important, particularly for the high-lysine and tryptophan maize products, commonly known as quality protein maize (QPM) which shows a higher incidence and severity of Fusarium kernel rot than the normal endosperm maize. The microbial contamination of grain tends to take place during cultivation, processing, storage and distribution, although generally, higher incidences are observed in hot and humid tropical and sub-tropical regions of the world (Widstrom, 1996) particularly where there are poor handling and storage practices (Oyeru and Oyefolu, 2010). In Zambia, Mukanga *et al*. (2010a) observed that *F. verticillioides* was among the most important ear rot causing organisms with incidences ranging between 2 and 21%. *F. verticillioides* incidence of 2-7% was also observed among what was seemingly healthy maize grain. The fumonisin levels were proportionally higher than other mycotoxins in that study. In a similar survey conducted in Zimbabwe by Gamanya and Sibanda (2001), incidences for *F. moniliforme* of 0.5% to 21% were observed throughout three agro-ecological regions.

F. verticillioides is air borne such that poor cob or ear coverage by the husks accelerates the spread of the conidia spores (Clements *et al*., 2004). It has been established that the pathway for infection is through localised infection with a possibility of systemic infection through infected seeds or stalks (Desjardins and Plattner, 1998). *F. verticillioides* affect maize throughout the growth stages as it can cause infection through seed, silk or wounds, resulting in ear rotting or in some cases no symptoms, but leaving behind a metabolite that is injurious to humans and animals. Despite the presence of the fungus on the grain or seed the ear rot symptoms may not be exhibited and that lack of symptoms has often reduced attention to it as the magnitude of its effect is underestimated (Munkvold and Desjardins, 1997c; Fandohan *et al*., 2003). Local infection is either through conidia resting on the silk (Munkvold *et al*., 1997b), or through injury caused on the ear or kernel by insect pests (Farrar and Davis, 1999).

Besides the embryo, or cytoplasm or the endosperm that has been attributed to *opaque-2* maize (Pixley and Bjarnason, 1992) and shrunken endosperm (sh_2) maize (Styre and Cantliffe, 1984), the silk, the aleurone layer, the pericarp and the placento-chalazal region (the black layer) of corn kernels have also been associated with resistance to local infection by *F. verticillioides*.

2.2.1.2 Fumonisins

Fumonisins have been discovered recently with the B_1 having been discovered in 1988 (Gelderblom *et al*., 1988) and have been found to contaminate maize in the USA, south Americas, China, Europe and Africa (Fandahan *et al*., 2003). Rheeder *et al*. (2002) revealed that 28 analogues of fumonisins had been identified and of these, what are mainly found in maize are FB_1, FB_2 and FB_3 (Rheeder *et al.*, 2002). Although *F. verticillioides* and *F. proliferatum* have been identified as the main *Fusarium* species, causing fumonisins, *F. nygamai, F. anthophilum, F. dlamini, F. napiformi, F. thapsinum* and *F. globosum* have also been implicated as causal with lower effects (Fandahan *et al*., 2003).

Fumonisins have been reported to be mainly produced in maize, although lower levels have also been reported in sorghum (Shetty and Bhat, 1997; Gamanya and Sibanda, 2001; Leslie and Marasas, 2001), in rice (Abbas *et al*., 1998; Tanaka *et al*., 2007), in spices (Pittet, 1998; Fandohan *et al*., 2003), in grapes (Somma *et al*., 2012) and in raisins (Mogensen *et al*., 2010).

Just as with other mycotoxins, fumonisins are detrimental to the well-being of humans and animals as they have been identified as agents for esophageal cancer in humans in South Africa, North East Italy, Iran and central China (Doko *et al*., 1995; Kimanya *et al*., 2009; Suleiman *et al*., 2013). Fumonisins have also been implicated in neural tube birth defects in humans with early reports on effects on new born babies in the Texas-Mexico border area (Stack, 1998; Suleiman *et al*., 2013), and in mice (Rheeder *et al.*, 1993; Clements *et al*., 2004; Robertson *et al*., 2006; Voss *et al*., 2006), equine leucoencephalomalacia in horses (Kellerman *et al*., 1990; Pitt, 2000; Williams and Windham, 2009), a serious disease that affects the brains of horses, donkeys, mules, and rabbits. Fumonisins have also been associated with pulmonary oedema syndrome

in pigs (Harrison *et al*., 1990; Pitt, 2000; Robertson *et al*., 2006; Williams and Windham, 2009) and hepatocarcinogenesis in rats (Gelderblom *et al*., 2001). In humans, fumonisins have also been causing stunted growth in children, an observation made in Tanzania (Kimanya *et al*., 2010).

In a short term carcinogenetic assay developed after studying fusarin C in rats, the cultured *F. verticillioides* MRC 826 caused development of lesions within the liver, which marks initiation of cancer development. It was therefore deduced that fumonisins produced by *F. verticillioides* interfere with biosynthesis of sphingolipids which essentially causes disruption of lipid metabolism in humans (Marasas, 2001).

In order to minimise the effects to human beings and their livestock, various institutions globally have put in place legislations and recommendations for maximum tolerable levels (MTL) (Mesterhazy *et al*., 2012). The US Food and Drug Administration (FDA) has set a maximum target of 4 ug g^{-1} in human foods and does not allow interstate commerce of feed grain containing more than 20 ug g^{-1} of aflatoxins (Park and Liang, 1993; Marasas, 2001; Williams and Windham, 2009; Clements *et al*., 2004). Switzerland does not allow more than 1 ug g^{-1} in dry maize products for human consumption. The United Nations agencies, the Food and Agricultural Organization (FAO) and the World Health Organisation (WHO) in 2002 jointly put a limit of provisional maximum tolerable daily intake (PMTDI) of 2 ug g^{-1} for B_1, B_2 and B_3 either individually or in combination (WHO, 2002).

Limits have also been set for animal feeds which are slightly higher than that of human beings and vary according to the species of animals. Viljoen (2003) recommended a maximum tolerance level of 4 µg g^{-1} for whole unclean maize, 2 µg g^{-1} for dry-milled maize products with fat content of ≥3.0% on a dry weight basis such as in sifted and unsifted maize meal, and 1µg g^{-1} with fat content of <3.0 on a dry weight basis such as in grits. These limits set as standards are too high to achieve and lead to high economic losses by farmers that have contaminated grain. Fumonisin has been found to be phytotoxic to emerging seedlings in maize (Scott, 1993; Lamprecht *et al*., 1994; Doehlet, 1994; Fandohan *et al*., 2003; Wicklow *et al*., 2011).

2.2.1.3 Aspergillus and aflatoxins

The fungus *Aspergillus flavus* (Link), like *A. parasiticus* (Speare) has been observed in South Africa but has been reported not to cause any ear-rotting in the country (Rheeder *et al*., 1994). *A. flavus* produces aflatoxins elsewhere (Busboom and White, 2004). In the southern maize growing regions of the USA, *A. flavus* causes extensive ear rots and accumulation of aflatoxins, particularly aflatoxin B_1 that is regarded as the most carcinogenic (Wild and Turner, 2002; Busboom and White, 2004; Brooks *et al*., 2005). The fifth most common cancer worldwide, herpatocellular carcinoma is reported to be largely caused by the consumption of aflatoxins (Wild and Turner, 2002). In some regions in the USA, the highest incidences are recorded in years when the rainfall is low, humidity is high and temperatures are high (36-38^{o}C). Apparently these are characteristics of agro-climatic regions where most of the poor farmers that produce and rely on maize as a staple food reside. Such areas are increasing in size with the advent of global warming. In Zambia, *A. flavus* was among the most prevalent ear rot causing fungi as 3-18% was recovered from seemingly healthy grain in a study conducted by Mukanga *et al*. (2010a). Aflatoxin can be indirectly ingested by humans as they were detected under ultra violet (UV) at 360 nm and subsequently extracted from animal products by Oyeru and Oyefolu (2010) using a thin layer chromatographic method. The actual concentration was further derived by using the absorbance values. The hydroxylated homologue of aflatoxin B_1, called M_1 may be found in milk or milk products from animals that consumed infected feed (Busboom and White 2004). However, the observed results in the meat products could be coming from the stalk infection that has been associated with incidences of mycotoxins as well (Mesterhazy *et al*., 2012).

2.2.2 Diplodia

Diplodia maydis (Berk.) Sacc. [=*Stenocarpella maydis* Berk.), is associated with ear-rots in maize, particularly in sub-Saharan Africa and world-wide, including Argentina and the USA (Wicklow *et al*., 2011). In a study conducted by Mukanga *et al.* (2010a), it was found to be one of the dominant causes of ear-rots in Zambia, with incidences reaching 37%. As alluded to earlier, *S. maydis* is rated among the major ear rot causing fungi in sub-Saharan Africa.

S. maydis infected maize grazed by cattle in southern Africa and Argentina, have been reported to have been affected by a neuromycotoxicosis. The *S. maydis* metabolites in the form of ethyl acetate extracts obtained from solid-substrate fermentations derived from numerous isolates in the USA, have been found to have phytotoxic, antifungal, and anti-insect activity in significant levels (Wicklow *et al*., 2011).

S. maydis has been known to exude metabolites such as diplodiatoxin, (all-E)-trideca-4,6,10,12-tetraene-2,8-diol and chaetoglobosins K and L.

2.2.3 Interaction between *Fusarium verticillioides* and fumonisins

A group of mycotoxins that causes cancer in rats, called fumonisins, was isolated from cultures of *F. verticillioides* (Rheeder *et al*., 1994). The fumonisin incidences tend to be higher in situations where there is moderate to higher levels of *F. verticillioides* ear rot severity (Gamanya and Sibanda, 2001; Mesterhazy *et al*., 2012). Fumonisin can also be found amongst healthy plants where the incidence is low and not visible (Munkvold, 1997a; b; Fandohan *et al*., 2003; Robertson *et al*., 2006; Reid *et al*. 2009). Menkir *et al*. (2008) also noted the existence of endophytic kernel attack by *Fusarium* species and *A. flavus*, and that significant amounts of fumonisin can be produced in symptomless plants or slightly rotten grain, a phenomenon that has been attributed to low phenotypic correlations between the Fusarium ear rot and fumonisin concentration.

Contrary to that association of infection and presence of mycotoxins, Brown *et al*. (2001) observed resistance to mycotoxins from grain that came from heavily infected plants. This was corroborated by Garcia *et al*. (2009) who concluded that the development of fungi does not necessarily suggest a proportionate development of the mycotoxin. This could be attributed to the fact that the conditions favourable for fungal growth may not be conducive for the synthesis of the mycotoxins (Mesterhazy *et al*., 2012). Despite that, more observations have associated presence of the fungi and the incidence of mycotoxins.

2.3 Pre-disposing factors

The fungi that cause mycotoxin find entry through various channels. The fungus could be in the soil or seed borne and may infect the new crop through a systemic movement. This may lead to the whole crop being a carrier, with or without symptoms showing.

2.3.1 Insects

Insects can be used as damage inducers, thus creating an entry point to mycotoxin causing fungi. Such insects include the lepidopteran stem and ear borers, *Ostrinia nubilalis, Sesamia calamistis, Eldana saccharina, Musidia negrivenela* and *Buseola fusca* (Cardwell *et al*., 2000; Ako *et al*., 2003; Fandohan *et al*., 2003). Such borers may result in wounds being created which will form the entry point for fungi and some have been associated with being carriers themselves. It was reported by Schulthess *et al*. (2002) that a positive correlation between *F. verticillioides* and *Eldana saccharina, Cryptophlebia leucotreta, Missidia negrivenella* and *Sessamia calamistis* existed. Inoculated maize resulted in increased egg laying, fecundity and survival of *Eldana saccharina* (Ako *et al*., 2003). Schulthess *et al*. (2002) further hypothesised that the presence of *F. verticillioides* attracts insects and further extrapolated that keeping the field free from fungi is an indirect way of keeping the crop free from insects. On the other hand, Riley and Norrid (1999) concluded that when the field is free from insects, the fungal load is drastically reduced.

It is not only the lepidopteras that damage or cause injury to the ear that are positively linked to an increase in fungal infection, but beetles too, such as the nitidulid, cucurlionid and *silvanid* spp. (Cardwell *et al*., 2000) which are equally positively associated with *F. verticillioides* infection.

2.3.2 Climatic conditions

The occurrence of fumonisins has been associated with weather conditions such that higher incidences occur during hot and dry conditions (Marasas, 2001). It has been observed that the occurrence is not consistent in one area of production, or in consecutive seasons, even when the same cultivar is used, something that has been attributed to variations in the weather conditions from one season to another (Hennigen *et al*., 2000). The stresses induced by unusual dry spells towards harvesting and just prior to pollination, have been associated with fumonisin production (Fandohan *et al*.,

2003). As for aflatoxin accumulation, drought tolerant maize varieties were associated with the production of significantly less aflatoxins when the crop is in a drought stricken field when compared with aflatoxin resistant cultivars. This therefore suggests a possible correlation of drought tolerance and aflatoxin resistance in maize (Brown *et al*., 2009).

As with most other stored products, maize is hygroscopic, meaning that it can absorb or lose moisture or humidity within the surrounding environment until it is in equilibrium with the ambient moisture content that leads to rapid deterioration in storage (Devereau *et al*., 2002).

Ambient temperature and moisture in storage has been associated with infection and development of *F. verticillioides*. Temperatures of between 18^{o}C and 25^{o}C have been associated with rapid development of *F. verticillioides* with a temperature of 15^{o}C having a lower effect on growth (Scott, 1993; Marin *et al*., 1999; Velluti *et al*., 2000).

2.3.3 Processing

When maize undergoes processing either through mechanical harvesting or mechanical shellers, cracks may develop which can be the entry points for the infecting fungi (Dharmaputra *et al*., 1996; Fandohan *et al*., 2003).

2.3.4 Use of susceptible cultivars

Most of the cultivars on the market do not have specific resistance to mycotoxins such as fumonisins and aflatoxins (Brooks *et al*., 2005) as breeding for resistance is a recent development. It is recent that sources of resistance have been identified and several breeding programmes are using them to introgress in the local germplasm (Brooks *et al*., 2005; Menkir *et al*., 2008; Warburton *et al*., 2009; Williams and Windham, 2009). In Africa for instance, the International Institute for Tropical Agriculture (IITA) has within their gene bank a substantial amount of lines that have a high level of resistance (Menkir *et al*., 2008).

2.4 Control

Although the most desirable and effective control of mycotoxin contamination and their causal organisms is through developing genetically resistant maize genotypes, the ultimate success in terms of management in the field and storage require host plant

resistance complimented by other management strategies that include appropriate nutrient availability such as nitrogen fertilization, correct plant population, insect management, and ensuring that enough water is available during active growth of the crop by irrigation as a drought mitigatory strategy (Tubajika and Damann, 2001).

2.4.1 Cultural

Fumonisin accumulation can be reduced or prevented by harvesting when moisture level is low, drying immediately after harvesting and managing storage facilities in such a way that the grain is kept moisture free, since some mycotoxin organisms such as *F. verticillioides* need a minimum of 18% moisture content to develop (Vincelli and Parker, 2002). It has been reported by Widstrom (1996) that various processing methods such as roasting, boiling, frying, baking or fermentation may not eliminate the aflatoxin and fumonisin effectively. However, since fumonisins are mainly concentrated on the pericarp and the germ, dehulling may significantly reduce contamination (Fandohan *et al*., 2006).

Implementation of a rotation programme that does not allow growing a host plant after another host plant of the mycotoxin causing fungi has proven to significantly reduce the infection rate. This includes ensuring that weeds which can host the fungi are controlled effectively (Fandohan *et al*., 2003).

2.4.2 Use of resistant cultivars

2.4.2.1 Breeding for resistance to mycotoxin causing fungi

Commercially available maize cultivars do not have specific resistance to mycotoxins such as fumonisins and aflatoxins (Brooks *et al*., 2005). Despite that, sources of resistance have been identified (Brooks *et al*., 2005; Menkir *et al*., 2008; Warburton *et al*., 2009; Williams and Windham, 2009).

Besides good cultural practices, use of *Aspergillus* and *Fusarium* species resistant maize in combination with cultural practices, can effectively reduce contamination of mycotoxin, although Robertson *et al*. (2006) ruled out any success in breeding for resistance to either causal fungi *Fusarium* species or fumonisin production itself. Breeding for resistant maize cultivars is the most effective as it has less environmental

effects while it can be applied in all socio-economic environments (Clements *et al.*, 2004; Warburton *et al.*, 2009; Menkir *et al.*, 2008). Breeding for resistance is therefore the most effective and economic way of managing the effects caused by mycotoxins (Busboom and White, 2004; Clements *et al.*, 2004). Resistance to *A. flavus* and *F. verticillioides* has been found, but most of the sources of resistance are poorly adapted and are agronomically poor (Menkir *et al*., 2008; Warburton *et al*., 2009). It was also observed by Warburton *et al*. (2009) that most available products on the market do not carry resistance to aflatoxins nor to fumonisins (Munkvold, 2003; Reinprecht *et al*., 2008). This is not surprising, as most of the lines in use are derivatives of B73 types, itself derived from the Reid Yellow Dent that is associated with a high susceptibility level. Although some resistance sources such as Mp717 (Warburton *et al*., 2009), Oh516 (Busboom and White, 2004) and Tex6 (Hamblin and White, 1999; Busboom and White, 2004) have been identified in the USA to have low susceptibility levels. Robertson *et al.* (2006) observed low accumulation in inbreds GE440 and NC3100 and in three hybrids out of 14 commercial hybrids widely grown in North Carolina in the USA, where *Fusarium* spp. infection was also low. It was found that there was high positive correlation between fumonisin and aflatoxin resistance (Robertson-Hoyt *et al*., 2007). They also found two QTLs that were associated with both mycotoxins. On the other hand, Williams and Windham (2009) observed that the GCA effects for maize inbred GA209 were highly significant and positive for aflatoxin accumulation in one study and significant and negative for fumonisin accumulation in another study, indicating that the genes for resistance could be different. Other lines that have been confirmed to carry resistance include the inbreds Mp715 and Mp717 that have resistance to both pathogens and mycotoxins (Williams and Windham, 2009). Selection for resistance to mycotoxins has centred on visual assessment of ears harvested with less rots and tight husk cover, although selection of those ears with less mycotoxins is an indirect way of selecting for plants with less ear rot infection.

Resistance breeding starts with the screening process to determine which lines are resistant and this resistance needs to be reliable and repeatable. Since the genotype by environment interaction (GxE) has been found to be high for the ear rot causing fungi, the need to use artificial inoculation becomes of paramount importance. Inoculation artificially ensures provision of the required dosage of the inoculum to the target plant part at the correct stage of plant development (Bolduan *et al*., 2009). Brown *et al*. (1995)

developed a rapid laboratory method for infecting and screening for resistance to aflatoxins which results are highly correlated to the field screening.

2.4.2.2 Breeding for resistance to insects positively correlated with mycotoxin causing fungi

The breeding effort has also been directed toward the development of maize genotypes with resistance to insects that can tunnel through the ear, thus creating an entry point for the fungi to enter. Insects that bore cobs such as *Ostrinia lubilalis, Diatraea grandiosella, Diabrotica virgifela, Helicoverpa zeae, Frankliniella* spp. have been associated with an increase in Fusarium infection (Archer *et al*., 2001). In South Africa, *Busseola fusca* and in some parts of Africa, *Chilo partellus* are some of the stem boring pests that cause the injury that subsequently act as entry points for the mycotoxin causing fungi. Use of transgenics in the management of such infection has proven to be effective, particularly for deoxynivalenol (DON), one of the three metabolites produced by *F. graminearum* that also include nivalenol (NIV) and zearalenone (ZEA) and other mycotoxins (Munkvold *et al*., 1997a; Munkvold, 2003). Ncube and Flett (2013) observed that there is a positive interaction between *B. fusca* and *F. verticillioides* that leads to an increase in the infection by *F. verticillioides*. Use of the transgenic Bt maize that carries the *Bt* gene, has proven to significantly reduce both the lepidopteran insects, the fungal infection and subsequently the accumulation of mycotoxins such as fumonisins (Ncube and Flett, 2013).

2.4.3 Fungicide use

Although some progress has been observed in the management of the ear rot causing fungi by use of fungicides, its use in sub-Sahara Africa is minimal where maize is used as food. This could partly be due to the additional expense as well as environmental effects that may be caused. Loffler *et al*. (2010) reported recent success in the use of fungicides in Europe while a reduction in mycoflora of 90% has been reported by Folcher *et al.* (2009).

2.5 Gene action and heritability

2.5.1 Gene action

Resistance to Fusarium ear rot causing species is polygenic (Perez-Brito *et al*., 2001) which confirmed earlier studies by Boling and Grogran (1965) and Ullstrup (1977).

Resistance to aflatoxin production has also been found to be quantitatively inherited (Walker and White, 2001; Busboom and White, 2004; Warburton *et al*., 2009). Busboom and White (2004) found that both additive and dominant gene effects played a major role in conferring resistance to *A. flavus*, which was verified by studies that identified at least four associated QTLs on chromosomes 1, 2, 4 and 5. The same chromosomes were identified in populations involving Tex6 and Mp313E. Dominant gene effects were responsible for about 50% of resistance to aflatoxins whereas Clements *et al*. (2004) reported complete dominance or over dominance of resistance alleles to fumonisin concentration.

Mukanga *et al.* (2010b) observed both GCA and SCA being important for ear rot causing organisms *A. flavus, F. verticillioides* and *S. maydis* while working with full-sib families. Williams and Windham (2009), however found only GCA to be significant for fumonisins. Additive gene effects have been reported to play a major role in conferring resistance, although some studies (Campbell *et al.,* 1997; Campbell and White 1995; Maupin *et al*. 2003; Clements *et al.* 2004; Busboom and White 2004; Mukanga *et al*., 2010b) reported dominance playing a major role also. Most studies have been done using a diallel mating scheme (Darrah *et al*., 1987; Gardener *et al*., 1987; Zuber *et al*., 1978; Gorman *et al*., 1992; Williams and Windham, 2009; Mukanga *et al*., 2010b). GCA had a greater effect than SCA on resistance to aflatoxin accumulation in grain (Zuber *et al*., 1978; Darrah *et al*., 1987). These were observed in some trials, as different results are obtained at different environments and when different genotypes are used (Falconer and Mackay, 1996; Busboom and White, 2004). Maternal effects were reported to be important in conferring resistance to the complex of ear rots in Southern Africa (Mukanga *et al*., 2010b). In a study conducted by Desjardins *et al*. (1992), a single gene or a group of closely linked genes, was found to be responsible for production of fumonisins, particularly fumonisin B_1. However, Widstrom *et al*. (1987) identified 2-5 QTLs that were additive in the four environments where they were phenotyped, with two QTLs being significant in at least three environments.

The mechanism of resistance to ear rot and production of aflatoxins has been attributed to the production of proteins that inhibit production of aflatoxins. Production of a high level of B-1-3-glucanase in kernels, was observed in the resistant Tex6 in culture as

opposed to what was observed in the susceptible maize inbred B73 (Hamblin and White, 1999). They also observed that the cross Mo17 x Tex6 exhibited higher dominance effects for susceptibility, something that was not observed in the cross of B73 x Tex6. The pericarp and the super pericarb structures have been associated with resistance or susceptibility to aflatoxin contamination (Brown *et al*., 1995). The wax and the cutin content have been suggested as responsible for the pericarp resistance while the resistance within the super pericarp could be due to internal pericarp tissues.

2.5.2 Heritability

Heritability of resistance to *F. verticillioides* ear rot has been reported to be moderate-to-low while the G x E interaction has been observed to be high, which complicates and retards breeding progress in the field (Warburton *et al*., 2009; Mukanga, *et al*., 2010b). Estimates of broad sense heritability for aflatoxin accumulation in a study by Brooks *et al*. (2005) showed a range of 0.27 and 0.42, suggesting a low heritability. In a study involving resistant maize inbred (Tex6) and two susceptible maize lines (Mo17 and B73), the broad sense heritability obtained from generation mean analysis for ear rot and aflatoxin production were 58% and 63% for crosses Mo17 x Tex6 and 66% and 73% for cross B73 x Tex6, respectively. The narrow sense heritability for ear rot and aflatoxin production for cross B73 x Tex6 was 39% and 43% (Hamblin and White, 1999). For resistance to *A. flavus*, Busboom and White (2004) recorded heritability as low as 11.3% among BCP_1S_1 families. Such a low heritability can be attributed to a low genetic variance within the population, which makes it imperative to use marker assisted selection for progress to be made as phenotypic selection will not be appropriate. Perez-Brito *et al*. (2001) observed low heritability in a study involving two highland maize populations in Mexico.

2.6 Mycological analysis

Mycological analysis for ear rot causing fungi has been done in different ways depending on the resources available. Although morphological analysis has been extensively and successfully used, recent advances in molecular tools have allowed more precise determination of fungal isolates. Moody and Taylor (1990) reported that it takes 2 days to weeks to distinguish the *A. flavus* isolates from the *A. parasiticus* using the degree of conidial roughening and from *A. nomius* using the diameters of colonies grown at elevated temperature as a basis.

2.6.1 Morphological methods for distinguishing fungal species

In a study on enumeration of fungi in barley by Rabie *et al*. (1997), the PDA, MSA and PCNB using unsoaked grain and disinfected by ethanol was seen to be simple and effective for examination of samples. The morphological tools start with surface sterilisation with either 3.5% sodium hypochloride (Rheeder *et al*.,1994; Rabie *et al*. 1997), or 0.16% NaOCl (Schaafsma *et al.* 2008), or 80% (v/v) ethanol in water (Rabie *et al*., 1997. The plates with the media and samples are incubated in environments which vary from dark (Rheeder *et al*.,1994), 12:12 hours light and darkness (Desjardins *et al*., 1992; Schaafsma *et al.*, 2008), or with ultra violet (UV) light (Gamanya and Sibanda, 2001). The developing fungi are then identified using a microscope and description that is achieved with the help of relevant books. The identification is centred on careful examination of the presence of macro and microconidia, spore shape, mono and/or polyphialides, phialidic development of spores, survival structures, sexual and asexual fruiting structures, pigmentation, exudate formation, the magnitude of mycelial growth, the characteristics of conidiogenous cells, as well as the presence or absence of chlamydospores (Lodolo *et al*., 1992),

2.6.2 Molecular tools in distinguishing fungal species

The inherent limitations or questionable data from the traditional ways of distinguishing species inhibits drawing of conclusive taxonomic verification of species, particularly of the Fusarium genus such as *F. verticillioides, F. nygamai and F. napiforme*. Neither pathogenicity of isolates or sexual compatibility will be adequate to distinguish the isolates. Failure to sporulate inhibits use of molecular markers as a tool for identification of morphological characteristics (Roux *et al*., 2001). Some fungal species such as *F. subglutinans* strains that are found in various hosts, tend to be indistinguishable when morphological characters are used but have been distinguished while using the β-tubulin gene (Steenkamp *et al*., 2000). Molecular markers such as restriction fragment length polymorphisms (RFLP) of the mtDNA and random amplified polymorphic DNA (RAPD) differentiated pine isolates of *F. subglutinans* from those of non-pine isolates (Correl *et al*., 1992; Viljoen *et al*., 1997).

RFLP of ribosomal deoxyribonucleic acid (rDNA), have been used to distinguish *Aspergillus* species (Moody and Tayler, 1990) and to separate *F. rodolens* from *F. oxysporum*. It was, however, observed that the rRNA internal transcribed spacer (ITS)

in combination with the RFLP (ITS-RFLP) technique could not distinguish *F. redolens* from *F. hostae*, its close relative (Baayen *et al*., 2001). Besides that, the ITS-RFLP is not only technically complicated, but expensive too (Bogale, 2007). Also RFLP profiles of histone *H3* gene has been used in a rapid and reliable way to distinguish *F. circinatum* from other *Fusarium* species (Steenkamp *et al.*, 1999; Jacobs *et al*., 2006). In an effort to verify the taxonomy of *Fusarium* species, Lodolo *et al*. (1992) successfully used RFLP to distinguish *F. moniliforme*, *F. nygamai* and *F. napiforme*. Bogale *et al*. (2006) used the Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeats (SSR) and DNA sequence analysis to study 32 strains of *F. oxysporum* from Ethiopia. These three methods all managed to classify the strains into the three lineages that were consistent with the known clades of *F. oxysporum*. Comparison of isolates of *Acacia grandis* and *Eucalyptus grandis* with *F. graminearum* isolates where β-tubulin and histone *H3* gene sequencing was used by Roux *et al*. (2001) to separate the isolates into clear phylogenetic and morphological species. Molecular tools are also being used as fast diagnostic tools for plant diseases (McCartney *et al.*, 2003).

2.7 Molecular characterization of inbred lines

Germplasm that is characteristically superior in terms of reaction to biotic factors have been associated with inferior agronomic performance. Exceptions have been observed such as the study of Busboom and White (2004) who described inbred line Oh516 as a source of resistance to *A. flavus* and aflatoxins. This line and Mp717 exhibited superior agronomic performance as compared with other sources such as Tex6 and MP313E that had extremely poor agronomic performance (Hamblin and White, 1999; Busboom and White 2004; Warburton *et al*., 2009). It is therefore important to identify the sources of resistance that can be crossed to those lines that are agronomically superior in order to develop lines that carry both resistance and superior agronomic performance. In maize breeding, such improvements are confined within heterotic groups. This necessitates classification of inbred lines into heterotic groups so that lines with resistance are crossed with lines with superior agronomic characteristics that belong to the same heterotic group.

In a breeding programme, it is always desirable to have a high level of genetic diversity and to know the level within the available gene pool. This is a starting point to ensure

attainment of higher genetic gain from selection. Use of diverse germplasm safeguards any breeding programme from genetic vulnerability in the event of a sudden outbreak of a new strain of disease or pest or sudden changes in climatic conditions. A low level of diversity may result in rendering the whole population base susceptible to an outbreak of either a biotic or abiotic stress. An increase in diversity in a breeding programme can best be achieved through introduction of new variation from a source that is diverse.

In maize breeding for example, similar background germplasm has been maintained within certain known heterotic groups crossed to another group to obtain high levels of heterosis. It is with this background that determination of level of diversity has become an important component of the maize breeding procedure. Traditionally, this has been achieved through test crossing, which is currently being complemented by molecular tools. Determination of genetic relatedness will also facilitate determination of potential to exhibit better agronomic performance as it has been hypothesised that the more diverse the lines are, the more is the heterosis in general.

Genetic diversity assessment is now being done using molecular tools. SSR markers have been extensively used in maize to determine the level of diversity (Senior *et al*., 1998; Warburton *et al*., 2002; Prasanna *et al*., 2002; Reif *et al*., 2003). The SSR markers have been the most preferred due to the inherent high level of polymorphism which offers good prospects for large-scale fingerprinting of maize genotypes. The genetic distance estimates measure genetic difference at either the sequence or allelic frequency level which is calculated between individuals, populations or species (Mohammadi and Prasanna, 2003). Genetic distance or similarity can be calculated from binary data in different ways, which include (i) Nei and Li's (Nei and Li, 1979) coefficient, (ii) Jaccard's (Jaccard, 1901) coefficient (iii) simple matching coefficient, and (iv) Modified Rogers' distance

Dudley *et al.* (1991) while using temperate maize germplasm, found significant but low correlation of Modified Rogers' distance (MRD), with SCA for yield. Betran *et al.* (2003) also recorded significant and positive correlation between SCA and mid parent heterosis (r = 0.47), and SCA and high-parent (r = 0.31) heterosis while Reif *et al.* (2003) found a significant (p<0.01) correlation between MRD^2 and pelmitic mid parent

heterosis (PMPH) of 0.63. The results reported in other crops have varied with Cheres *et al*. (2000) reporting significant correlation between the hybrid performance and genetic distance (GD) in sunflower (*Helianthus annus* L.) and no correlation between diversity values and hybrid performance in wheat (Martin et al., 1995)

Heterosis is sometime observed among lines of the same heterotic group which explains why significant GD and SCA can occur, but with a general weak correlation ($r<0.5$). This therefore suggests that determination of GD cannot solely replace evaluation of hybrids for SCA. Such distortions are due to the fact that several factors contribute to heterosis and these include dominance, over-dominance, biochemical, and molecular factors.

With new developments in molecular technology, single nucleotide polymorphism (SNP) markers are becoming more popular due to several factors that are attributed to them. Genotyping using SNP markers is becoming more cost effective per data point besides having a high genomic abundance. Use of SNPs is more preferred because of a high degree of precision during genotyping coupled with its locus-specificity and codominance characteristic in addition to its high potential for automation that results in high throughput (Rafalski, 2002; Schlotterer *et al*., 2004; Chagne *et al*., 2007). Besides the wide application in genetic diversity studies, SNP technology has found wide use in other molecular applications, which include various mapping studies (Semagn *et al*., 2012).

There are various SNP platforms which offer high throughput. These include Illumina which is a chip based technology with various multiplexing possibilities, and the KASpar. Uniplex platforms are also available which are ideal where a few SNPs are needed over a larger number of samples that are normally involved in mapping projects, marker assisted recurrent selection and backcrossing and in quality control. In uniplex however, it is important to identify and use the best SNPs so that a good level of discrimination can be achieved (Low *et al*., 2006).

2.8 Genotype by environment interaction

In maize breeding, the differential performance of genotypes from one environment to the other often complicates the process of selection. While the role of crop improvement through breeding is to accumulate the most favourable alleles for traits of interest, the final product needs to withstand the vagaries of different environments at micro or macro level. The analysis of various genotypes in diverse environments, allows classification of genotypes for specific or a wide range of environments. After the realisation of its confounding effects, much work has been devoted towards its quantification and understanding (Lin *et al*., 1986; Yan and Kang, 2003).

The current focus is on the matching between genotypes and environments often achieved through biplot analysis. This offers a two way graphical display of the row and column factors and their interactions that can be simultaneously analysed. In maize breeding, GGE biplot analysis is often being used at the IITA (Badu-Apraku *et al*., 2011a; b).

2.9 Conclusions

The literature reviewed reveals preceded risk as a result of non-availability of cultivars of maize that are resistant to mycotoxins. Various surveys conducted provide adequate information indicative of the most occurring fungi and in some cases, the mycotoxins associated with them. The risk level is clearly elaborated from various studies conducted. WHO regards aflatoxins as a class-1 carcinogen (Martinez *et al*., 2011) and B_1 as class-2. The revealed maginude of the problems associated with mycotoxins promulgated into both FAO and the WHO setting up the maximum allowable levels of various mycotoxins for various grains. Despite that, many nations, particularly in sub-Saharan Africa like Zimbabwe, do not consider when accepting grain delivered and supplied to food processors, despite the availability of legislation stating the limits (FAO, 2004). As a result, surveys have revealed presence of mycotoxins in stored grain, both in the farm storages and in national strategic reserve storage facilities. In order to limit occurrence of both the mycotoxins and the causal fungi, studies have been conducted to elucidate type of gene action responsible for resistance to the mycotoxins and their causal fungi. The most common approach used being to breed for resistance to the causal fungi. Most breeding programs select maize lines or final products based on the incidences for ear rots based on a subjective score or by counting ears that have

no visible symptoms of the infection by various fungi. This does not take into consideration the possibility that at one stage, the fungus occurred and left these toxic metabolites that cannot be seen by a naked eye. This is the reason why mycotoxins are sometimes observed in asymptomatic samples. The occurrence of the mycotoxin causing fungi such as *F. verticillioides* is variable, therefore to ensure successful screening there is need to artificially inoculate. This allows for breeding to take place as there is evidence of availability of sources of resistance which may be of temperate origin but can be introgressed into the local germplasm to transfer the resistance genes. Inheritance of resistance has been found to be both additive and non-additive as some studies found GCA and SCA being significant, hence exploitation of both additive gene action by use of resistant lines only and non-additive gene effects by selecting specific combiners will go a long way in breeding for resistance to both the fungi and various mycotoxins. The general conclusions by various publications that the QTLs responsible for resistance of one species of fungi within either the same genus such as the Fusarium or across other genuses such as the Aspergillus are the same, simplify the effort in developing resistant genotypes that cut across regions. This is more so where the various sources of resistance from one region such as central and West Africa are used in conjunction with lines from southern Africa where mycotoxin causing fungi are different. The possibility of using such germplasm is further made possible with genetic diversity studies that culminate in the identification of lines that belong to the same grouping which is further confirmed by the line x tester analysis in the form of the North Carolina Design II. Apart from classification of the germplasm, such a mating design further elucidates the gene action pertaining to the germplasm in use as the results obtained elsewhere with different germplasm may differ when another set of germplasm is used.

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Chapter 3

Diversity of fungal isolates in storage facilities in Zimbabwe

Abstract

Maize production is affected by both biotic and abiotic stresses. Some of the biotic factors continue to affect maize even during storage where they produce some toxic metabolites with high health risks to end-users. The need to identify the extent of fungal load in storage cannot be over emphasized as the results may give an indication of the historical handling of the maize and the extent to which consumers may be exposed to mycotoxins associated with some of the fungi. The objective of this study was to identify and quantify the viable fungi that are associated with maize in storage facilities in Zimbabwe and to determine the associated level of mycotoxin. Stratified samples of maize were drawn from 23 grain storage facilities in maize producing areas in Zimbabwe in 2011. Six of the locations had grain from both the current and the previous year, resulting in 29 collected samples. The samples were plated on Potato Dextrose Agar (PDA) and Malt Salt Agar (MSA) media where viable fungi were allowed to sporulate. Due to the presence of cryptic species in Fusarium that can only be identified based on DNA sequence comparisons, the Translation Elongation Factor 1-α gene sequences of the *Fusarium* species in the maize were compared to those of known species. The samples were further evaluated for the presence of ear rot and fumonisin contamination. In total, 33 fungal species were identified from the maize using morphological characteristics with some only observed on PDA, some only on MSA, and others in both media. *Fusarium verticillioides* was the most abundant field fungus while *Eurotium repens* had the highest incidence among the storage fungi. The two fungi could be identified using the two media, although *F. verticillioides* had a preference to sporulate on PDA, whereas *E. repens* preferred MSA. The field and storage fungi were distinguished using the two different media. The occurrence of *Aspergillus flavus* seemed to correlate with a reduced incidence level of *F. verticillioides*, and was only observed in the samples from the 2010 season. *Fusarium verticillioides* did not show a specific pattern in terms of geographical distribution, whereas *A. flavus* was more prevalent in the northern parts of Zimbabwe. The morphologically identified *F. verticillioides* isolates were confirmed by using DNA

sequence comparisons with a few exceptional cases. The correlation between the kernel rot and total fumonisin was negative and low while that between the *F. verticillioides* incidence and fumonisin levels were positive and low.

Introduction

The Grain Marketing Board (GMB) of Zimbabwe requires a limited maximum level of moisture content to accept grain from farmers for storage and trading to the various users. Some other countries have also fixed the maximum acceptable levels (MAL) of certain mycotoxins before the grain is accepted for consumption (FAO, 2004). Ear rot causing fungi render maize grain unsuitable for consumption by decreasing the nutritional quality, increasing the levels of mycotoxins, and influencing the aesthetic appearance of maize. The fungi that produce toxins in the grain are divided into those that emanate from the field and those developing during storage (Barney *et al*., 1995). Some fungi may be carried over from the field undetected. Field fungi can survive when relative humidity (RH) is more than 80%, moisture content (MC) is 22% to 33% and temperature is 10±35^{o}C (Williams and McDonald, 1983; Montross *et al*., 1999). Fungi developing in the field may lose their viability when seed or grain is brought to storage facilities, although some may continue to survive (Sanchis *et al*., 1982). The fungi important during storage may become dominant and outgrow the field fungi, although both can originate from the field (Reed *et al*., 2007). Most of the grain buying authorities, such as the GMB in Zimbabwe, require a moisture level of 12.5% and lower for grain storage. In addition, maize is inherently hygroscopic (Suleiman *et al.*, 2013) that leads to the uptake of water from the environment during storage until equilibrium is reached under ambient conditions (Samuel *et al*., 2011) while keeping the dry matter constant (Devereau *et al*., 2002). Yakubu (2009) also reported that fluctuations of temperature and humidity, particularly within the tropical regions, impact negatively on stored grains as the infestation of fungi and insects tend to increase under such conditions. In Zimbabwe these conditions are found in regions with higher altitude, higher rainfall and comparatively cooler environments, including the Natural Regions I, II, and III where Gamanya and Sibanda (2001) sampled. Poor storage conditions further exacerbates deterioration of stored products. This may include stored grain that came in contact with water or damage that was caused by both the grain weevil

(*Sitophilus* spp.) and the Angomous Grain Moth (*Sitotroga cerealella*) (Campbell, 2002). These can further lead to the secondary development of various fungi during storage. The grading systems in some buying agencies such as GMB, takes into consideration visual weevil damage and fungal infestation. On the other hand, contaminated and damaged maize grain can be delivered to buying agencies without any visual signs. Some fungi only become apparent when the maize is put onto nutritive media such as Potato Dextrose Agar (PDA) and Malt Salt Agar (MSA), with the former being ideal for plant pathogenic fungi associated with field infections, whereas the latter being for opportunistic fungi associated with poor storage conditions (Rabie *et al*., 1997).

Besides weevils and natural entry points, such as silks, ear rot causing fungi can enter maize systemically. *Fusarium verticillioides*, for example, has been found to infect the stalk and can be mobile to infect the kernels through translocation. Physical damage due to poor mechanical handling of the grain is another entry point for fungi into the kernel (Dharmaputra *et al*., 1994).

There are various stalk and cob rot causing fungi, including *Stenocarpella maydis*, *Fusarium* spp., and *Aspergillus* spp. which are associated with mycotoxin production, causing some health disorders in humans and animals (Marasas, 1977; Marasas *et al*., 1981; Gelderblom, *et al*., 1992; Julian, *et al*., 1992). Fusaric acid has been found to be emitted by *F. verticillioides* and attributed to birth defects in rats (Porter *et al*., 1995).

Correct identification of problem fungi assists in managing and solving the problems they cause. Effective and appropriate management methods previously developed can be applied, including chemical control methods, and developing and planting resistant plant varieties. Accurate identification also impacts on diagnosis of diseases as appropriate molecular tools for fast diagnosis can be identified (McCartney *et al*. 2003), which in turn aid studies elucidating the epidemiology of the disease or contamination problem (McCartney, *et al*., 2003; Nalim, 2004). Phenotypically similar species (based on morphology, ecology, pathology) were shown to often represent more than one species based on DNA sequence data. To be more confident in distinguishing true species, it is thus necessary to obtain better representation of the genome by sequencing, preferably by more than one gene. Taylor *et al*. (2000) described this phylogenetic

method to distinguish morphologically similar but genetically distinct species as the Genealogical Concordence Phylogenetic Species Recognition (GCPSR) and it is based on the independent support of more than one gene for the distinction of different species. A single marker from the DNA sequence may only be used when the species has been identified on several markers, and the single marker is then thought to be representative and distinctive. The intron-rich regions of the genes that code for single copy, household proteins are the preferred choice of markers for use in the species level phylogenetics in fungi due to the high level of DNA sequence polymorphisms in these areas (Geiser, 2003), while the presence of conserved exon regions offer easy alignment (Bruns *et al*., 1991; Geiser *et al*., 2004). For instance, the Translocation Elongation factor (TEF) *1-α* gene region encodes an important portion of the protein translation mechanism and has a high utility in phylogenetics because it is highly informative. Benefits include that there are no orthologous copies in this region, universal primers exist that work across the whole spectrum of the fungi, and it is especially useful for some genera such as Fusarium. Traditional taxonomy has often been found to be challenging based on poor morphological characteristics, culture variability and mutations, environmental conditions, and the stage of the life cycle of fungi when identified (Geiser *et al*., 2004). Furthermore, morphological characterisation was over simplified leading to mycotoxicologists and pathologists occasionally incorrectly naming some fungi and their toxins, or drawing erroneous conclusions (Geiser *et al*., 2004). The use of such methods also needs a high level of experience and expertise for accurate identification to be obtained. It has been found that single morphological species may constitute several biological and phylogenetic species (Taylor *et al*., 2000), while the species that are being identified by molecular means have proven to be so complicated and diverse to the extent of being impossible to be morphologically identified (Aoki *et al*., 2003).

In order to determine the level of fungi that exist in stored maize grain and associated mycotoxin levels, samples were collected in 2011 from various storage facilities in Zimbabwe.

A study was undertaken to determine the level of fungal infestation in stored maize from various localities in Zimbabwe. In addition, an attempt was made to identify the most prominent fungi associated with maize produced in Zimbabwe, and determine the

mycotoxins that are of importance that can affect human and animal health if consumed. The presence of mycotoxigenic fungi in Zimbabwean maize is not thoroughly studied, and the question exists to what extent fungi such as *F. verticillioides* are associated with food commodities destined for human consumption in Zimbabwe. The objective of this study was to determine the most dominant fungi and their associated mycotoxins in Zimbabwean maize to target breeding for resistance against mycotoxin accumulation.

3.1 Materials and methods

3.1.1 Sampling area

Random samples were collected from grain silos and stacks in maize growing areas in Zimbabwe. These areas represent three of the five agro-ecological regions in Zimbabwe, also known as the Natural Regions (NR) I, II and III, although grain from NR IV and V could have also find their way into these storage facilities since most of them are provincial centres. Twenty three GMB facilities were visited across five provinces, including Mashonaland Central, Mashonaland West, Mashonaland East, Manicaland and the Midlands provinces (Table 3.1 and Figure 3.1). Mashonaland West and Mashonaland Central are regarded as the bread-basket of the country where most of the maize is produced and distributed to other regions. Restricting sampling to these provinces in this study reduced chances of re-sampling the same grain. This is due to the fact that maize might have been transferred from these two regions to the other drier regions for household consumption, where GMB facilities mainly operate as distribution centres.

Table 3.1 Provinces and locations where samples were taken

Mashonaland Central	Mashonaland West	Mashonaland East	Manicaland	Midlands
Centenary	Banket	Marondera	Macheke	Kwekwe
Concession	Chinoyi	Hwedza	Rusape	Gweru
Bindura	Lions Den	Murehwa	Mutare	
Glendale	Mhangura			
Mvurwi	Doma			
	Chegutu			
	Kadoma			
	Norton			
	Karoi			
	Magunje			

Figure 3.1 Map of Zimbabwe indicating the various provinces

3.1.2 Sampling

A total of 23 locations were sampled (Figure 3.2). Grain samples from both the 2010 and 2011 seasons were collected in Marondera, Macheke, Rusape, Norton, Chegutu and Kadoma. Those samples drawn in Murehwa, Hwedza, Mvurwi, Banket, Chinhoyi, Lions Den, Mhangura, Doma, Karoi, Magunje and Mutare were from grain delivered in 2011. The samples taken at Centenary, Bindura, Glendale, Concession, Kwekwe and Gweru came from the 2010 delivered grain. Stratified sampling was done at each

location. Where the sample was drawn from stacked bags, sampling was done through probing the stacks that were picked at regular intervals. In cases where scooping from conveyer belts was done, a method described by Davis *et al*. (1980), called stream sampling, was used. This is where a small sample is scooped at specified intervals. In Lions Den, several bags containing maize drawn from individual deliveries of the 2011 season were sampled by probing. Originally, the maize in these bags had been analysed for moisture content and grading, and were therefore representative of all the maize delivered during the 2011 season. The process involved taking a small amount of grain per probe that was further aggregated to make approximately 1.5 kg in quantity per location as recommended by Davies *et al*. (1980).

A sub-set of 1 kg maize was sent to the University of the Free State for the quantitative identification of fungi causing ear rot in the grain. The remaining samples were stored at 4^{o}C for subsequent analysis of mycotoxins that was done at Trilogy laboratory in the USA.

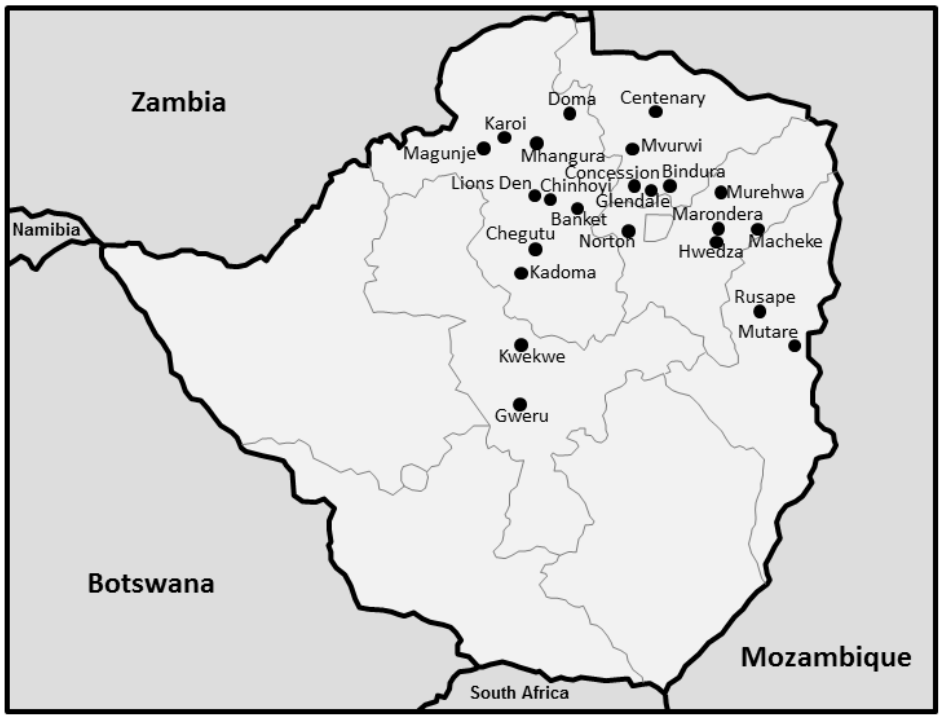

Figure 3.2 Map of Zimbabwe indicating the localities where samples were taken in 2011 from the 2010 and 2011 seasons

3.1.3 Media preparation

The PDA was prepared by adding 39 g of PDA to 1000 ml of demineralised water. The medium was autoclaved for 20 min at 100 kPa steam pressure. The PDA was then cooled to 50^{o}C before pouring approximately 20 ml medium into 90 mm Petri plates.

The MSA was prepared by adding 50 g malt extract agar and 5 g Agar Bacteriological to 700 ml distilled water. In a separate container 90 g sodium chloride was mixed with 300 ml distilled water (Rabie *et al*., 1997; Mathur and Kongdal, 2003; Dawlal, 2010). The two containers were separately autoclaved for 20 min, cooled down to approximately 50^{o}C, and then mixed together. After cooling, the MSA was poured into 90 mm diameter Petri plates in quantities that just covered the bottom, which approximates 15 – 20 ml per Petri plate, and was left overnight in a laminar flow cabinet at room temperature to set. Petri plates were stored at 4-8^{o}C until used.

3.1.4 Mycological analysis

In order to determine the fungal populations associated with maize in Zimbabwe and the possible levels of mycotoxins, 1 kg sub-samples were put in bags and taken to the University of the Free State Pathology Laboratory for fungal enumeration. The presence of fungi such as *F. verticillioides* and *A. flavus* further necessitated for the analyses of mycotoxins.

About 200 maize kernels were taken from the sub-sample and surface sterilised for one minute by immersing it in 76% ethanol in a flask and shaken for 60 seconds. The sample was then rinsed twice with sterilised distilled water for 60 seconds before it was left to dry on a sterile paper towel. Kernels were randomly taken by a pair of forceps that was sterilised by dipping it into 96% alcohol and dried by passing it through a flame. Five kernels were evenly distributed on 90 mm diameter Petri plates. A total of 20 Petri plates of MSA and PDA respectively were prepared to represent 100 kernels on each medium. The placement of the maize kernels on each plate was carefully done with minimum opening period of the Petri plates in a sterile laminar flow cabinet. The plates were immediately placed in an incubator with mixed light at a temperature of 25$^{\pm}$2^{o}C for 14 days.

3.1.4.1 Morphological identification of fungal species

Fungal identification was done based on morphological characteristics including the presence of macro and microconidia, spore shape, phialidic development of spores, survival structures, sexual and asexual fruiting structures, pigmentation, exudate formation, the magnitude of mycelial growth, and the characteristics of conidiogenous cells. Several referencing and taxonomic books were used for identification (Ellis, 1971; Sutton, 1980; Carmichael *et al*. 1980; Klich, 2002; Leslie and Summerbell, 2006; Domsch *et al*. 2007; Seifert *et al.,* 2011; Guarro *et al.* 2012).

Out of the 100 kernels per sample, the number of fungi observed on each kernel was counted and expressed as a percentage of kernels infested with each of the fungal species identified (Rabie *et al.*, 1997; Ghiasian *et al.,* 2004).

Determination of the extent of ear rot causing fungi in each maize sample was done by examining a total of 100 kernels. This was done by counting the number of kernels that were visibly infected with fungi. The samples were sent to Trilogy laboratory in the USA for fumonisin analyses.

3.1.4.2 Identification using DNA sequence comparisons

Verification of the identity of the most common mycotoxin producing fungi, including *F. verticillioides* and those that are morphologically similar, was done using DNA sequence comparisons. Single-spore isolations were made from cultures that were morphologically identified as *F. verticillioides* and *F. graminearum* (Table 3.2). Mycelia originating from single spore cultures were scraped with a spatula from one-week-old cultures grown on PDA and transferred to Eppendorf tubes. A sample size of approximately 1 mm^3 was derived.

3.1.4.2.1 DNA extraction

DNA was extracted from the mycelia of the isolates (Table 3.2) using the KAPA Robust DNA extraction kit according to the instructions of the manufacturer (KAPA Biosystems, Lasec, South Africa). It involved four steps, the reaction setup, lysis, heat inactivation and sample recovery.

In a thin walled PCR tube, 10 µl of 10X KAPA Express Extract Buffer, 2.0 µl of 1 U μl^{-1} KAPA extract Enzyme, 100 µl PCR-grade water and the sample were mixed by vortex. The lysis procedure involved incubation in the thermocycler for 10 min at 75^{o}C. The process resulted in the degradation of the nucleases and proteins and the ultimate release of the DNA. The inactivation of the thermostable KAPA Express protease was achieved by heat-inactivation that involves incubation for 5 min at 95^{o}C. The reaction product was further vortexed for 2-3 sec before centrifuging at high speed for 1 min, resulting in pelleting of the debris. The supernatant, containing the DNA, was transferred to a clean tube. Without quantification, 1 µl of DNA extract was used directly in a 25 µl PCR. After the dilution in a TE Buffer at a ratio of 1:10, the samples were stored at -20°C.

Table 3.2 Fungal isolates analysed and the geographic locations from which the grain samples were derived

Grain sample code	Location	Year of grain delivery
1.1d	Murehwa	2011
3.1c	Mvurwi	2011
3.2a	Mvurwi	2011
4.2a	Banket	2011
6.2c	Lions Den	2011
7.2d	Mhangura	2011
8.2e	Doma	2011
10.1f	Magunje	2011
10.2h	Magunje	2011
11.1d	Mutare	2011
12b.2e	Marondera	2010
13A.1d	Macheke	2011
13a.2a	Macheke	2011
13b.1h	Macheke	2010
14b.1j	Rusape	2010
15A.1i	Norton	2011
15A.2c	Norton	2011
16A.1a	Chegutu	2011
17A.1b	Kadoma	2011

3.1.4.2.2 DNA sequencing

The Translation Elongation Factor 1-alpha (TEF *1α*) gene region was amplified with PCR using the Primers EF-1 (5'-ATGGGTAAGGG(A/G)GACAAGAC-3') and EF-2 (5'-GGA(G/A)GTACCAGT(G/C)ATCATGTT-3') from O'Donnell *et al*. (1998). This gene region is known to be used in all of the current species complexes of Fusarium and able to distinguish between species (O'Donnell *et al*., 2010). PCR reactions and programme parameters were done with the Robust PCR kit (KAPA Biosystems) following the instructions of the manufacturer, except that the annealing temperature of the PCR programme was 61°C. In brief, 1-5 µl DNA extract was added to the 25 µl KAPA2G Robust HotStart PCR. After denaturing, the annealing was done for 15 sec which was followed by another 15 sec of extension at 72°C for 40 cycles. Amplification products were visualised on 1% agarose gels (Cleaver Scientific, AEC-Amersham, South Africa) containing Gelred DNA stain (Biotium, Anatech, South Africa) under UV illumination using a Geldoc XR+ imaging system (Bio-Rad, South Africa).

PCR amplicons were purified using the EXO/SAP Amplicon Purification system (Werle *et al.*, 1994). The purified PCR product (upto 20 ng^{-1}µl) was used in sequencing reactions consisting of BigDye Terminator v3.1 cycle sequencing ready reaction kit (Applied Biosystems, Foster City, CA, USA). Sequencing reactions were purified with EDTA/Ethanol precipitation and run on an ABI 3130XL genetic analyser (Applied Biosystems). Chromatograms were compiled in contigs and manually verified with Geneious v. 7.0.6 (Biomatters, New Zealand). Resultant DNA sequences were compared to DNA sequences of valid *Fusarium* species found in Genbank (http://www.ncbi.nlm.nih.gov/genbank), the dedicated Fusarium DNA databases FUSARIUM ID v1.0 database (http://isolate.fusariumdb.org/blast.php (Geiser *et al*., 2004) and the Fusarium Multilocus Sequence Typing (MLST) Database (http://www.cbs.knaw.nl/fusarium) in order to establish the appropriate species complex for the queried sequences.

Complete DNA datasets for the appropriate species complex identified from the internet searches were built. A complete DNA dataset for the *Fusarium fujikuroi* species complex (FFSC) were requested from Dr. Kerry O'Donnell (USDA, USA) for isolates comparing to *F. verticillioides* and others that were closely related and

grouping in this complex. Additional sequences of *F. verticillioides* from various origins and substrates were downloaded from the Fusarium-ID database and included in a separate dedicated *F. verticillioides* dataset.

3.1.5 Data analysis

A t-test analysis was conducted to separate means for the maize fungal incidence, prevalence of fungal species and mycotoxin concentrations (Rheeder *et al*., 1994; Mukanga *et al.*, 2010). Square root transformation was performed to correct for possible bias of the fumonisin data. Pearson correlation coefficients were conducted by Genstat 16th Edition (Genstat, 2013) between fumonisin content and Fusarium incidence. Phylogenetic analyses for the accurate placement of isolates in species were done on the individual datasets in MEGA v. 6.06 (http://www.megasoftware.net/). DNA sequences obtained in this study were incorporated in the appropriate datasets, aligned using the Muscle function of Mega and the alignments were manually verified. The appropriate evolutionary model for each dataset was determined with Mega v. 6.06 and Maximum Likelihood analyses were done in Mega v. 6.06 with the respective model parameters. A 1000 replicate bootstrap analysis was done to determine the confidence levels of branches.

3.2 Results

3.2.1 Fungal enumeration based on morphology

Twenty genera, representing 33 different fungal species and some bacteria were identified using morphological characteristics from all the maize samples tested (Figure 3.3 and Figure 3.4). Amongst these, *Aspergillus flavus*, *Aspergillus niger* group, *Cladosporium cladosporioides*, *Epicoccum sorghinum*, *Eurotium chevalieri* group, *Eurotium repens*, *F. verticillioides*, *Khuskia oryzae*, *Penicillium* spp., *Rhizopus oryzae*, *Stenocarpella maydis* and *Syncephalastrum racemosum* were dominant in most samples. The most abundant fungi were *E. repens* followed by *F. verticillioides*.

Members of *Fusarium* spp. found in the samples tested included *F. graminearum*, and one unidentified *Fusarium* spp. The most frequent genera observed were Aspergillus followed by Fusarium (Figure 3.3). Besides *A. flavus*, other species of the genus

Aspergillus were also found, including *A. clavatus*, *A. ochraceus* group, *A. versicolor*, and those identified as *Aspergillus* spp. as identification to the species level could not be ascertained.

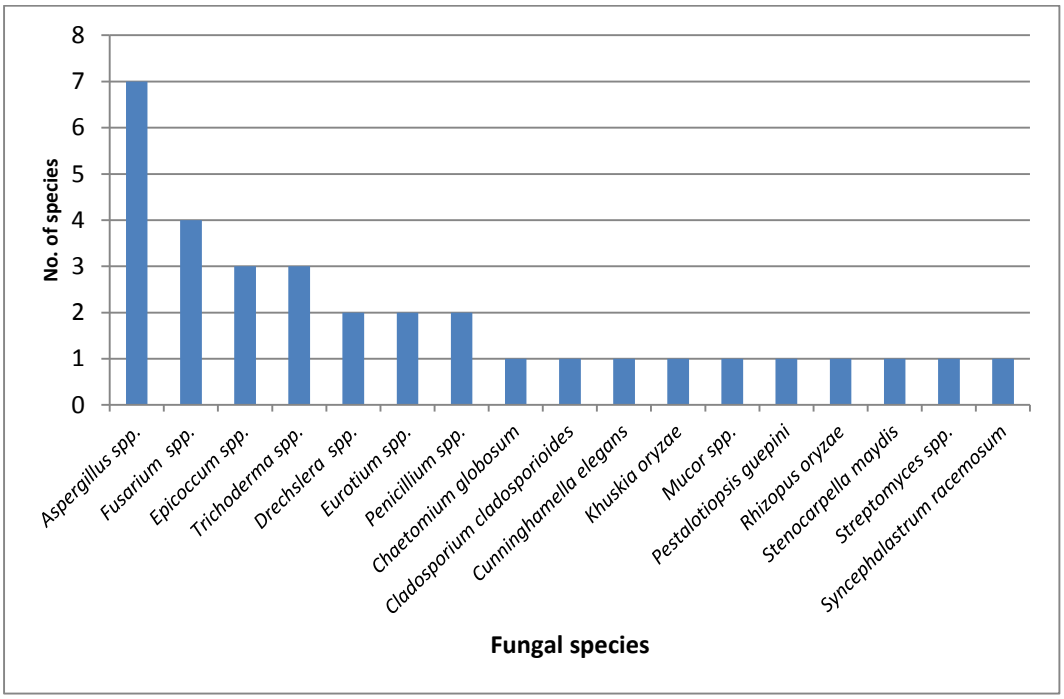

Figure 3.3 Number of fungal species identified from the samples collected from 23 Grain Marketing Board storage facilities in Zimbabwe in 2011

At the national level, *F. verticillioides* was the most abundant on PDA medium (Figure 3.4 and Figure 3.5), but the differences in level of infestation between locations were not significant (Appendix 1). The t-test revealed some significant ($P=\leq 0.05$) differences in incidences of several fungi between locations which were mainly sporadic where one site could have high incidence of a particular fungus compared with the rest of the fungal species including *Aspergillus niger* group, *Stenocarpella maydis*, *Penicillium* spp., *Epicoccum sorghinum*, *Rhizopus oryzae*, *Epicoccum nigrum*, *Trichoderma viride*, *Mucor* spp., *Fusarium graminearum*, *Drechslera halodes*, *Aspergillus ochraceus* group, *Fusarium oxysporum*, *Cunninghamella elegans*, *Chaetomium globosum*, *Pestalotiopsis guepinii*, *Drechslera hawaiiensis*, *Aspergillus clavatus* and some *Fusarium* spp. (Figure 3.4 and Appendix 1).

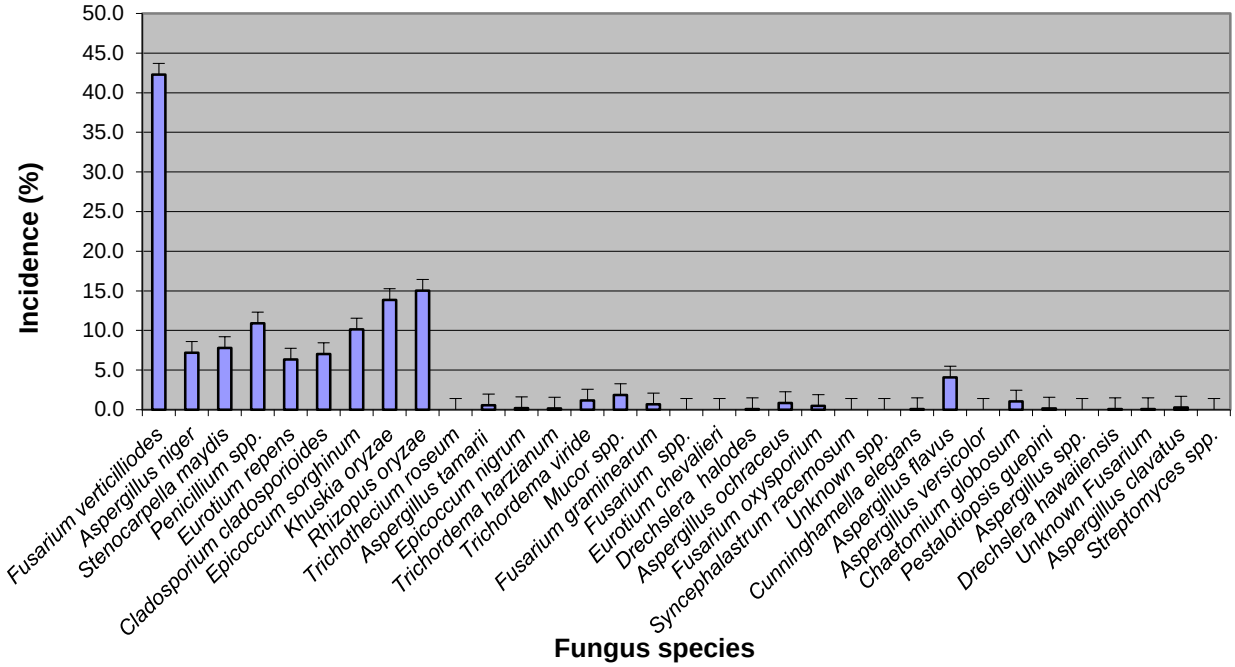

Figure 3.4 National average incidences of fungi based on fungal enumeration

Eurotium repens was the most frequently occurring fungus on MSA (Table 3.3; 3.4; and Figure 3.6) with no significant differences (t-test $P>0.05$) observed between any two means (Table 3.3 and 3.4). The incidences of *Cladosporium cladosporioides* were highly significant ($P\leq0.01$) at Mvurwi and Banket, so was *Khuskia oryzae* in Mhangura and Marondera 2011 delivered grain. At Rusape, both the 2010 and 2011 delivered grain had significantly higher incidences ($P\leq0.01$) of *Eurotium chevalieri* while significantly higher incidences ($P\leq0.01$) were observed for *Aspergillus tamarii* and *Aspergillus flavus* in Lions Den and Bindura respectively than in the other locations (Table 3.3 and Table 3.4).

Table 3.3 Incidences (%) of fungi identified on MSA in 2011 grain

Origin	*Eurotium repens*	*Cladosporium cladosporioides*	*Khuskia oryzae*	*Aspergillus tamarii*	*Eurotium chevalieri*	*Syncephalastrum racemosum*	*Aspergillus flavus*
Murehwa	10.0	12.0	0.0	0.0	0.0	0.0	0.0
Hwedza	72.0	22.0	0.0	6.0	0.0	0.0	0.0
Mvurwi	70.0	70.0**	54.0	0.0	0.0	0.0	0.0
Banket	50.0	64.0**	26.0	0.0	18.0	0.0	0.0
Chinhoyi	82.0	16.0	22.0	0.0	4.0	0.0	0.0
Lions Den	82.0	4.0	16.0	42.0***	4.0	0.0	0.0
Mhangura	36.0	20.0	70.0**	2.0	8.0	0.0	0.0
Doma	36.0	4.0	60.0	0.0	0.0	2.0	0.0
Karoi	74.0	8.0	40.0	0.0	4.0	0.0	0.0
Magunje	40.0	6.0	24.0	0.0	4.0	0.0	0.0
Mutare	58.0	16.0	14.0	12.0	20.0	0.0	0.0
Marondera	44.0	18.0	78.0**	0.0	8.0	0.0	0.0
Macheke	94.0	2.0	0.0	0.0	22.0	0.0	0.0
Rusape	100.0	0.0	2.0	0.0	58.0**	0.0	0.0
Norton	90.0	14.0	8.0	8.0	10.0	0.0	0.0
Chegutu	98.0	0.0	8.0	2.0	14.0	0.0	0.0
Kadoma	100.0	0.0	4.0	8.0	0.0	0.0	0.0
Mean	**66.8**	**16.2**	**25.1**	**4.7**	**10.2**	**0.1**	**0.0**
SD	**27.1**	**20.5**	**25.9**	**10.3**	**14.3**	**0.5**	**0.0**

*****=T- test P≤0.001; **=T – test P≤0.01; *=T – test P≤0.05; SD=standard deviation**

Table 3.4 Incidences (%) of fungi identified on MSA in 2010 grain

Origin	*Eurotium repens*	*Cladosporium cladosporioides*	*Khuskia oryzae*	*Aspergillus tamarii*	*Eurotium chevalieri*	*Syncephalastrum racemosum*	*Aspergillus flavus*
Marondera	100.0	2.0	38.0	0.0	4.0	0.0	0.0
Macheke	96.0	0.0	8.0	2.0	24.0	0.0	4.0
Rusape	100.0	0.0	0.0	0.0	74.0**	0.0	0.0
Norton	100.0	4.0	0.0	2.0	26.0	0.0	0.0
Chegutu	100.0	4.0	2.0	0.0	6.0	0.0	0.0
Kadoma	54.0	2.0	0.0	0.0	18.0	0.0	0.0
Centinery	100.0	0.0	0.0	0.0	10.0	0.0	0.0
Bindura	70.0	0.0	0.0	16.0	0.0	38.0	84.0***
Glendale	100.0	0.0	0.0	4.0	26.0	0.0	2.0
Concession	100.0	0.0	0.0	0.0	18.0	0.0	0.0
Kwekwe	62.0	10.0	22.0	0.0	2.0	0.0	0.0
Gweru	2.0	2.0	16.0	0.0	0.0	0.0	0.0
Mean	**82.0**	**2.0**	**7.2**	**2.0**	**12.2**	**3.2**	**7.5**
SD	**30.4**	**3.0**	**12.2**	**4.6**	**10.5**	**11.0**	**24.1**

*****=T- test P≤0.001; **=T – test P≤0.01; *=T – test P≤0.05; SD=standard deviation**

everal species, including *Aspergillus tamarii, A. flavus, A. niger, Cladosporium cladosporioides, Epicoccum sorghinum, Eurotium chevalieri, E. repens, F. verticillioides, Khuskia oryzae, Mucor* spp., *Penicillium* spp., *Rhizopus oryzae* and *S. maydis,* could be identified in significant levels. The genus, Penicillium, was observed in several samples with lower incidences. *Eurotium chevalieri* group was positively identified on MSA with high incidences in some samples (58% and 74% in Rusape for the 2011 and 2010 samples, respectively). The detection of *S. maydis* and *Epicoccum sorghinum* on the other hand, could only be achieved on PDA medium (Figure 3.5). Other species found at relatively low levels were *Chaetomium globosum, Cunninghamella elegans, Drechslera halodes, Drechslera hawaiiensis, Epicoccum nigrum, Pestalotiopsis guepinii, Trichoderma harzianum, Trichothecium roseum, Trichoderma viride, Streptomyces* spp. and *Syncephalastrum racemosum.*

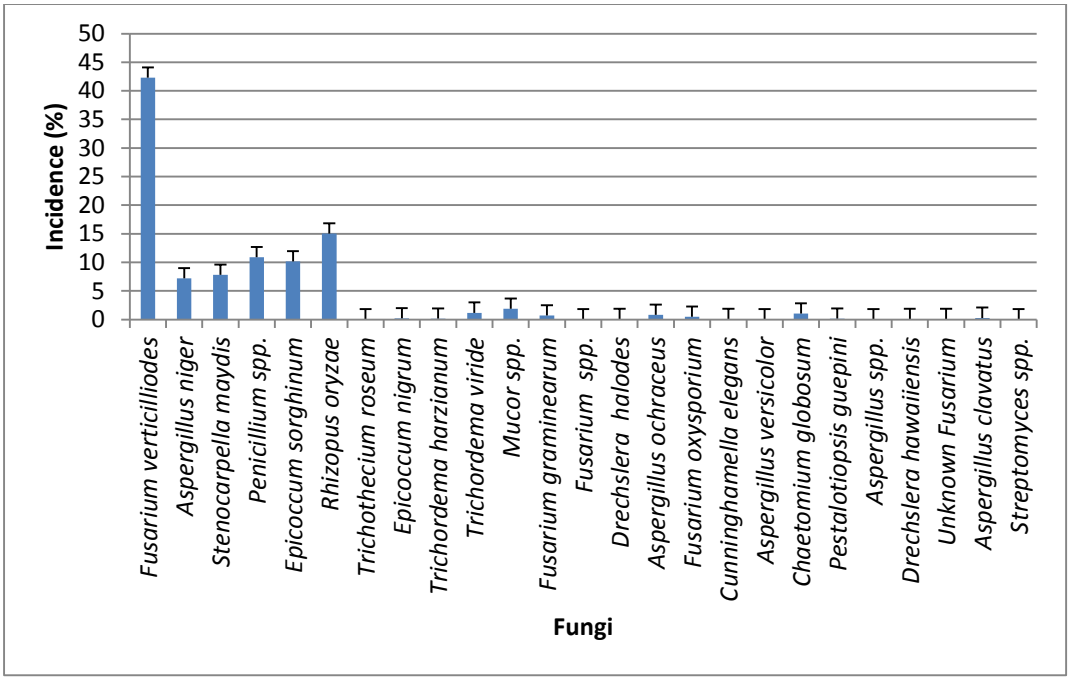

Figure 3.5 Mean fungal incidences obtained from the fungal enumeration for the maize grain samples collected in Zimbabwe in 2011 at 23 locations from PDA medium

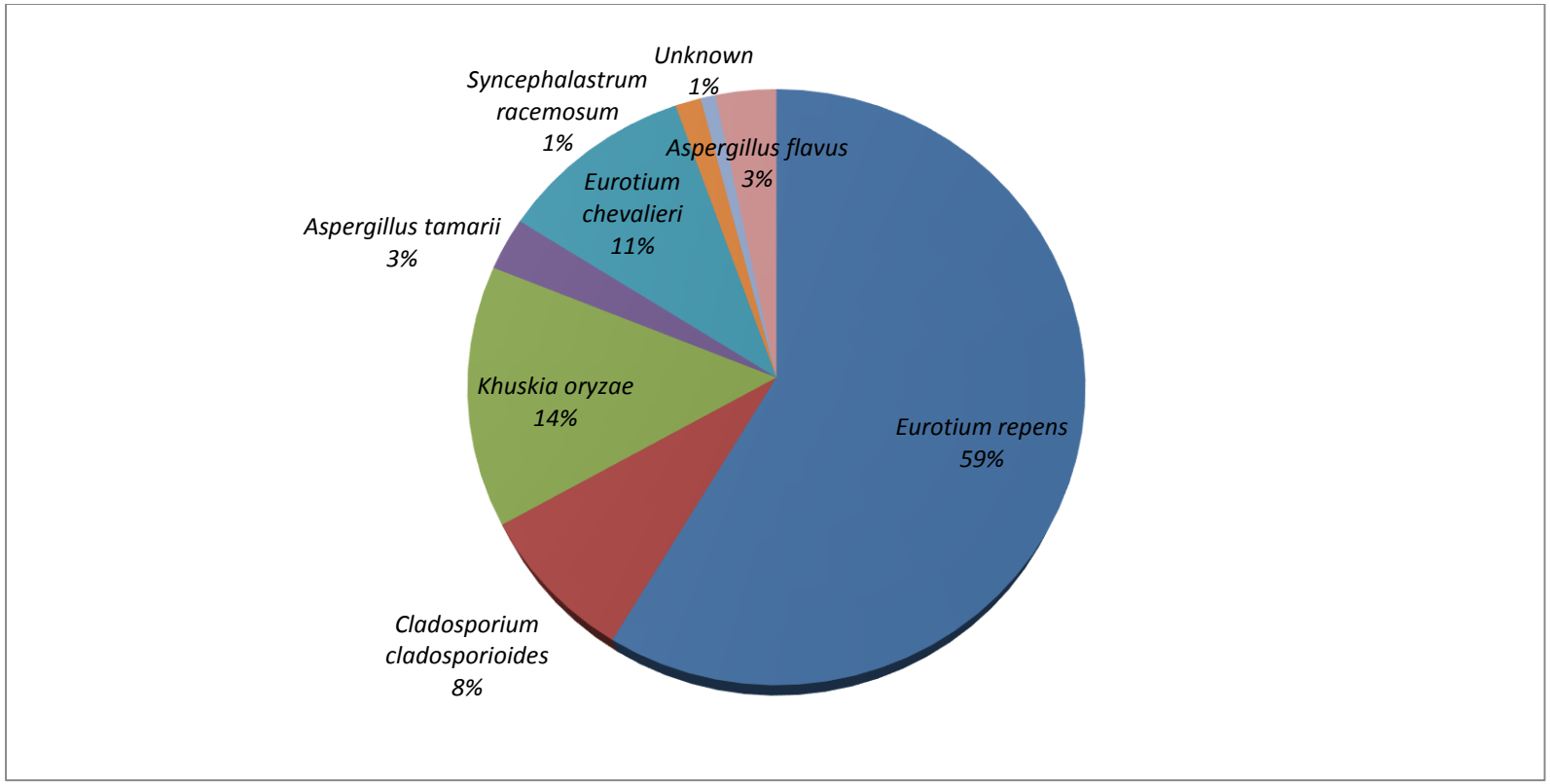

Figure 3.6 Mean fungal incidences obtained from the fungal enumeration for the maize grain samples collected in Zimbabwe in 2011 at 23 locations from MSA medium

3.2.1.1 *Fusarium verticillioides*

The fungus *F. verticillioides* (Figure 3.7) was found in all the samples analysed, being the most abundant in Gweru and Mutare, but the least abundant in Bindura and Glendale (Figure 3.7 and Table 3.5). Where grain samples were collected in 2010 and 2011 in Marondera, Macheke, Rusape, Norton, Chegutu and Kadoma, the 2011 samples had a significantly higher incidence of *F.verticillioides* than the 2010 samples, except in Marondera and Kadoma where lower levels were observed. The lowest incidence for 2011 was in Mvurwi (20%), which was not significantly different from those samples obtained in Kadoma and Hwedza for the same year.

The incidences in the samples drawn from the 2010 grain at Gweru (Figure 3.8 and Table 3.5) were the highest (98%), followed by Marondera and Kadoma with respective incidences of 78% and 59%. The lowest incidence recorded was in Bindura and Glendale, which was not significantly different (P>0.05) from those obtained in Concession.

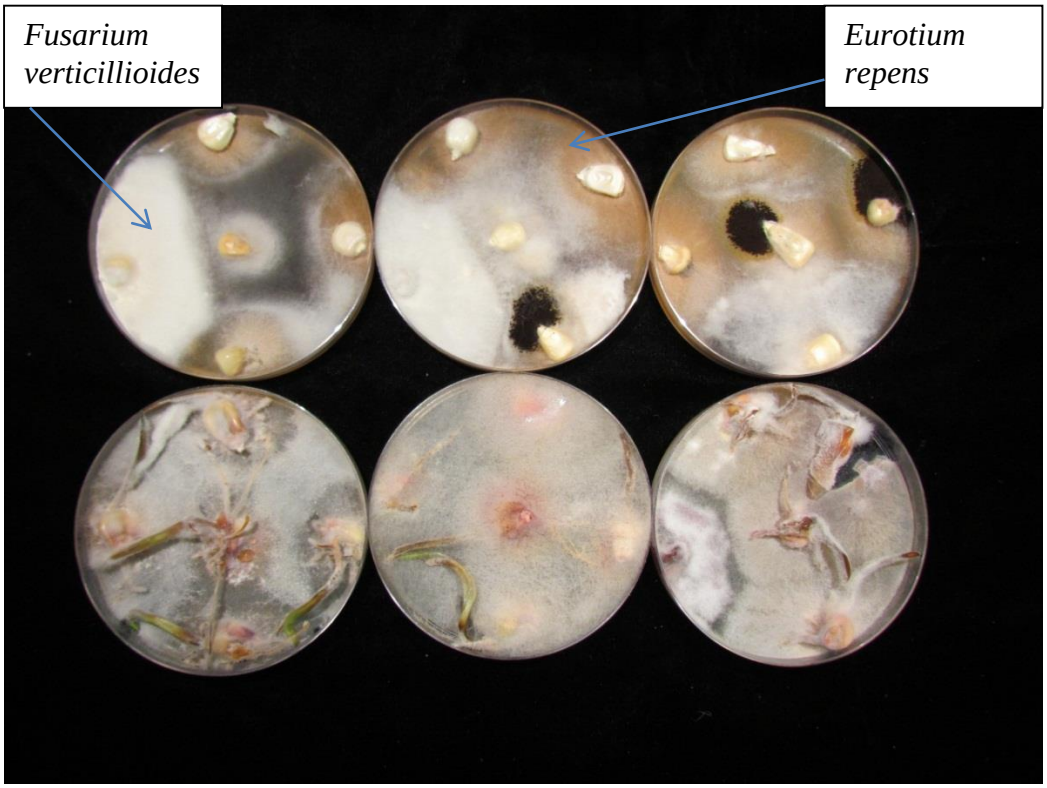

Figure 3.7 Various fungi including *Fusarium verticillioides* and *Eurotium repens* identified on the Gweru 2010 sample on MSA (top three petri plates) and PDA (bottom three plates)

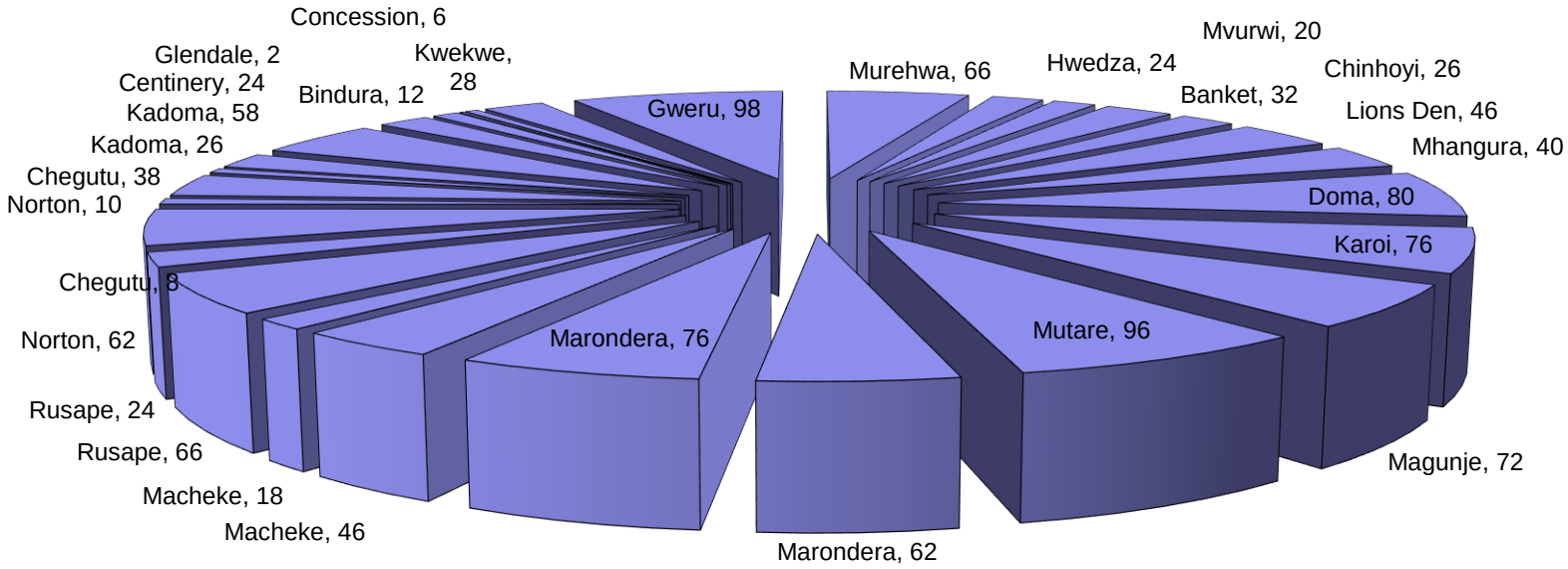

Figure 3.8 Total incidences (%) of *F. verticillioides* at different localities recorded from samples collected in Zimbabwe in 2011

Table 3.5 Incidences of *F. verticillioides* on the kernel rots scores, fumonisin content, the standard deviation and the correlations between the incidences

Location	*F. verticilliodes*	Ear rot score	Fumonisin			
	Incidence mean	Mean	B_1	B_2	B_3	Total
	%	%	ppm	ppm	ppm	ppm
Murehwa	65.0	10.3	0.0	0.0	0.0	0.0
Hwedza	12.0	12.0	0.0	0.0	0.0	0.0
Mvurwi	14.0	6.3	1.2	0.4	0.2	1.8
Banket	21.0	5.7	0.0	0.0	0.0	0.0
Chinhoyi	20.0	4.3	0.0	0.0	0.0	0.0
Lions Den	26.0	4.7	0.0	0.0	0.0	0.0
Mhangura	25.0	2.0	0.1	0.0	0.0	0.1
Doma	74.0	3.3	0.0	0.0	0.0	0.0
Karoi	58.0	3.0	0.3	0.1	0.0	0.4
Magunje	56.0	5.3	0.8	0.6	0.3	1.7
Mutare	65.0	5.0	1.0	0.2	0.0	1.2
Marondera	39.0	4.7	0.2	0.0	0.0	0.2
Marondera	63.0	5.0	0.2	0.0	0.0	0.2
Macheke	27.0	6.7	0.5	0.1	0.0	0.6
Macheke	10.0	9.0	0.2	0.0	0.0	0.2
Rusape	44.0	10.3	0.8	0.2	0.1	1.1
Rusape	13.0	8.7	0.2	0.0	0.0	0.2
Norton	39.0	5.3	0.2	0.0	0.0	0.2
Norton	7.0	12.0	0.0	0.0	0.0	0.0
Chegutu	21.0	4.0	0.2	0.0	0.0	0.2
Chegutu	4.0	7.0	0.0	0.0	0.0	0.0
Kadoma	15.0	2.3	0.4	0.1	0.0	0.5
Kadoma	39.0	5.0	0.2	0.0	0.0	0.2
Centinery	14.0	7.0	0.4	0.2	0.0	0.6
Bindura	6.0	29.7	0.0	0.0	0.0	0.0
Glendale	1.0	8.7	0.3	0.0	0.0	0.3
Concession	3.0	15.0	0.0	0.0	0.0	0.0
Kwekwe	24.0	5.7	0.5	0.2	0.0	0.7
Gweru	96.0	4.7	0.2	0.0	0.0	0.2
Mean	**31.0**	**7.3**	**0.3**	**0.1**	**0.0**	**0.4**
SD	**25.0**	**5.3**	**0.3**	**0.1**	**0.1**	**0.5**
r(KR%, total fum)	**-0.4**	**1.0**	**-0.2**	**-0.1**	**-0.1**	**-0.2**
r(KR%, total fum)	**1.0**	**-0.4**	**0.2**	**0.1**	**0.1**	**0.2**

r=correlation coefficients; KR=kernel rot; fum=fumonisin; SD=standard deviation; ppm=parts per million; B_1=fumonisin B_1 analogue; B_2=fumonisin B_2 analogue; B_3=fumonisin B_3 analogue

The highest incidences of *F. verticillioides* were in the extreme north and east as depicted in (Figure 3.9 and 3.10).

The correlations between the Fusarium incidence derived from the culture score and the ear rot score obtained by visually assigning a score based on the counted kernels perceived to be infected, were moderately low and negative (-0.38, Table 3.5). It was equally low and negative (-0.21) between the kernel rot score and fumonisin B_1 just as with subsequent analogues B_2 and B_3, and their summation (-0.14, -0.05, and -0.18 respectively). Between incidence of the *F. verticillioides* derived from the culture score and the fumonisin analogues B_1, B_2, B_3 and the sum of the three were positive and low (0.17, 0.14, 0.11 and 0.16 respectively).

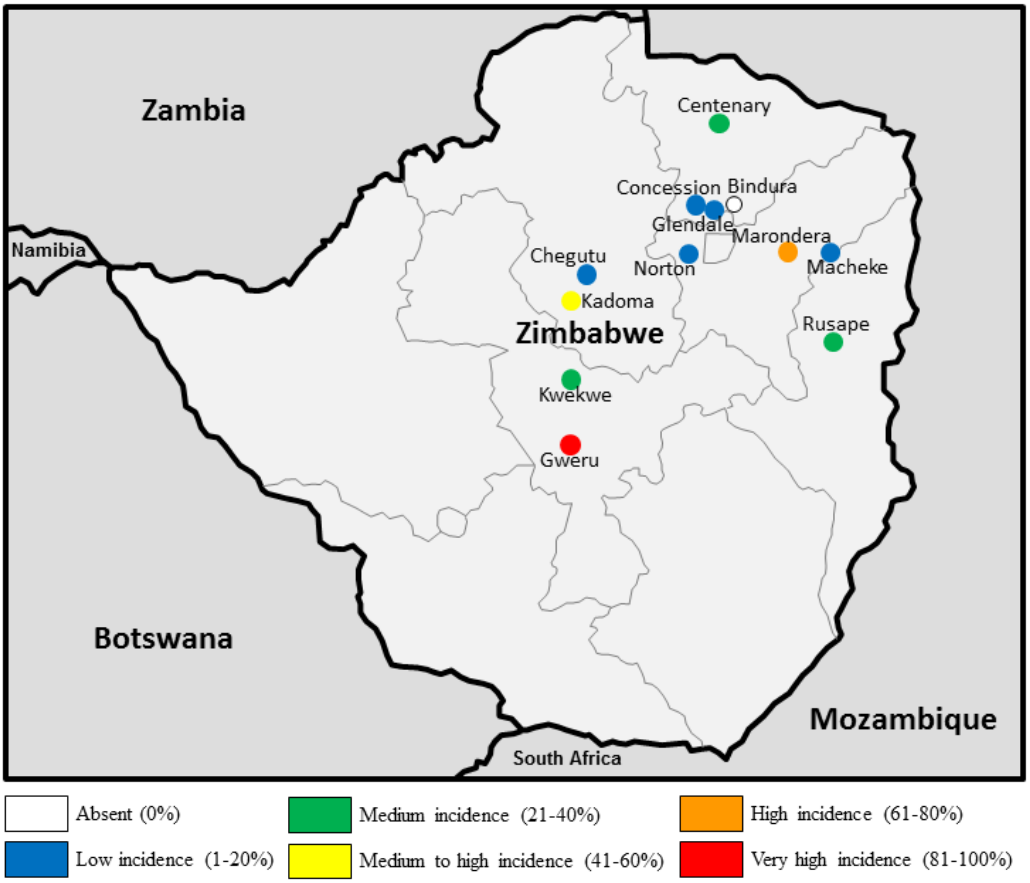

Figure 3.9 *F. verticillioides* incidences in different storage facilities in Zimbabwe from the 2010 season delivered grain samples that were sampled in 2011

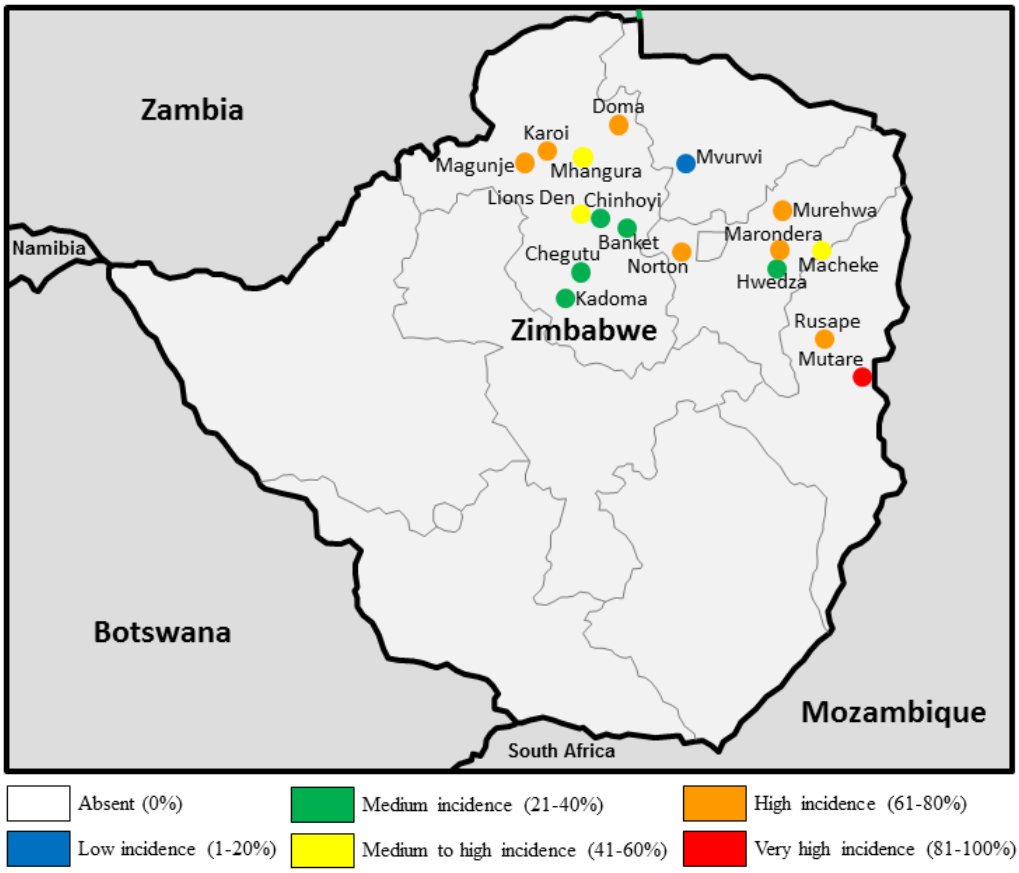

Figure 3.10 *F. verticillioides* incidences in different storage facilities in Zimbabwe from the 2011 season delivered grain sampled in the same year

3.2.1.2 *Aspergillus flavus*

The 2010 samples from Macheke, Bindura, and Glendale were the only three sites where *A. flavus*, the aflatoxin producing fungus, was observed. Relatively low levels were recorded in Chegutu in both 2010 and 2011 samples, and in Kadoma in the 2011 samples (Figures 3.11; 3.12).

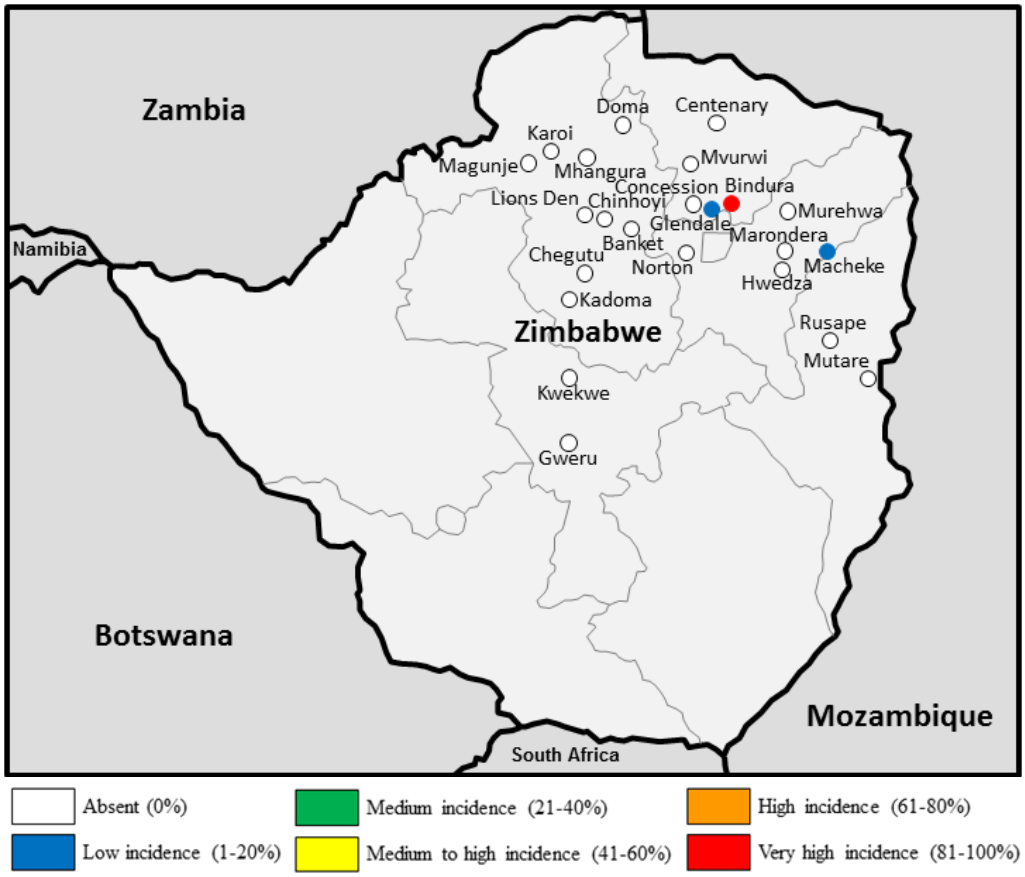

Figure 3.11 Incidence of *A. flavus* in the samples collected in Zimbabwe from both the 2010 and 2011 delivered grain

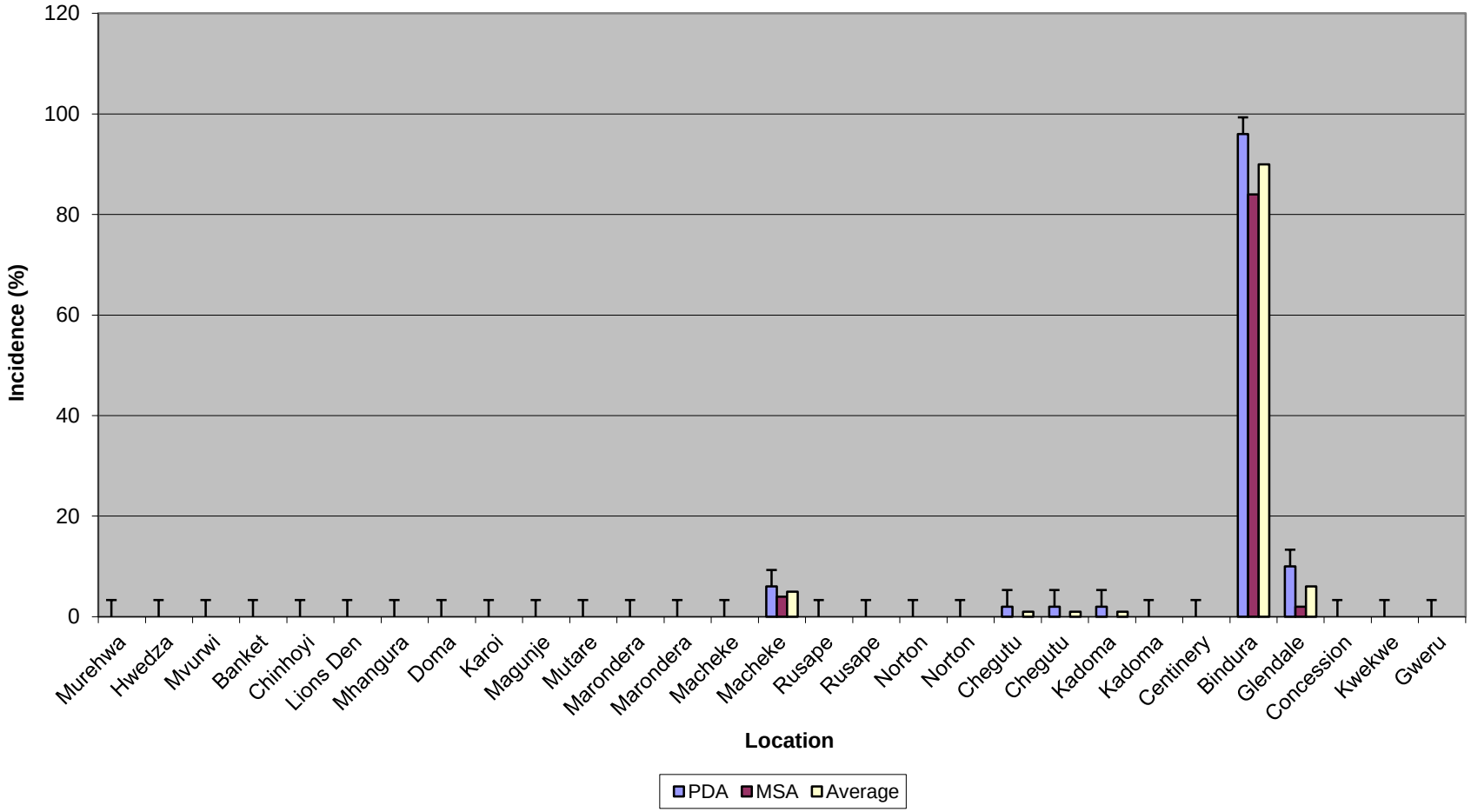

Figure 3.12 The incidences of *A. flavus* observed in 2011 from the grain delivered to in sampled Grain Marketing Board depots in Zimbabwe from both the 2010 and 2011 delivered grain

(Where a site appears twice, the first is from the 2011 season while the second is from the 2010 season)

The grain in Bindura appeared heavily infected with ear rotting fungi, making it undesirable for use based on its aesthetical appearance (Figure 3.13; 3.14). Mycological analysis and fungal enumeration indicated that *A. flavus* was the most abundant with an incidence rate of 96% in Bindura. The samples from Glendale and Macheke had incidence levels of 10% and 6%, respectively.

Figure 3.13 Samples drawn from Bindura representing the 2010 season, showing extensive fungal and insect damage

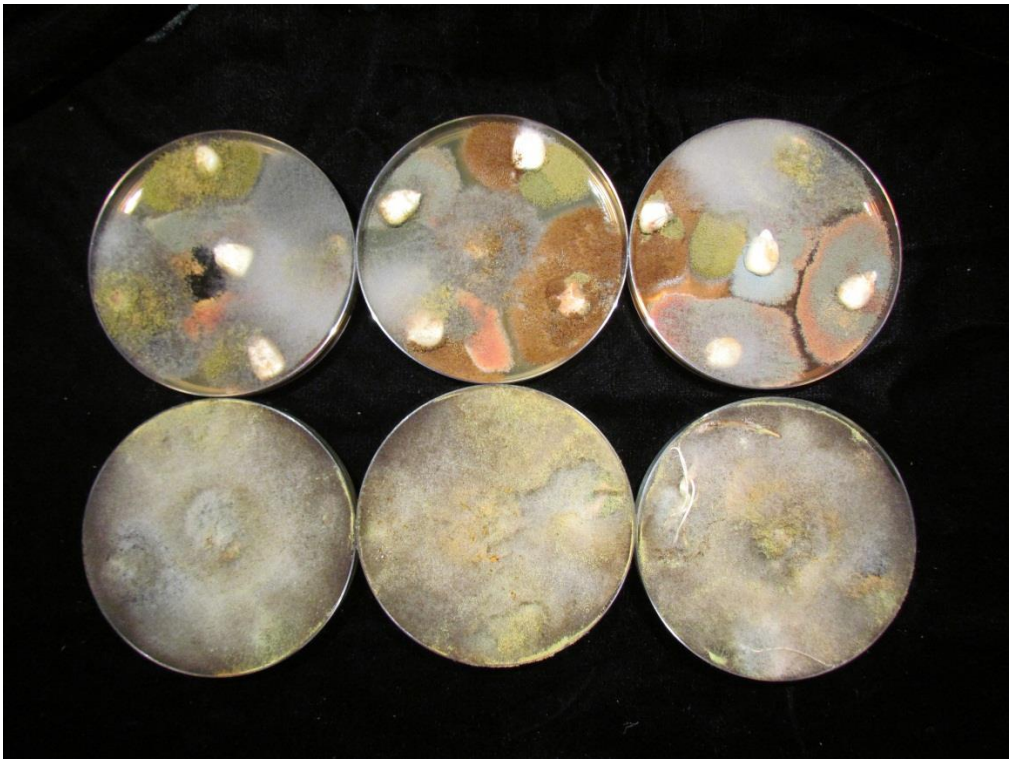

Figure 3.14 Petri plates representing the results of the Bindura sample on MSA (top three petri plates) and PDA (bottom three plates)

3.2.1.3 *Eurotium repens*

The incidence of *Eurotium repens* was above 10% at all the sites except for Gweru (Figures 3.15, 3.16 and 3.17). In Marondera, Rusape, Norton, Chegutu, Kadoma, Centenary, Concession and Glendale, the incidences were all 100%. This fungus was observed on both the 2010 and 2011 delivered grain samples with identical incidences at Macheke, Rusape and Chegutu while there were differences between the 2010 and 2011 delivered grain at Marondera and Norton. At Marondera the 2011 crop had a lower incidence whereas at Norton the 2010 grain had a lower incidence. The lowest incidence was recorded at Gweru from the 2010 season. The other samples from the 2010 season that showed relatively lower incidences included Kadoma, Kwekwe and Bindura. Incidences from the northern GMB depots from the 2011 season generally were lower, including Chinhoyi, Banket, Lions-Den, Mhangura, Doma, Karoi and Magunje.

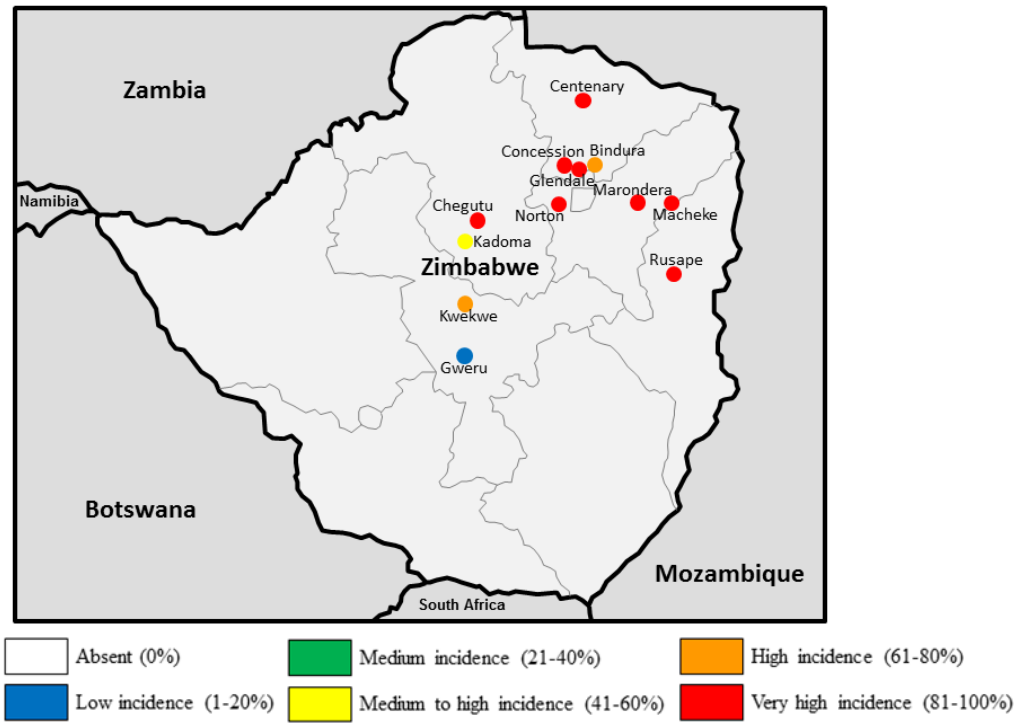

Figure 3.15 Incidence of *E. repens* in samples collected in Zimbabwe from the 2010 season

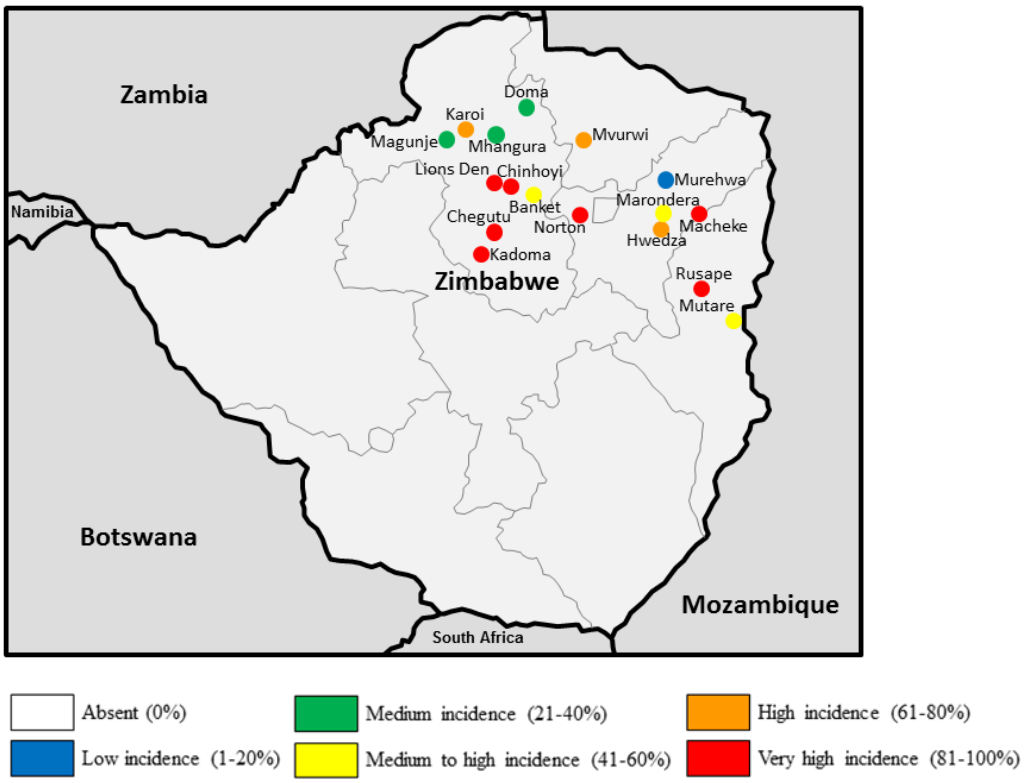

Figure 3.16 Incidence of *E. repens* in samples collected in Zimbabwe from the 2011 season

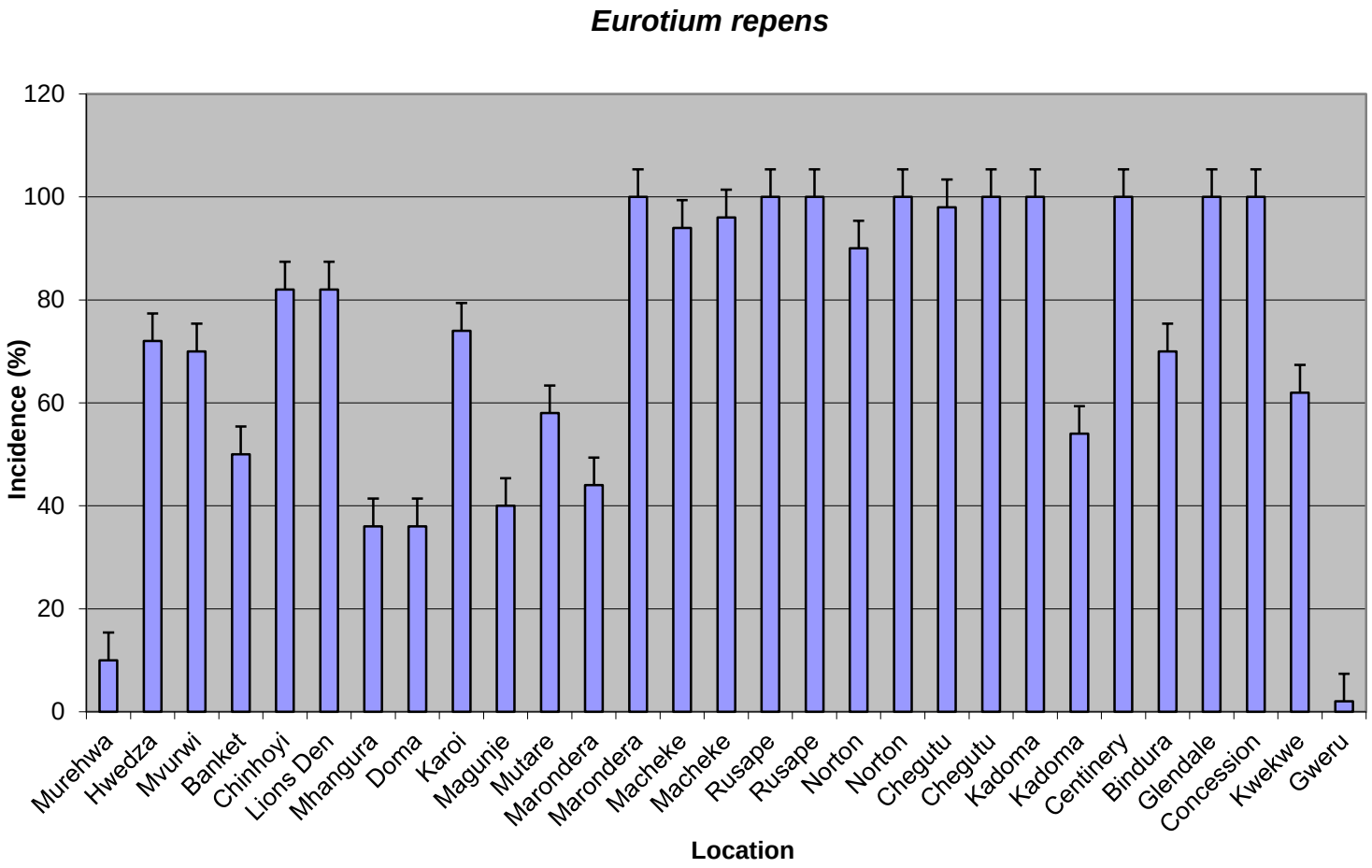

Figure 3.17 Incidences of *E. repens* obtained based on fungal enumeration

3.2.2 Fumonisin contamination

Fumonisins were detected in 19 samples out of the 29 submitted, constituting 65.5% (Table 3.5). Fumonisin B_1 (FB_1) was the most dominant mycotoxin at Mvurwi, Magunje, and Rusape, although fumonisins B_2 (FB_2) and B_3 (FB_3) were also detected in these samples. Mutare also had high levels of FB_1, but no FB_2 and FB_3 were detected. The highest total fumonisin levels were found at Mvurwi (1.8 ppm), Magunje (1.7 ppm) and Rusape (1.1 ppm). At all the locations where sampling was done from both the 2010 and 2011 seasons (Marondera, Macheke, Rusape, Norton, Chegutu and Kadoma), the highest levels were observed during the 2011 season. In contrast, samples taken from the 2010 season at Chegutu and Norton had no fumonisins.

3.2.3 Sequencing

Table 3.6 Summary of fungal identification based on DNA sequencing

Field Code	Location	Year of grain production	Laboratory Code	Morphological Identification	DNA sequence based identification based on phylogenetic analysis
1.1d	Murehwa	2011	2508 A	*F. verticillioides*	*F. verticillioides*
3.2a	Mvurwi	2011	2501 C	*F. verticillioides*	*F. verticillioides*
11.1d	Mutare	2011	2523 K	*F. verticillioides*	*F. verticillioides*
15A.2c	Norton	2011	2520 R	*F. verticillioides*	*F. verticillioides*
16A.1a	Chegutu	2011	2511 S	*F. verticillioides*	*F. verticillioides*
6.2c	Lions Den	2011	2503 U	*F. verticillioides*	*F. verticillioides*
8.2e	Doma	2011	2518 G	*F. verticillioides*	*F. verticillioides*
17A.1b	Kadoma	2011	2516 T	*F. verticillioides*	*F. verticillioides*
12B.2e	Marondera	2010	2507 L	*F. verticillioides*	*F. verticillioides*
14B.1j	Rusape	2010	2509 P	*F. verticillioides*	*F. verticillioides*
10.2h***	Magunje	2011	2505 J	*F. verticillioides*	*F. verticillioides*
7.2d***	Mhangura	2011	2514 E	*F. verticillioides*	*F. verticillioides*
13B.1h	Macheke	2010	2513 Q	*F. verticillioides*	*F. verticillioides*
15A.1i	Norton	2011	2517 Q	*F. verticillioides*	*F. subglutinans*
13A.1d	Macheke	2011	2527 N	*F. graminearum*	*F. pseudoanthophilum*
3.1c	Mvurwi	2011	2529 B	*F. graminearum*	FGSC
4.2a	Banket	2011	2530 D	*Fusarium* spp.	FGSC
10.1f	Magunje	2011	2525 H	*F. graminearum*	FGSC
13A.2a	Macheke	2011	2528 M	*F. graminearum*	FGSC

FGSC= *F. graminearum* species complex

To confirm morphological identification, 19 Fusarium isolates, representing 14 locations, were rondomly selected and sequenced (Table 3.6). Three isolates, representing Marondera, Macheke and Rusape were from the 2010 season. Sequencing results of the isolates from the 2011 season from Murehwa, Mvurwi, Doma, Mutare, Norton, Chegutu and Kadoma, as well as those from the 2010 season from Marondera and Rusape confirmed that they were *F. verticillioides*. Isolates from the 2011 season from Mhangura, Magunje and Macheke were a distant apart from the other isolates, although all grouping within the *F. verticillioides* sub-group (Figure 3.18).

DNA searches on three international databases containing DNA sequence data, revealed that all isolates included were species of *Fusarium*. The majority of isolates were similar to isolates of *F. verticillioides*, with the remainder grouping within the greater *F. fujikuroi* species complex (FFSC), *F. chlamydosporum* species complex (FCSC) and *F. graminearum* species complex (FGSC). Appropriate DNA datasets for each species complex were prepared for phylogenetic analyses based on these results.

Isolates resembling *F. verticillioides* and isolate 2517 from Norton 2011 delivered grain (Table 3.6) were included in a dataset including most species in the FFSC. Maximum likelihood (ML) analyses indicated that isolate 2517 grouped with *F. pseudoanthophyllum*, while the remainder all resided within the clade containing true *F. verticillioides* isolates (Fig. 3.18). Within this clade, isolates from Zimbabwe were identical to isolates from other isolates in the database. A second sub-clade of isolates also existed in *F. verticillioides*, consisting of isolates from Magunje, Macheke and Mhangura. The remainder of isolates showed some level of sequence variation in the form of SNP's that were different from those grouping in the first sub-clade. Isolates 2529, 2525 and 2528, corresponding with Mvurwi (2011), Magunje (2011) and Macheke (2011) grouped in the FGSC, while isolate 2530 (Banket 2011) grouped within the *F. chlamydosporum* species complex (Figure 3.19). In all, the Fusarium ID managed to identify 13 isolates as *F. verticillioides*. DNA sequence comparisons of two isolates resembling *F. verticillioides* (isolate 2517) and *F. graminearum* (isolate 2527) from Norton and Macheke 2011 delivered grain samples respectively also revealed that these grouped in the *Fusarium fujikuroi*

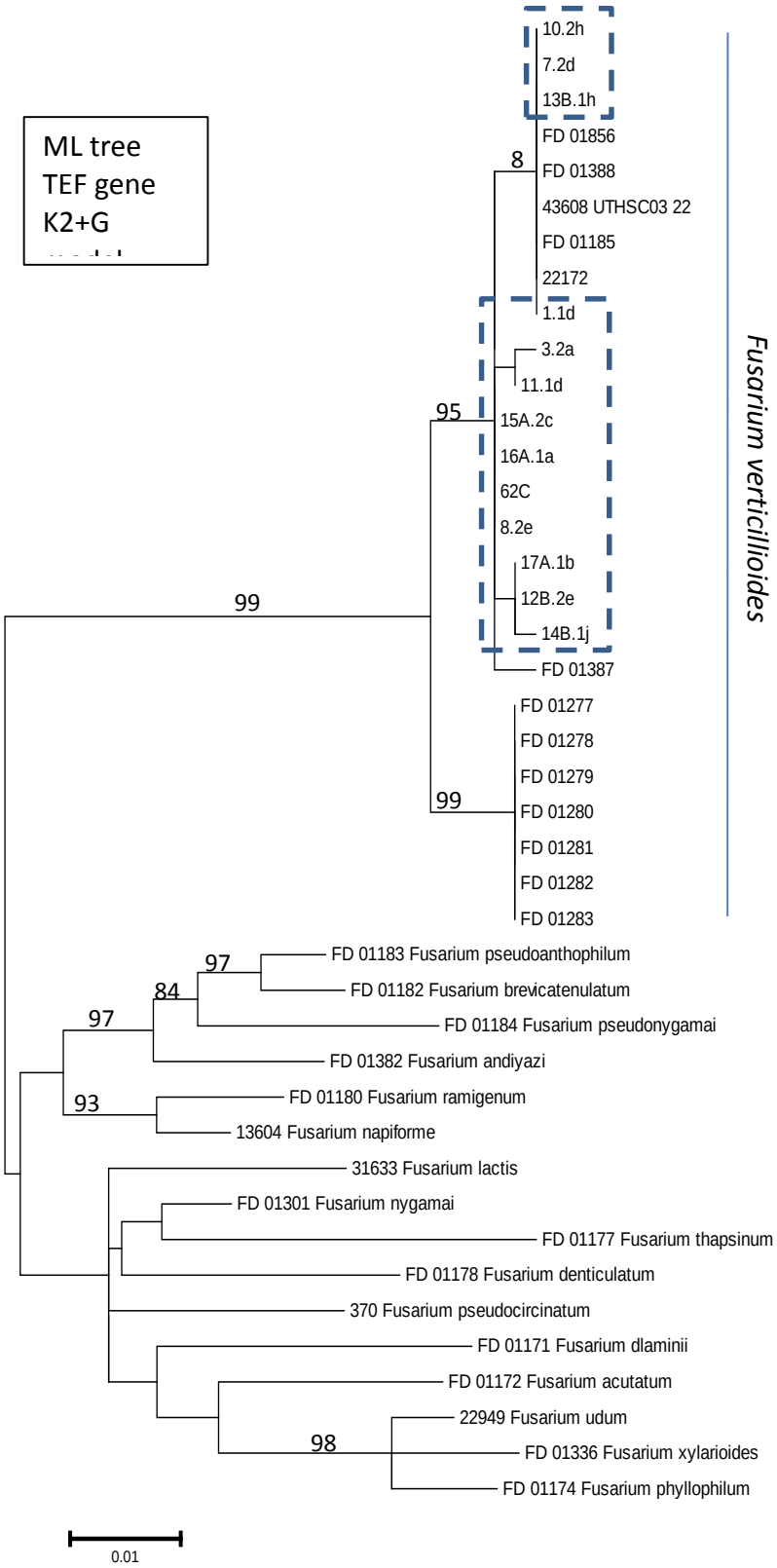

Figure 3.18 Phylogram for the *F. fujikuroi* species complex (including *F. verticillioides* and closely related species)

species complex, which includes *F. verticillioides*. These represented *F. subglutinans* and *F. pseudoanthophyllum* respectively (Figure 3.19).

DNA sequence data of the TEF *1-α* gene derived from Maximum Likelihood (ML) analyses are given in Figure 3.18. Confidence levels of the branches obtained from 1000 replicate Bootstrap analyses are indicated on the branches. Isolates obtained from this study are indicated in the boxes, while other isolates were obtained from the international databases Genbank and Fusarium ID.

The phylogram for the greater *Fusarium fujikuroi* species complex based on DNA sequence data of the TEF *1-α* gene presented in Figure 3.19, was derived from ML analyses. Confidence levels of the branches obtained from 1000 replicate Bootstrap analyses are indicated on the branches. Isolates obtained from this study are included in the boxes, while other isolates were obtained from a published dataset (Nirenberg and O'Donnell, 1998).

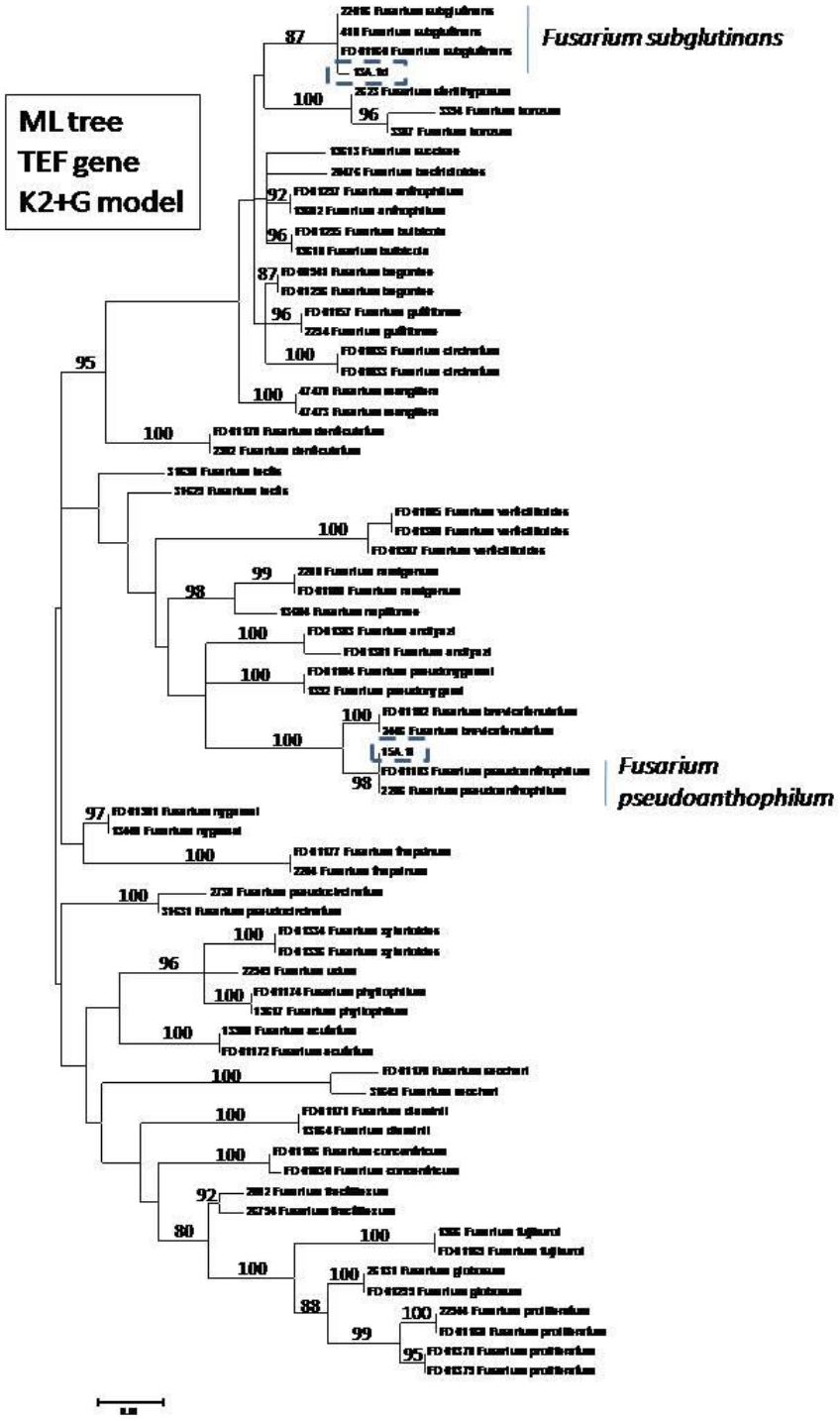

Figure 3.19 Phylogram for the greater *F. fujikuroi* species complex based on DNA sequence data of the Translation Elongation Factor 1-α (TEF) gene

3.3 Discussion

When grain or seed is propagated on a medium such as PDA and MSA, fungi will sporulate enabling morphological identification of the viable fungi present. This study identified 33 fungi from both media used. Eighteen species identified (*A. flavus, A. niger* group*, Cladosporium cladosporioides, Epicoccum sorghinum, Eurotium chevalieri* group*, Eurotium repens, F. verticillioides, Khuskia oryzae, Penicillium* spp.*, Rhizopus oryzae, Stenocarpella maydis, Syncephalastrum racemosum*) were found in significant quantities, while *Aspergillus tamarii, Aspergillus niger* group*, Cladosporium cladosporioides, Epicoccum sorghinum, Eurotium chevalieri* group*, Eurotium repens, F. verticillioides, Khuskia oryzae, Mucor* spp.*, Penicillium* spp.*, Rhizopus oryzae* and *Stenocarpella maydis,* could be identified using both PDA and MSA media. *Eurotium chevalieri* group could only be identified to species level using MSA with high incidence in some samples, whereas *Stenocarpella maydis* and *Epicoccum sorghinum* were detected using PDA. The ability of *S. maydis* to continue to grow under storage conditions are less as the viability tends to diminish with maturity as long as the moisture content is kept below 14% (Steckel, 2003). Dawlal (2010) classified *Epicoccum sorghinum* as a field pest, which is consistent with the results obtained in this study. The fungus, *E. chevalieri,* is widely known as a soil borne fungus but is known to be a problem in stored paddy rice and stored maize seeds. Kocakaya and Coksöyler (2002) found it to be resistant to heat and hence it can survive well under storage conditions with a wide temperature range. In addition to the above fungi, *Chaetomium globosum, Cunninghamella elegans, Drechslera halodes, Drechslera hawaiiensis, Epicoccum nigrum, Penicillium* spp.*, Pestalotiopsis guepinii, Streptomyces* spp. *Syncephalastrum racemosum, Trichoderma harzianum, Trichoderma viride, Trichothecium roseum,* were found in low levels, suggesting that their occurrence is insignificant.

Most of these fungi have previously been identified in similar studies. Several species within the genus, *Penicillium* were found in more than 50% of the samples, albeit at lower incidence of between 12% and 15%, which corresponds with observations by Fandohan *et al*. (2005). *Trichoderma* and *Mucor* spp. were found in less than 5% of the samples while *Colletotrichum graminicola* and *A. niger* group were also found. *Colletotrichum graminicola* was found in all the agro-ecological regions sampled, while *A. niger* group was found only in the northern regions of Zimbabwe.

Most of these species were also found elsewhere in southern Africa. Dawlal (2010) in South Africa identified 40 species as being associated with maize cobs and kernels (Rabie and Marais, 2000). In Zambia, Mukanga *et al*. (2010) isolated 15 fungal species of which some were present in very low levels. In this study the ear rot causing fungi of importance included *F. verticillioides, S. maydis, A. flavus, F. graminearum, A. niger* group, *Penicillium* spp., *Botryodiplodia* and *Cladosporium* spp. in that order of importance, which was consistent with Mukanga *et al*. (2010). In India, Sreenivasa *et al*. (2010) reported 19 fungal genera in the survey conducted in Karnataka State on sorghum where samples were drawn from local stores, agricultural cooperatives and in the field. Some of the fungi found were similar to those found in this study despite working on a different crop and different environment.

The incidences of *Fusarium* species were high in this study, which is consistent with the observations by Sreenivasa *et al*. (2010) who found these fungi in all samples analysed. In this study, *F. verticillioides* was the most abundant field fungus that is consistent with several other findings. For example this was observed by Scot (1993) who reported that *Fusarium* spp. were considered the most abundant. Mukanga *et al*. (2010) also observed high incidences of *F. verticillioides*. High incidence of *F. verticillioides* was equally observed in animal and poultry feeds derived from maize and sorghum (Dass *et al*., 2007). High incidences were observed on grain with both symptomatic and asymptomatic infections (Suleiman *et al*., 2013).

The occurrence of *F. verticillioides* in relation to the region from which the sample originated is generally consistent with other findings with a few exceptions such as Gweru. The highest incidences were from NRI (Mutare and Rusape) and NRII (Murehwa, Doma, Karoi, Magunje, Marondera and Norton). This is in agreement with findings by Gamanya and Sibanda (2001) who observed a trend of *F. verticillioides* and *Fusarium* spp. and the associated FB_1 levels decreasing from regions characterised by high rainfall and moderate annual temperature to low rainfall region. Several other studies such as Miller (2001), Logrieco *et al*. (2002), Fandohan *et al*. (2005) and Mukanga *et al*. (2010) observed the same trend. The skewness in this study where there is no clear cut trend on incidences and the NR could be attributed to the sampling techniques used that differed substantially from those used elsewhere. For instance, Mukanga *et al*. (2010) collected some ears

or cobs which had just been harvested or still in the field. In this study, sampling was done from national storage facilities where grain moves from one region to another, apart from being the grain in storage facilities and not in the field.

The incidence *F. verticillioides* in the harvested crop of the 2011 season was higher than the incidences in the previous year crop (2010) in most cases. Fandohan *et al*. (2005) reported the same trend where in the three years of testing, the incidences were highly variable while differences within the regions were not significant. This is typical with most diseases, hence the need to artificially inoculate for reliable phenotyping. Where breeding for such diseases is concerned, the use of molecular markers may also assist under such circumstances as markers are not affected by the environmental conditions.

Khuskia oryzae and *Cladosporium cladosporioides* demonstrated regional preferences as they were predominantly found in the northern parts of the country. The ear rot causing fungus *Stenocarpella maydis* had a low incidence, the highest being 26% at Murehwa and Macheke. In Zambia, it was the most occurring disease with an incidence of 37% (Mukanga *et al*. 2010). The fungus, *A. flavus* equally showed regional preference, being the most abundant in the north in Bindura and Glendale. It is known to be a storage fungus that occurred in the grain from the 2010 season only and not from the 2011 delivered crop. The grain damage and possibly poor storage conditions in Bindura could have exacerbated its incidence.

The existence of *F. verticillioides* and *A. flavus* appear to be antagonistic. The incidences of *F. verticillioides* were observed to be low where *A. flavus* existed. Where there were high incidences of *A. flavus*, the incidences of *F. verticillioides* were low. This therefore suggests that the *F. vertcillioides* is a field fungus while the *A. flavus* is a storage fungus in Zimbabwean conditions. The correlations were, however, low but negative. Marin *et al*. (1998) reported on the competitiveness of *F. verticillioides* and *F. proliferatum* that are both dominant against *A. flavus* as their presence precludes the occurrence of *A. flavus*. This therefore suggests that *F. verticillioides* has a dominant role where its presence reduces the occurrence of other fungi such as *A. flavus* in this case.The non occurrence of either of the two fungi where the other fungi occurs could be further attributed to the favourable environments for each of these to occur. This could

be in terms of moisture activities and the ambient temperature in the storage facilities. The grain is legally accepted at most 12.5% moisture content but increases in moisture could take place when storage facilities are not water proof. In the contrary, storage may be so good that no moisture or temperature can go beyond acceptable level thus reducing the occurrence of either fungus or both of them.

Eurotium repens, on the other hand, is mainly a storage fungus with the highest incidence on MSA, which was also reported by Dawlal (2010). It has been observed that the most frequently occurring fungi have been *E. repens* where incidences of up to 100% were recorded at eight sites. This fungus is known to produce the mycotoxin, sterigmatocystin, which is a precurser of aflatoxin (Gniadek, 2012). It is a storage fungus that tends to develop when the moisture content of grain is above 14% for a long time. The other possibility could be due to poor storage as maize absorbs high humidity and moisture in the storage environment until equilibrium is reached, resulting in rapid deterioration in storage (Devereau *et al*., 2002). However, Reed *et al*. (2007) reported that despite the observation that both the storage and field fungi originate from the field, the fungi important during storage replace the dominance of field fungi when in storage facilities. The magnitudes of the two types of fungi are therefore reversed.

There was a negative correlation between the kernel rot attributed to Fusarium and *F. verticillioides* incidences. This suggests that either the association of kernel rotting to *F. verticillioides* was not correct or a significant occurrence of the fungus without showing visual infection. Fumonisin can also be found amongst apparently healthy plants where the incidence is low and not visible (Munkvold, 1997a; b; Fandohan *et al*., 2003; Robertson *et al*., 2006; Reid *et al*. 2009). Such a result was consistent when the score was correlated with all the fumonisins analogues B_1, B_2, and B_3, where it was negative and low, suggesting that perceived ear rotting does not translate into higher incidence of fumonisins as other pathogens could be playing a role in development of rotting. On the other hand, incidences of *F. verticillioides* obtained through fungal enumeration had positive but low correlations with fumonisins. This confirms the identification *F. verticillioides* as its presence resulted in the contamination of the grain by the fumonisin, albeit at low levels. Menkir *et al*. (2008) also noted the existence of endophytic kernel attack by Fusarium and *A. flavus*, and that significant amounts of fumonisin can be produced in symptomless plants

or slightly rotten grain, a phenomenon that has been attributed to low phenotypic correlations between Fusarium ear rot and fumonisin levels.

The samples from nine locations (Mvurwi, Karoi, Magunje, Mutare, Macheke, Rusape, Kadoma) sampled from the 2011 season, Centinary, Glendale and Kwekwe from the 2010 delivered grain, had fumonisin levels that were above the recommended limit 5 μg kg^{-1} (FAO, 2004) while the other sites had a total contamination below 0.2 ppm. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) set maximum tolerable daily intake (PMTDI) of 2 μg g^{-1} for B_1, B_2, and B_3, while the US Food and Drug Administration (FDA) set 4 μg g^{-1} for all types of fumonisins (Marasas, 2001; WHO, 2002). WHO regards aflatoxins as a class-1 carcinogen (Martinez *et al*., 2011) and fumonisin B_1 as class-2. The fumonisin incidences tend to be higher in situations where there is moderate to higher levels of *F. verticillioides* ear rot severity (Gamanya and Sibanda, 2001; Mesterhazy *et al*., 2012). This pauses a serious health hazard as maize constitutes the bulk of the food consumed by the majority of the population. The low incidence or absence of any fumonisin detected in the 2010 is contrary to the observation that once the grain is contaminated with fumonisins, it remains on the grain despite deterioration or viable reduction in the incidence of the causal organism *F. verticillioides*. With low but positive correlations observed between *F. verticillioides* incidence and total fumonisins, it may be concluded that in this study, fumonisins existed where the causal organism was observed to be present. This could be attributed to the fact that the conditions favourable for fungal growth may not necessarily be conducive for the production of mycotoxins (Mesterhezy *et al*., 2012).

Out of the 19 isolates analysed by PCR based sequencing, 13 were confirmed to be *F. verticillioides* out of the 14 that had been identified as *F. verticillioides* during morphological analysis. Those morphologically identified as *F. graminearum* were equally found to belong to the *F. graminearum* species complex (FGSC) with one exception that fell within the *F. subglutinans*. It can be concluded that the morphological characterisation was accurately done, in contrast with observations by Geiser (2004) that some fungi appear morphologically similar while they are basically unrelated. One exceptional case was when morphologically, one isolate was identified as *F. graminearum* when sequencing results classified it as *F. subglutinans* which is consistent with observations that some strains of *F. subglutinans* that are found in various hosts tend to be

indistinguishable when morphological characters are used. However, these could be distinguished and described as distinct species when using DNA sequencing such as β-tubulin gene sequences (Steenkamp *et al*., 2000). Despite these exceptional cases, the overall results indicated classification of the isolates into the *F. fujokori* clade as the *F. verticillioides* isolates collected from the Zimbabwe samples compared positively with DNA sequence comparisons with those within the data base. Hence such isolates were used in the inoculation studies in the following chapters. Bogale *et al*. (2006) used other molecular tools including AFLP, SSR and DNA sequence analysis to study 32 strains of *F. oxysporum* from Ethiopia. All the three methods used managed to classify the strains into the three lineages that were consistent with the known clades of *F. oxysporum*. Roux *et al*. (2001) reported of another success while comparing isolates of *Acacia grandis* and *Eucalyptus grandis* with *F. graminearum* isolates where β-tubulin and histone *H3* gene sequencing was used as the isolates were separated into clear phylogenetic and morphological species.

3.4 Conclusions

Maize grain has to be stored either for household consumption or as strategic grain reserves. Besides individuals storing their own grain, the government of Zimbabwe does that through the GMB, which also plays a role in distribution to the non-maize growing areas. Various fungi are found in the stored maize in Zimbabwe that reduce quality, loss of the stored product, and contamination by some mycotoxins produced by some fungi found in storage. This study revealed that 33 fungi could be isolated from maize samples collected from 23 grain storage depots in the main maize growing areas of Zimbabwe. The fungus *Fusarium verticillioides* was the most abundant field fungus, while *Eurotium repens* was the most common fungus under storage conditions. The other mycotoxin producing fungus of importance, *A. flavus* that produces aflatoxins was found at three depots that stored maize from the 2010 season. *F. verticilliodes* and *E. repens*, did not show clear patterns in terms of geographical distribution. On the other hand *A. flavus* was found in the north of the country. It was also observed that *F. verticillioides* incidences generally decrease in storage as illustrated by high incidences from samples collected from the grain of the 2011 season and the incidences from the grain of the 2010 season. It was also interesting to note the high incidences of *A. flavus* where *F. verticillioides* incidences were low to absent, an observation atrributted to the dominance effect of *F. verticillioides* which give rise to

occurrence of other fungi when it is absent. Alternatively, this can be explained in terms of the environmental contions that may be favourable to the other as each of them require different conditions to thrive. The correlations between ear rot scores and incidence of *F. verticillioides* were negative and low to intermediate, suggesting presence of asymptomatic fungi on the grain or wrongly ascribing rotting to *F. verticillioides*. Positive correlations between the incidences of *F. verticillioides* and fumonisins are suggestive of eminent contamination of grain by the fungus when present. The high incidences of *F. verticillioides* justify investing in breeding for resistance to reduce fumonisin contamination of the grain that is mainly used as human food. What had been identified as *F. verticillioides* were confirmed using DNA sequencing of the TEF *1-α* gene. These isolates were further used for artificial inoculation for the studies on inheritance of resistance.

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Chapter 4

Performance of maize F1 hybrids generated from ear rot resistant tropical lowland and mid altitude inbred lines

Abstract

In developing countries such as Zimbabwe, little effort has been made to reduce mycotoxin levels in food and agricultural products. The breeding programmes have not paid any attention to this problem either. One hundred and forty four F1 maize hybrids developed from 12 maize inbred lines from the southern African sub-tropical region and 12 lines from central and West African tropical region that were mated in a North Carolina design II, were evaluated together with six check varieties from these two respective regions. Alongside these hybrid trials were the parent trials with the 24 inbred lines. These were evaluated at three sites in Zimbabwe in the 2012/13 and 2013/14 seasons with one site in the 2012/13 season artificially inoculated with *Fusarium verticillioides*. Both trials were tested in a 0.1 alpha lattice design with two replications but were analysed as a randomized complete block design. The objectives of the study were to observe performance of crosses made between the mid-altitudes cental and West Africa, and southern Africa maize inbred lines with various levels of ear rot causing fungi in *per se* agronomic performance, their reaction to *F. verticillioides* ear rot and fumonisin contamination. The infection levels were low, although there were significant differences for ear rot incidence and fumonisin content for the F1 hybrids. No significant differences were observed for the inbred lines for these traits and the grain disease score (GDS). F1 hybrids SC 2/IITA 7, SC 3/IITA 1, SC 3/IITA 10, SC 5/IITA 10 and SC 10/IITA 2 had the lowest *F. verticillioides* ear rot incidences *per se* and the lowest fumonisin contamination. Inbred line IITA 4 appeared frequently among the best hybrids in terms of grain yield and had the highest yield in the parent trial. The F1 hybrids with *per se* low infection levels for ear rot, including the lines that also had low ear rot infection, were identified. Such lines can be associated with a high potential of contributing towards low infection and high yield.

4.1 Introduction

Maize germplasm is basically grouped into lowland, mid-altitude and highland mega environments despite the existence of various other classifications based on usage, colour, texture, maturity, constitution of the kernel and endosperm and origin (Lu *et al*., 2009). In Africa, all these types exist and inter crossing among them to capture certain desired genes is a common practice. This is not unique to Africa as Bolduan *et al*. (2009) used inbred lines such as CO430 and CO441 that were highly resistant to *F. graminearum* (Reid *et al*., 2003), and two inbred lines reported to have resistance to *F. verticillioides*, CQ201 and CG1 from Canada, in conjunction with local European inbred lines. It is done in some cases to increase genetic diversity within a breeding programme and to introduce adaptability in some cases. Genetic variation at intra or inter-specific or inter-genetic level is required for any trait (Martinez *et al*., 2011). For instance, most of the commercial maize cultivars in several parts of the world do not have specific resistance to mycotoxins such as fumonisins and aflatoxins (Abbas *et al*., 2002; Brooks *et al*., 2005). Warburton *et al*. (2009) observed that most available cultivars on the market in the USA do not carry resistance to aflatoxins. This is not surprising as most of the lines in use are derivatives of B73 types, themselves derived from the Reid Yellow Dent that is associated with high susceptibility levels. In southern Africa, basically mid-altitude material commonly used are derived from the N3 heterotic group that has been observed to be susceptible to ear rot causing fungi such as the *Fusarium* species. As with the USA material, this inbred has a high combining ability for yield. Use of germplasm belonging to the same heterotic group has been a practice in most breeding programmes where breeders recycle a limited number of elite inbred lines (Lu and Bernado, 2001; Hallauer *et al*., 2001; Smith *et al*., 2004).

Use of breeding material that ranges across different classifications such as mega-environments, can not only lead to the development of resistant material, but can result in development of cultivars that cut across regions. Dhliwayo *et al.* (2009) reported the possible use of two CIMMYT mid altitude maize inbred lines in West Africa and use of one line from West Africa in the southern African mid-altitude. Breeding for resistance as a tool for control of both biotic and abiotic factors, is the most effective, with the added advantage of having less environmental effects while its application cuts across the whole spectrum of the socio-economic environments (Busboom and White, 2004; Clements *et al.*, 2004; Menkir *et al*., 2008; Warburton *et al.*, 2009). The inherent

poor agronomic traits found in some germplasm resistant to *Aspergillus flavus* and *Fusarium verticillioides* that has been reported to exist, has not been as desirable due to several reasons. Chief among these is the lack of ability to adapt, with a tendency of being late maturing and having poor combining ability for yield. The lack of adaptation, particularly in the tropical and mid-altitude areas, emanates from the fact that most of such sources originate from temperate regions (Menkir *et al*., 2008; Warburton *et al*., 2009). However, Dhliwayo *et al*. (2009) observed that mid altitude maize inbred lines from southern Africa tended to be earlier when grown under the lowland tropical environments that characterise West Africa.

Robertson-Hoyt *et al*. (2007) found that there was high positive correlation between fumonisin and aflatoxin resistance and that there were two QTLs that affected both mycotoxins, which makes it attractive to observe crosses made between the West African germplasm that is predominately lowland, with southern African, mainly mid-altitude germplasm. *Aspergillus flavus*, the fungus that causes aflatoxins is regarded as field fungi in West Africa while in southern Africa, it is basically a storage pest, although occasionally found in the field (Mukanga *et al*., 2010). The fungus *F. verticillioides* on the other hand, is common as field pathogen in southern Africa but can remain on grain in storage for some time. This suggests possible existence of resistance to these fungi in the germplasm originating from these respective regions, cutting across such regions.

Since mycotoxins such as fumonisins are metabolites of fungal infection which is equally undesirable in any breeding programme, it has been assumed that selection for resistance to ear rots would also be linked to selection for mycotoxins. Selection for ear rots from a breeding point of view involves visual assessment of ears harvested with less rots and other attributes such as loose husks and good husk or tip cover (Butrón *et al*., 2006) and maturity as well as the silk channel length. By the same token, selection of those ears with less mycotoxins is an indirect way of selecting for the cultivars with less ear rot infection. The presence of mycotoxins, in other words, is indicative that the causal fungi has been on the grain at one point, although it may not show symptoms, as these two are not always correlated, despite Vigier *et al*. (2001), Robertson *et al*. (2006) and Bolduan *et al*. (2009) finding strong correlation between disease severity and the concentration of mycotoxins.

Several sources of resistance to *A. flavus* and aflatoxins have been identified (Menkir *et al*., 2008; Warburton *et al*., 2009) including those adapted to central and West Africa, and southern Africa. Utilisation of germplasm from environments with some similarities (such as tropical environments that characterise southern and West Africa) or priority traits that are common (such as resistance to mycotoxins) can result in attainment of medium term objectives (Mickelson *et al*., 2001). The objective of this study was to observe performance of crosses made between the central and West African mid-altitude and southern African mid-altitude maize inbred lines with various levels of ear rot causing fungi in *per se* agronomic performance, their reaction to various ear rot causing fungi that emit some toxic metabolites associated with contamination with various mycotoxins and the mycotoxin fumonisin produced by the most frequently observed fungi on stored grain in Zimbabwe.

4.2 Materials and methods

The maize inbred lines used in this study were classified according to their known reaction to Fusarium and *Diplodia maydis* (Berk.) Sacc. [=*Stenocarpella maydis* (Berk.) Sutton] ear rot and for mycotoxin resistance and known heterotic pattern and origin in terms of geographical location, whether from southern or central and West Africa. The 12 maize inbred lines from the major southern African mid-altitude mega environment included four lines that are resistant to Fusarium and *S. maydis* ear rot (SC 1, SC 2, SC 3 and SC 4), four lines very susceptible (SC 5, SC 6, SC 7 and SC 8), and those whose reaction to most ear rot causing fungi is not known (SC 9, SC 10, SC 11 and SC 12). Also included were 12 lines from central and West Africa, that were developed by the International Institute for Tropical Agriculture (IITA) which include those developed and observed to carry resistance to aflatoxins, IITA 1, IITA 2, IITA 3 and IITA 4, moderately resistant lines IITA 5, IITA 6, IITA 7 and IITA 8, and susceptible lines IITA 9, IITA 10, IITA 11 and IITA 12 (Table 4.1). These parental lines were crossed in a modified North Carolina Design II mating design (Comstock and Robinson, 1948) where the southern African lines were used as female lines while the central and West African derived lines were used as male testers.

The southern African lines came from the heterotic groups listed in Table 4.2 while the heterotic pattern of the central and West African inbred lines was unknown. The IITA maize inbred lines were of mixed origin but classified by their reaction to aflatoxin from earlier evaluations.

Table 4.1 Maize inbred lines used in the study from southern Africa (SA) and West Africa (WA) and their known reaction to either *Diplodia maydis* ear-rot or to mycotoxins

Inbred	HG	Origin	Reaction to Fusarium/Diplodia	Inbred	Code	Reaction to aflatoxin	Origin
SC 1	PH	SA	Resistant	TZMI758	IITA 1	Resistant	WA
SC 2	PI	SA	Resistant	TZMI733	IITA 2	Resistant	WA
SC 3	NO	SA	Resistant	TZMI743	IITA 3	Resistant	WA
SC 4	SS	SA	Resistant	TZMI407	IITA 4	Resistant	WA
SC 5	PO	SA	Very susceptible	TZMI755	IITA 5	Intermediate	WA
SC 6	NN	SA	Susceptible	TZMI757	IITA 6	Intermediate	WA
SC 7	SS	SA	Very susceptible	TZMI746	IITA 7	Intermediate	WA
SC 8	NO	SA	Very susceptible	TZMI102	IITA 8	Intermediate	WA
SC 9	OO	SA	Unknown	TZMI744	IITA 9	Susceptible	WA
SC 10	OCO	SA	Unknown	TZMI749	IITA 10	Susceptible	WA
SC 11	OH	SA	Unknown	TZMI750	IITA 11	Susceptible	WA
SC 12	NO	SA	Unknown	TZMI756	IITA 12	Susceptible	WA

HG=heterotic group

Table 4.2 Southern African heterotic groups and their description

Heterotic group	Heterotic group description
N3	Salisbury White
P	Potchefstroom Pearl
H	Pride of Saline (Old K group)
SC	Southern Cross

4.2.1 Formation of F1 hybrids

The 24 inbred lines were crossed in summer 2011/12 and winter 2012 to make the F1 hybrids in a North Carolina Design II mating design at Kadoma Research Centre (18^0 20’ E and 30^0 6’ S at an altitude of 1149 m), Rattray Arnold Research Station (17^0 40’ E and 31^0 13’ S) at an altitude of

1341 m) and in winter at a low altitude site in Muzarabani in Zimbabwe. The F1 hybrids considered to have inadequate quantities of seed for the second year evaluation, were further made in summer 2012/13 and winter 2013 to ensure seed availability for the second year of testing in 2013/14.

Based on the origin of the seed, whether from southern Africa or central and West Africa and known reaction to *A. flavus*/aflatoxin or ear rot causing fungi, the lines were further divided into six groups, three groups from each region, each group having four lines. The crosses in the crossing nurseries were achieved using paired crossing (Vivek *et al*., 2009) planted in such a way that both the female and male lines were in six rows each, 0.75 m apart and 4 m long. The male parent rows were divided such that two rows were planted a week before the female, the other two rows planted on the same day as the female and the last two rows planted a week after the female to ensure synchronisation of pollen and silk emergence.

The three groups from southern Africa were mated with each of the three groups from central and West Africa such that all possible intergroup matings across origin of the germplasm was achieved (Dhliwayo *et al*., 2009) excluding mating groups within the same origin group. Every line from one group was crossed with all four lines in the other groups and resulted in nine sets of 16 hybrid combinations per set.

The seed of the parent lines were increased simultaneously to enable evaluation of lines in replicated trials alongside the F1 trials (Vivek *et al*., 2009).

4.2.2 Phenotyping

The 144 F1 hybrid crosses and the 24 parental lines were evaluated in replicated trials side by side at each of the sites. The commercial hybrids SC535 and SC719 (resistant) and SC537 (susceptible) from Seed Co in southern Africa, and some experimental hybrids from IITA in central and West Africa M0826-1, M1124-29 (resistant) and M0926-8 (susceptible) were included in the hybrid trials to make a total of 150 entries. The experimental design for all trials was an alpha-lattice (Patterson *et al*., 1978). The hybrids were subjected to a field evaluation to determine their reaction to ear rot in summer 2012/13 and 2013/14 at three locations in Zimbabwe and at Seed Co West African Research Centre (WARC) in Nigeria where only the 2013 trial for the F1 hybrids was

successfully conducted. The hybrids and the parent trials were planted in different replicated trials (Ribaut *et al*., 1997) with two replications, two-row plots, 5 m long spaced 0.75 m apart at a final population density of 53 000 plants per hectare achieved through over planting 3-4 kernels per station followed by thinning two weeks after plant emergence (Badu-Apraku, 2011). This was the practice in both 2012/13 and 2013/14 summer seasons at a hot spot area at Stapleford Research Centre (latitude 17º48' S, longitude, 31º02' E and altitude, 1470 masl) near Harare, where heavy natural epidemics of *Fusarium* ear rots normally occur, at Rattray Arnold Research Station (RARS), latitude, 17º40' S, longitude, 31º13' E and altitude, 1341 masl, at Kadoma Research Centre (KRC), latitude, 18º32' S, longitude, 30º90' E and altitude, 1155 masl and at WARC latitude, 8º80' N, longitude, 7º3' E and altitude, 230 masl.

4.2.3 Agronomic practices

The traditional agronomic practices on maize were followed which included ploughing and discing the land to prepare the seed bed. Fertilizer was applied starting with compound fertilizer that was incorporated by the disc harrow as basal application prior to planting in Zimbabwe. A total of 400 kg ha^{-1} basal application of maize fertilizer (7% N, 15% P_2O_5, and 8% K_2O) was made. Nitrogen fertilizer was further added as a top dressing approximately four weeks after crop emergence (WACE) to supply 138 kg ha^{-1} nitrogen.

4.2.4 Weed and pest control

The weed population was suppressed by application of pre-emergence herbicides according to Long *et al*. (2004) or by hand weeding to control weeds that emerged later. A systemic insecticide was applied at planting to protect the crop from early pest damage, at 4 kg ha^{-1} in Zimbabwe. As stalk borer (*Busseola fusca* and *Chilo partellus*) is known to be a problem pest, prophylactic application of a granular contact insecticide into the whorl was done at four WACE.

4.2.5 Artificial inoculation

The hybrids and their parent lines were artificially inoculated with *F. verticillioides* at RARS only in the 2012/13 season and *F. verticillioides* ear rot severity scores were obtained at all the locations, while fumonisin concentration was determined only for the RARS 2012/13 location and season due to huge costs involved. Inoculum was prepared from the infected grain collected in Zimbabwe

in 2011 from the major maize growing areas which was used in the fungal enumeration and phylogenetic studies in Chapter 3.

The inoculum was prepared according to Clements *et al*. (2004) and Warburton *et al*. (2009) from the isolates of *F. verticillioides*. In brief, the inoculum was produced from isolates of *F. verticillioides* that was identified from most of the samples collected in the major maize growing areas of Zimbabwe. The preparation and maintenance of the isolates was done at the University of the Free State Pathology laboratory in 2012. The isolates were further increased at the University of Zimbabwe from where the purification and drying, to enable easy transportation to the trial site, was done.

The isolates were grown on potato dextrose agar (Biolab, Wadeville, Gauteng). The agar was prepared as described for the enumeration study in section 3.2.4.

The *F. verticillioides* isolates identified from the various samples were blended together in equal proportions in distilled water to form a bulk of fungal blend that represented the pathotypes existing in the country. Use of a blend of isolates has been successfully done to differentiate the reaction of commercial hybrids to *F. verticillioides* ear and kernel rot and fumonisin accumulation (Clements *et al*., 2003; 2004).

The increase of fungal inoculum was achieved through further propagation on sterile PDA, in Petri-plates. The conidia were washed from the agar dextrose using 500 ml sterile distilled water. At RARS site in Zimbabwe, artificial inoculation was conducted in 2012/13 season using the method described by Williams and Windham (2009). In brief, 10 ml of *F. verticillioides* propagule suspension was applied through the Zummo and Scott (1989) technique through the husk of the primary ear at seven days after the emergence of the silks on every plant within the plot (R2 growth stage which coincides with approximately two weeks after mid-pollen, that is when the silk has emerged in 50% of the plants in the plot) (Busboom and White, 2004; Clements *et al*., 2004; Williams and Windham, 2009; Warburton *et al*., 2009). The application was accomplished through the side needle technique where a 14-gauge needle was used with 3×10^8 conidia.

Natural fungal epidemic was anticipated to further increase the disease pressure, particularly in hot-spot areas such as SRC

4.2.6. Measurements

Table 4.3 describes the agronomic traits that were recorded and derived before harvesting according to Poehlman and Sleper (1995), and Badu-Apraku *et al*. (2011). Harvesting was done at physiological maturity which is approximately 60 days after mid-silking when the grain moisture content is approximately 18%. The ears were subjectively counted for visual infection by *Fusarium* spp. and infection expressed as a proportion of the total number of ears harvested. After drying the ears to 14% or less moisture after harvesting, a grain disease score (GDS) was also obtained at RARS, SRC, KRC where a sample was drawn from shelled grain and given a subjective score in equal increments of one (1) where 1 represented no visible ear rot while 9 was for complete infection of the harvested grain kernels exhibiting *F. verticillioides* ear rot infection. Infection severity of a score of 20% or less (1 – 2) was considered as "good", 25% - 30% (3 – 5) as "intermediate", and 35% or higher (6-9) as "poor". *F. verticillioides* ear rot was further evaluated by counting the number of ears from each plot of the hybrid and the parent trials found infected which were then expressed as a proportion of the total ears harvested.

The grain from each of the 2012/13 plots at RARS where artificial inoculation was done had fumonisin levels determined through high-performance liquid chromatography (HPLC) where the fumonisin analogues B_1, B_2 and B_3 were determined. After shelling, samples were drawn from each F1 hybrid plot and parent trial plots and submitted to Trilogy (www.trilogy.co) lab in the USA for quantification of fumonisin. The grain from the whole plot was bulked and milled according to the Trilogy protocol (J. AOAC Int. 79, 688, 1996). In brief, the sample was ground to particles that allow retention of ca 90% through a mesh screen of 500-250 µm. A 50 g test sample was put into a plastic based 250 ml centrifuge bottle. Methanol and 100 ml water was added and homogenised for 3 min at a speed setting of 60% of the normal full speed. The mixture was centrifuged for 10 min at 500 xg and a fluted filter paper was used to collect the supernatant.

Table 4.3 Pre-harvest measured and derived traits

Trait	Measurement procedure
Root lodging (RL)	The proportion of plants with an inclination of 30^{o} or more at the base of the plant
Stem lodging (SL)	Proportion of plants at harvest with stalks broken below the ear level
Foliar disease scores	Subjectively assigned on a 1 to 9 scale where 1 = no symptoms observed, while 9 = completely blighted foliage by Grey leaf spot (GLS) *Cercospora zeae-maydis*, Tehon and Daniels, *Exserohilium turcicum* (HT), *Puccinia sorghi* (PS), *Puccinia polysora* (PP) and *Phaeosphaeria* spp, Henn (PLS)
Days to mid pollen shed (DMP)	Days from planting to when 50% of plants shed pollen,
Days to mid pollen shed (DMS)	Days from planting to when 50% of plants silk
Anthesis to silking interval (ASI)	The difference between the DMS and DMP (i.e. ASI = DMS-DMP)
Plant height (PHT)	The distance between the base of a plant and the position of the first tassel branch
Cob height (CHT)	The distance between the base of a plant to the position of the top ear insertion
Ears per plant (EPP)	The proportion of ears harvested over number of plants at harvest time
Ear position (EPO)	The position of the ear on the plant at harvesting time (CHT divided by PHT)
Husk cover	The number of plants with open tips (HC) expressed as a proportion of the total number of ears harvested (EC)

The filtrate was maintained at a pH of 5.8 which was adjusted by use of 1M NaOH. The SPE cartridge was then fitted to the SPE manifold which was conditioned by washing consistently in order to condition it with 5 ml methanol that was followed by another 5 ml methanol-water solution. Steadily, 10 ml of the extract from the filter was administered at a constant rate of ≤ 2 ml min^{-1}. The cartridge was then washed with 5 ml methanol and water solution and then with 3 ml methanol while keeping the cartridge wet. Fumonisins were then extracted with 10 ml solution made by acetic acid and methanol achieved at a rate of ≤ 1 ml min^{-1}. The extract or eluate was collected from a 20 ml glass vial.

The aliquot of eluate was then transferred to a 4 ml glass vial, concurrently using a nitrogen stream at ca 60^{o}C to dry up the solvent through evaporation. Methanol (1 ml) and 4 ml of rinsing solvent were used to rinse the collection vial while accumulating the residue at the base by washing the sides of the vial. The additional methanol was evaporated, which also ensured a thorough evaporation of the acetic acid.

Derivatisation and liquid chromatograph (LC) analysis was then performed following the Trilogy protocol. Derived standard fumonisin solution (25µl) was transferred to a small test tube where 225 µl *o*-phthaldialdehyde (OPA) reagent was added before mixing and injecting 10 µl and immediately 10 µl was injected into the LC system. The sensitivity settings of the fluorescence detector were adjusted to enable fumonisin B_1 standard-OPA derivative to yield at least 80% of the recorded response.

The residue was dissolved in 200 µl methanol before transferring 25 µl solution to the base of a small test tube followed by an additional 225 µl of OPA reagent. This was then mixed before injection of 10 µl of the solution into the LC system also immediately within a min of adding OPA reagent. This caused all the fumonisin peaks to be on scale and the peak identity was confirmed through retention time comparison of the extracts with the observed standard of individual fumonisins. The fumonisin content was obtained from the readings supplied for B_1, B_2 and B_3 and the total of the three.

The ear aspect (EASP) was a subjective score on a scale of 1-9 where 1 represented a large ear that is insect damage and disease free and appears to be well covered with well filled grains, while 9 represented an ear with all sorts of undesirable characteristics (Badu-Apraku, 2011).

Yield data was obtained following hand harvesting and weighing the actual grain yield per plot after shelling and moisture determination, which was standardised to a moisture content of 125 g kg^{-1} H_2O. This was done at all the sites for both the F1 and the parent trials. Grain moisture was measured with a moisture meter.

4.2.7 Experimental design and data analysis

Experimental design for the F1 and the parent trials was alpha-lattices (Patterson *et al*., 1978), an incomplete block design that can be analysed as randomised complete block design. With the wide range of values for fumonisins that were below the limit (<0.1 ppm), the data were transformed by square root transformation ($\sqrt{(x-0.5)}/2$) such that where no detection of the fumonisin were recorded as 0.4. Campbell *et al*. (1997) used transformation when some undetectable values (<2 ng g^{-1}) were obtained.

The fungal incidence and severity and other agronomic traits, including the fumonisin content for RARS, were subjected to analysis of variance (ANOVA) using AGROBASE Version II (2010) with replications and incomplete blocks considered random while genotypic variance among the hybrids and the parent inbreds was considered fixed. Each trial was analysed individually with checks included, before an across site analysis was conducted. The interaction with the environment was used as an error term for the across environment analysis.

4.3 Results

The data appearing in the means tables constitute a portion of the data for the whole set of hybrids and are selected on the basis of either the best 20 or those within the LSD (5%) value suggesting that they are statistically the same with the best entry. Also presented are the worst 20 or those within the LSD (5%) with the poorest. The full data set is presented in Appendices 2 and 3. The *F. verticillioides* ear rot and fumonisin data for RARS in 2012/13 season is presented separately, as it was the only site and season where artificial inoculation was done and where fumonisin data were obtained and analysed. Data for fumonisin is presented as square root transformed data.

4.3.1 F1 hybrid performance

Grain yield (GY) in t ha^{-1}, DMP, DMS, ASI, rust and traits directly associated with *F. verticillioides* in causing ear rotting (ER), as measured by grain diseases score (GDS), were included in the across site analysis. The mean squares for GY, DMP, DMS and ASI were highly significant ($P \leq 0.001$), while ER incidence and rust were significant ($P \leq 0.05$) (Table 4.4). For all the traits, locations were highly significant ($P \leq 0.001$). The interaction of block and year was highly significant for GDS, GY, DMP, DMS and ASI ($P \leq 0.001$) but significant for ER ($P \leq 0.05$). All the traits except for GY and ASI mean squares for year, were highly significant ($P \leq 0.001$) while location and entry interaction was highly significant for DMP and DMS ($P \leq 0.001$) and rust ($P \leq 0.01$). The mean squares for the location by year interaction were highly significant ($P \leq 0.001$) for all the traits while for location, entry and year they were highly significant for DMP, DMS ($P \leq 0.001$), GDS, GY and rust ($P \leq 0.01$) (Table 4.4).

Analysis of variance and F1 hybrid performance in terms of *F. verticillioides* ear rot, grain disease score and fumonisin

At RARS in 2012/13 where the F1 hybrids were inoculated by *F. verticillioides*, the ear rot incidence was low with a mean of 1.4% but *P* values were significant ($P \leq 0.05$) as the incidence ranged between 0% and 7.6% while fumonisin B_1 and B_2 were highly significant ($P \leq 0.001$ and $P \leq 0.01$ respectively). The total fumonisin *P* values were highly significant ($P \leq 0.001$) (Table 4.5). Out of the 144 F1 hybrids, 69 had no fumonisin analogue detected where inbred lines SC 8 and SC12 appeared in nine and seven hybrids respectively out of a possible 12 while SC 3, SC 4, SC 9 and SC 10 constituted six F1 hybrids (Table 4.5). The most frequently occurring testers were

IITA 2, IITA 10 that appeared in nine F1 hybrids, IITA 8 and IITA 2 that constituted seven hybrids and IITA 7 and IITA 1 that were in six out of a possible 12 hybrids.

Table 4.6 shows F1 hybrids that had the highest amount of total fumonisins at RARS. The poorest F1 hybrids were SC 5/IITA 12, SC 5/IITA 6, and SC 6/IITA 2 which had total fumonisin values of 3.9, 3.2 and 3.1 respectively (Table 4.6). Among the poorest were commercial checks SC537 and SC719 that had a respective total amount of fumonisins of 3.2 ppm and 3.0 ppm respectively. Inbred lines SC 5, SC 4 and SC 11 used as females, appeared more frequently among these F1 hybrids with respective frequency of five, four and three out of a total possible 12. The testers that appeared more frequently were IITA 11, IITA 9 and IITA 12 each having a frequency of four for the former and three for the latter two (Table 4.6), also out of a possible 12.

The mean performances of the best F1 hybrids in terms of *F. verticillioides* ear rot across sites are given in Table 4.7 while performance at RARS in 2012/13 season where artificial inoculation was conducted is exhibited in Appendix 2. All the entries presented had a mean incidence below the mean of 0.8% and were among the 72 entries that had incidences below the mean value across sites and years. Hybrids SC 2/IITA 7, SC 3/IITA 1, SC 3/IITA 10, SC 5/IITA 10 and SC 9/IITA 3 had a mean value of 0%.

The poorest hybrids in terms of mean incidences for *F. verticillioides* across sites and years are shown in Tables 4.8, while the poorest at RARS in 2012/13 season are in Appendix 2. In the combined analysis, the F1 hybrids SC 11/IITA 2, SC 9/IITA 9, (incidence of 1.9%), SC 7/IITA 12 (incidence of 1.8%), SC 2/IITA 9, SC 7/IITA 4 and SC 11/IITA 12 (incidence of 1.7%) were among the worst.

Table 4.4 Combined analysis of variance of six sites in the 2012/13 and 2013/14 seasons

	DF	ER	GDS	GY	DMP	DMS	ASI	Rust
		%	1-9	t ha^{-1}	days	days	days	1-9
Location	2	72.04***	1.48***	2223.18***	10835.66***	4887.15***	1211.95***	23.15***
Entry	149	2.56*	0.03ns	3.81***	19.17***	23.64***	3.89***	0.05*
Block in location x year	2	10.65*	1.14***	139.93***	59.64***	50.03***	96.3***	0.03ns
Year	1	172.24***	1.33***	0.08ns	627.76***	530.29***	4.11ns	4.96***
Location x entry	298	2.41ns	0.03ns	1.49ns	3.35***	4.51***	2.11ns	0.05**
Location x year	2	271.54***	2.05***	169.40***	2182.98***	1420.35***	319.45***	4.03***
Entry x year	149	2.79ns	0.03**	1.95**	4.95***	6.80***	2.53ns	0.04**
Location x entry x year	298	2.62ns	0.03ns	1.79*	3.5***	4.44***	2.22*	0.04ns
Error	898	2.55	0.03	1.49	2.42	3.34	1.91	0.04
Total	1799							

*****P≤0.001; **P≤0.01; *P≤0.05; DF=degrees of freedom; ER=ear rot; GDS=grain disease score; GY=grain yield; DMS=days to mid pollen; DMS=days to mid silking; ASI=anthesis to silking interval**

Table 4.5 Results of square root transformed fumonisins, Fusarium ear rot, and grain disease score from the 2012/13 season trial conducted at Rattray Arnold Research Station showing F1 hybrids with the least total fumonisins

Entry	Pedigree	ER %	GDS 1-9	B_1 ppm	B_2 ppm	B_3 ppm	Total Fumonisin ppm
1	SC 1/IITA 1	0.0	1.1	0.4	0.4	0.4	1.1
2	SC 1/IITA 2	0.6	1.1	0.4	0.4	0.4	1.1
8	SC 1/IITA 8	2.5	1.1	0.4	0.4	0.4	1.1
10	SC 1/IITA 10	1.3	1.1	0.4	0.4	0.4	1.1
15	SC 2/IITA 3	0.0	1.1	0.4	0.4	0.4	1.1
16	SC 2/IITA 4	2.2	1.1	0.4	0.4	0.4	1.1
19	SC 2/IITA 7	0.0	1.1	0.4	0.4	0.4	1.1
21	SC 2/IITA 9	2.0	1.5	0.4	0.4	0.4	1.1
25	SC 3/IITA 1	0.0	1.1	0.4	0.4	0.4	1.1
26	SC 3/IITA 2	0.0	1.1	0.4	0.4	0.4	1.1
27	SC 3/IITA 3	1.9	1.5	0.4	0.4	0.4	1.1
29	SC 3/IITA 5	2.1	1.1	0.4	0.4	0.4	1.1
32	SC 3/IITA 8	0.7	1.1	0.4	0.4	0.4	1.1
34	SC 3/IITA 10	0.0	1.5	0.4	0.4	0.4	1.1
37	SC 4/IITA 1	0.7	1.1	0.4	0.4	0.4	1.1
38	SC 4/IITA 2	1.1	1.1	0.4	0.4	0.4	1.1
39	SC 4/IITA 3	0.6	1.1	0.4	0.4	0.4	1.1
41	SC 4/IITA 5	4.7	1.1	0.4	0.4	0.4	1.1
42	SC 4/IITA 6	1.1	1.1	0.4	0.4	0.4	1.1
43	SC 4/IITA 7	0.0	1.1	0.4	0.4	0.4	1.1
50	SC 5/IITA 2	0.6	1.1	0.4	0.4	0.4	1.1
52	SC 5/IITA 4	0.7	1.1	0.4	0.4	0.4	1.1
55	SC 5/IITA 7	0.0	1.1	0.4	0.4	0.4	1.1
59	SC 5/IITA 11	1.6	1.1	0.4	0.4	0.4	1.1
67	SC 6/IITA 7	0.7	1.1	0.4	0.4	0.4	1.1
68	SC 6/IITA 8	0.6	1.1	0.4	0.4	0.4	1.1
70	SC 6/IITA 10	0.7	1.1	0.4	0.4	0.4	1.1
71	SC 6/IITA 11	0.0	1.1	0.4	0.4	0.4	1.1
74	SC 7/IITA 2	0.7	1.1	0.4	0.4	0.4	1.1
75	SC 7/IITA 3	2.1	1.1	0.4	0.4	0.4	1.1
78	SC 7/IITA 6	0.6	1.1	0.4	0.4	0.4	1.1
80	SC 7/IITA 8	1.2	1.1	0.4	0.4	0.4	1.1
82	SC 7/IITA 10	1.3	1.1	0.4	0.4	0.4	1.1
84	SC 7/IITA 12	3.8	1.1	0.4	0.4	0.4	1.1
85	SC 8/IITA 1	1.2	1.1	0.4	0.4	0.4	1.1
86	SC 8/IITA 2	2.7	1.1	0.4	0.4	0.4	1.1
87	SC 8/IITA 3	4.7	1.1	0.4	0.4	0.4	1.1

Table 4.5 (continued) F1 hybrids with the least fumonisins and their Fusarium ear rot and grain disease scores from the 2012/13 season at the Rattray Arnold Research Station trial

Entry	Pedigree	ER %	GDS 1-9	B_1 ppm	B_2 ppm	B_3 ppm	Total Fumonisin ppm
88	SC 8/IITA 4	0.0	1.1	0.4	0.4	0.4	1.1
89	SC 8/IITA 5	3.9	1.1	0.4	0.4	0.4	1.1
90	SC 8/IITA 6	0.6	1.1	0.4	0.4	0.4	1.1
91	SC 8/IITA 7	0.7	1.1	0.4	0.4	0.4	1.1
92	SC 8/IITA 8	3.1	1.1	0.4	0.4	0.4	1.1
94	SC 8/IITA 10	2.0	1.1	0.4	0.4	0.4	1.1
97	SC 9/IITA 1	1.4	1.1	0.4	0.4	0.4	1.1
98	SC 9/IITA 2	1.3	1.1	0.4	0.4	0.4	1.1
99	SC 9/IITA 3	2.7	1.1	0.4	0.4	0.4	1.1
100	SC 9/IITA 4	0.6	1.1	0.4	0.4	0.4	1.1
106	SC 9/IITA 10	0.7	0.8	0.4	0.4	0.4	1.1
108	SC 9/IITA 12	4.9	1.1	0.4	0.4	0.4	1.1
109	SC 10/IITA 1	1.3	1.1	0.4	0.4	0.4	1.1
110	SC 10/IITA 2	1.2	1.1	0.4	0.4	0.4	1.1
112	SC 10/IITA 4	1.3	1.1	0.4	0.4	0.4	1.1
116	SC 10/IITA 8	1.2	1.1	0.4	0.4	0.4	1.1
118	SC 10/IITA 10	2.7	1.1	0.4	0.4	0.4	1.1
119	SC 10/IITA 11	2.4	1.1	0.4	0.4	0.4	1.1
125	SC 11/IITA 5	0.7	1.1	0.4	0.4	0.4	1.1
126	SC 11/IITA 6	0.0	1.1	0.4	0.4	0.4	1.1
128	SC 11/IITA 8	0.6	1.1	0.4	0.4	0.4	1.1
130	SC 11/IITA 10	1.2	1.1	0.4	0.4	0.4	1.1
134	SC 12/IITA 2	0.0	1.1	0.4	0.4	0.4	1.1
135	SC 12/IITA 3	0.7	1.1	0.4	0.4	0.4	1.1
138	SC 12/IITA 6	1.2	1.1	0.4	0.4	0.4	1.1
139	SC 12/IITA 7	0.6	1.1	0.4	0.4	0.4	1.1
142	SC 12/IITA 10	0.6	1.1	0.4	0.4	0.4	1.1
143	SC 12/IITA 11	1.2	1.1	0.4	0.4	0.4	1.1
144	SC 12/IITA 12	0.0	1.1	0.4	0.4	0.4	1.1
146	SC535	1.9	1.1	0.4	0.4	0.4	1.1
148	M0826-1	0.0	1.5	0.4	0.4	0.4	1.1
149	M0926-8	1.5	1.5	0.4	0.4	0.4	1.1
Mean		**1.4**	**1.1**	**0.6**	**0.4**	**0.4**	**1.3**
SE		**3.2**	**0.4**	**0.7**	**0.2**	**0.1**	**1.0**
LSD (5%)		**2.7**	**0.3**	**0.6**	**0.2**	**0.1**	**0.9**
P value		*****	**ns**	*******	******	**ns**	*******

*****P≤0.001; **P≤0.01; *P≤0.05; ns=not significant; LSD=least significant differences; P value=F probability; ER=ear rot; GDS=grain disease score; B_1=fumonisin B_1 analogue; B_2=fumonisin B_2 analogue; B_3=fumonisin B_3 analogue; ppm=parts per million**

Table 4.6 F1 hybrids with the most fumonisins and their Fusarium ear rot and grain disease scores from the 2012/13 season Rattray Arnold Research Station trial

Entry	Pedigree	ER	GDS	B_1	B_2	B_3	Total Fumonisin
		%	1-9	ppm	ppm	ppm	Ppm
141	SC 12/IITA 9	3.7	1.5	0.9	0.5	0.4	1.7
31	SC 3/IITA 7	0.6	1.5	1.0	0.5	0.4	1.8
53	SC 5/IITA 5	2.3	1.1	0.9	0.5	0.5	1.8
56	SC 5/IITA 8	2.8	1.1	0.9	0.5	0.4	1.8
57	SC 5/IITA 9	2.5	1.1	0.8	0.6	0.4	1.8
131	SC 11/IITA 11	1.2	1.1	0.9	0.6	0.4	1.8
40	SC 4/IITA 4	1.2	1.1	1.1	0.5	0.4	1.9
83	SC 7/IITA 11	2.6	1.1	1.0	0.5	0.5	1.9
44	SC 4/IITA 8	1.3	1.5	1.0	0.6	0.5	2.0
48	SC 4/IITA 12	1.2	1.1	1.0	0.6	0.5	2.0
121	SC 11/IITA 1	0.0	1.1	1.1	0.6	0.5	2.1
124	SC 11/IITA 4	1.3	1.1	1.1	0.6	0.5	2.1
7	SC 1/IITA 7	2.5	1.1	1.3	0.6	0.5	2.3
36	SC 3/IITA 12	2.5	1.5	1.2	0.6	0.5	2.3
107	SC 9/IITA 11	0.6	1.1	1.3	0.7	0.5	2.5
47	SC 4/IITA 11	0.7	1.1	1.4	0.7	0.5	2.6
69	SC 6/IITA 9	0.7	1.1	1.6	0.7	0.6	2.8
147	SC719	0.0	1.1	1.7	0.8	0.6	3.0
62	SC 6/IITA 2	0.7	1.1	1.9	0.8	0.5	3.1
54	SC 5/IITA 6	1.3	1.1	2.0	0.8	0.5	3.2
145	SC537	1.8	1.5	1.9	0.8	0.6	3.2
60	SC 5/IITA 12	3.5	1.1	2.5	0.9	0.6	3.9
Mean		**1.4**	**1.1**	**0.6**	**0.4**	**0.4**	**1.3**
SE		**3.2**	**0.4**	**0.7**	**0.2**	**0.1**	**1.0**
LSD (5%)		**2.7**	**0.3**	**0.6**	**0.2**	**0.1**	**0.9**
P value		*****	**ns**	*******	******	**ns**	*******

*****P≤0.001; **P≤0.01; *P≤0.05; ns=not significant; LSD=least significant differences; P value=F probability; ER=ear rot; GDS=grain disease score; B_1=fumonisin B_1 analogue; B_2=fumonisin B_2 analogue; B_3=fumonisin B_3 analogue; ppm=parts per million**

Table 4.7 Performance of the best F1 hybrids in terms of *F. verticillioides* ear rot and other traits across six sites in 2012/13 and 2013/14 seasons

Entry	Pedigree	ER %	GDS 1 - 9	STB &	GY t ha-1
19	SC 2/IITA 7	0.0	1.1	4.6	4.295
25	SC 3/IITA 1	0.0	1.1	3.3	4.437
34	SC 3/IITA 10	0.0	1.2	3.2	4.486
58	SC 5/IITA 10	0.0	1.2	7.3	4.671
111	SC 9/IITA 3	0.0	1.1	0.7	5.419
22	SC 2/IITA 10	0.1	1.2	6.7	4.891
45	SC 4/IITA 9	0.1	1.2	4.1	4.000
50	SC 5/IITA 2	0.1	1.1	6.1	5.226
137	SC 12/IITA 5	0.1	1.1	4.8	3.355
9	SC 1/IITA 9	0.2	1.2	3.6	3.691
23	SC 2/IITA 11	0.2	1.1	0.9	3.751
28	SC 3/IITA 4	0.2	1.1	1.2	5.202
64	SC 6/IITA 4	0.2	1.1	2.4	5.549
68	SC 6/IITA 8	0.2	1.1	6.8	4.707
85	SC 8/IITA 1	0.2	1.2	10.3	3.384
110	SC 9/IITA 2	0.2	1.1	1.4	5.555
112	SC 9/IITA 4	0.2	1.1	1.8	5.842
113	SC 9/IITA 5	0.2	1.1	3.1	4.790
139	SC 12/IITA 7	0.2	1.2	2.2	4.469
17	SC 2/IITA 5	0.3	1.2	10.4	5.100
71	SC 6/IITA 11	0.3	1.1	3.8	4.639
78	SC 7/IITA 6	0.3	1.1	5.5	4.341
80	SC 7/IITA 8	0.3	1.3	12.1	4.362
93	SC 8/IITA 9	0.3	1.1	3.3	4.623
142	SC 12/IITA 10	0.3	1.1	5.9	4.614
Mean		**0.8**	**1.1**	**4.3**	**4.477**
LSD (5%)		**1.1**	**0.1**	**4.6**	**0.819**
P value		**ns**	**ns**	******	*******

*****P≤0.001; **P≤0.01; *P≤0.05; ns=not significant; LSD=least significant differences; P value=F probability; ER=ear rot; GDS=grain disease score; STB=stalkborer; GY=grain yield (t ha^{-1}); ppm=parts per million**

Table 4.8 Performance of the poorest F1 hybrids in terms of *F. verticillioides* ear rot and other traits from the combined analysis of variance

Entry	Pedigree	ER %	GDS 1 - 9	STB %	GY t ha^{-1}
6	SC 1/IITA 6	0.8	1.1	5.6	3.793
30	SC 3/IITA 6	0.8	1.1	3.5	4.230
51	SC 5/IITA 3	0.8	1.1	3.3	5.292
70	SC 6/IITA 10	0.8	1.1	5.0	4.804
74	SC 7/IITA 2	0.8	1.2	7.4	4.637
97	SC 9/IITA 1	0.8	1.1	3.3	3.663
104	SC 9/IITA 8	0.8	1.2	0.7	4.961
117	SC 9/IITA 9	0.8	1.2	3.3	4.216
123	SC 11/IITA 3	0.8	1.1	8.1	3.584
129	SC 11/IITA 9	0.8	1.1	1.7	4.361
135	SC 12/IITA 3	0.8	1.1	3.2	4.916
143	SC 12/IITA 11	0.8	1.2	2.4	4.544
3	SC 1/IITA 3	0.9	1.1	8.7	4.303
16	SC 2/IITA 4	0.9	1.1	4.6	5.141
43	SC 4/IITA 7	0.9	1.2	4.2	3.920
59	SC 5/IITA 11	0.9	1.2	7.6	4.820
81	SC 7/IITA 9	0.9	1.2	6.5	3.636
82	SC 7/IITA 10	0.9	1.2	3.8	4.449
95	SC 8/IITA 11	0.9	1.2	6.7	4.752
96	SC 8/IITA 12	0.9	1.1	6.2	4.742
101	SC 9/IITA 5	0.9	1.2	3.1	3.612
106	SC 9/IITA 10	0.9	1.2	6.7	3.463
125	SC 11/IITA 5	0.9	1.2	5.7	4.773
130	SC 11/IITA 10	0.9	1.1	2.9	4.744
138	SC 12/IITA 6	0.9	1.2	4.8	3.788
148	M0826-1	0.9	1.2	4.0	3.386
149	M0926-8	0.9	1.3	1.2	4.142
150	M1124-29	0.9	1.1	3.7	3.849
35	SC 3/IITA 11	1.0	1.2	3.8	4.685
61	SC 6/IITA 1	1.0	1.1	7.1	3.672
65	SC 6/IITA 5	1.0	1.2	4.6	4.267
92	SC 8/IITA 8	1.0	1.1	2.7	5.220
108	SC 9/IITA 12	1.0	1.0	3.2	3.613
115	SC 9/IITA 7	1.0	1.1	3.0	4.852
127	SC 11/IITA 7	1.0	1.3	7.9	4.819
136	SC 12/IITA 4	1.0	1.1	3.5	4.969
15	SC 2/IITA 3	1.1	1.2	7.0	4.579
41	SC 4/IITA 5	1.1	1.1	0.8	4.653
73	SC 7/IITA 1	1.1	1.2	2.8	3.902

Table 4.8 (continued) Performance of the poorest F1 hybrids in terms of *F. verticillioides* ear rot and other traits from the combined analysis of variance

Name	Pedigree	ER %	GDS 1 - 9	STB %	GY t ha^{-1}
118	SC 9/IITA 10	1.1	1.1	1.4	4.723
134	SC 12/IITA 2	1.1	1.1	4.2	5.324
140	SC 12/IITA 8	1.1	1.1	1.6	3.884
2	SC 1/IITA 2	1.2	1.2	3.8	4.997
29	SC 3/IITA 5	1.2	1.2	5.1	4.443
52	SC 5/IITA 4	1.2	1.1	3.7	5.938
12	SC 1/IITA 12	1.3	1.2	0.6	4.251
18	SC 2/IITA 6	1.3	1.1	2.9	4.838
38	SC 4/IITA 2	1.3	1.2	6.1	4.812
86	SC 8/IITA 2	1.3	1.1	6.6	5.198
99	SC 9/IITA 3	1.3	1.1	4.1	4.058
102	SC 9/IITA 6	1.3	1.2	2.7	3.605
89	SC 8/IITA 5	1.4	1.2	7.4	4.852
94	SC 8/IITA 10	1.4	1.1	3.8	5.228
126	SC 11/IITA 6	1.4	1.1	3.3	4.755
5	SC 1/IITA 5	1.5	1.2	7.3	4.623
24	SC 2/IITA 12	1.5	1.1	5.5	4.354
42	SC 4/IITA 6	1.5	1.2	4.7	4.431
120	SC 9/IITA 12	1.5	1.1	2.1	5.066
10	SC 1/IITA 10	1.6	1.2	9.1	3.801
66	SC 6/IITA 6	1.6	1.2	4.0	4.994
87	SC 8/IITA 3	1.6	1.1	6.4	5.021
21	SC 2/IITA 9	1.7	1.2	4.4	5.047
76	SC 7/IITA 4	1.7	1.2	5.3	5.436
132	SC 11/IITA 12	1.7	1.2	2.5	4.088
84	SC 7/IITA 12	1.8	1.1	7.8	4.206
105	SC 9/IITA 9	1.9	1.2	4.9	3.895
122	SC 11/IITA 2	1.9	1.2	3.6	3.925
Mean		**0.8**	**1.1**	**4.3**	**4.477**
LSD (5%)		**1.1**	**0.1**	**4.6**	**0.819**
P value		**ns**	**ns**	******	*******

*****P≤0.001; **P≤0.01; *P≤0.05; ns=not significant; LSD=least significant differences; P value=F probability; ER=ear rot; GDS=grain disease score; STB=stalkborer; GY=grain yield (t ha^{-1})**

Table 4.9 Performance of the best F1 hybrids in terms of *F. verticillioides* ear rot and total fumonisins from combined analysis of variance

Name	Pedigree	ER %	GDS 1 - 9	STB %	GY ha^{-1}	B_1	B_2	B_3	Total fumonisin
118	SC 9/IITA 10	1.1	1.1	1.4	4.723	0.1	0.1	0.1	0.2
134	SC 12/IITA 2	1.1	1.1	4.2	5.324	0.1	0.1	0.1	0.2
140	SC 12/IITA 8	1.1	1.1	1.6	3.884	0.1	0.1	0.1	0.2
2	SC 1/IITA 2	1.2	1.2	3.8	4.997	0.1	0.1	0.1	0.2
29	SC 3/IITA 5	1.2	1.2	5.1	4.443	0.1	0.1	0.1	0.2
52	SC 5/IITA 4	1.2	1.1	3.7	5.938	0.1	0.1	0.1	0.2
12	SC 1/IITA 12	1.3	1.2	0.6	4.251	0.1	0.1	0.1	0.2
18	SC 2/IITA 6	1.3	1.1	2.9	4.838	0.1	0.1	0.1	0.2
38	SC 4/IITA 2	1.3	1.2	6.1	4.812	0.1	0.1	0.1	0.2
86	SC 8/IITA 2	1.3	1.1	6.6	5.198	0.1	0.1	0.1	0.2
99	SC 9/IITA 3	1.3	1.1	4.1	4.058	0.1	0.1	0.1	0.2
102	SC 9/IITA 6	1.3	1.2	2.7	3.605	0.1	0.1	0.1	0.2
89	SC 8/IITA 5	1.4	1.2	7.4	4.852	0.1	0.1	0.1	0.2
94	SC 8/IITA 10	1.4	1.1	3.8	5.228	0.1	0.1	0.1	0.2
126	SC 11/IITA 6	1.4	1.1	3.3	4.755	0.1	0.1	0.1	0.2
5	SC 1/IITA 5	1.5	1.2	7.3	4.623	0.1	0.1	0.1	0.2
24	SC 2/IITA 12	1.5	1.1	5.5	4.354	0.1	0.1	0.1	0.2
42	SC 4/IITA 6	1.5	1.2	4.7	4.431	0.1	0.1	0.1	0.2
120	SC 9/IITA 12	1.5	1.1	2.1	5.066	0.1	0.1	0.1	0.2
10	SC 1/IITA 10	1.6	1.2	9.1	3.801	0.1	0.1	0.1	0.2
66	SC 6/IITA 6	1.6	1.2	4.0	4.994	0.1	0.1	0.1	0.2
87	SC 8/IITA 3	1.6	1.1	6.4	5.021	0.1	0.1	0.1	0.2
21	SC 2/IITA 9	1.7	1.2	4.4	5.047	0.1	0.1	0.1	0.2
76	SC 7/IITA 4	1.7	1.2	5.3	5.436	0.1	0.1	0.1	0.2
132	SC 11/IITA 12	1.7	1.2	2.5	4.088	0.1	0.1	0.1	0.2
84	SC 7/IITA 12	1.8	1.1	7.8	4.206	0.1	0.1	0.1	0.2
105	SC 9/IITA 9	1.9	1.2	4.9	3.895	0.1	0.1	0.1	0.2
122	SC 11/IITA 2	1.9	1.2	3.6	3.925	0.1	0.1	0.1	0.2
Mean		**0.8**	**1.1**	**4.3**	**4.477**	**0.1**	**0.1**	**0.1**	**0.2**
LSD (5%)		**1.1**	**0.1**	**4.6**	**0.819**	**0.1**	**0.0**	**0.0**	**0.1**
P value		**ns**	**ns**	******	*******	*******	*******	*****	*******

*****P≤0.001; **P≤0.01; *P≤0.05; ns=not significant; LSD=least significant differences; P value=F probability; ER=ear rot; GDS=grain disease score; STB=stalkborer; GY=grain yield; B_1=fumonisin B_1 analogue; B_2=fumonisin B_2 analogue; B_3=fumonisin B_3 analogue; ppm=parts per million**

When both the fumonisin and *F. verticillioides* ear rot resistance are considered (Table 4.9), F1 hybrids SC 9/IITA 10, SC 12/IITA 2 and SC 12/IITA 8 were the best among the selected hybrids with 1.1% ER across site and years incidence while their total fumonisin content determined from RARS 2012/13 was 0.2 ppm.

The poorest performing F1 hybrids are shown in Table 4.10 where SC 5/IITA 12 (with total fumonisin 0.6 ppm), SC 6/IITA 9, SC 6/IITA 2, SC 5/IITA 6, SC719, SC537 (0.5 ppm total fumonisins), SC 3/IITA 12, SC 1/IITA 7, SC 9/IITA 11 and SC 4/IITA 11 (0.4 ppm total fumonisins) had the most fumonisins.

Table 4.10 Performance of the poorest F1 hybrids in terms of *F. verticillioides* ear rot and total fumonisins from combined analysis of variance

Name	Pedigree	ER %	GDS 1 - 9	STB %	GY ha^{-1}	B_1	B_2	B_3	Total fumonisin
131	SC 11/IITA 11	0.2	1.1	0.7	4.378	0.1	0.1	0.1	0.3
53	SC 5/IITA 5	0.4	1.2	6.9	4.512	0.1	0.1	0.1	0.3
121	SC 11/IITA 1	0.4	1.1	3.1	4.032	0.2	0.1	0.1	0.3
63	SC 6/IITA 3	0.5	1.2	5.0	4.833	0.1	0.1	0.1	0.3
20	SC 2/IITA 8	0.6	1.2	5.1	5.078	0.1	0.1	0.1	0.3
44	SC 4/IITA 8	0.6	1.2	6.0	4.676	0.2	0.1	0.1	0.3
57	SC 5/IITA 9	0.6	1.2	4.3	4.128	0.1	0.1	0.1	0.3
56	SC 5/IITA 8	0.7	1.2	6.4	5.157	0.2	0.1	0.1	0.3
133	SC 12/IITA 1	0.9	1.1	5.0	3.896	0.1	0.1	0.1	0.3
14	SC 2/IITA 2	1.0	1.2	6.1	5.015	0.1	0.1	0.1	0.3
124	SC 11/IITA 4	1.1	1.1	4.4	4.593	0.2	0.1	0.1	0.3
141	SC 12/IITA 9	1.1	1.2	2.7	3.512	0.1	0.1	0.1	0.3
31	SC 3/IITA 7	1.2	1.2	1.5	4.461	0.2	0.1	0.1	0.3
48	SC 4/IITA 12	1.6	1.2	2.3	4.160	0.2	0.1	0.1	0.3
83	SC 7/IITA 11	1.7	1.2	6.6	4.371	0.2	0.1	0.1	0.3
40	SC 4/IITA 4	1.9	1.1	2.6	5.077	0.2	0.1	0.1	0.3
47	SC 4/IITA 11	0.1	1.2	4.8	4.223	0.2	0.1	0.1	0.4
107	SC 9/IITA 11	0.3	1.1	3.4	4.516	0.2	0.1	0.1	0.4
7	SC 1/IITA 7	0.4	1.2	6.8	4.667	0.2	0.1	0.1	0.4
36	SC 3/IITA 12	0.4	1.2	3.1	3.815	0.2	0.1	0.1	0.4
145	SC537	0.5	1.3	2.9	4.225	0.3	0.1	0.1	0.5
147	SC719	0.5	1.2	6.6	5.361	0.3	0.1	0.1	0.5
54	SC 5/IITA 6	0.7	1.2	2.6	4.615	0.3	0.1	0.1	0.5
62	SC 6/IITA 2	1.0	1.1	4.7	4.999	0.3	0.1	0.1	0.5
69	SC 6/IITA 9	1.2	1.2	7.4	3.687	0.3	0.1	0.1	0.5
60	SC 5/IITA 12	0.9	1.1	6.2	3.898	0.4	0.2	0.1	0.6
Mean		**0.8**	**1.1**	**4.3**	**4.477**	**0.1**	**0.1**	**0.1**	**0.2**
LSD (5%)		**1.1**	**0.1**	**4.6**	**0.819**	**0.1**	**0.0**	**0.0**	**0.1**
P value		**ns**	**ns**	******	*******	*******	*******	*****	*******

*****P≤0.001; **P≤0.01; *P≤0.05; ns=not significant; LSD=least significant differences; P value=F probability; ER=ear rot; GDS=grain disease score; STB=stalkborer; GY=grain yield; B_1=fumonisin B_1 analogue; B_2=fumonisin B_2 analogue; B_3=fumonisin B_3 analogue; ppm=parts per million**

The subjective GDS was not significant across all sites.

4.3.1.1 Analysis of variance and F1 hybrid performance in terms of grain yield and other agronomic traits

The combined mean performances of the best 20 F1 hybrids in terms of grain weight in t ha^{-1} are presented in Table 4.11 and Appendix 3. The best hybrid in terms of yield was SC 5/IITA 4 although it was statistically the same with the other 18 hybrids as they all fell within the LSD (5%) value of 0.819. Despite that, the other hybrids SC 9/IITA 4, SC 9/IITA 2, SC 6/IITA 4, SC 1/IITA 4, SC 7/IITA 4, and SC 9/IITA 3 had mean yields above the best commercial check, SC719, which was among the best 20 hybrids. Their days to mid pollen shed and days to mid silking were earlier than that of SC719 with the exception of SC 6/IITA 4 and SC 9/IITA 2 that were the same as SC719 in terms of DMP and DMS respectively.

The poorest hybrids are presented in Table 4.12 and Appendix 3 where F1 hybrids SC 8/IITA 1, SC 1/IITA 1 and SC 12/IITA 5 had a mean yield of 3.4 t ha^{-1}, the same yield as the check hybrid M0826-1. All 20 poorest hybrids were within the LSD (5%) value.

Besides grain yield, DMP, DMS, ASI, PHT, CHT, SL, EASP, EPO, EPP, HC, TEXT, GLS and HT mean squares were highly significant ($P \leq 0.001$).

Table 4.11 Performance of the best 20 F1 hybrids in terms of grain yield from combined analysis of variance

Pedigree	GY	DMP	DMS	ASI	PHT	CHT	RL	SL	EASP	EPO	EPP	HC	TEXT	GLS	HT	MSV	PLS	RUST
SC 5/IITA 4	5.938	69.0	70.0	2.0	1.9	1.1	0.0	20.0	2.2	0.5	0.7	1.3	1.8	2.1	1.6	1.0	0.2	1.2
SC 9/IITA 4	5.842	70.0	71.0	1.0	1.9	1.2	1.9	4.5	3.3	0.5	0.7	1.2	0.5	2.5	1.2	1.0	0.2	1.2
SC 9/IITA 2	5.555	70.0	72.0	1.0	1.8	1.0	0.0	1.4	3.0	0.5	0.8	1.5	0.8	2.6	1.3	1.2	0.2	1.2
SC 6/IITA 4	5.549	71.0	71.0	1.0	1.9	1.1	0.0	10.6	2.4	0.5	0.7	1.8	1.9	2.6	1.3	1.1	0.2	1.2
SC 1/IITA 4	5.524	68.0	70.0	2.0	2.0	1.2	0.0	6.6	3.0	0.5	0.7	1.4	1.0	2.3	0.9	1.0	0.2	1.2
SC 7/IITA 4	5.436	70.0	71.0	1.0	2.1	1.3	1.4	21.0	2.8	0.5	0.8	1.3	1.5	1.9	1.0	1.0	0.2	1.3
SC 9/IITA 2	5.419	69.0	70.0	1.0	1.8	1.0	0.2	6.7	3.1	0.5	0.7	1.2	0.7	2.8	0.9	1.0	0.2	1.2
SC719	5.361	71.0	72.0	1.0	2.0	1.2	0.0	22.5	2.6	0.5	0.7	1.5	1.9	2.1	1.2	1.0	0.2	1.2
SC 12/IITA 2	5.324	71.0	71.0	1.0	1.9	1.1	0.0	3.8	3.0	0.5	0.7	1.5	1.7	2.3	1.3	1.0	0.2	1.2
SC 5/IITA 2	5.292	67.0	69.0	1.0	1.8	0.9	1.0	10.8	2.7	0.4	0.8	1.6	1.7	2.2	1.3	1.0	0.2	1.2
SC 9/IITA 8	5.239	70.0	71.0	1.0	2.0	1.1	0.4	2.8	3.3	0.5	0.7	1.6	0.5	2.4	0.8	1.1	0.2	1.2
SC 8/IITA 10	5.228	68.0	69.0	1.0	1.9	1.0	0.2	14.5	3.1	0.5	0.7	1.4	0.7	2.1	0.9	1.0	0.2	1.2
SC 5/IITA 2	5.226	67.0	68.0	1.0	1.8	1.0	0.3	14.3	3.3	0.5	0.8	1.3	2.2	1.6	1.1	1.0	0.2	1.2
SC 8/IITA 8	5.220	70.0	70.0	0.0	2.0	1.1	0.0	12.2	3.8	0.4	0.8	1.7	0.9	3.0	0.8	1.0	0.2	1.1
SC 3/IITA 4	5.202	69.0	71.0	1.0	1.9	1.2	0.0	6.0	2.5	0.5	0.7	1.5	1.3	2.9	1.5	1.1	0.2	1.2
SC 8/IITA 2	5.198	70.0	70.0	0.0	1.9	1.0	0.0	9.9	3.3	0.5	0.7	1.3	1.2	2.1	0.8	1.0	0.2	1.1
SC 5/IITA 8	5.157	69.0	69.0	0.0	2.0	1.0	0.0	18.2	3.4	0.4	0.7	1.6	1.0	2.5	1.0	1.0	0.2	1.1
SC 2/IITA 4	5.141	68.0	70.0	2.0	2.0	1.1	0.2	12.6	2.3	0.5	0.7	1.3	1.7	2.5	1.2	1.0	0.2	1.2
SC 9/IITA 6	5.108	69.0	71.0	2.0	1.8	1.0	2.0	7.3	3.2	0.5	0.7	1.5	0.5	2.3	0.9	1.0	0.2	1.2
SC 2/IITA 5	5.100	69.0	70.0	1.0	1.9	1.1	0.3	18.7	3.3	0.5	0.8	1.3	1.8	2.1	1.0	1.0	0.2	1.3
Mean	**4.477**	**68.9**	**70.2**	**1.3**	**1.9**	**1.1**	**0.4**	**11.3**	**3.3**	**0.5**	**0.7**	**1.5**	**1.2**	**2.2**	**1.0**	**1.0**	**0.2**	**1.2**
LSD (5%)	**0.819**	**1.0**	**1.2**	**0.9**	**0.1**	**0.1**	**1.3**	**9.5**	**0.5**	**0.0**	**0.1**	**0.4**	**0.3**	**0.5**	**0.3**	**0.1**	**0.1**	**0.1**
P value	***	***	***	***	***	***	ns	***	***	***	***	**	***	***	***	ns	ns	*

*****P≤0.001; **P≤0.01; *P≤0.05; ns=not significant; LSD=least significant differences; P value=F probability; GY=grain yield (t ha^{-1}); DMP=days to mid pollen; DMS=days to mid silking; ASI=anthesis to silking interval; PHT=plant height; CHT=cob height; RL=root lodging; SL=stalk lodging; EASP=ear aspect; EPO=ear position; EPP=ears per plant; HC=husk cover; TEXT=grain texture; GLS=grey leaf spot; HT=*Turcicum* leaf bight; MSV=maize streak virus; PLS= *Phaeosphaeria* leaf spot; B_1=fumonisin B_1 analogue; B_2=fumonisin B_2 analogue; B_3=fumonisin B_3 analogue**

Table 4.12 Performance of the poorest 20 F1 hybrids in terms of grain yield from combined analysis of variance

Pedigree	GY	DMP	DMS	ASI	PHT	CHT	RL	SL	EASP	EPO	EPP	HC	TEXT	GLS	HT	MSV	PLS	RUST
SC 1/IITA 6	3.8	69.0	70.0	1.0	1.8	1.0	0.0	10.9	4.0	0.5	0.7	1.3	0.6	2.4	0.9	1.0	0.2	1.3
SC 12/IITA 6	3.8	69.0	70.0	1.0	1.8	1.1	0.7	9.6	3.6	0.5	0.6	1.8	2.0	2.3	0.8	1.1	0.2	1.2
SC 2/IITA 11	3.8	66.0	66.0	0.0	1.8	1.0	0.5	3.5	2.2	0.5	0.8	1.6	2.0	2.0	1.8	1.0	0.2	1.3
SC 4/IITA 1	3.7	71.0	72.0	1.0	2.0	1.3	0.0	15.1	3.4	0.6	0.7	1.5	1.0	2.3	1.1	1.0	0.2	1.2
SC 1/IITA 9	3.7	70.0	72.0	3.0	1.8	1.1	0.0	2.8	3.8	0.5	0.6	1.8	0.9	2.1	1.0	1.0	0.2	1.3
SC 6/IITA 9	3.7	70.0	72.0	2.0	1.9	1.1	0.2	6.1	3.3	0.5	0.6	1.3	1.6	2.6	1.1	1.0	0.2	1.2
SC 6/IITA 1	3.7	70.0	72.0	2.0	1.9	1.1	1.3	12.1	3.5	0.5	0.6	1.2	2.0	2.4	0.9	1.0	0.2	1.3
SC 9/IITA 1	3.7	70.0	71.0	1.0	1.9	1.1	0.0	24.9	3.7	0.5	0.7	1.6	0.5	2.2	0.8	1.0	0.2	1.2
SC 7/IITA 9	3.6	70.0	71.0	2.0	2.0	1.1	0.0	23.8	3.4	0.5	0.6	1.5	1.3	2.3	1.3	1.0	0.2	1.3
SC 9/IITA 12	3.6	69.0	70.0	1.0	1.6	0.9	0.2	6.1	4.0	0.5	0.7	1.6	1.0	2.1	1.0	1.0	0.3	1.2
SC 9/IITA 5	3.6	71.0	72.0	1.0	2.0	1.1	0.5	13.2	3.1	0.5	0.6	1.6	1.3	2.0	0.8	1.0	0.2	1.3
SC 9/IITA 6	3.6	68.0	69.0	1.0	1.7	1.0	0.4	22.5	3.4	0.5	0.7	1.8	0.8	2.3	0.9	1.0	0.2	1.2
SC 6/IITA 12	3.6	69.0	70.0	2.0	1.8	1.0	0.2	6.0	4.1	0.5	0.7	1.4	1.8	2.3	1.2	1.1	0.2	1.2
SC 11/IITA 2	3.6	67.0	69.0	1.0	1.9	1.1	0.5	6.9	3.8	0.5	0.7	1.3	1.2	1.9	1.0	1.0	0.2	1.2
SC 12/IITA 9	3.5	70.0	72.0	2.0	1.9	1.2	0.0	5.5	3.4	0.5	0.6	1.3	1.8	2.2	1.3	1.0	0.2	1.3
SC 9/IITA 10	3.5	69.0	71.0	3.0	2.0	1.2	0.0	18.2	3.2	0.5	0.7	1.5	1.1	2.5	0.8	1.0	0.2	1.3
M0826-1	3.4	70.0	71.0	1.0	2.0	1.1	0.0	13.1	4.1	0.4	0.6	1.3	1.3	2.0	1.4	1.0	0.2	1.2
SC 8/IITA 1	3.4	70.0	71.0	1.0	1.9	1.1	1.3	23.7	3.7	0.5	0.6	1.8	1.2	2.1	0.9	1.1	0.2	1.2
SC 1/IITA 1	3.4	69.0	71.0	2.0	2.0	1.1	0.8	16.9	3.9	0.5	0.7	1.3	0.6	2.3	0.9	1.0	0.2	1.3
SC 12/IITA 5	3.4	69.0	70.0	1.0	2.0	1.2	0.5	16.0	3.0	0.5	0.6	1.4	1.6	2.1	1.2	1.0	0.2	1.2
Mean	**4.5**	**68.9**	**70.2**	**1.3**	**1.9**	**1.1**	**0.4**	**11.3**	**3.3**	**0.5**	**0.7**	**1.5**	**1.2**	**2.2**	**1.0**	**1.0**	**0.2**	**1.2**
LSD (5%)	**0.8**	**1.0**	**1.2**	**0.9**	**0.1**	**0.1**	**1.3**	**9.5**	**0.5**	**0.0**	**0.1**	**0.4**	**0.3**	**0.5**	**0.3**	**0.1**	**0.1**	**0.1**
P value	***	***	***	***	***	***	ns	***	***	***	***	**	***	***	***	ns	ns	*

*****P≤0.001; **P≤0.01; *P≤0.05; ns=not significant; LSD=least significant differences; P value=F probability; GY=grain yield (t ha^{-1}); DMP=days to mid pollen; DMS=days to mid silking; ASI=anthesis to silking interval; PHT=plant height; CHT=cob height; RL=root lodging; SL=stalk lodging; EASP=ear aspect; EPO=ear position; EPP=ears per plant; HC=husk cover; TEXT=grain texture; GLS=grey leaf spot; HT=*Turcicum* leaf bight; MSV=maize streak virus; PLS=** *Phaeosphaeria* leaf spot

4.3.2 Inbred line performance

4.3.2.1 Analysis of variance for the parent inbred trials

The mean squares for various traits recorded on the parent trials are presented in Table 4.13. The location mean squares were highly significant (P≤0.001) for the seven traits, ER,GDS, GY, DMP, DMS, ASI and GLS, so were mean squares for entries, except for ER and GDS. Block in location x year were highly significant for DMS and GLS (P≤0.001) and GDS (P≤0.01) and significant for DMS (P≤0.05). For year, the mean squares were highly significant for DMP, DMS, ASI, GDS and GLS (P≤0.001) and were significant for ER (P≤0.05). The mean squares for the interaction of location and entry were highly significant for GY, DMP, DMS (P≤0.001) and GLS (P≤0.01) and significant for GDS and ASI (P≤0.05) while all the traits' mean squares for location x year were highly significant (P≤0.001). The mean squares for the entry x year interactions were highly significant (P≤0.001) for GY, DMP (P≤0.001) and DMS (P≤0.01) but significant (P≤0.05) for GDS while that of the location x entry x year were highly significant for GY, DMP, DMS (P≤0.001) and ASI P≤0.01) and again for the GDS it was significant (P≤0.05).

4.3.2.2 Parent inbred line performance in terms of *F. verticillioides* ear rot, grain disease score and fumonisin

At RARS, there were no significant differences among the *F. verticillioides* ear rots, the grain diseases score and the fumonisin measured by B_1, B_2, B_3 and their summation (Table 4.14). This was despite the *F. verticillioides* ear rot incidence range of 0% to 38.9% where inbreds IITA 10, IITA 5, IITA 2, SC 7 and SC 3 had incidences below 5% (0.0%, 1.4%, 2.2%, 3.5% and 5.0% respectively). Maize inbred lines with the highest incidences were SC 4, IITA 12, IITA 1 and SC 1 that had incidences above 20% (23.1%, 25.3%, 27.8% and 38.9% respectively).

Table 4.13 Combined analysis of variance for parent lines at six sites in the 2012/13 and 2013/14 seasons

	DF	ER	GDS	GY	DMP	DMS	ASI	GLS
		%	1-9	t ha^{-1}	Days	days	days	1-9
Location	2	1543.31***	4.82***	75.09***	544.42***	196.22***	101.09***	284.42***
Entry	23	58.84	0.15	2.09***	35.08***	46.32***	10.77***	1.96***
Block in location x year	2	118.41	0.59**	0.43	20.31***	15.7*	2.42	6.52***
Year	1	239.71*	7.67***	0.14	124.05***	355.03***	72.00***	238.35***
Location x entry	46	58.68	0.21*	0.74***	6.50***	10.96***	5.39*	1.12**
Location x year	3	914.20***	1.50***	1.99***	171.03***	156.13***	43.97***	224.96***
Entry x year	23	63.41	0.25*	0.61***	6.06***	8.99**	4.63	1.00
Location x entry x year	46	33.22	0.17	0.32***	6.36***	9.16***	5.95**	0.88
Error	142	47.57	0.14	0.155	2.46	4.26	3.39	0.64
Total	287							

*****P≤0.001; **P≤0.01; *P≤0.05; DF=degrees of freedom; ER=ear rot; GDS=grain disease score; GY=grain yield; DMS=days to mid pollen; DMS=days to mid silking; ASI=anthesis to silking interval; GLS=grey leaf spot**

Table 4.14 Performance of parent lines at Rattray Arnold Research Station in the 2012/13 season where artificial inoculation was done and square root transformed fumonisin data derived

Entry	Name	ER %	B_1 ppm	B_2 ppm	B_3 ppm	Total ppm	GDS 1 - 9
10	IITA 10	0.0	0.9	0.8	0.8	2.6	1.0
5	IITA 5	1.4	0.9	0.8	0.8	2.6	1.0
3	IITA 3	2.2	1.1	0.9	0.9	2.9	1.0
18	SC 7	3.5	0.8	0.8	0.8	2.5	1.0
15	SC 3	5.0	0.8	0.8	0.8	2.5	1.5
14	SC 2	5.9	1.0	0.9	0.8	2.7	1.0
2	IITA 2	6.0	0.9	0.8	0.8	2.6	1.0
20	SC 9	6.1	1.1	1.0	0.9	3.0	1.0
7	IITA 7	7.8	0.9	0.8	0.8	2.6	1.0
6	IITA 6	7.9	1.0	0.9	0.8	2.8	1.0
9	IITA 9	8.3	1.0	0.8	0.8	2.7	1.0
21	SC 10	9.3	1.0	0.9	0.8	2.7	1.0
17	SC 6	10.1	0.9	0.8	0.8	2.6	1.0
23	SC 12	11.6	0.8	0.8	0.8	2.5	1.0
11	IITA 11	12.3	0.9	0.9	0.9	2.6	1.0
22	SC 11	12.6	0.9	0.8	0.8	2.6	1.5
4	IITA 4	12.7	0.8	0.8	0.8	2.5	1.0
19	SC 8	13.5	1.1	0.9	0.9	2.9	1.0
16	SC 5	17.3	0.9	0.8	0.8	2.6	1.0
8	IITA 8	19.1	0.9	0.8	0.8	2.6	1.0
24	SC 4	23.1	1.0	0.9	0.8	2.7	1.0
12	IITA 12	25.3	0.8	0.8	0.8	2.5	1.0
1	IITA 1	27.8	0.9	0.8	0.8	2.6	1.0
13	SC 1	38.9	1.0	0.9	0.8	2.7	1.0
	Mean	**12.0**	**0.9**	**0.8**	**0.8**	**2.6**	**1.0**
	SE	**32.4**	**0.3**	**0.1**	**0.1**	**0.4**	**0.4**
	LSD (5%)	**27.7**	**0.2**	**0.1**	**0.1**	**0.4**	**0.3**
	P value	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**

*****P≤0.001; **P≤0.01; *P≤0.05; ns=not significant; SE=standard error; LSD=least significant differences; P value=F probability; ER=ear rot; GDS=grain disease score; B_1=fumonisin B_1 analogue; B_2=fumonisin B_2 analogue; B_3=fumonisin B_3 analogue**

The inbred line IITA 4 had the highest yield of 2.6 t ha^{-1} across sites and years which was significantly higher than the second inbred IITA 10 that had a yield of 2.1 t ha^{-1} which in turn, statistically, had the same yield as inbreds IITA 12, IITA 7, IITA 3 and SC 12. The lowest yielding inbred was SC 1 whose yield of 0.988 t ha^{-1} was not significantly different from that of SC 6, SC 9, IITA 6, SC 7, IITA 9 and IITA 1.

The mean square for the incidence of stalk-borer (STB) was highly significant (P≤0.01) where inbred lines IITA 12, IITA 8 and IITA 9 had incidences below 2% (Table 4.15).

Table 4.15 Performance of parent lines in terms of *F. verticillioides* ear rot, grain disease score and other agronomic traits across sites and y

Entry	Name	GY	MOI	ER	GDS	STB	DMP	DMS	ASI	PHT	CHT	EASP
		t ha^{-1}	%	%	1-9	%	Days	Days	Days	cm	cm	1-9
4	IITA 4	2.643	11.7	3.3	1.3	5.6	73.0	73.0	0.0	130.0	79.2	2.1
10	IITA 10	2.113	10.4	0.5	0.9	2.8	73.0	75.0	2.0	124.6	72.1	2.6
12	IITA 12	2.106	10.8	4.7	1.0	1.5	72.0	73.0	2.0	117.1	57.9	3.8
7	IITA 7	1.945	10.1	1.8	1.3	3.8	73.0	74.0	1.0	152.9	82.1	2.8
3	IITA 3	1.884	9.6	0.9	1.1	7.9	71.0	72.0	0.0	119.6	69.2	3.3
23	SC 12	1.859	10.6	2.2	0.9	2.8	73.0	74.0	1.0	121.3	67.5	2.3
14	SC 2	1.834	10.1	1.0	1.0	12.6	69.0	72.0	3.0	123.3	66.7	3.3
22	SC 11	1.719	10.0	2.8	1.1	7.0	72.0	75.0	3.0	127.5	62.9	3.3
15	SC 3	1.654	10.7	0.8	1.0	2.4	72.0	74.0	2.0	113.8	60.8	2.8
5	IITA 5	1.599	9.4	0.6	1.0	4.2	74.0	76.0	2.0	144.2	83.8	2.5
21	SC 10	1.528	11.6	2.7	1.1	9.2	73.0	76.0	3.0	130.4	68.8	3.1
16	SC 5	1.52	10.1	3.6	1.0	9.1	70.0	71.0	1.0	124.2	70.8	3.7
8	IITA 8	1.475	9.7	3.8	1.1	1.3	75.0	75.0	1.0	141.3	80.4	3.3
19	SC 8	1.436	9.8	2.3	1.0	10.7	75.0	77.0	1.0	115.4	58.8	3.4
2	IITA 2	1.425	11.4	2.1	1.0	3.6	75.0	77.0	2.0	120.4	65.8	3.3
24	SC 4	1.314	10.3	3.8	1.1	7.1	74.0	74.0	0.0	123.3	80.0	3.2
11	IITA 11	1.264	9.9	2.3	1.0	3.4	74.0	74.0	0.0	123.3	67.5	2.8
17	SC 6	1.193	11.0	2.4	1.1	5.3	73.0	75.0	2.0	133.8	71.7	3.7
20	SC 9	1.157	10.9	8.8	1.3	5.5	71.0	72.0	1.0	112.9	62.1	3.5
6	IITA 6	1.105	10.9	2.3	1.2	2.2	71.0	71.0	0.0	99.6	57.5	3.2
18	SC 7	1.091	10.7	3.0	1.3	7.0	74.0	77.0	3.0	141.7	81.3	3.3
9	IITA 9	1.073	11.5	4.1	1.2	1.1	75.0	77.0	2.0	112.1	72.9	2.7
1	IITA 1	0.999	8.9	5.0	0.9	0.0	76.0	78.0	2.0	109.6	67.9	3.2
13	SC 1	0.988	8.9	9.0	1.1	2.0	73.0	76.0	3.0	120.4	61.7	2.4
Mean		**1.538**	**10.4**	**3.1**	**1.1**	**4.9**	**73.0**	**74.5**	**1.5**	**124.3**	**69.5**	**3.0**
SE		**0.787**	**4.1**	**13.8**	**0.7**	**15.7**	**3.1**	**4.1**	**3.7**	**41.5**	**26.0**	**1.3**
LSD (5%)		**0.266**	**1.4**	**4.7**	**0.2**	**5.3**	**1.1**	**1.4**	**1.2**	**14.0**	**8.8**	**0.4**
P value		*******	*****	**ns**	**ns**	******	*******	*******	*******	*******	*******	*******

*****P≤0.001; **P≤0.01; *P≤0.05; ns=not significant; SE=standard error; LSD=least significant differences; P value=F probability; GY=grain yield (t ha^{-1}); MOI=moisture content; ER=ear rot; GDS=grain disease score; STB=stalkborer; DMP=days to mid pollen; DMS=days to mid silking; ASI=anthesis to silking interval; PHT=plant height; CHT=cob height; EASP=ear aspect; TEXT=grain texture**

The mean squares for other agronomic traits that included DMP, DMS, ASI, PHT, CHT and EASP were highly significant (P≤0.001).

4.4 Discussion

The ER incidence was low while its mean square was significant even for RARS where artificial inoculation was done in the 2012/13 season where hybrids SC 2/IITA 7, SC 3/IITA 1, SC 3/IITA 10, SC 5/IITA 10 and SC 9/IITA 3 had a mean *F. verticillioides* incidence value of 0%. Naturally, this site records lower incidences which can be attributed to the environment that may not be conducive for the sporulation of the fungus, even after inoculation. This is contrary to Bolduan *et al*. (2009) who observed higher severity of Gibberella ear rot and Fusarium ear rot where artificial inoculation was done when compared with where the crop was left to natural infection. However, *F. verticillioides* has been observed to exist in high incidences in asymptomatic form as infection may not be seen by a naked eye while its occurrence may be in substantial amount (Suleiman *et al*., 2013). Such observations have been attributed to low correlations between occurrence of fumonisins and *F. verticillioides* ear rot incidences. The observed fumonisin analogues are suggestive of the presence of the fungi as it can only occur when the fungi exist or once existed. Most of the mean squares for traits in the F1 hybrid trials were significant, indicating enough variability within the germplasm. Locations as well as the interaction of the location with years were significant for all the traits. This could be due to known variability within the locations such as KRC which is known to be located within the region where rainfall is low and heat and drought generally characterise the site. Such a site is characteristic of what Bottalico (1998) described as favourable for Fusarium ear rot, since it is warm and dry which induces stress that Miller (2001) attributed to elevation of severity. SRC is a site associated with natural occurrence of ear rotting causing fungi such as *F. verticillioides* with high rainfall potential while at RARS, probably because it is located in a high rainfall zone, the occurrence of ear rot causing fungi is not prevalent (Bolduan *et al*., 2009). Variation for reaction to *F. verticillioides* ear rot and fumonisin content existed within the F1 hybrids. Level of resistance to fumonisin was high for 69 out of 150 hybrids that included 144 F1 hybrids and six check hybrids having a total of nil to undetectables

levels of fumonisin analogues B_1, B_2 and B_3. Inbred lines used as lines or females such as SC 10, SC 8, SC 12, SC 4, SC 3 and SC 9, appeared in at least six hybrids out of a maximum possible 12 of these resistant F1 hybrids. The tester inbred lines IITA 2, IITA 3, IITA 7, IITA 10 and IITA 1 also prominently appeared with high frequencies in these F1 hybrids with nil to undetectable levels of fumonisins. Inbred lines SC 8, IITA 2 and IITA 10 appeared in nine F1 hybrids and this suggests high general combining ability for resistance to fumonisins. Inversely, the inbred lines contributing more to susceptibility were SC 5, SC 4, SC 11, IITA 9, IITA 11 and IITA 12 as they were parents of F1 hybrids that were most susceptible that included SC 5/ IITA 12, SC 5/ IITA 6 and SC 6/ IITA 2. Resistance to *F. verticillioides* ear rot was substantial in 72 hybrids, with incidences below the mean in the combined analysis of the trials across sites and years. Among the hybrids with resistance were SC 2/ IITA 7, SC 3/ IITA 1, SC 3/ IITA 10, SC 5/IITA 10 and SC 9/ IITA 3 that had a mean incidence of 0%. The poorest hybrids included SC 11/ IITA 2, SC 9/ IITA 9, SC 7/ IITA 12, SC 2/ IITA 9, SC 7/ IITA 4 and SC 11/ IITA 12. The frequencies of inbred lines SC 7, SC 11, IITA 4, IITA 9 and IITA 12 are conspicuous in the most *F. verticillioides* ear rot susceptible F1 hybrids.

The F1 hybrids that had both low fumonisin content and low *F. verticillioides* ear rot incidences at RARS 2012/13 season included SC 2/ IITA 7, SC 3/ IITA 1, SC 3/ IITA 10, SC 5/ IITA 10 and SC 9/ IITA 3. Incidentally, these were the best hybrids when *per se* performance under *F. verticillioides* ER was considered. This is consistent with findings by Robertson *et al*. (2006) where they reported the existence of high genotypic correlations between Fusarium ear rot symptoms and fumonisins within the Corn Belt germplasm of the USA. The inbreds SC 3 and IITA 10 appeared more frequently within the resistant hybrids while the inbred line SC 6 was the most frequently occurring inbred among the F1 hybrids that had the least combinations for both ER incidence and fumonisin contamination. The inbred lines Mp715 and Mp717, bred in Mississippi, were reported to have cross resistance to both the ear rot causing pathogens and mycotoxins (Williams and Windham, 2009). Inbred lines SC 3, SC 10, IITA 10, IITA 3, IITA 7 and IITA 1 appeared in the best hybrids at RARS where artificial inoculation was done, when *per se*

performance under *F. verticillioides* ER was considered and when both ER and fumonisin analogues were taken into consideration. By the same token, lines SC 4, IITA 9, IITA 11 and IITA 12 frequently appeared in the poorest hybrids at RARS, in the combined analysis and where both *F. verticillioides* ER and fumonisins were considered. Inbred line SC 5 was a parent in some of the poorest hybrids at RARS and when both fumonisin and ER were considered.

F1 hybrids SC 9/IITA 10, SC 12/IITA 2 and SC 12/IITA 8 were the best among the selected hybrids with low ER across site and years incidence and low total fumonisin content. On the other hand, the poorest performing F1 hybrids in terms of fumonisin content were SC 5/IITA 12, SC 6/IITA 9, SC 6/IITA 2, SC 5/IITA 6, SC719, SC537, SC 3/IITA 12, SC 1/IITA 7, SC 9/IITA 11 and SC 4/IITA 11.

The GDS scores mean squares were not significant which is consistent with Campbell and White (1995) who observed that the evaluation of ear rots gave a more reliable estimate of aflatoxin accumulation compared with evaluation of kernels as is the practice when the GDS is done.

The hybrid SC 5/ IITA 4 had the highest yield. This however, was within the LSD (5%) value with 18 F1 hybrids including SC 6/ IITA 4, SC 1/ IITA 4, SC 7/ IITA 4 and SC 10/IITA 3 that had mean yields exceeding that of the best commercial check, SC719. These flowered earlier than SC719 while SC 6/IITA 4 and SC 9/IITA 2 had the same days to flowering as SC719. Of the best 20 hybrids, the most frequently occurring inbred lines were IITA 4 (in seven hybrids), SC 10 (in five hybrids), SC 5 and IITA 2 (in four hybrids), SC 8 and IITA 8 (in three hybrids) and inbreds SC 3 and IITA 2 (in two hybrids). Inbred IITA 4 appeared in combination with SC 5, SC 10, SC 6, SC 1, SC 7, SC 3 and SC 2 while the line SC 10 was in combination with IITA 4, IITA 2, IITA 3, IITA 8 and IITA 6. It therefore implies high general combining ability for grain yield, hence these lines have a high breeding value that can be used for yield improvement. Some lines also appeared more frequently among the poorest yielding 20 hybrids and these included SC 9 and IITA 1 that

appeared in five hybrids, IITA 9 that constituted four hybrids, SC 12, SC 8, SC 1 and IITA 6 that were in three hybrids, and IITA 5 and IITA 12 that were in two hybrids .

Inbred line SC 5, although observed among the highest yielding hybrids was a constituent of the worst hybrids in terms of *F. verticillioides* ear rot at RARS. This corroborates earlier observation that resistant lines tend to have poor agronomic traits (Menkir *et al*., 2008; Warburton *et al*., 2009).

For the inbred line trial that was conducted adjacent to the F1 hybrid trial, the locations and the interaction of the location and the year were highly significant for all the traits just as with the hybrid trial. The entry effect was significant for all traits except the *F. verticillioides* ear rot incidence and the GDS score.

Where artificial inoculation was done at RARS in the 2012/13 season, *F. verticillioides* ER, GDS and the fumonisin B_1, B_2, B_3 and the total of the three did not differ significantly suggesting all inbreds reacted equally to the fungus and the mycotoxin. This could be as a result of low incidence. The GDS was too low for differences to be observed.

Differences between most other agronomic traits were highly significant across sites and years. The inbred line IITA 4 from central and West Africa had the highest yield that was significantly above that of the next inbred line, IITA 5 also from the IITA. This inbred, IITA 4, is the line constituting most of the best F1 hybrids in terms of yield, followed by inbred IITA 3 that combined well with SC 10. The inbred SC 1 was the lowest yielding line and was one of the most frequently occurring lines in the lowest yielding F1 hybrids. It therefore suggests the importance of GCA in yield, which is associated with additive gene effects. Where additive effects are important, selection can be done at line level where a line exhibiting traits of interest can be used in combination with other good lines with a high probability of observing higher advantage in the offspring due to additivity.

Despite prophylactic control measures, stalk-borer incidences were high which may contribute towards high incidences of fungi such as *F. verticillioides* that, in turn, may increase the level of fumonisins and GDS. Apparently this was not the case in this study, probably due to low levels of the pathogen itself or poor environment for the disease to thrive. Insects such as stalk-borers form part of several infection pathways by *F. verticillioides* that include infection through silks and transmission systemically from the soil or seed through the root to the kernel (Munkvold and Carlton, 1997; Sobek and Munkvold, 1999).

4.5 Conclusions

Maize production in Zimbabwe has improved with the adoption of high yielding hybrids without focusing on devastating catastrophic effects that are caused by fungal metabolites such as *F. verticillioides*, the fumonisins. This study has revealed enough variability within the germplasm used in terms of its reaction to *F. verticillioides* ear rot and the fumonisins themselves, despite low incidences observed. Artificial inoculation was effective in creation of significant differences in terms of *F. verticillioides* ear rot among the F1 hybrids although no significant differences were observed in the inbred line trial. Potential single crosses as well as inbred lines have been identified that can be used in further development of resistance to the causal pathogen and the fumonisins themselves. Inbred lines such as those from the southern African mid-altitude SC 10, SC 5, SC 2 and SC 3 and those from central and West African mid-altitude IITA 3, IITA 7, IITA 10 and IITA 1 have been identified as sources of resistance to both the fumonisins and the visible ear rots caused by *F. verticillioides*. Their combinations in making single cross hybrids SC 2/ IITA 7, SC 3/ IITA 1, SC 3/ IITA 10, SC 5/ IITA 10 and SC 9/ IITA 3 resulted in both low incidences of ear rots and low levels of fumonisins. In terms of yield, the inbred line IITA 4 was superior to all other hybrids and occurred most among the highest yielding single crosses that included SC 5/188, SC 9/ IITA 4, SC 9/IITA 2, SC 6/IITA 4, SC 1/IITA 4, SC 7/IITA 4 and SC 10/IITA 3. The IITA inbred lines can therefore be used in combination with southern African mid-altitude inbred lines (Dhliwayo *et al*., 2009), hence superior performance in terms of yield against the best commercial check hybrid SC719. The high

heterosis can be explained by high diversity between the lines as they originated from distant regions. It may also be attributed to the poor performance of the inbred lines when grown in a different environment where it is not adapted to. Makumbi *et al*. (2011) reported high heterosis when both the F1 and inbred lines were grown under drought stress conditions which they associated with poor performance of inbred lines under stress.

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Chapter 5

Line x Tester analysis of mid-altitude inbred lines from southern Africa, central and West Africa for *Fusarium verticillioides* infection and fumonisin accumulation

Abstract

Prevalence of ear rotting fungi, some of which produce metabolites that cause various diseases in both consumers and their livestock, is rampant in Zimbabwe. The objectives of this study were to determine the inheritance of resistance to *Fusarium verticillioides* and its metabolite fumonisins and other agronomic traits of importance. Twelve inbred lines each from Seed Co and IITA were mated using the modified NCDII mating design analysed as a line x tester design. The resulting 144 F1 hybrids and six check hybrids were evaluated at three sites in Zimbabwe in the 2012/13 and 2013/14 seasons and in Nigeria in 2013. Artificial inoculation with *F. verticillioides* was done at RARS in 2012/13. The GCA and SCA effects for *F. verticillioides* incidence and fumonisin contamination were variable across sites for both the lines and testers. Significant GCA effects for grain yield, days to mid pollen, days to mid silk and anthesis to silking interval were observed across all sites. Both additive and non-additive gene effects had a role in conferring resistance to ear rots and fumonisins with additive gene effects playing a major role in the fumonisins, particularly for the southern African inbred lines. Additive gene effects played a major role in agronomic traits such as grain yield and flowering related traits such as days to mid-pollen, days to mid-silking and anthesis to silking interval. The southern African inbred lines SC 2, SC 3, SC 4, SC 9, SC 11 and SC 12 had desirable GCA for *F. verticillioides* ear rot and can be used as a source for resistance. Among these, SC 2, SC 3 and SC 12 had negative GCA for fumonisins. Outstanding testers in terms of ear rots and fumonisins were IITA 1, IITA 3, IITA 4, IITA 5, IITA 6, IITA 7 and IITA 8 that had negative GCA for *F. verticillioides* ear rot and for fumonisins. Good agronomic attributes were shown by lines SC 7, SC 9 and SC 10 and testers IITA 2 and IITA 4. Both additive and non-additive effects were implicated in resistance to ear rot caused by *F. verticillioides* and potential lines were identified that can be used in these regional breeding programmes.

5.1 Introduction

Commercially available maize cultivars in most parts of the world do not have specific resistance to mycotoxins including fumonisins and aflatoxins (Brooks *et al*., 2005). This could be attributed to the general observation that maize cultivars widely cultivated in the USA, and other countries were developed from a narrow germplasm base that are known to be susceptible to both *Aspesgillus flavus* and *Fusarium* species that produce mycotoxins. In spite of this, diverse sources of resistance to aflatoxin (Busboom and White, 2004; Brooks *et al*., 2005; Menkir, 2008; Warburton *et al*., 2009; Williams and Windham, 2009) and fumonisin (Busboom and White, 2004; Robertson *et al.*, 2006) production have been found, although as few as three hybrids out of 14 commonly grown in North Carolina (USA), have been reported to show low levels of both the Fusarium ear rots as well as fumonisin accumulation (Busboom and White, 2004; Robertson *et al.*, 2006).

The known sources of resistance originating from the temperate zone tend to exhibit poor adaptability and have poor agronomic traits, particularly in sub-Saharan Africa. These lines also have poor combining ability for yield (Menkir *et al*., 2008; Warburton *et al*., 2009). However, some sources of resistance have been identified (Menkir *et al*., 2008; Warburton *et al*., 2009) including those adapted to central and West Africa and southern Africa (Menkir *et al*., 2008).

Among the control measures, good cultural practices are recommended to reduce mycotoxin build up and this includes the use of maize varieties resistant to ear rot causing fungi including *Aspergillus* and *Fusarium* species. When the resistant varieties are used in combination with cultural practices, the level of mycotoxins can significantly be reduced as maize varieties with complete resistance to *Fusarium* species and fumonisin production are not available (Robertson *et al*., 2006).

Breeding for resistant germplasm is the most effective strategy as it has less detrimental environmental effects while it can be applied in all socio-economic environments

(Busboom and White, 2004; Clements *et al.*, 2004; Menkir *et al*., 2008; Warburton *et al.*, 2009).

Robertson-Hoyt *et al*. (2007) observed positive correlation between resistance to aflatoxins and fumonisins caused by *A. flavus*, an ear rot fungus that occurs mostly in the central and West African region, and *F. verticillioides* that is the main cause of ear rotting in southern Africa, respectively. It therefore suggests that the genes controlling these mycotoxins occurring in the southern, central and West African regions could be the same, as two QTLs were also associated with resistance to both mycotoxins (Robertson-Hoyt *et al*., 2007).

Since the cost of analysing various mycotoxins is high, selection for resistance to the causal fungi has been done indirectly through visual assessment of ears harvested with less ear rots, loose husk cover that allows for a fast dry-down, good husk cover that allows less direct contact of water with kernels and ears that droop after physiological maturity that limits accumulation of moisture in the cob and silk characteristics. When resources permit, selection of ears with less mycotoxins is an indirect way of selecting for plants with less ear rot infection. This could explain the observation by Brown *et al*. (1995) that in some genotypes, the resistance to colonisation by fungi is linked to resistance to aflatoxin accumulation. However, selection for mycotoxins based on ear rots may be misleading as some cultivars, although being asymptomatic in terms of causal organisms, could still be carrying the causal fungi and possibly the mycotoxin exuded by that particular fungi.

The breeding process starts with screening to determine which lines are resistant directly or using the above indirect traits. This needs to be reliable and repeatable. Since genotype by environment (GxE) interaction is high for the ear rot causing fungi (Mukanga *et al*., 2010), artificial inoculation becomes of paramount importance (Windham *et al*., 2003). Brown *et al*. (1995) developed a rapid laboratory method for infecting and screening for resistance to aflatoxins, which is highly correlated to field screening.

When sources of resistance have been identified, they can be used in breeding for resistance as additive and dominant gene effects have been associated with resistance to mycotoxin causing fungi (Mukanga *et al*., 2010). Additive gene effects have been attributed to resistance to mycotoxins such as aflatoxins (Campbell *et al*., 1997) who also observed relatively high heritability from generation mean analysis (66%). Gorman *et al.* (1992) found both non-additive effects and additive gene effects playing a significant role in the resistance to aflatoxins. An interaction of GCA effects with different types of aflatoxins was found (Gorman *et al*., 1992) implying that introgression of resistance to one form of aflatoxin would lead to introduction of resistance to the other types. Additive gene effects have been reported to play a larger role as GCA was found to have a greater effect than SCA on resistance to aflatoxin accumulation in grain (Zuber *et al*., 1978; Darrah *et al*., 1987). While working on aflatoxins and secondary traits to evaluate occurrence of *A. flavus*, Maupin *et al*. (2003) reported dominance playing a major role. Additive gene effects were attributed to resistance to *A. flavus* while non-additive gene effects, epistasis specifically, were associated with aflatoxin resistance (Walker and White, 2001). The additive and non-additive effects were further explained in terms of QTL analysis by Perez-Brito *et al*. (2001) who found in one population, on chromosomes 1, 2, 3, 4, 6, 7 and 10 nine QTLs that explained 30-44% of the variance associated with the phenotype, while in the other population, on chromosomes 1, 2, 3, 4, 5, 6 and 7, 11-26% of the variance was explained by seven QTLs. Three QTLs that were common in both populations were found on chromosomes 3 and 6. Earlier on, Widstrom *et al*. (1987) identified 2-5 QTLs of which two QTLs were significantly associated with resistance. Such observations all point to the importance and significance of both additive and non-additive gene effects.

The objectives of this study were to determine the inheritance of resistance to *Fusarium verticillioides* and its metabolite fumonisins and other agronomic traits of importance using a North Carolina Design II (NCDII) mating design between the central and West African mid-altitude and southern African mid-altitude maize inbred lines with varying levels of resistance to ear rot causing fungi. It was also aimed at determining the usability of such inbred lines in terms of agronomic performance across these regions.

5.2 Materials and methods

Twenty four mid-altitude lines were used for this study. These included twelve from southern Africa and twelve from central and West Africa developed by Seed Co and IITA respectively. The southern Africa lines were selected on the basis of their reaction to to *F. verticillioides* and other ear-rot causing fungi including *Diplodia maydis* (Berk.) Sacc. [=*Stenocarpella maydis* (Berk.) Sutton] while those from central and West Africa were selected from their reaction to ear rots and aflatoxin. Details of the lines are presented in section 4.2. The lines were classified based on their known reaction to ear rot causing fungi such as *Stenocarpella maydis,* Fusarium species and aflatoxin production and geographic origin. These parental lines were crossed in a modified NCDII mating design (Comstock and Robinson, 1948).

The three groups from southern Africa were mated to each of the three groups from central and West Africa such that all possible intergroup mating across origin of the germplasm was achieved (Dhliwayo *et al*., 2009) excluding mating lines within the same origin group. Every line from one group was crossed with all four lines in the other groups resulting in nine sets of 16 hybrid combinations per set.

The seed of the parent lines were increased simultaneously to enable evaluation of lines in replicated trials alongside the F1 trials (Vivek *et al*., 2009).

5.2.1 Phenotyping

The 144 F1 hybrids and the 24 parental lines were evaluated in replicated trials side by side at each of the sites. The details of the phenotyping process, management, artificial inoculation, fumonisin analysis and measurements taken and derived are all provided in sections 4.2.1 to 4.2.6 and the evaluation trials were conducted at the same sites mentioned in these sections.

5.2.2 Experimental design and data analysis

The NCDII mating design was used. Although there were nine sets of hybrids as a result of each of the three groups from southern Africa mating with each of the three groups from central and West Africa, the sets were combined and analysed in a line x tester design where the former lines were used as female lines while the latter lines were used as male testers. The experimental design for the F1 and the parent trials were 5 m long x 0.75 m wide alpha-lattice (Patterson *et al*., 1978) with two replications each.

All the traits recorded, including the fungal incidence and severity and other agronomic traits, were subjected to ANOVA using AGROBASE Version II (2010) with replications and incomplete blocks considered random while genotypic variance among the hybrids and the parent inbreds were considered fixed. Each trial was analysed individually first, and then across sites and years as described in Chapter 4. This was followed by ANOVA according to the NCDII where hybrids were nested within sets of each environment. The variation among hybrids was split into that caused by males and females within the sets (parents) and their interaction pooled across the sets. The variance among the parents, which is the GCA effects for both the male and the female parents, was tested for significance using the interaction effects as an error term while the SCA (the interaction between parents) was tested using the error variance pooled across all sets.

The GCA and SCA effects were computed using SAS (SAS Institute, 2002). following the Hallauer and Miranda (1988) model:

$$Y_{ijk} = \mu + m_1 + f_j + (m \times f)_{ij} + e_{ijk}$$

Where:

Y_{ijk} is the kth observation on i x jth progeny

μ is the general mean

m_1 is the effect of the ith male

f_j is the effect of the jth female

$(m \times f)_{ij}$ is the interaction effect

e_{ijk} is the error associated with each observation

Estimation of the GCA effects

$GCA_f = X_f - \mu$

$GCA_m = X_m - \mu$

Where:

X_f is the mean for female parent

X_m is the mean for male parent

GCA_f is the GCA for female parent

GCA_m is GCA for male parent

μ is the grand mean of the crosses

Estimation of the SCA effects

$SCA_x = X_x - E(X_x) = X_x - [GCA_f + GCA_m + \mu]$

Where:

SCA_x is SCA for a cross

X_x is the observed mean value for the cross

$E(X_x)$ is the expected value of cross based on the GCAs of its parents (GCA_f, GCA_m)

5.3 Results

The ANOVA and the derived means for various agronomic traits, including ear rot incidence, grain disease scores and the fumonisin content across the environments and for specific environments are presented in Chapter 4.

5.3.1 General combining ability for *F. verticillioides* ear rot

5.3.1.1 Combining ability across all sites in the 2012/2013 and 2013/14 seasons

Of the southern African lines used as lines and females, inbred line SC 1 had the highest GCA for the female line (GCA_f) effects for *F. verticillioides* ear rot infection (0.97) while SC 10 and SC 3 had the lowest and negative GCA_f effects of -0.59 and -0.55 respectively (Table 5.1). The GCA_f effects were not significant across sites. Among testers which were lines from the IITA of central and West Africa used as males, inbred line IITA 12 had the highest GCA_m effect of 0.83 while the lowest were inbred lines IITA 1 and IITA 7 that had negative GCA_m values of -0.44 and -0.41 respectively (Table 5.2).

5.3.1.2 Fusarium ear rot incidences at RARS and WARC in the 2012/13 season

At RARS, despite the artificial infestation, the ear rot incidence among hybrids was not significant while at the WARC, the incidence was highly significant ($P<0.001$) among the hybrids (Table 5.3). At RARS SC 11 and SC 6 had the highest negative GCA effects for the ER (-0.49 and -0.38) respectively, while the lines with the highest positive effects were SC 8 and SC 1 with respective effects of 0.53 and 0.49 (Table 5.3). At WARC, the incidences were highly significant ($P\leq0.001$) and two inbred lines, SC 9 and SC 2 had the highest negative GCA effects of -0.44 each. SC 1 was also among the two inbred lines with the highest positive GCA effects (0.44) and SC 6 that had a GCA value of 0.51.

The GCA effects for the testers at RARS were significant ($P\leq0.05$) with the highest negative GCA effects for the testers observed on IITA 1 and IITA 7 that had respective effects of -0.71 and -0.62. Testers IITA 12 and IITA 5 had the highest GCA effects at RARS (1.85 and 0.59 respectively). At WARC the highest negative effects were observed on testers IITA 4 and IITA 7 with -0.27 and -0.25 respectively while the highest effects of 0.60 and 0.45 were on tester lines IITA 9 and IITA 10 respectively.

Table 5.1 Inbred line general combining ability effects for *F. verticillioides* ear rot across sites in 2012/2013 and 2013/14 for the lines

Line	Line code	Line mean	GCA	T_Value	Prob_T	GCA Rank
1	SC 1	0.75	0.97	1.22	ns	1
2	SC 2	0.00	0.05	0.06	ns	4
3	SC 3	0.25	-0.55	-0.69	ns	11
4	SC 4	0.25	-0.04	-0.05	ns	7
5	SC 5	0.25	-0.07	-0.09	ns	8
6	SC 6	1.00	-0.29	-0.36	ns	9
7	SC 7	0.42	0.36	0.45	ns	3
8	SC 8	0.17	0.50	0.62	ns	2
9	SC 9	0.00	-0.01	-0.01	ns	5
10	SC 10	0.58	-0.59	-0.74	ns	12
11	SC 11	0.00	-0.02	-0.03	ns	6
12	SC 12	0.44	-0.32	-0.40	ns	10
Grand mean		**1.15**				
GCA SE		**0.80**				

GCA=general combining ability; Prob_T=probability for T-Test; SE=standard error

Table 5.2 Inbred line general combining ability effects for *F. verticillioides* ear rot across sites in 2012/2013 and 2013/14 for the testers

Line	Line code	Line mean	GCA	T_Value	Prob_T	GCA Rank
1	IITA 1	0.71	-0.44	-0.78	ns	12
2	IITA 2	1.13	-0.02	-0.04	ns	6
3	IITA 3	0.79	-0.36	-0.63	ns	9
4	IITA 4	1.61	0.45	0.80	ns	2
5	IITA 5	1.51	0.36	0.63	ns	3
6	IITA 6	1.30	0.15	0.26	ns	4
7	IITA 7	0.74	-0.41	-0.73	ns	11
8	IITA 8	0.79	-0.36	-0.63	ns	10
9	IITA 9	1.09	-0.06	-0.10	ns	7
10	IITA 10	1.04	-0.11	-0.19	ns	8
11	IITA 11	1.13	-0.02	-0.04	ns	5
12	IITA 12	1.98	0.83	1.45	ns	1
GCA SE		0.57				

GCA=general combining ability; Prob_T=probability for T-Test; SE=standard error

5.3.1.3 Fumonisin content at RARS in the 2012/13 season

The fumonisin as measured by the value of B_1, B_2 and B_3 analogues was highly significant for B_1 ($P \leq 0.001$) and B_2 ($P \leq 0.01$) and significant for B_3 ($P \leq 0.05$, Tables 5.3 and 5.4). The GCA_f estimates were highly significant ($P \leq 0.01$) for B_1 and B_2 ($P \leq 0.01$) and significant ($P \leq 0.05$) for B_3 (Table 5.3). Inbred lines SC 8 had the highest negative effects (-0.22, -0.06 and -0.02 respectively) for all three fumonisin B_1, B_2 and B_3 analogues. Inbred SC 10 and SC 7 both had the highest negative GCA effects for B_3. The highest positive GCA effects were recorded for inbred line SC 5 for B_1, B_2 and B_3 where they had respective effects of 0.38, 0.10 and 0.04 (Table 5.3).

The GDS recorded at RARS was not statistically significant ($P > 0.05$) among the lines, neither were the GCA effects for both the lines (Table 5.3) and the testers (Table 5.4).

In 2012/2013, the SCA effects for Fusarium ear rot were not significant at RARS, while at WARC the SCA effects were highly significant (P≤0.001, Tables 5.3 and 5.4). For the fumonisins, SCA effects for analogues B_1, B_2 and B_3 were highly significant (P≤0.01), significant (P≤0.05) and not significant respectively (Table 5.3 and 5.4).

The Fusarium ear rot scores for the F1 trials conducted in 2012/2013 (Table 5.4) were not significant at RARS for the testers while they were highly significant for the fumonisins B_1 (P≤0.001) and B_2 (P≤0.01) and significant for B_3 (P≤0.05). The highest negative GCA_m effects for ER were observed on maize inbred line IITA 1 (-0.71) and IITA 7 (-0.62) while the highest positive values were on IITA 12 (1.85) and IITA 5 (0.59) for RARS (Table 5.4). At the WARC site at Sheda, inbred tester IITA 10 and IITA 4 had the highest positive and negative effects respectively, with effects of 0.45 and -0.27 for the ER. The GCA_m effects for the testers for mycotoxin fumonisin B_1, B_2 and B_3 were all not significant. IITA 10 had negative values for B_1, B_2 and B_3 (-0.21, -0.06 and -0.02 respectively). The highest positive effects were on IITA 12 and IITA 11 (Table 5.4).

In 2012/2013, the ear rot SCA effects for the F1 hybrids were not significant (P>0.05) for RARS and at WARC (Table 5.4). The SCA effects for the fumonisin B_1, B_2 and B_3 were highly significant (P≤0.01), significant (P≤0.05) and not significant respectively (Table 5.4).

5.3.2 General combining ability for yield and other traits across all environments in 2012/2013 and 2013/14

The ANOVA for the lines and the F1 hybrids are presented in sections 4.3 to 4.16. The GCA for the lines which were used as females (GCA_f) was highly significant (P≤0.001) for GY, DMP, DMS, ASI, EASP and MSV. For GLS, HT and rust, there were no significant differences. The line SC 10 had the highest GCA_f effects (0.41) for yield (Table 5.5).

The GCA_m for GY, DMP, DMS ASI and EASP, were highly significant (P≤0.001) while GLS, HT, MSV and rust were not significant. Inbred line IITA 4 had the highest positive

GCA effects for GY and highest negative GCA for EASP which were both significant ($P \leq 0.05$ and $P \leq 0.001$ respectively) among the male lines (Table 5.6).

Table 5.3 The GCA effects for lines for the Fusarium ear rot and fumonisin B_1, B_2 and B_3 in 2012/13 for RARS, and GCA effects for ear rots for SRC, KRC and WARC

		Fusarium ear rot		GDS	Fumonisin		
Lines	**Code**	**RARS**	**WARC**	**RARS**	**B_1**	**B_2**	**B_3**
1	SC 1	0.49	0.44	-0.05	-0.02	-0.01	-0.01
2	SC 2	0.15	-0.44	0.08	-0.07	-0.02	-0.01
3	SC 3	-0.30	-0.16	0.16	0.00	-0.01	-0.01
4	SC 4	-0.35	-0.15	0.00	0.15	0.05	0.01
5	SC 5	0.12	0.39	-0.05	0.38	0.10	0.04
6	SC 6	-0.38	0.51	0.00	0.18	0.06	0.02
7	SC 7	-0.16	0.02	0.00	-0.09	-0.03	-0.01
8	SC 8	0.53	0.18	-0.05	-0.22	-0.06	-0.02
9	SC 9	0.29	-0.44	-0.05	-0.09	-0.02	-0.01
10	SC 10	0.32	0.04	0.00	-0.15	-0.06	-0.02
11	SC 11	-0.49	-0.16	-0.05	0.04	0.02	0.01
12	SC 12	-0.23	-0.23	0.00	-0.11	-0.03	-0.01
Mean		**1.48**	**0.44**	**1.05**	**0.27**	**0.06**	**0.02**
P value Hybrids		**ns**	*******	**ns**	*******	******	*****
P value GCA Lines		**ns**	*******	**ns**	******	******	*****
P value SCA		**ns**	*******	**ns**	******	*****	**ns**

*****P≤0.001; ** P≤0.01; P≤0.05; ns=not significant; RARS=Rattray Arnold Research Station; SRC=Stapleford Research Centre; KRC=Kadoma Research Centre; WARC=West Africa Research Centre; GDS=grain disease score; GCA=general combining ability; SCA= specific combing ability; B_1=fumonisin B_1 analogue; B_2=fumonisin B_2 analogue; B_3=fumonisin B_3 analogue**

Table 5.4 The GCA effects for testers for the Fusarium ear rot, GDS and fumonisin B_1, B_2 and B_3 for RARS, and GCA effects for ear rots for SRC, KRC and WARC in 2012/2013

Entry	Code	Fusarium ear rot		GDS	Fumonisin		
		RARS	WARC	RARS	B_1	B_2	B_3
1	IITA 1	-0.71	-0.10	0.00	-0.07	-0.01	-0.01
2	IITA 2	-0.36	0.29	-0.09	0.00	0.00	0.00
3	IITA 3	0.03	-0.20	0.00	-0.16	-0.04	-0.01
4	IITA 4	-0.52	-0.27	-0.05	-0.02	-0.01	0.00
5	IITA 5	0.59	-0.02	0.04	-0.06	-0.03	-0.01
6	IITA 6	0.18	-0.19	0.00	0.06	0.00	0.00
7	IITA 7	-0.62	-0.25	0.08	-0.03	-0.02	-0.01
8	IITA 8	0.30	-0.24	0.00	-0.07	-0.01	0.00
9	IITA 9	-0.11	0.60	0.08	0.10	0.03	0.01
10	IITA 10	-0.53	0.45	-0.05	-0.21	-0.06	-0.02
11	IITA 11	-0.10	-0.13	0.00	0.20	0.07	0.02
12	IITA 12	1.85	0.05	0.00	0.26	0.06	0.03
Mean		**1.48**	**0.44**	**1.05**	**0.27**	**0.06**	**0.02**
P value hybrids		**ns**	**ns**	**ns**	*******	******	*****
P value GCA testers		*******	**ns**	*******	**ns**	**ns**	**ns**
P value SCA		**ns**	**ns**	**ns**	******	*****	**ns**

*****P≤0.001; ** P≤0.01; P≤0.05; ns=not significant; RARS=Rattray Arnold Research Station; SRC=Stapleford Research Centre; KRC=Kadoma Research Centre; WARC=West Africa Research Centre; GDS=grain disease score; GCA=general combining ability; SCA=specific combining ability; B_1=fumonisin B_1 analogue; B_2=fumonisin B_2 analogue; B_3=fumonisin B_3 analogue**

Table 5.5 General combining ability (GCAf) effects across environments in 2012/2013 and 2013/14 for lines on yield and other traits

Entry	Code	GY	DMP	DMS	ASI	EASP	GLS	HT	MSV	Rust
1	SC 1	-0.20	-0.64	-0.19	0.46	0.51*	0.09	-0.14	-0.01	0.06
10	SC 10	0.41	0.79	1.25*	0.47	-0.29	0.00	0.06	0.03	0.03
11	SC 11	-0.08	-0.83	-1.20*	-0.37	0.34	0.10	0.16	-0.02	-0.03
12	SC 12	-0.03	0.26	-0.16	-0.41	-0.30	-0.10	0.10	-0.01	-0.02
2	SC 2	0.08	-1.45**	-1.25*	0.19	0.29	-0.23	0.13	-0.01	0.10
3	SC 3	-0.22	-0.03	0.00	0.03	-0.05	0.19	-0.04	0.00	-0.20**
4	SC 4	0.03	1.21*	1.05*	-0.16	-0.23	-0.16	-0.11	0.00	0.02
5	SC 5	0.28	-1.25*	-1.33*	-0.09	-0.24	-0.09	0.12	-0.01	-0.05
6	SC 6	-0.06	0.53	0.49	-0.04	0.03	0.26	0.06	0.05	-0.05
7	SC 7	-0.17	0.85	1.189*	0.34	-0.09	0.02	-0.02	-0.02	0.05
8	SC 8	0.24	0.49	-0.04	-0.53	0.08	0.00	-0.13	0.02	-0.02
9	SC 9	-0.27	0.07	0.18	0.11	-0.05	-0.11	-0.18	-0.01	0.10
Mean		**4.75**	**67.08**	**68.17**	**1.09**	**4.88**	**2.47**	**1.27**	**1.02**	**1.34**
GCA SE		**0.34**	**0.48**	**0.51**	**0.32**	**0.25**	**0.13**	**0.17**	**0.03**	**0.07**
P value GCA_f		***	***	***	***	***	ns	ns	***	ns

*****P≤0.001; P≤0.05; ns=not significant; GY=grain yield; DMP=days to mid pollen shedding; DMS=days to silking; ASI=anthesis to silking interval; EASP=ear aspect; GLS=grey leaf spot; HT=*helminosporium turcicum*; MSV=maize streak virus; PHA=*Phaeosophaerea leaf spot*; GCA=general combining ability; SCA=specific combining ability**

Table 5.6 General combining ability (GCAm) effects across environments in 2012/2013 and 2013/14 for testers on yield and other traits

Entry	Code	GY	DMP	DMS	ASI	EASP	GLS	HT	MSV	Rust
1	IITA 1	-0.56	0.59	0.74	0.15	0.33	-0.04	0.01	-0.01	0.05
2	IITA 2	0.35	0.38	0.93	-0.05	0.19	-0.03	0.05	0.02	0.01
3	IITA 3	0.03	-0.75	-0.66	-0.20	0.16	-0.06	-0.04	-0.01	-0.09
4	IITA 4	0.71*	0.20	0.00	0.15	-0.71***	0.14	0.22	0.01	-0.02
5	IITA 5	-0.02	0.70	0.17	0.23	-0.18	-0.10	0.03	-0.01	0.09
6	IITA 6	0.00	-0.56	1.6**	-0.10	0.05	0.00	-0.17	0.01	0.05
7	IITA 7	0.13	0.21	-0.39	-0.22	-0.32	-0.10	-0.04	0.01	0.02
8	IITA 8	0.12	0.73	-1.16*	-0.56	0.31	0.34**	-0.15	0.00	-0.17
9	IITA 9	-0.29	1.05*	-0.97	0.57	0.17	-0.06	0.06	0.01	0.03
10	IITA 10	-0.08	-0.73	0.33	0.34	0.04	-0.06	-0.17	0.00	0.05
11	IITA 11	-0.05	-0.89*	-0.95	-0.25	-0.59**	-0.14	0.06	-0.01	0.05
12	IITA 12	-0.36	-0.92*	0.36	-0.07	0.53**	0.09	0.15	-0.01	-0.06
Mean		**4.75**	**67.08**	**68.17**	**1.09**	**4.88**	**2.47**	**1.27**	**1.02**	**1.34**
GCA SE		**0.34**	**0.41**	**0.57**	**0.37**	**0.18**	**0.10**	**0.15**	**0.02**	**0.06**
P value GCA_m		*******	*******	*******	*******	*******	**ns**	**ns**	*******	**ns**

*****P≤0.001; P≤0.05; ns=not significant; GY=grain yield; DMP=days to mid pollen shedding; DMS=days to silking; ASI=anthesis to silking interval; EASP=ear aspect; GLS=grey leaf spot; HT=*helminosporium turcicum*; MSV=maize streak virus; PHA=*Phaeosophaerea leaf spot*; GCA=general combining ability; SCA=specific combining ability**

5.3.3 Combining ability for yield and other traits in the 2013 season at WARC at Sheda

At WARC site (Table 5.7) in the 2013 main season, the F1 hybrids mean squares were significant (P≤0.001) for GY, EASP, HC, rust, grain texture (TEXT), and DMP (P≤0.01) while RL was significant (P≤0.05).

The traits with highly significant GCA_f (P≤0.001) mean squares included GY, DMP, EASP, HC score, RL, rust and Text while DMP and DMS were highly significant (P≤0.01). GCA_m were highly significant (P≤0.01) for GY, EASP, HC, RL, rust, TEXT, DMP and DMS. PHT and CHT were significant (P≤0.05, Table 5.8). The highest GCA_f and GCA_m for GY were for inbred SC 4 (0.68) and IITA 6 (0.79) respectively (Table 5.7 and Table 5.8).

At WARC in Nigeria in 2013, the SCA mean square for GY was significant (P≤0.05). Highly significant SCA mean squares were observed for the traits Text (P≤0.001), EASP and HC (P≤0.01).

Table 5.7 General combining ability (GCAf) effects at WARC 2013 for lines for yield and other traits

Entry	Code	GY	DMP	DMS	ASI	CHT	EASP	EPP	HC	PHT	RL	Rust	Text
1	SC 1	-0.28	-0.10	1.05	1.15	0.03	0.67	-0.04	0.96	0.04	-1.37	-0.11	-0.90
2	SC 2	-0.87	-0.94	-0.83	0.11	0.01	0.84	-0.01	0.46	0.04	-0.30	0.43	0.68
3	SC 3	-0.68	-0.35	-0.41	-0.06	0.01	0.17	-0.01	0.04	0.00	1.14	0.60	-0.65
4	SC 4	0.68	1.15	0.00	-1.14	0.00	-0.53	0.00	-0.29	0.01	-0.05	-0.94	-0.96
5	SC 5	0.14	-0.90	-1.08	-0.18	-0.05	-0.12	0.01	0.42	-0.08	2.98	-0.44	1.10
6	SC 6	-0.83	0.73	0.96	0.23	0.03	0.51	-0.02	0.88	0.06	-2.93	0.43	1.50
7	SC 7	-0.20	1.65	1.17	-0.48	-0.02	-0.24	0.01	-0.83	-0.02	-0.51	0.39	-0.40
8	SC 8	0.22	-0.19	-0.33	-0.14	-0.01	0.30	0.01	0.50	0.01	1.14	0.27	0.35
9	SC 9	0.56	-0.94	-1.00	-0.06	-0.03	-0.33	0.02	-0.88	-0.07	6.13	0.31	0.77
10	SC 10	0.14	0.73	2.09	1.36	0.01	-0.49	0.02	-0.71	0.04	-4.11	0.14	-2.13
11	SC 11	-0.01	-0.81	-1.33	-0.52	0.02	0.13	0.04	-0.17	-0.03	-0.79	-0.52	-0.73
12	SC 12	1.14	-0.02	-0.29	-0.27	0.01	-0.91	-0.02	-0.38	-0.01	-1.34	-0.57	1.37
Mean		**6.25**	**55.98**	**55.87**	**-0.11**	**1.22**	**4.83**	**0.97**	**2.79**	**2.23**	**8.94**	**7.23**	**3.17**
P value hybrids		***	**	ns	ns	ns	***	ns	***	ns	*	***	***
P value GCA lines		***	***	**	ns	ns	***	ns	***	ns	*	***	***
P value SCA		*	ns	ns	ns	ns	**	ns	**	ns	ns	ns	***

*****P≤0.001; ** P≤0.01; P≤0.05; ns=not significant; GY=grain yield; DMP=days to mid pollen shedding; DMS=days to silking; ASI=anthesis to silking interval; CHT=cob height; EASP=ear aspect; EPP=ears per plant; HC=husk cover; PHT=plant height; RL=root lodging; TEXT=grain texture; GCA=general combining ability; SCA=specific combining ability.**

Table 5.8 General combining ability (GCAm) effects at WARC 2013 for testers for yield and other traits

Entry	Code	GY	DMP	DMS	ASI	CHT	EASP	EPP	HC	PHT	RL	Rust	Text
1	IITA 1	-0.91	0.69	1.13	0.44	-0.04	0.59	0.01	-0.17	-0.03	-4.18	0.56	0.08
2	IITA 2	0.09	-0.31	0.75	1.07	0.02	0.38	0.07	0.58	0.01	-3.36	-0.32	-0.02
3	IITA 3	-0.22	-1.15	-1.66	-0.52	-0.01	0.30	-0.02	-0.29	-0.07	0.51	0.77	0.06
4	IITA 4	0.47	0.10	0.25	0.15	0.02	-0.70	0.00	-0.79	0.00	-1.28	0.27	0.79
5	IITA 5	0.29	0.31	0.55	0.23	-0.01	-0.41	0.01	0.17	0.00	5.48	-0.27	0.58
6	IITA 6	0.79	-0.73	-1.50	-0.77	0.10	-0.53	0.02	0.21	0.15	-1.01	0.64	-0.09
7	IITA 7	0.60	-0.23	-0.41	-0.18	0.03	-0.49	-0.01	-0.92	-0.01	-1.36	-2.11	-0.19
8	IITA 8	-0.29	0.94	-0.29	-1.23	0.02	0.22	-0.01	0.08	-0.03	0.12	0.73	-0.86
9	IITA 9	0.56	0.81	1.17	0.36	-0.12	-0.12	-0.02	0.92	-0.11	-2.52	-1.52	0.43
10	IITA 10	-0.89	0.06	1.63	1.57	-0.02	0.59	0.00	0.71	0.01	1.20	0.93	-1.15
11	IITA 11	-0.10	0.06	0.42	0.36	-0.02	-0.28	-0.03	0.29	0.03	8.87	-0.23	0.18
12	IITA 12	-0.41	-0.56	-2.04	-1.48	0.02	0.47	-0.03	-0.79	0.04	-2.48	0.56	0.18
Mean		**6.25**	**55.98**	**55.87**	**-0.11**	**1.22**	**4.83**	**0.97**	**2.79**	**2.23**	**8.94**	**7.23**	**3.17**
P-value hybrids		***	**	ns	ns	ns	***	ns	***	ns	*	***	***
P value GCA testers		***	**	***	ns	*	***	ns	***	*	***	***	***
P value SCA		*	ns	ns	ns	ns	**	ns	**	ns	ns	ns	***

*****P≤0.001; ** P≤0.01; P≤0.05; ns=not significant; GY=grain yield; DMP=days to mid pollen shedding; DMS=days to silking; ASI=anthesis to silking interval; CHT=cob height; EASP=ear aspect; EPP=ears per plant; HC=husk cover; PHT=plant height; RL=root lodging; TEXT=grain texture; GCA=general combining ability; SCA=specific combining ability**

5.3.4 Specific combining ability

5.3.4.1 Specific combining ability for yield across all sites

For grain yield, the SCA effects were not significant across all the environments except the SCA mean square for SC 11/IITA 3 that was significant ($P \leq 0.05$) with the highest negative SCA effect of -1.06 (Table 5.9). The GCA effects for the constituting line SC 11 was negative and low (-0.08) while that of the tester IITA 3 was low and positive (0.03). The highest positive SCA was on F1 hybrid SC 3/IITA 1 (0.74) which was not significant.

5.3.4.2 Specific combining ability for *F. verticillioides* ear rot across all sites in the 2012/13 and 2013/14 seasons

F1 hybrid SC 7/IITA 2 had the highest negative SCA effect (-1.19) which was not significant. The highest effects (not significant) were on F1 hybrid SC 8/IITA 9 (Table 5.10).

5.3.4.3 Specific combining ability for *F. verticillioides* ear rot at RARS in the 2012/13 season

The SCA for the ER incidences at RARS where artificial inoculation was carried out, were not significant (Table 5.11) although the F1 hybrid SC 12/IITA 12 and SC 10/IITA 5 had the lowest negative effects (-3.10 and -2.93 respectively). The highest positive effects were on SC 10/IITA 12 (3.95), SC 1/IITA 6 (3.40), SC 4/IITA 5 (2.98) and SC 3/IITA 11 (2.39).

Table 5.9 Grain yield general combining ability for the females (lines) and males (testers) and specific combining ability across all sites in the 2012/13 and 2013/14 seasons

	Line												
Tester	**SC 1**	**SC 2**	**SC 3**	**SC 4**	**SC 5**	**SC 6**	**SC 7**	**SC 8**	**SC 9**	**SC 10**	**SC 11**	**SC 12**	**GCA**
IITA 1	-0.44	0.32	0.74	0.00	0.07	-0.31	0.04	-0.69	0.05	0.27	0.17	-0.22	**-0.56**
IITA 2	0.26	-0.01	-0.57	0.09	0.07	0.16	0.02	0.10	0.27	0.30	-0.77	0.08	**0.35**
IITA 3	0.07	-0.23	-0.19	-0.09	0.30	0.21	-0.17	0.25	-0.13	0.35	**-1.06***	0.70	**0.03**
IITA 4	0.26	-0.34	0.28	-0.01	0.26	0.28	0.31	-0.47	-0.04	0.09	-0.53	-0.09	**0.71**
IITA 5	0.18	0.30	0.17	0.14	-0.19	-0.24	0.16	-0.23	-0.12	-0.01	0.42	-0.58	**-0.02**
IITA 6	-0.20	0.20	-0.23	0.27	-0.21	0.41	0.30	-0.46	-0.52	0.36	0.44	-0.36	**0.00**
IITA 7	0.20	-0.25	0.10	-0.41	-0.01	0.52	-0.38	0.01	-0.08	-0.45	0.40	0.36	**0.13**
IITA 8	-0.22	0.19	-0.43	-0.04	0.19	-0.38	-0.10	0.19	0.71	0.16	0.24	-0.51	**0.12**
IITA 9	-0.21	0.60	0.03	-0.05	-0.17	-0.18	-0.33	0.31	0.19	-0.19	0.33	-0.31	**-0.29**
IITA 10	-0.64	-0.04	-0.12	0.08	0.16	0.20	-0.05	0.49	-0.67	-0.23	0.39	0.43	**-0.08**
IITA 11	0.52	-0.70	0.36	-0.11	0.14	-0.14	0.07	0.02	0.53	-0.90	-0.05	0.26	**-0.05**
IITA 12	0.22	-0.03	-0.15	0.13	-0.62	-0.52	0.11	0.50	-0.19	0.25	0.04	0.26	**-0.36**
GCA	**-0.20**	**0.08**	**-0.22**	**0.03**	**0.28**	**-0.06**	**-0.17**	**0.24**	**-0.27**	**0.41**	**-0.08**	**-0.03**	

GCA=general combining ability

Table 5.10 *F. verticillioides* ear rot general combining ability for the females (lines) and males (testers) and specific combining ability across all sites in the 2012/13 and 2013/14 seasons

	Line												
Tester	**SC 1**	**SC 2**	**SC 3**	**SC 4**	**SC 5**	**SC 6**	**SC 7**	**SC 8**	**SC 9**	**SC 10**	**SC 11**	**SC 12**	**GCA**
IITA 1	-0.38	0.81	-0.02	0.15	-0.31	0.46	0.08	-1.03	0.01	0.26	-0.37	0.35	**-0.44**
IITA 2	0.57	0.53	0.46	0.52	-0.58	-0.39	-1.19	-0.65	0.55	-0.40	0.86	-0.28	**0.36**
IITA 3	1.98	-0.39	-0.18	-0.92	-0.25	0.67	-0.07	0.94	-0.15	-0.71	-0.60	-0.29	**0.15**
IITA 4	-1.08	0.00	-0.02	0.94	0.33	-0.27	0.51	-0.78	-0.32	0.03	0.24	0.42	**-0.41**
IITA 5	-0.49	-0.59	0.75	0.19	-0.33	0.43	-0.66	-0.11	0.76	-0.02	0.24	-0.16	**-0.36**
IITA 6	0.28	0.00	0.10	0.26	-0.08	0.59	-0.26	-1.08	-0.01	0.04	0.16	0.01	**-0.06**
IITA 7	0.56	-1.09	0.54	0.54	-0.60	-0.39	0.09	0.18	-0.55	0.73	0.56	-0.56	**-0.11**
IITA 8	-0.86	0.80	0.09	0.22	-0.48	-0.67	1.45	-0.79	0.34	-0.23	0.00	0.11	**-0.02**
IITA 9	0.78	-0.59	-1.05	-0.59	1.13	0.37	-0.34	2.23	-0.34	-0.69	-0.20	-0.71	**0.83**
IITA 10	-0.51	0.77	-0.58	-0.47	-0.10	-0.13	0.24	-0.14	-0.39	0.57	-0.12	0.77	**-0.02**
IITA 11	-1.14	-0.68	0.59	-0.66	0.80	-0.08	0.29	0.93	-0.55	0.13	-0.60	0.96	**-0.36**
IITA 12	0.29	0.44	-0.71	-0.19	0.45	-0.59	-0.13	0.30	0.63	0.29	-0.16	-0.63	**0.45**
GCA	**0.97**	**0.05**	**-0.55**	**-0.04**	**-0.07**	**-0.29**	**0.36**	**0.50**	**-0.01**	**-0.59**	**-0.02**	**-0.32**	

GCA=general combining ability

Table 5.11 *F. verticillioides* ear rot general combining ability for the females (lines) and males (testers) and specific combining ability at RARS in the 2012/13 season

	Line												
Tester	**SC 1**	**SC 2**	**SC 3**	**SC 4**	**SC 5**	**SC 6**	**SC 7**	**SC 8**	**SC 9**	**SC 10**	**SC 11**	**SC 12**	**GCA**
IITA 1	-1.25	-0.92	-0.46	0.23	0.47	0.96	0.10	-0.09	0.35	0.22	-0.28	0.67	-0.71
IITA 2	-1.01	0.48	-0.81	0.33	-0.68	-0.09	-0.31	1.05	-0.15	-0.24	2.32	-0.89	-0.36
IITA 3	-0.54	-1.65	0.65	-0.60	1.08	0.18	0.71	2.67	0.91	-1.82	-1.01	-0.57	0.03
IITA 4	-0.90	1.04	-0.65	0.54	-0.37	0.02	-0.15	-1.48	-0.69	0.03	0.78	1.83	-0.52
IITA 5	0.09	-0.47	0.29	2.98	0.07	0.21	-0.21	1.25	0.25	-2.39	-0.93	-1.14	0.59
IITA 6	3.40	2.59	0.45	-0.21	-0.47	0.77	-0.90	-1.58	-0.64	-1.98	-1.17	-0.28	0.18
IITA 7	1.10	-1.01	0.05	-0.51	-0.97	0.17	-0.10	-0.73	1.61	-1.18	1.58	-0.03	-0.62
IITA 8	0.23	0.42	-0.82	-0.13	0.91	-0.80	-0.42	0.75	0.44	-0.95	-0.69	1.05	0.30
IITA 9	-0.71	0.43	-0.41	-1.02	0.97	-0.29	-1.21	-0.05	-1.65	2.31	-0.88	2.51	-0.11
IITA 10	-0.19	-1.10	-0.65	0.55	-1.07	0.08	0.51	0.47	-0.54	1.38	0.69	-0.12	-0.53
IITA 11	-1.32	-0.38	2.93	-0.38	0.06	-1.00	1.38	-1.20	-1.11	0.65	0.31	0.05	-0.10
IITA 12	1.08	0.57	-0.57	-1.78	0.01	-0.20	0.58	-1.05	1.24	3.95	-0.74	-3.10	1.85
GCA	**0.49**	**0.15**	**-0.30**	**-0.35**	**0.12**	**-0.38**	**-0.16**	**0.53**	**0.29**	**0.32**	**-0.49**	**-0.23**	

GCA=genera combining ability; RARS=Rattray Arnold Research Station

5.3.4.4 Specific combining ability for total fumonisin content across all sites in the 2012/13 and 2013/14 seasons

Fumonisin content SCA was significant ($P \leq 0.01$) for B_1, B_2 and the summation of the three. The highest negative SCA effects involved tester IITA 11 crossed to SC 5 (-1.16) and SC 6 (-0.90). The highest positive effects were on SC 5/IITA 12 (2.73), SC 6/IITA 2 (2.29) and SC 5/IITA 6 (2.12) (Table 5.12). The mean squares for the *F. verticillioides* ear rot, GDS and fumonisins B_1, B_2 and B_3 are presented in Table 5.13. For the ear rot the mean squares for GCA_f were highly significant at WARC in 2013, and in the 2013/14 season at RARS and KRC ($P \leq 0.001$). At RARS in 2012/13 and at SRC in both years, there were no significant differences. The GCA_m mean squares were highly significant at RARS in 2012/13 and 2013/14, WARC in 2013 ($P \leq 0.001$) and SRC in 2013/14 ($P \leq 0.01$) and were significant at KRC in 2013/14 ($P \leq 0.05$). The GDS values were not significant while the fumonisin B_1 and B_2 were highly significant ($P \leq 0.01$) and B_3 was significant ($P \leq 0.05$) for the GCA_f but none was significant for the testers.

For the other agronomic traits including GY, the mean squares are presented in Table 5.14. The GY mean squares were highly significant for GCA_f in 2013 at KRC and SRC, and WARC and in 2014 at RARS and KRC ($P \leq 0.001$) and at SRC ($P \leq 0.01$). There were no significant differences at RARS in 2012/13 and at SRC in the 2013/14 season. Testers were highly significant at RARS, SRC and WARC in the 2012/13 season, and at RARS and SRC in 2013/14 ($P \leq 0.001$) but were significant ($P \leq 0.05$) at KRC in the 2013/14 season. There were no significant differences at KRC in the 2012/13 season.

The mean squares for GCA for DMP and DMS were highly significant at all sites except for SRC in 2013/14 for both the lines and the testers ($P \leq 0.001$). At WARC in 2013 the testers for DMP and both lines and testers for DMS were significant ($P \leq 0.01$). PHT and CHT were highly significant ($P \leq 0.001$) for both lines and testers with the exception of lines for SRC in 2012/13 and SRC in 2013/14 ($P \leq 0.01$) except at WARC in 2013 where there were no significant differences for the lines for both traits while the testers were significant ($P \leq 0.05$) for both traits. Where texture was recorded at WARC in 2013 and at the three Zimbabwe sites in 2013/14, the GCA mean squares for both the lines and the testers were highly significant ($P \leq 0.001$).

Table 5.12 Fumonisin general combining ability for the females (lines) and males (testers) and specific combining ability at RARS in the 2012/13 season

	Line												
Tester	**SC 1**	**SC 2**	**SC 3**	**SC 4**	**SC 5**	**SC 6**	**SC 7**	**SC 8**	**SC 9**	**SC 10**	**SC 11**	**SC 12**	**GCA**
IITA 1	-0.17	0.19	-0.25	-0.48	-0.69	-0.07	-0.04	0.04	-0.15	-0.04	1.11	0.54	**-0.71**
IITA 2	-0.20	0.56	-0.33	-0.56	-0.82	2.29	-0.22	-0.05	-0.23	-0.07	-0.17	-0.19	**-0.36**
IITA 3	0.07	-0.02	-0.11	-0.34	-0.55	0.36	0.00	0.18	-0.01	0.50	-0.10	0.03	**0.03**
IITA 4	-0.17	-0.21	-0.15	0.62	-0.84	-0.32	0.36	-0.01	-0.20	-0.04	1.01	-0.06	**-0.52**
IITA 5	0.25	0.06	-0.23	-0.36	0.18	-0.41	0.08	0.06	0.02	0.23	-0.32	0.46	**0.59**
IITA 6	-0.06	0.00	0.01	-0.52	2.12	-0.47	-0.18	-0.10	-0.04	-0.08	-0.43	-0.25	**0.18**
IITA 7	1.41	-0.19	0.78	-0.45	-0.76	-0.55	0.04	0.06	-0.07	0.14	-0.27	-0.13	**-0.62**
IITA 8	-0.23	0.58	-0.21	0.81	0.16	-0.53	-0.15	0.03	-0.06	-0.05	-0.35	-0.02	**0.30**
IITA 9	-0.28	-0.37	-0.36	-0.34	0.05	1.71	-0.15	-0.07	-0.26	-0.05	-0.40	0.53	**-0.11**
IITA 10	-0.01	0.15	0.01	-0.07	-0.43	-0.32	0.07	0.30	0.11	0.17	-0.08	0.10	**-0.53**
IITA 11	-0.14	-0.28	-0.22	1.30	-1.16	-0.90	0.74	-0.18	1.43	-0.41	0.29	-0.48	**-0.10**
IITA 12	-0.45	-0.45	1.07	0.39	2.73	-0.81	-0.57	-0.25	-0.53	-0.32	-0.27	-0.54	**1.85**
GCA	**0.49**	**0.15**	**-0.30**	**-0.35**	**0.12**	**-0.38**	**-0.16**	**0.53**	**0.29**	**0.32**	**-0.49**	**-0.23**	

GCA=general combining ability

Table 5.13 General combining ability mean squares of *F. verticillioides* ear rot, grain diseases score and fumonisins for the female and male lines at individual locations

	RARS13		SRC13		KRC13		WARC13		RARS14		SRC14		KRC14	
	GCA_f	GCA_m	GCA_f	GCA_m	GCA_f	GCA_m	GCA_f	GCA_m	GCA_f	GCA_m	GCA_f	GCA_m	GCA_f	GCA_m
ER	3.08ns	11.97***	0.00ns	0.00ns	nr	nr	2.50***	2.07***	8.25***	3.05***	9.74ns	13.90**	169.91***	86.61*
GDS	0.09ns	0.06ns	nr	nr	nr	nr	nr	nr	0.03ns	0.02ns	0.53ns	0.40ns	0.01ns	0.01ns
B1	0.68**	0.45ns	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
B2	0.06**	0.06ns	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
B3	0.01*	0.01ns	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	Nr

RARS=Rattray Arnold Research Station; SRC=Stapleford Research Centre; KRC=Kadoma Research Centre; WARC=West Africa Research Centre; ER=*Fusarium verticillioides* ear rot; GDS=grain disease score; GCA=general combining ability; ***P≤0.001; **P≤0.01; *P≤0.05; ns=P>0.05; nr=not recorded

Table 5.14 General combining ability mean squares for yield and other agronomic traits for the female and male lines at individual locations

	RARS13		SRC13		KRC13		WARC13		RARS14		SRC14		KRC14	
	Line	**Tester**	**Line**	**Tester**	**Line**	**Tester**	**Line**	**Tester**	**Line**	**Tester**	**Line**	**Tester**	**Line**	**Tester**
GY	1.05ns	7.38***	4.64**	13.14***	2.25***	1.20ns	9.15***	7.76***	6.66***	4.45***	1.37ns	5.58***	7.01***	4.04*
DMP	15.65***	29.72***	23.77***	22.65***	27.75***	21.81***	18.50***	9.69**	29.95***	29.34***	9.44ns	5.11ns	48.43***	35.27***
DMS	20.84***	37.65***	25.17***	22.31***	54.49***	52.88***	27.75**	34.50**	35.47***	30.92***	7.14ns	4.77ns	32.29***	34.81***
ASI	1.69**	2.10**	3.32**	1.31ns	14.47**	16.21***	11.26ns	18.80ns	1.76***	0.85**	0.80ns	0.65ns	11.20***	10.06***
PHT	nr	nr	0.09***	0.14***	0.09***	0.07***	0.05ns	0.10*	0.15***	0.48***	0.15***	0.57***	0.50***	0.16***
CHT	nr	nr	0.07**	0.15***	0.15***	0.07***	0.01ns	0.06*	0.18***	0.25***	0.06**	0.08***	0.17***	0.11***
RL	nr	nr	0.72ns	1.19ns	17.13ns	13.81ns	172.78*	340.70***	8.00***	5.11ns	1.09ns	1.09ns	2.46ns	3.13ns
SL	nr	nr	7989.12***	4584.85***	773.85***	851.67***	nr	nr	nr	nr	1.09ns	1.09ns	14.39ns	7.82ns
EASP	5.15***	6.85***	nr	nr	nr	nr	6.74***	5.41***	2.85***	3.55***	0.81ns	4.59***	4.18***	3.76***
EPO	nr	nr	0.01**	0.01***	0.03***	0.01***	nr	nr	0.02***	0.01***	0.00ns	0.01ns	0.02***	0.01***
EPP	nr	nr	0.00ns	0.00ns	0.16***	0.12***	0.12ns	0.02ns	0.04***	0.01ns	0.05**	0.06***	0.09**	0.13***
GLS	1.97***	1.31***	0.45ns	0.84ns	nr	nr	nr	nr	1.85***	1.20***	3.44ns	2.60ns	0.09ns	0.06ns
RUST	0.48**	0.48**	nr	nr	nr	nr	6.05***	22.02***	0.77**	0.40ns	nr	nr	0.05ns	0.05ns
HC_score	nr	nr	nr	nr	nr	nr	9.89***	8.84***	4.23***	2.23**	0.05ns	0.06***	7.62***	3.56
TEXT	nr	nr	nr	nr	nr	nr	30.31***	7.25***	25.42***	8.15***	6.97***	3.50***	15.04***	5.51***

RARS=Rattray Arnold Research Station; SRC=Stapleford Research Centre; KRC=Kadoma Research Centre; WARC=West Africa Research Centre; ER=*Fusarium verticillioides* ear rot; GDS=grain disease score; GCA=general combining ability; GY=grain yield; DMP=days to mid pollen shedding; DMS=days to silking; ASI=anthesis to silking interval; PHT=plant height; CHT=cob height; RL=root lodging; SL=stalk lodging; EASP=ear aspect; EPO=ear position; EPP=ears per plant; GLS=first grey leaf spot; HC_score=husk cover score; TEXT=grain texture; ***P≤0.001; **P≤0.01; *P≤0.05; ns=P>0.05; nr=not recorded

5.4 Discussion

The ANOVA results across sites and years indicated no significant differences for *F. verticillioides* in hybrids and parents. This could be attributed to the environment in terms of precipitation that has some correlation with humidity as these two years of testing were characterised by low rainfall. SRC, a location regarded as a hot spot for *F. verticillioides* had low incidences of the disease, so had RARS that was artificially inoculated but did not express any significant differences for the disease. Although the inoculum was applied, its interaction with the environment impacted negatively on its effect on the study material. Disease manifestation requires the pathogen, host plant and a conducive environment, which in this case was not present. Despite that, inbred lines SC 10, SC 3 and SC 6 had the highest negative GCA_f while SC 1 had the highest positive GCA_f effects across sites and seasons in the 2012/13 and 2013/14 seasons for *F. verticillioides* ear rot. Significant differences were observed at the Nigerian site, WARC hence, in as far as *F. verticillioides* ear rot results are concerned, conclusions can be made based on results from WARC where there were significant differences. Of the four resistant lines, three inbred lines used as females (SC 2, SC 3 and SC 4) had the highest negative GCA_f effects at WARC while the four susceptible lines all had positive effects. Among the lines with unknown reaction to various ear rots, three had negative effects (SC 9, SC 11 and SC 12). The resistant inbred lines, SC 3 and SC 4 also had negative GCA_f for *F. verticillioides* ear rot across sites while the susceptible inbreds SC 5 and SC 6 had negative GCA_f. All the lines whose reaction was unknown had negative GCA_f. This suggests that SC 9, SC 11 and SC 12 whose reaction to various ear rot causing fungi in southern Africa was unknown, but which had negative GCA_f at both WARC and across sites, can be classified as inbred lines that can significantly contribute towards resistance. Negative GCA effects are indicative of the ability of the lines to contribute resistance to fungus causing ear rots.

Despite the artificial inoculation at RARS in 2013, the incidence of *F. verticillioides* ear rot remained low with a mean incidence of 1.48%, hence there were no significant differences for the hybrids themselves and for the GCA_f. However, the GCA_m effects were significant. Such an observation can be attributed to normal selections for resistance since inbreds used as lines were developed at RARS, hence would naturally carry a similar response unlike the testers that were foreign introductions having been

bred in West Africa. The best testers that can be of use include IITA 1 and IITA 7 that had the highest negative GCA_m effects.

Despite lack of significant differences for the *F. verticillioides* ear rot and grain disease score at RARS, there were significant differences for the fumonisins ranging from highly significant for B_1 to significant for B_3. Such a result is consistent with observations that symptomatic expression of infection does not correlate with presence of the fumonisins (Scott, 1993; Suleiman *et al*., 2013). Inbred lines SC 5, and SC 6 that had a positive GCA_f for ear rots at WARC but negative values across sites, had positive GCA_f for B_1, B_2 and B_3 fumonisin analogues. The same trend was observed for the lines SC 12, SC 9 and SC 3 that had the negative GCA_f for Fusarium ear rot at WARC and across all the environments for B_1, B_2 and B_3 fumonisin analogues. SC 8 and SC 1 that had negative GCA_f for ear rots at WARC but positive effects across environments, were negatively correlated with all the fumonisins B_1, B_2 and B_3. A negative relationship was also observed for SC 11 and SC 4 at both WARC and across sites where the GCA_f for ear rots were not corresponding with the observed positive GCA_f for fumonisins. Such results are consistent with the observations by Brown *et al*. (1995) who concluded that some genotypes' reaction to Aspergillus infection had direct correspondence with the amount of aflatoxins obtained on the kernels.

For the testers, on the other hand, the GCA_m mean squares were significant for the ear rots, suggesting clear cut classification of testers as being resistant, moderately resistant and susceptible. This is not surprising as these lines have been screened for aflatoxin resistance, hence the classification was based on the objective quantified analysis of the lines. It shows consistency with findings by Robertson-Hoyt *et al*. (2007) that lines found resistant to aflatoxins caused by *A. flavus* were equally resistant to fumonisins, metabolites from *F. verticillioides*. In this case, additive gene effects can be attributed to the gene action responsible for *F. verticillioides*. The tester inbred lines IITA 4, IITA 7 and IITA 8 had the highest negative GCA_m for ear rots at RARS and WARC except IITA 8 which had negative effects at WARC only. These hybrids and IITA 1 and IITA 5, apart from having negative GCA_m for ear rots at WARC, their GCA for fumonisins at RARS were equally negative. IITA 1, IITA 7 and IITA 8 also had negative GCA_f across all the environments in the 2012/13 and 2013/14 seasons. Positive GCA_m for both ear rots and fumonisins were observed on lines IITA 12 and IITA 9 at WARC,

with the former having a positive effect across sites. The tester IITA 12 contributed most towards susceptibility to ear rots and fumonisins as it had the highest positive GCA effects. Contribution towards resistance to ER was observed to be highest for testers IITA 6 and IITA 11 that had negative GCA_m for ER at WARC with the latter also having a negative effect across all sites. However, both lines had positive GCA_m for all the fumonisins. Such observations were in direct contrast with tester IITA 10 that had the highest positive GCA_m effects for ear rots at WARC but had negative effects across all environments while all the fumonisins were negative. Such results showing negative GCA_m for ear rots while having some of the highest GCA_m for fumonisins concur with observations by Scott (1993) and Suleiman *et al*. (2013) that fumonisins may exist in asymptomatic kernels.

The normal breeding process leads to selection of lines and hybrids resistant to the most frequently occurring diseases. Therefore selection for ear rots at RARS can happen indirectly. At KRC, a normally dry and hot site, diseases seldom occur, hence products developed from such sites may succumb to various diseases. It might be deduced that different pathotypes exist between the two locations leading to specificity when resistance is considered. The highest negative effects were observed on SC 10 across environments, a line that has been associated with drought and wide adaptability. Inbred lines SC 5 and SC 12 had negative ear rot GCA_f while line SC 7 was consistently positive at all the sites, implying that the former lines contributed significantly towards resistance and the latter towards susceptibility to *F. verticillioides* ear rot.

For the testers, inbred lines IITA 1 and IITA 11 had negative GCA_m across all the sites, indicating contribution of resistance to *F. verticillioides* ear rot. The results for IITA 1 are consistent with known resistance to aflatoxins, but the results observed for IITA 11 came as a surprise as *per se*, the line is susceptible to aflatoxins. IITA 6, despite having negative GCA_m effects at WARC, had a positive GCA_m across sites. Inbred testers IITA 2 and IITA 9 had positive GCA_m effects at WARC and across all the sites, suggesting high contribution to susceptibility to *F. verticillioides* ear rots.

Both additive and non-additive effects were important in conferring resistance to fumonisins. Both the GCA_f and GCA_m were significant for fumonisins but not significant for *F. verticillioides* ear rots at RARS in 2012/13 season where artificial

infection was conducted. SCA is associated with non-additive gene effects such as dominance. Maupin *et al*. (2003) found dominance to play a major role in conferring resistance. In a different report on resistance to *A. flavus* ear rot and aflatoxin production, Walker and White (2001) attributed resistance to additive gene effects. It can therefore be deduced that ear rot resistance can be attributed to additive gene effects as the GCA_f and GCA_m were both significant at WARC in the 2012/13 season, and at RARS in 2013/14. Paul *et al*. (2003) studied QTLs associated with aflatoxin resistance using the resistant inbred Tex6 and susceptible B73. They identified loci from both parents that contributed to resistance to aflatoxins. Chromosomes 3, 4, 5 and 10 were associated with the resistance QTLs, suggesting multi-genic activities playing a bigger role in resistance, although they also found a high magnitude of environmental effects that contributed towards making most QTLs significant in one year only. In this study, the environmental effects were equally important, as they largely contributed to variation. Mukanga *et al*. (2010) reported high GxE interaction for the ear rot causing fungi that necessitates use of artificial inoculation which Windham *et al*. (2003) found to play a crucial role in such studies. Earlier, Desjardins *et al*. (1992) postulated that one or two loci are capable of controlling the synthesis of some fungal toxins, an observation that further attributes resistance to accumulation of the mycotoxin to a single or few genes. The gene action associated with additive and dominant gene effects for the resistance to mycotoxins such as aflatoxins has been reported by Campbell *et al.* (1997) who found relatively high heritability from the generation mean analysis (66%). Non-additive and additive gene effects have also been reported by Gorman *et al*. (1992) to determine resistance to aflatoxins as they found various GCA effects interacting with different types of aflatoxins.

For grain yield and other agronomic traits, across all sites in 2012/2013 and 2013/14 seasons, the yield mean squares *per se* were significant ($P \leq 0.05$), as were the GCA_f effects. Inbred line SC 10 had the highest GCA_f for yield. It is the same line that had generally exhibited negative GCA_f for *F. verticillioides* ear rot and fumonisins across sites. As with SC 10, the line SC 5 had positive GCA_f across all the sites. Several hybrids with the recurrent parent SC 5 with wide adaptability exist in southern Africa,. SC 9, SC 3, SC 1 and SC 7 had negative GCA_f effects for yield across sites in the two years of testing.

The tester line IITA 4 had the highest and significant GCA_m effects for GY across all sites in 2012/13 and in 2013/14. This was followed by IITA 2 that had positive effects at all seven sites. This therefore implies high contribution to yield by additive gene effects. IITA 1, IITA 12 and IITA 9 had negative effects across sites and years for GY GCA_m.

Grain yield GCA for both the lines and the testers exhibited significant differences across environments for both the lines (GCA_f) and for the testers (GCA_m). The inverse is true for the SCA where significant differences were observed at WARC in 2013 while there were no significant differences across all sites. Observations of both GCA and SCA that respectively explain additive and non-additive gene effects, have been reported by Long *et al*. (2004) who found SCA effects being more important than GCA effects for yield.

The SCA mean squares for grain yield and ear rots across sites was not significant, although in a cross between SC 11 and IITA 3, both lines that recorded low GCA for yield, were significant and negative. Yield is generally a function of both additive and non-additive effects, especially dominance that has been attributed to heterosis. For traits associated with flowering, the SCA for DMP, DMS and ASI across all sites in 2012/13 and in 2013/14 seasons were significant, implying non-additive gene effects controlling duration to flowering. Differences in flowering were prominent from one site to the other which is consistent with Betran *et al.* (2003) who observed significant differences for days to anthesis for different environments. Dhliwayo *et al*. (2009) observed significant changes in flowering when germplasm is moved from one region to the other.

The magnitudes of the mean squares for the ear rots were inconsistent with the GCA_f values being greater than that of the GCA_m except for the RARS in the 2012/13 season. This trend was also observed for the fumonisins where the mean squares for GCA_f were either higher or equal to that of GCA_m.

For GY, the GCA_f mean squares were higher than GCA_m mean squares in 2012/13 season at KRC, WARC and in 2013/14 at RARS. The flowering related traits (DMP, DMS and ASI), had GCA_f mean squares that were less than the GCA_m mean squares at

RARS in 2012/13 and at KRC 2013/14 seasons while at other sites, the former was more than the latter. The mean squares for height related traits, the PHT and CHT were variable from site to site in terms of GCA_f and GCA_m. Lack of a clear cut trend in the magnitude of mean squares for such traits as GY, flowering and height related traits implies lack of maternal effects which suggests that maternal effects were not important for such traits. For leaf disease ratings of GLS and rust, the mean squares for the GCA_f were always greater than or equal to that of the GCA_m and such results would indicate marginal or lack of maternal effects for such traits. The maternal effects were important for the texture score where the general trend observed was that the GCA_f mean squares were always higher than that of the GCA_m.

5.5 Conclusions

Maize production suffers from abiotic and biotic stress factors of which some contribute towards production of secondary metabolites that are detrimental to the consumers in sub-Saharan countries including Zimbabwe. It has been demonstrated from the results obtained that sources of resistance to Fusarium ear rot and the metabolites produced by these fungi, the fumonisins, exist which include those screened and adapted to the central and West African tropical mid-altitude and southern African mid-altitude. Such lines have proven to be useful in both regions in terms of agronomic performance such as yield. The mating design in which some selected southern African lines were used as lines and central and West African lines were used as testers revealed the type of gene action responsible for resistance to both *F. verticillioides* ear rot and the fumonisins B_1, B_2 and B_3 where both GCA and SCA effects were found to be important. This implies that both additive and non-additive gene effects were important for conferring resistance to the fungus and the mycotoxin production. The southern African inbred lines SC 2, SC 3, SC 4, SC 9, SC 11 and SC 12 frequently had negative GCA effects for *F. verticillioides* ear rot and that can be used as resistance sources. Among these, SC 2, SC 3 and SC 12 had negative GCA for fumonisins although not significant. Testers that can be of high utility include IITA 4, IITA 8, IITA 3, IITA 5, IITA 7, IITA 6 and IITA 1 that had negative GCA for *F. verticillioides* ear rot and for fumonisins. Occurrence of *F. verticillioides* ear rots but without fumonisins was also observed in SC 8, IITA 10 and IITA 2. All other lines had negative GCA for ear rots, but the GCA for fumonisins were positive with the exception of SC 9 that had negative GCA for both traits including the GDS, hence can be a useful line in the introgression of resistance to

both fumonisin causing fungus and the fumonisins themselves. In terms of other agronomic traits, inbred lines SC 10, SC 9 and SC 7 were outstanding among the southern African lines used as lines. The tester lines IITA 4 and IITA 2 exhibited possible utility in these two mega environments with high GCA_m. The lack of consistency in the magnitude of the mean squares for the *F. verticillioides* ear rots suggests either marginal or lack of maternal effects. Because the mean squares for GCA_f were either higher or equal to that of GCA_m for the fumonisins, it can be concluded that a certain level of maternal effects exist. The agronomic traits exhibited marginal or lack of maternal effects except for the texture where GCA_f mean squares were always higher than that of the GCA_m where strong maternal effects existed. Maternal effects were also observed in leaf disease scores where there was a trend that mean squares for the GCA_f always being greater than or equal to that of the GCA_m.

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Chapter 6

Genetic diversity analysis of the southern African and central and West Africa maize inbred lines associated with ear rot resistance and mycotoxins

Abstract

In a maize breeding programme, characterisation of germplasm is important as it assists in the management of available germplasm and any introduced exotic germplasm which impacts positively in the exploitation of heterosis. Acquisition of germplasm from public institutions like CIMMYT, IITA and other institutions that Seed Co collaborate with remains important in increasing diversity within the gene pool while introducing additional alleles of importance. The objective of this study was to establish the genetic relationship that may exist between and among elite lines from Seed Co southern Africa and IITA central and West African inbred lines with various known reaction to ear rot causing fungi including *Fusarium verticillioides* or aflatoxin contamination. A total of 24 inbred lines of which 12 lines were from central and West Africa while the other 12 were from southern Africa were evaluated using 1140 SNP markers of which 998 markers were used in the final analysis. The genetic diversity level was moderate as the dissimilarity average was 0.29. The average genetic distance based on Rogers' dissimilarity coefficients was 0.297 which is equally low. The lines IITA 12 and SC 11 had the highest divergence of 0.3782. Despite that, the dendrogram based on the Rogers' dissimilarity coefficients indicated three main clusters, where cluster one contained the Seed Co germplasm, cluster two the IITA lines and cluster three contained an odd line with some temperate pedigree that was converted to white and quality protein maize in southern Africa. The subgroups, particularly from Seed Co represented the four heterotic groups used while the IITA group had two subgroups that could possibly represent two opposite heterotic groups. The results from this analysis were consistent with the pedigree information, hence will assist in utilizing the resistance sources for both the mycotoxin and their causal fungi within the Seed Co breeding programme.

6.1 Introduction

In a breeding programme, a high level of genetic diversity is desired as well as the background on the genetic diversity level within the available gene pool. This is a starting point, especially where new alleles are desired (Warburton *et al*., 2009). Use of germplasm with a wide diversity safeguards any breeding programme from genetic vulnerability in the event of a sudden outbreak of a new strain of disease or pest or sudden changes in climatic conditions. The lack of genetic diversity may result in rendering a product susceptible to an outbreak of biotic stresses or changes in abiotic stress (Singh, 2005). An increase in diversity in a breeding programme can best be achieved through the introduction of new variation from diverse sources. In maize breeding, inbred lines derived from the same populations are considered similar due to the same genetic background. These lines would be maintained within certain groups known as heterotic groups and when mated across another group, will achieve high levels of heterosis (Parentoni *et al*. 2001). The determination of genetic diversity has become an important component of the breeding procedure in a maize breeding programme, because the genetic progress depends on the existence of genetic variability. Traditionally, this has been achieved through test crossing (Badu-Apraku *et al.*, 2013) which is currently being complemented by molecular tools.

Molecular tools have enabled breeders to determine genetic diversity more accurately with different types of analyses of which the genetic distance (GD) between and among individuals play an important role (Betran *et al*., 2003; Menkir *et al*., 2010). The GD can be defined as a quantitative measure of genetic difference at either the sequence or allelic frequency level which is calculated between individuals, populations or species (Mohammadi and Prasanna, 2003). Genetic distance or similarity (GS) using binary data can be measured in different ways including: i) Nei and Li's coefficient (GDNL) (Nei and Li, 1979), ii) Jaccard's coefficient (GDJ) (Jaccard, 1901), iii) simple matching coefficient (GDSN), and Modified Rogers' distance (MRD). The MRD is a measure of genetic distance that indicates no diversity when it is zero (0) to no similarity when the value is one (1). This is determined by the square root fraction of the heterozygous loci of the hybrids with homozygous loci. Dudley *et al.* (1991) used this measurement with the analysis of temperate maize germplasm and found significant but low correlation of MRD, with specific combining ability (SCA) for yield (0.35 with 66 loci and 0.25

with 29 loci). Reif *et al*. (2003) found a significant (p<0.01) correlation between MRD^2 and pelmitic mid parent heterosis (PMPH) of 0.63.

Genetic distance has been correlated to combining ability or heterosis in maize and other crops. While using temperate maize germplasm, Betran *et al.*, (2003) recorded significant and positive correlation between SCA and mid parent (r = 0.47), and SCA and high-parent (r = 0.31) heterosis. In other crops besides maize, the results reported have been mixed with Cheres *et al*. (2000) reporting significant correlation between the hybrid performance and GD in sunflower (*Helianthus annus* L.) and no correlation between diversity values and hybrid performance in wheat (Martin *et al*., 1995) with low correlation (r = 0.07) between yield of F2 hybrids, heterosis and GD. High correlation indicates that determination of genetic distance will provide adequate information to design crosses for optimum expression of heterosis in hybrids at a minimum cost (Menkir *et al.*, 2010).

Heterosis is often observed among lines of the same heterotic group which explains why significant GD and SCA can be obtained, but with a general weak correlation (r<0.5). This suggests that determination of GD cannot solely replace evaluation of hybrids for SCA (Shieh and Thseng, 2006; Benchimol *et al*., 2008; Menkir *et al*., 2010). This distortion can be explained by several factors that contribute to heterosis and these include theories of dominance and over-dominance, as well as biochemical factors (Crow, 2010).

Various molecular markers have been used to evaluate genetic diversity, but simple sequence repeats (SSR) have been extensively used in maize for this purpose (Senior *et al*., 1998; Warburton *et al*., 2002; Prasanna *et al*., 2002; Reif *et al*., 2003, Dhliwayo *et al*., 2009). The SSR markers have been the most preferred molecular markers due to the inherent high level of polymorphism which offers high prospects for large-scale fingerprinting of maize genotypes (Mitchell *et al*. 1997; Warburton *et al*., 2002).

With new developments in marker technology, SNP markers are becoming more popular due to their abundance in the genome, low cost, high degree of precision during genotyping, locus-specificity and co-dominance characteristics as well as high potential for automation (Rafalski *et al*., 2002; Schlotterer, 2004; Chagne *et al*., 2007).

There are various SNP platforms which offer high throughput of which the chip based technologies, Illumina and KASP (formerly KASpar), provide multiplexing possibilities and high throughput. Uniplex platforms are also available which are suitable where a few SNPs are needed over a larger number of samples that are normally involved in mapping projects, marker assisted recurrent selection (MARS), marker assisted backcrossing (MABC) as well as quality control. In uniplex however, identifying the best SNPs is important for achieving good level of discrimination (Low *et al*., 2006).

The objective of this study was to assess the extent of genetic diversity of 24 maize inbred lines using SNP markers and to determine the level of homozygosity among these lines.

6.2 Materials and methods

6.2.1 Germplasm

The maize inbred lines used in the North Carolina Design II for the inheritance studies in Chapter 4 as listed in Table 4.1 were used in this study. In brief, these comprised of 12 maize inbred lines that are commonly used and adapted to the southern African mid-altitude region, and 12 lines from International Institute for Tropical Agriculture (IITA) that are adapted to tropical mid-altitude in central and West Africa. The southern African lines were selected based on reaction in terms of resistance to ear rot caused by *Stenocarpella maydis* and *Fusarium verticillioides,* while selection for the central and West African inbred lines was based on their reaction to aflatoxin production and *Aspergillus flavus* infection.

6.2.2 Sample preparation

Seed from the 24 inbred lines were planted in plastic trays in the greenhouse until 3-4 leaf stage which is 2-3 weeks after planting. Leaf tissue from 10 plants per each inbred line were punched and the collected leaf disks were pooled (Dhliwayo *et al*., 2009; George *et al*., 2011; Strigens *et al*., 2013) into 96 well sample tubes supplied by LGC Genomics (LGC). A packaging protocol supplied by LGC was followed to securely seal the samples before shipping to LGC laboratories. This was done in two replications to ensure that at least one of the replications could be successfully prepared and shipped to produce quality DNA. The lines were shipped to LGC laboratory Hoddesdon, Herts,

UK, for both DNA extraction and SNP genotyping as leaf tissue enclosed with a supplied dessicant.

6.2.3 Deoxyribonucleic acid (DNA) extraction

DNA extraction was done at LGC according to their protocol which, is an automated magnetic extraction process. The leaf tissue were centrifuged to bring the sample to the bottom of the well. The leaf samples were ground into a fine powder using a GenoGrinder at 1500 revolutions per minute (rpm) for 30 seconds. The lysis buffer (buffer L1) was added to the ground leaf material and vortexed to mix the sample thoroughly with the buffer. The plates were incubated for 30 seconds at 55^{o}C while shaking gently at regular intervals. The samples were transferred to a new set of deep well plates with magbeads in each well and mixed until the attainment of a uniform colour. The samples were incubated for a further 15 minutes at 55^{o}C. A magnet was used to pellet the silica containing the DNA to the bottom of the wells and a gentle shaking process ensured the magbeads were pelleted from the lid. The supernatant was carefully discarded before adding wash buffer (A1) while mixing gently until attainment of a uniform colour. A magnet was used to draw the silica containing the DNA to the base of the well using the magbeads. After the removal of the supernatant, a wash buffer (buffer W1) was added into the well before sealing that was preceded by mixing thoroughly to a uniform colour. Pelleting of the magbeads was further achieved by following the same procedure of using the magnetized silica and gentle shaking. The supernatant was carefully removed while the pellets were dried by placing the tubes into an oven at 55^{o}C before adding the elution buffer (buffer E1). The samples were mixed by vortexing for two minutes and incubated for 30 minutes at 55^{o}C while being shaken gently, before finally being vortexed briefly. This was followed by centrifuging and the eluted DNA was transferred to fresh tubes before the plates were kept into -20^{o}C freezer.

The procedure involved two steps of binding to an adapter which allows specific binding of nucleic acids. It then used pure water to wash before yielding pure and high quality DNA.

6.2.4 Single nucleotide polymorphism (SNP) genotyping

The validation of the assays was achieved through the use of LGC's KASP genotyping chemistry based on the competitive allele-specific PCR (KASP) genotyping chemistry. This incorporates oligo extension and a fluorescence resonant energy transfer (FRET) quencher cassette that had a fluorescence of either 2´chloro-7´-phenyl-1,4-dichloro-6-carboxyfluorescein (VIC) or fluorophores 6-carboxyfluorescein (FAM) fluorophores (Kumpatla *et al*., 2012). The KASP system is capable of bi-allelic scoring at specific loci SNPs, insertions and deletions (Semagn *et al*., 2014).

A total of 1250 already developed maize SNP markers were used. These SNPs markers were developed at Cornel University and converted as a joint effort with International Maize and Wheat Improvement Centre (CIMMYT). Out of these 1250, 106 failed the in-house test of LGC where automatic quality of the data was done per marker basis, resulting in 1144 markers to continue the SNP analysis.

The process involved inclusion of the no template controls (NTCs) which facilitated determination of contamination or amplifications that are non-specific. The selected markers had a call rate >90% with minor allele frequency > 2% except for the SNPs that were known to have low frequencies.

DNA was arrayed in a 96 well microtitre PCR plate. Two components of the protocol were used that included the SNP-specific KASP Assay mix and the universal KASP Master mix.

The assay mix comprised of three unlabeled primers, the SNP specific component of the system that included two allele-specific forward primers and a reverse primer that was common. Each of the allele-specific primers had a peculiar tail sequence matching the universal fluorescence resonant energy (FRET) transfer cassette where one had a FAM dye label while the other had a HEX dye.

The universal master mix included the totality of other required components such as the reporting system based on the universal fluorescent. The master mix had the universal FRET cassettes, a passive reference dye called ROX, taq polymerase, free nucleotides

and $MgCl_2$ contained in buffer solution that was optimized. These two were added to the DNA samples before thermal cycling was initiated.

The sample arraying in a microtitre 96-well PCR plate was the next step. The reaction on the PCR had a total volume of 8 µl with 20 ng μl^{-1} template DNA, reaction mix of 2 µl, assay mix of 0.11 µl, 0.064 µl $MgCl_2$, a volume of 0.026 µl of KTaq polymerase, and a volume of 1.8 µl of H_2O. The reaction mix had a 2.2 mM $MgCl_2$ concentration.. A liquid dispenser was used to dispense the assay mix and reaction mix over the DNA samples. The plates were sealed by the fusion laser welding mechanism which was followed by an end-point fluorescent reading.

The LGC "Duncan" water bath cycler was used for the PCR cycling. The optimised cycling conditions used were one cycle at 94°C for 15 min, followed by 20 cycles at 94°C for 10 seconds, before going down to an annealing temperature of 57°C for 5 seconds and extension at 72°C for 10 seconds. This was followed by 18 cycles of denaturing at 94°C for 10 seconds, with the same annealing and extension temperatures as for the first step but for longer durations for 20 seconds at 57°C and for 40 seconds at 72°C respectively. The plates were read using a fluorescence resonance energy transfer (FRET) plate reader. The FAM and VIC were used to distinguish between genotypes and for the passive reference, ROX was used as the reference dye. Homozygous genotypes generate only one of the two possible fluorescent signals while heterozygous genotypes caused a mixture of fluorescent signal to be emitted.

6.2.5 Data analysis

The SNP data were analysed using PowerMarker version 3.25 (Liu and Muse, 2005) to calculate various statistics as presented in Appendix 2. These included number of major alleles, number of genotypes, and proportion of samples without missing data, gene diversity, observed heterozygosity, and polymorphic information content (PIC).

The PIC estimate was derived at by:

$$PIC_i = 1 - \sum_{j=1}^{n} P^2_{ij} - \sum_{j=1}^{n-1} \sum_{k=j+1}^{n} 2 P^2_{ij} P^2_{ik}$$

where:

P_{ij} and P_{ik} = marker $_i$ allele frequencies of $_j$th and $_k$th respectively while the summation cuts across *n* alleles (Botstein *et al*., 1980).

For the genetic distance analysis, the Modified Rogers Genetic distance (MRD) between each pair of lines was calculated following Wright (1978) and Goodman and Stuber (1983).

MRD was calculated between each pair of inbred lines as

$$\text{MRD} = \sqrt{1/2m \sum_{I=1}^{m} \sum_{j=1}^{a} (p_{ij} - q_{ij})^2}$$

Where:

p_{ij} and q_{ij} = allele frequencies of the *j*th allele at the *j*th marker in the two lines under consideration.

a_i = number of alleles at the *i*th marker
m = number of markers

Average linkage clustering analysis of the SNP data was based on, MRD dissimilarity coefficients (Rogers, 1972) between pairs of lines. The analysis of the similarity matrix was done using the neighbour-joining algorithm in PowerMarker version 3.25 (Liu and Muse, 2005) which generated dendrograms that were visualized in MEGA version 6 (Tamura *et al*., 2013). The minor allele frequencies were derived by determination of the difference between one and the major allele frequencies.

6.3 Results

6.3.1 Quality of SNP analysis

One set of the samples submitted had sufficiently high DNA quality and was used in the SNP analysis. A total of 1250 SNP markers were used to characterize the inbred lines, but only 1144 markers were advanced to further analysis andthose with low calling values (<0.95) were excluded. Further more, markers that were either monomorphic (146 markers) or had missing data points (?%) that were more than 20%

including those with minor allele frequencies that were lower than 5% were excluded and only 998 markers constituting 87% remained for the diversity assessment.

Despite exclusion of markers with minor allele frequencies that were lower than 5 %, in the final analysis, the minor allele frequencies (Appendix 4) ranged from 0.01 to 0.50. The majority of the markers (32%) had minor allele frequencies between 0.0 and 0.10, while minor allele frequencies between 0.11 to 0.5, rangedfrom 15% to 18% (Figure 6.1).

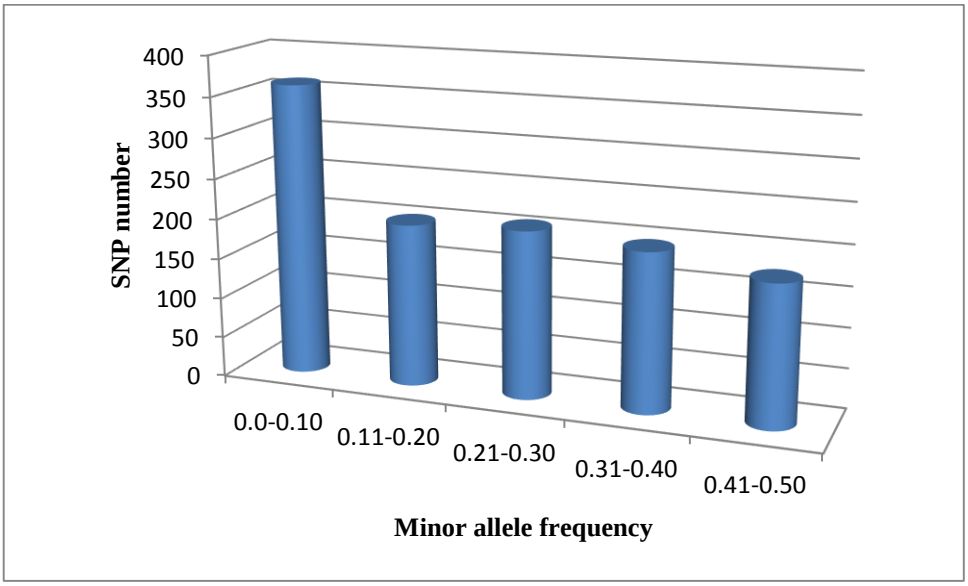
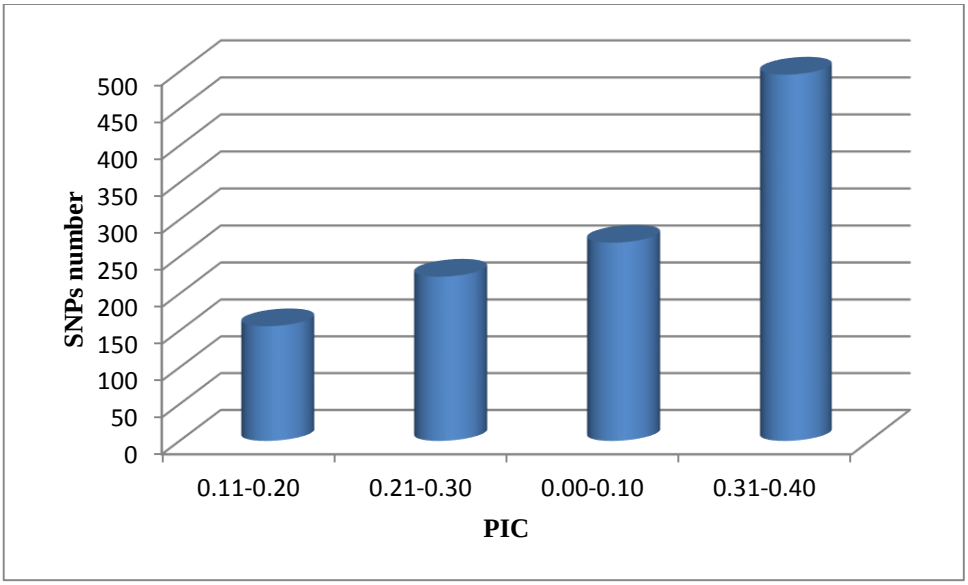


Figure 6.1 Single nucleotide polymorphism (SNP) minor allele frequency distribution among the 24 inbred lines based on 998 SNP markers

PIC demonstrates how informative the SNP loci are and their potential to detect differences among inbred lines based on their genetic relationships. The PIC values (Appendix 5) for the 998 SNP loci ranged between 0.040 and 0.375 with an average value of 0.23. The gene diversity which is the expected heterozygosity ranged between 0.0408 and 0.500, the mean being 0.2861. The highest frequency distribution was within the range of 0.31 to 0.40 (Figure 6.2) that comprised of 43% of the SNPs.



SNP=single nucleotide polymorphism; PIC=polymorphic information content

Figure 6.2 Polymorphic information content (PIC) frequency distribution among the 24 maize inbred lines based on 998 SNP markers

The calculation of the number of heterozygous loci per inbred line of the 13 lines identified, resulted in nine lines having a proportion of heterozygosity of 0.01, two (SC 1 and IITA 3) with 0.02 and one each with 0.03 and 0.05 for inbred lines SC 3 and SC 6, respectively. The other 11 lines had a 0.00 proportion of heterozygosity (Table 6.1) and are therefore regarded as homozygous since they were 100% homozygous.

Table 6.1 Missing single nucleotide polymorphism (SNP) data, number of heterozygous loci and proportion of heterogeneity of the 24 maize inbred lines

Taxa Name	No. of Sites	No. of SNPs with mising data	Proportion Missing	No. of heterogenous SNPs	Proportion Heterogeneity
SC 10	1144	29	0.03	3	0.00
SC 12	1144	25	0.02	12	0.01
SC 5	1144	21	0.02	5	0.00
SC 4	1144	20	0.02	8	0.01
SC 2	1144	14	0.01	3	0.00
IITA 1	1144	37	0.03	15	0.01
SC 3	1144	188	0.16	24	0.03
SC 8	1144	76	0.07	3	0.00
SC 7	1144	11	0.01	5	0.00
SC 6	1144	65	0.06	56	0.05
SC 1	1144	43	0.04	21	0.02
SC 9	1144	83	0.07	8	0.01
SC 11	1144	28	0.02	7	0.01
IITA 8	1144	36	0.03	4	0.00
IITA 4	1144	18	0.02	7	0.01
IITA 2	1144	23	0.02	3	0.00
IITA 3	1144	36	0.03	21	0.02
IITA 9	1144	19	0.02	9	0.01
IITA 7	1144	24	0.02	3	0.00
IITA 10	1144	31	0.03	6	0.01
IITA 11	1144	19	0.02	5	0.00
IITA 5	1144	36	0.03	0	0.00
IITA 12	1144	21	0.02	4	0.00
IITA 6	1144	36	0.03	11	0.01

SNP=single nucleotide polymorphism

6.3.2 Genetic distances of the maize inbred lines generated from single nucleotide polymorphism (SNP) markers

The genetic distances (GD) among the maize inbred lines based on Rogers dissimilarity coefficient of the SNP data ranged between 0.0310 and 0.3782 with an average distance of 0.2971 (Table 6.2).

The lines IITA 12 and SC 11 had the highest distance of 0.3782. The other high distances recorded were 0.3678 (between SC 6 and SC 7), 0.3673 (between SC 8 and SC 7), 0.3643 (between SC 11 and IITA 1), 0.3620 (between SC 11 and SC 7) and 0.3618 (between SC 11 and IITA 7).

The lowest GD was 0.0310 recorded between lines IITA 3 and IITA 9. Among the pairs with low GD values were IITA 9 and IITA 6 (0.0949), SC 5 and SC 2 (0.0960) and IITA 3 and IITA 6 (0.0981).

Visualisation of the GD based on the PowerMarker 3.25 neighbour-joining cluster analysis that was done in MEGA version 6 (Temura *et al.*, 2013) showed three major groups (Figure 6.3). Lines clustered based on origin. The first group was made up of SC 6, SC 8, SC 1, SC 7, SC 4, SC 3, SC 2, SC 5, SC 9 and SC 11. The second group had maize inbred lines IITA 9, IITA 3, IITA 6, IITA 1, IITA 12, IITA 4, IITA 10, SC 12, IITA 5, IITA 8, IITA 7, IITA 2 and IITA 11. The third and last was made up of a single line SC 10.

Within these groups, some sub-groupings existed especially in the first group made up of lines from southern African mid-altitude mega environment where three sub-groups consisting of lines SC 6, SC 8 and SC 1 formed the first sub-group, SC 7, SC 4 and SC 3 (sub-group 2) and SC 2, SC 5, SC 9 and SC 11 being the last sub-group 3. The first sub-group (1A) has SC 6, an N3 line converted to QPM to develop SC 8 which used *opaque-2* gene donor from the same source as SC 1. These lines clustered closely as expected and was confirmed by the genetic distances between the lines. The sub-group 2 (1B) is made up of SC 7 and SC 4 that belong to the SC heterotic group and SC 3 that is a total misplace as it has always been considered an N3 from the pedigree records. However, it is important to note that this is the line that has a slightly higher heterozygosity implying a lack of purity hence could be suggestive of the reasons for misplacement. The third sub-group (1C) comprises of the "P" heterotic group with the inbred line SC 5 being a QPM version of SC 2 while the QPM donor for SC 5 was also used on SC 9.

Table 6.2 Rogers' genetic distances estimates based on the single nucleotide polimorphism data

	SC10	SC12	SC5	SC4	SC2	IITA 1	SC3	SC8	SC7	SC6	SC1	SC9	SC11	IITA 8	IITA 4	IITA 2	IITA 3	IITA 9	IITA 7	IITA 10	IITA 11	IITA 5	IITA 12	IITA 6
SC10																								
SC12	0.3074																							
SC5	0.3326	0.3200																						
SC4	0.3236	0.3117	0.3265																					
SC2	0.3338	0.3090	0.0960	0.3329																				
IITA 1	0.3219	0.3075	0.3359	0.3271	0.3251																			
SC3	0.3124	0.3151	0.3080	0.2985	0.3088	0.3169																		
SC8	0.3369	0.2839	0.3384	0.3427	0.3472	0.3439	0.2947																	
SC7	0.3502	0.3395	0.3378	0.2065	0.3375	0.3447	0.2452	0.3673																
SC6	0.3474	0.3013	0.3514	0.3402	0.3536	0.3402	0.2732	0.1601	0.3678															
SC1	0.3250	0.3140	0.3246	0.3175	0.3122	0.3246	0.2835	0.3079	0.3546	0.2876														
SC9	0.3056	0.2733	0.2925	0.3078	0.2846	0.3150	0.2879	0.3290	0.3251	0.3588	0.3188													
SC11	0.3552	0.3306	0.3373	0.3484	0.3391	0.3643	0.3165	0.3538	0.3620	0.3450	0.3419	0.3137												
IITA 8	0.3275	0.2751	0.3352	0.3054	0.3362	0.2989	0.2616	0.2941	0.3247	0.3031	0.3023	0.2999	0.3364											
IITA 4	0.3100	0.2894	0.3314	0.3339	0.3360	0.1973	0.2950	0.3276	0.3411	0.3437	0.3312	0.2975	0.3491	0.2758										
IITA 2	0.3005	0.2855	0.3194	0.2969	0.3258	0.2592	0.2699	0.3063	0.3234	0.3184	0.2982	0.2983	0.3309	0.1702	0.2233									
IITA 3	0.3326	0.3028	0.3173	0.3410	0.3234	0.1732	0.3130	0.3345	0.3455	0.3400	0.3308	0.3267	0.3558	0.2953	0.1976	0.2440								
IITA 9	0.3222	0.3011	0.3176	0.3360	0.3228	0.1661	0.3051	0.3330	0.3402	0.3352	0.3223	0.3160	0.3588	0.2939	0.1899	0.2511	0.0310							
IITA 7	0.3294	0.2855	0.3391	0.3102	0.3400	0.2696	0.2755	0.3171	0.3225	0.3365	0.3165	0.2965	0.3618	0.1402	0.2181	0.1277	0.2509	0.2529						
IITA 10	0.3258	0.2941	0.3250	0.3425	0.3302	0.2551	0.2965	0.3228	0.3359	0.3279	0.3096	0.3006	0.3384	0.2636	0.2216	0.2187	0.2391	0.2343	0.2255					
IITA 11	0.3077	0.2738	0.3050	0.3043	0.3195	0.3046	0.2926	0.3156	0.3399	0.3234	0.3176	0.3150	0.3570	0.2278	0.2662	0.2201	0.2917	0.3008	0.2296	0.2596				
IITA 5	0.2983	0.2845	0.3240	0.3061	0.3283	0.2790	0.2718	0.2985	0.3128	0.3294	0.3170	0.2823	0.3458	0.1315	0.2459	0.1491	0.2760	0.2768	0.1319	0.2482	0.2266			
IITA 12	0.3297	0.3060	0.3362	0.3285	0.3212	0.1783	0.2962	0.3554	0.3377	0.3490	0.3273	0.3129	0.3782	0.3006	0.2270	0.2767	0.1929	0.1857	0.2818	0.2765	0.3142	0.2885		
IITA 6	0.3187	0.2917	0.3186	0.3281	0.3136	0.1552	0.3002	0.3420	0.3418	0.3348	0.3168	0.3142	0.3562	0.2905	0.1961	0.2325	0.0981	0.0949	0.2546	0.2366	0.2844	0.2839	0.1807	

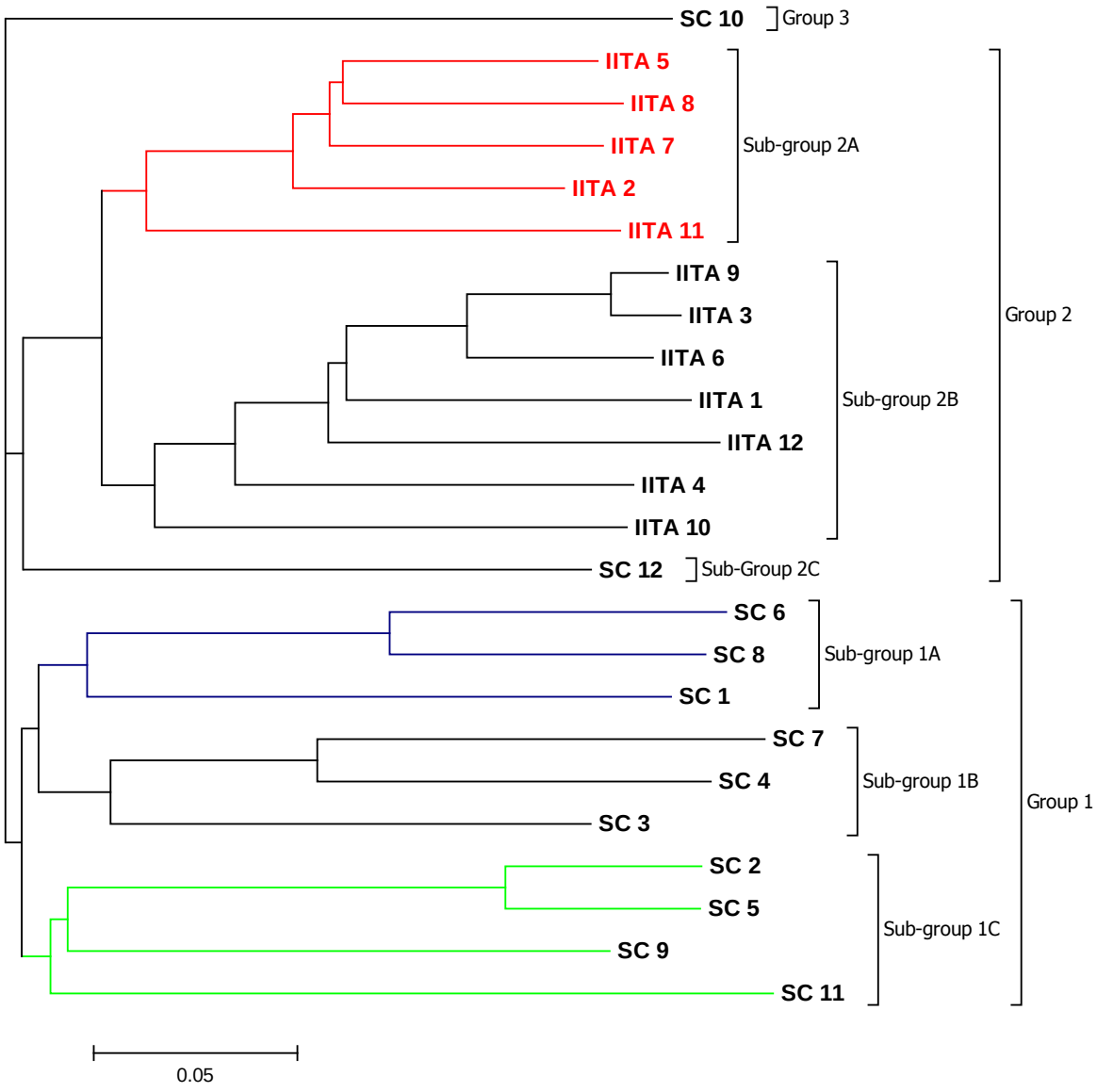


Figure 6.3 Maize inbred lines neighbour-joining cluster analysis from genetic distances estimated by Rogers dissimilarity coefficient using 1144 single nucleotide polymorphism (SNP) markers

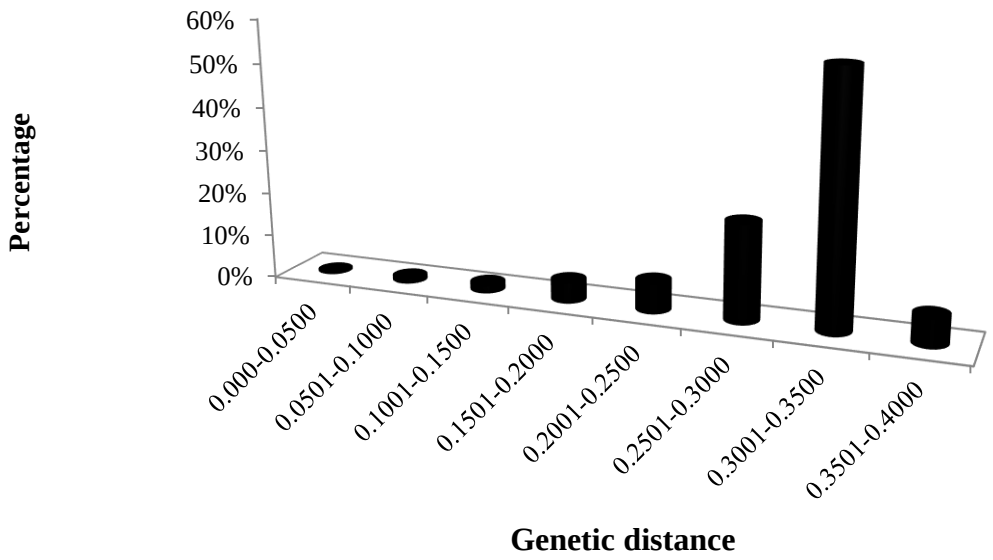


Figure 6.4 Total expected pairwise genetic distance

Table 6.3 Frequency of the genetic distance on a scale of 0.05

Genetic distance	Frequency (%)	Frequency
0.0000-0.0500	0.36	1
0.0501-0.1000	1.09	3
0.1001-0.1500	1.81	5
0.1501-0.2000	4.71	13
0.2001-0.2500	6.88	19
0.2501-0.3000	22.1	61
0.3001-0.3500	56.52	156
0.3501-0.4000	6.52	18
Total	**100**	**276**

The frequencies above were based on a GD scale of 0.05. However, when the GD frequency is looked at on a scale of 0.1, the highest frequency is between a scale of 0.3 and 0.4 constituting 63.5% (Table 6.4 and Figure 6.5).

Table 6.4 Frequency of the genetic distance on a scale of 0.1

Genetic distance	Frequency
0.0000-0.1000	1.45
0.1001-0.2000	6.52
0.2001-0.3000	28.99
0.3001-0.4000	63.04
Total	**100.00**

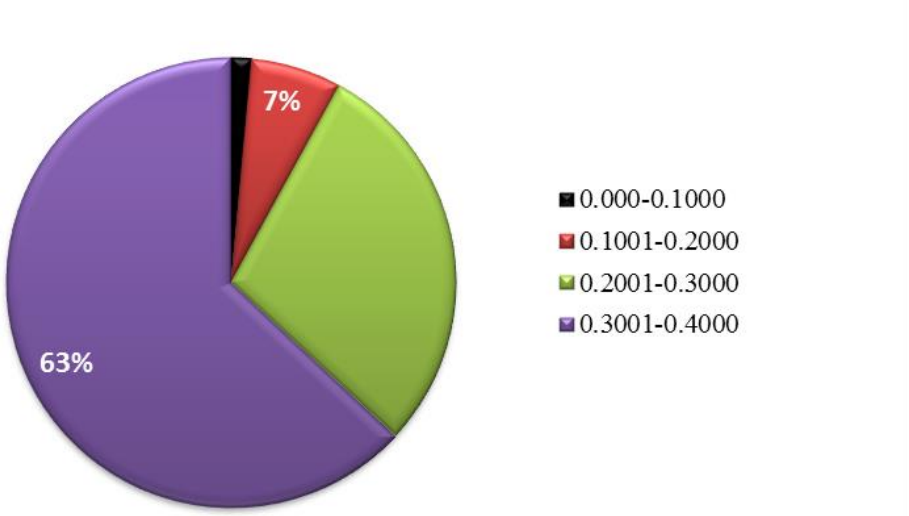


Figure 6.5 Total expected pairwise genetic distance frequencies on a scale of 0.1

The frequencies of the GD based on GD scale of 0.05 are illustrated in Figure 6.3 and Table 6.4 where the highest frequency was occurring between the GD of 0.3001 and 0.3500 (156 or 56.5%).

6.4 Discussion

Genetic variability is a basic requirement in any breeding programme as this facilitates genetic gain. This is attained through introductions or through inter-crossing germplasm that offers required traits for any breeding objectives. Some institutions such as those in the temperate regions have done a substantial amount of work on maize in accumulating favourable alleles for priority traits of interest such as yield (Hoisington *et al*., 1999; Vigouroux *et al*., 2005). That process has led into reduced diversity within the breeding pool within the past century (Duvick *et al.*, 2004). As such, this improved germplasm found its way into various breeding programmes around the world. Private seed companies are working across diverse environments. Hence use of introduction such as maize developed in different environments *per se* or in combination with existing germplasm has become a common feature or practice in their breeding programmes.

In this study, where 24 maize inbred lines coming from two mega environments, the mid-altitude central and West Africa tropics and the mid-altitude southern African for possible use across such environments, were analysed for their genetic relatedness using SNP markers. Out of the screened 1250 SNP markers, 106 markers that had a low call rate of less than 0.95 and were disarded. Exclusion of some markers has been reported by Strigens *et al*. (2013) who initially had 56,110 SNPs, and used 24,572 SNP. Lu *et al*. (2009) used 0.25 as a no call threshold for the lower bound Illumina GoldenGate for genetic analyses after considering the call rate and the quality check. This included the magnitude of the frequency of the missing values. Missing alleles are those lacking completely in a specific subset of germplasm but occurring at regular frequency in all germplasm (Lu *et al*., 2009). Semagn *et al*. (2012) used the criterion similar to this to select some SNPs to use for the final analysis which constituted 69.3 % of the initial SNPs. While using chip based technologies, Wen *et al*. (2011) and Lu *et al*. (2009) discarded 18 % and 33 % respectively. In this study, of the 1144 SNP markers selected, 998 were used in the final analysis of the relatedness of these inbred lines. The lower rate of discarded markers can be attributed to the pre-selection of SNP markers normally done with KASP users (Semagn *et al*., 2014). To genetically differentiate inbred lines of intermediate relatedness, typical of the samples used in this study, Tivang *et al*. (1994) showed that about 150 alleles would be sufficient. The implication of such results includes selection of SNP markers that are polymorphic for future use while the effort of starting with a higher number remains commendable. This is important to ensure that what remains usable will

be high enough to successfully validate the results finally obtained. The bi-allelic nature of SNP markers were proven in this study as two alleles per locus were observed while Dhliwayo *et al*.(2009) as they were working with SSR markers on CIMMYT and IITA lines obtained and amplified 209 alleles using 62 SSR markers thus giving an average of 3.4 alleles per marker. Yan *et al*. (2009) reported that for an equivalence of a single SSR, there is a need of ten or more SNPs in terms of the detected alleles in genetic diversity studies. As such, the SNPs in this study were more powerful in determination of the GD.

The missing number constituted a mean of 3% which is slightly less than what Dhliwayo *et al*., 2009 who obtained 4.5% per SSR marker while working with CIMMYT and IITA inbred lines which were different from the ones used in this study. With more markers used and less missing markers, this suggests consistency with other work done elsewhere and adds credibility to the genotyping process done in this study. Those used in this study were coming from a private company Seed Co while the IITA lines were selected on the basis of their reaction to aflatoxin resistance with a high probability that they might have been derived from the temperate germplasm. Sources of aflatoxin resistance have been reported in the USA Corn Belt germplasm (Hamblin and White, 1999; Busboom and White, 2004; Warburton *et al*., 2009).

The majority of the markers had the minor allele frequencies of 0.0-0.10 and these constituted 32% of the markers with 15-18% of markers falling between minor allele frequencies of 0.11 to 0.5. Lu *et al*. (2009) reported 8.8% of minor allele frequencies below 0.05 while Semagn *et al*. (2012) reported that within the minor allele frequencies of 0.051 and 0.20, there were 37.7% SNP markers. These results show that 68% of the minor alleles had frequencies above 0.11 with 15% being closer to 0.5 similar to the result of 18.7% obtained by Lu *et al*. (2009). This therefore suggests that most of the markers could be used in a maize breeding programme for diversity purposes as they had a high minor allele frequency and demonstrates that the germplasm came from diverse sources.

The PIC values obtained were consistent with other work done by Lu *et al*. (2009) which was 0.23. This value indicates the magnitude of genetic diversity or the value for each marker relative to the level of polymorphism. The obtained value however appear to indicate less genetic diversity within the used germplasm or that it is slightly informative (<0.25) if the ranges put up by Botstein *et al*.

(1980) while using restriction fragment length polymorphisms (RFLP) markers were to be followed. Dhliwayo *et al*. (2009) while using SSR markers had PIC values ranging from 0.00 to 0.77 with an average of 0.43 that was less than what Betrán *et al*. (2003) obtained on 17 inbred lines, Xia *et al*. (2004) while working with tropical germplasm, and what was obtained by Senior *et al*. (1998), and Barata and Carena, (2006) on temperate maize lines.

Due to the bi-allelic characteristic of SNP markers, the maximum obtainable PIC value is 0.50 (Kota *et al*., 2008) which is consistent with these results where the value ranged between 0.04 and 0.375. Botstein *et al*. (1980) as they worked on RFLP markers, described the PIC values as highly informative when >0.50, reasonably informative when between 0.25 and 0.50 and slightly informative when <0.25. However, the PIC values for SNPs are low being the reason why the marker density is usually high (Lu *et al*., 2011). In this study, 43 % of the markers had a PIC value greater than 0.3 suggesting that the markers were sufficiently informative.

The expected heterozygosity expressed as gene diversity mean of 0.286 was consistent with what has been obtained in maize (Lu *et al*., 2009; Semagn *et al*., 2012; Strigens *et al*., 2014). The gene diversity of 0.29 for SNPs is regarded as good since SNP markers also tend to give low gene diversity (Lu *et al*., 2009; Semagn *et al*., 2012). Dhliwayo *et al*. (2009) had a lower diversity which was attributed to the sample size of 18 lines and the relatedness of some of the lines that were used.

All the lines had more than 95% homozygosity indicating that the maize inbred lines used were genetically pure. At F6, the expected heterozygosity is 3.125% (Poehlman and Sleper, 1995). In the study conducted by Lu *et al*. (2009) using germplasm from CIMMYT, Brazil and China that was very variable, they obtained within the genetic loci, a mean heterozygosity of 4.3% which inversely expresses a homozygosity of 95.7%. While working on DH lines, Strigens *et al*. (2013), the inbred lines that had more than 2% heterozygosity were not included in the final analysis. It is expected that inbred lines are pure although allele frequencies may change during the regeneration of the seed, during the process of line maintenance, while bulking the lines or through contamination both physically or through foreign pollen (Heckenberger *et al*., 2002; Warburton *et al*., 2010). Lack of purity of a line in the form of changes in the form of genetic constitution may lead into significant negative effects on performance of the line in question (Semagn *et al*., 2014).

The neighbour-joining clustering analysis of the level of diversity showed genetic relationship among the lines. The GDs amongst these 24 inbred lines ranged between 0.0310 and 0.3782 which are consistent with those obtained by Semagn *et al*. (2012) for lines from eastern and southern Africa, and Lu *et al*. (2009) for germplasm from CIMMYT, China and Brazil. However, a pair-wise modified Rogers' distance ranging between 0.45 and 0.52 was obtained by Strigens *et al*. (2013) after using 24,572 SNP on landraces and European improved germplasm while Dhliwayo *et al*. (2009) recorded the GD of 0.15 - 0.67 for the most related pair with an average MRD of 0.50 that was less than what Xia *et al*. (2004; 2005) while working on tropical maize germplasm. The largest GD was between the southern African mid-altitude line and the mid-altitude central and West African maize inbred line. In the study by Lu *et al*. (2009), it was between the Chinese temperate and the Brazil and CIMMYT tropical and subtropical maize inbred lines that had the largest GD, while their smallest was observed from predominately tropical to mid-altitude found within the CIMMYT and Brazilian materials which is not surprising as the later relies on the former as a source of germplasm. The results obtained in this study are not surprising as recycling in pedigree breeding within the southern African program is quite rampant including use of the same germplasm in conversion programmes to traits such as quality protein maize (QPM) through backcrossing where such lines appear with the recurrent parents. This is more so with the southern African maize inbred lines within the first group that has been identified to have even more sub-groups. The preponderance of sub-groups within the southern African lines could also be attributed to several heterotic groupings within the germplasm such as derivatives of the SC, N3, Iodine, M37W, the P (Potchefstroom Pearl) and the H that was previously classified as "K", also known as Pride of Saline. High level of diversity was not only observed between the Seed Co and IITA lines, but also within the Seed Co lines. For instance, among the pairs that had the highest genetic distances, IITA 12 and SC 11 (0.3782), SC 6 and SC 7 (0.3678), SC 8 and SC 7 (0.3673), SC 11 and IITA 1 (0.3643), SC 11 and SC 7 (0.3620) and between SC 11 and IITA 7 (0.3618), three were between Seed Co lines. All these three involved a line SC 7 that belongs to the "SC" heterotic group. This is consistent with multiplicity of heterotic groups within the Seed Co breeding programme. The existence of four to six major heterotic groups within the Chinese maize lines (George *et al*., 2011) were equally proven when 187 Chinese inbred lines were analysed which resulted in the identification of six subgroups (Xie *et al*., 2008).The line SC 12, although being a

line developed and used in southern Africa, its origin is traced back to population 43 which could be the same source of lines in group 2 which is basically a central and West Africa germplasm group just as inbred lines in the third group. The third group with a single line is unique as a QPM conversion of an originally yellow temperate material where the QPM donor is most probable to be an M37W. George *et al*. (2011) observed highest genetic divergence between the temperate and the tropical/subtropical inbred lines which was followed by that observed between germplasm with different kernel colour which explains the outlier observed as group 3 that was derived from yellow temperate germplasm. The lines used in this study were basically mid altitude unlike with other studies where tropical lowland, mid altitude and temperate germplasm were included. The two seemingly outliers from the southern African mid altitude mega environment seem to adapt very well to the West African lowland tropics according to personal observation (data not available).
The IITA derived central and West African mid altitude lines were in group two that had two subgroups which could suggest the two major heterotic opposite groups within that germplasm. The groups were quite unique and conspicuous.

6.5 Conclusions

Traditionally, maize breeders have used test crossing as a way of classifying and characterising germplasm into heterotic groupings. Breeders have relied on pedigree data to classify their germplasm which is then followed by testcrossing to confirm the pedigree information available. This has remained complicated as sources of certain traits may come from exotic germplasm where the breeder may not know where it belongs and resort at classifying the material to the group from which the known recurrent line belongs to. In this study, lines from southern Africa and central and West African mid-altitude mega environments have been selected due to high possibility of either being used in the other mega environment. The lines were generally homozygous as expected of fixed maize inbred lines. The neighbour-joining algorithm clustering was able to divide the germplasm from the mid-altitude southern Africa from the lowland central and West African maize inbred lines with a few exceptions that are attributable to sources of such germplasm despite the fact that it is being used in the southern African mid-altitude mega environment. The sub-groupings were able to classify the germplasm into known heterotic groups based on known pedigrees. The mid-altitude central and West African germplasm whose pedigree is unknown were divided into two sub-groups, perhaps representing the two heterotic groups existing within that

breeding program. In a maize breeding programme, introgression of a new trait is done within the same heterotic groups hence correct classification as enunciated by this study will immensely contribute towards that. Although not always true, lines from distant relationships lead into higher heterosis hence the information generated may assist in the prediction of the performance of the single cross hybrids made amongst these inbred lines.

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Chapter 7

The relationship of genetic distance with heterosis and specific combining ability in southern African and central and West African mid-altitude maize inbred lines

Abstract

Use of inbred lines in a maize breeding programme has become important especially in this era of exploitation of heterosis in hybrids or where synthetics are regarded as alternatives to the traditional landraces in some parts of Africa. This study was conducted to evaluate the correlation between genetic distance, specific combining ability and heterosis for various agronomic traits, *Fusarium verticillioides* ER incidence and fumonisin production in Zimbabwe and in cental and West African inbred lines and F1 hybrids derived from crosses among these lines. Twelve maize mid-altitude inbred lines from each of the two regions were crossed in a modified NCDII resulting in 144 F1 hybrids. The inbred lines and the F1 hybrids were evaluated at five sites in the 2012/13 and at three sites 2013/14 seasons in Zimbabwe and one site each in Ghana and Nigeria. The three sites in Zimbabwe were the same for both years. The lines were genotyped using SNP markers. Heterosis averaging 199% and 163% for the MPH and HPH respectively, were obtained across the three Zimbabwe sites in two years. DMP, DMS and ASI had mean MPH of -5%, -6% and -2% while the average HPH were -4%, -7% and -35% respectively. The ear rot and fumonisin accumulation had respective MPH of -84% and -87% and HPH of -89% and -87%. This suggests high level of heterosis between the southern African and central and West African maize inbred lines. Crossing of the sub-regional lines also reduced the incidence of Fusarium ear rot and the amount of fumonisins accumulated. The correlations between GDs GY *per se* and with MPH were not significant and low. These correlations were positive for GD against MPH and GY mean while negative between GD and HPH. The correlation between GD and SCA for yield was low and not significant while that of GY and SCA and *per se* mean GY, MPH and HPH were significant. SCA for FER and fumonisin had significant and positive correlation with MPH and HPH suggesting non-additive gene effects being important in the inheritance of these traits. Inbred lines from southern Africa SC 10 and SC 5, and the West African IITA 4, IITA 2, IITA 3 and IITA 4 were superior in terms of yield with the best yielding hybrid SC 5/IITA 4 also showing resistance to both FER and fumonisin accumulation. The GD was effective in classifying the lines into heterotic groups in line with the known pedigree.

7.1 Introduction

Maize breeding programmes rely on heterotic group to maximize heterosis in hybrids. Testers from opposite groups are often used on lines developed from material whose pedigree is known to be different to establish the heterotic groups of the newly developed lines or introductions. The results from such testcrossing provide the level of combining ability and classification of the germplasm being test-crossed. Significant heterosis obtained when a specific tester is used would indicate that the test germplasm belongs to opposite heterotic group of the tester used (Hallauer and Miranda, 1988). The second stage of testcrossing involves use of multiple testers to determine the gene action involved in controlling a particular trait. GCA effects are considered important when the germplasm undergoing test exhibit high level of heterosis in crosses with most of these testers. The GCA effects are associated with additive gene action for the trait in question while SCA is associated with non-additive effects such as dominance, over dominance and epistasis. Testcrossing has been a traditional process and remains so even to date during the molecular tool era. It is an important step especially when exotic germplasm is introduced in a breeding programme. Improvement of traits through pedigree breeding or population improvement including reciprocal recurrent selection involves confinement of germplasm to heterotic groups (Hallauer and Miranda, 1988, Lu *et al*., 2009). Where the trait is introduced from exotic germplasm, testers have been used to confirm the heterotic group of the introduced line through evaluation of the final product which carries both alleles of the recurrent and donor parents.

Germplasm is introduced to increase diversity in a breeding programme and to introduce certain desired alleles for target traits. Knowledge of the trait plays an important role for the breeder to be able to develop strategies that will lead to breeding progress. For instance, breeding for resistance to Fusarium ear rots that cause the production of some mycotoxins requires a full knowledge of the type of gene action which, according to Perez-Brito *et al*. (2001) is polygenic, confirming earlier reports by Boling and Grogran (1965) and Ullstrup (1977). The mode of resistance is not different from another mycotoxin producing fungi, *Aspergillus flavus* that produces aflatoxins which resistance is also quantitatively inherited (Walker and White, 2001; Busboom and White, 2004; Warburton *et al*., 2009). Despite the numerous reports associating resistance to additive gene effects, both additive and non-additive effects, including dominant gene action, have been found to play a major role in conferring resistance to *A. flavus* (Darrah *et al*., 1987; Zuber *et al*., 1978;

Campbell and White, 1995; Campbell *et al.*, 1997; Maupin *et al*., 2003; Clements *et al.*, 2004; Busboom and White, 2004). Mukanga *et al.* (2010) reported that both the general combining ability (GCA) and specific combining ability (SCA) play a major role in the inheritance of resistance for ear rot causing organisms *A. flavus*, *F. verticillioides* and *Stenocarpella maydis* while working with full-sib families in southern Africa. The results indicate non-additive gene effects as a result of the magnitude of the SCA effects. Such observations linking non-additive gene action were consistent with observations by Desjardins *et al*. (1992) of a single gene or a group of closely linked genes which were attributed to the production of fumonisins, especially fumonisin B_1. Higher dominance effects for susceptibility were observed in the cross of Mo17 x Tex6 (Hamblin and White, 1999) which was not observed in the cross of B73 x Tex6. These demonstrate the importance of non-additive gene effects or lack of quantitative inheritance.

Not only were the additive and non-additive gene effects important, but maternal effects were also equally important in conferring resistance to various complex fungi causing ear rot in southern Africa (Mukanga *et al*., 2010).

Narrow sense heritability, particularly which measures the importance of additive gene effects, has been reported to be moderate-to-low with high G x E interaction, which slows breeding progress in the field (Warburton *et al*., 2009; Mukanga *et al*., 2010). In a different study, a range of 0.27 and 0.42 estimates of broad sense heritability for aflatoxin accumulation have been reported (Brooks *et al*., 2005) while heritability as low as 11.3% among BCP_1S_1 families were recorded for resistance to *A. flavus* (Busboom and White, 2004). In a study involving resistant maize inbred (Tex6) and two susceptible maize lines (Mo17 and B73), the broad sense heritability obtained from generation mean analysis for ear rot and aflatoxin production were 58% and 63% for crosses Mo17 x Tex6 and 66% and 73% for cross B73 x Tex6, respectively. The narrow sense heritability for ear rot and aflatoxin production for cross B73 x Tex6 was 39% and 43% (Hamblin and White, 1999). Perez-Brito *et al*. (2001) observed low heritability in two highland maize populations in Mexico. The medium to low heritability recorded is suggestive of non-additive gene effects playing a significant role in resistance to these fungi, hence justifies the importance of SCA.

Utilisation of known sources of resistance involves introductions from different mega environments that come with challenges on adaptability as well as lack of knowledge of the heterotic pattern of such introductions. It becomes necessary to classify such introductions into heterotic groups in maize which, when crossed with lines from the opposite group, high levels of heterosis are expected.

Heterosis is the superiority of a cross bred individual over and above the parents in a target trait. It can be manifested in terms of an increase in size, vigour, rate of growth and yield (Singh, 2005; Xu, 2010). In maize breeding, this has become an important component, particularly in hybrid development. Heterosis can further be explained in terms of whether that additional performance is above the better parents (heterobeltiosis) commonly known as high parent heterosis (HPH) or above the mean of the parents (relative heterosis) commonly known as mid parent heterosis (MPH) (Dabholkar, 1999). Exploitation of heterosis requires a high level of genetic diversity within the breeding programme and knowledge of that diversity.

Germplasm with wide genetic base is important inreducing the level of effects paused new biotic and abiotic threats such as oubreaks of new diseases and pests (Singh, 2005), and climatic changes. More heterosis is hypothesised to emanate from the use of diverse germplasm which facilitates determination of potential to exhibit better agronomic performance in general. The measurement of diversity is centered on the determination of genetic difference at the sequence or allelic frequency level which is calculated between individuals, populations or species (Mohammadi and Prasanna, 2003). Various ways can be used to determine genetic diversity using binary data and these include Nei and Li's (Nei and Li, 1979) coefficient, Jaccard's (Jaccard, 1901) coefficient, simple matching coefficient and Modified Rogers' distance (Rogers, 1972). Several studies including Dudley *et al.* (1991), Betran *et al.* (2003), Reif *et al*. (2003) and Gutierrez *et al*. (2002) found varied results when MRD and GD were correlated with various forms of heteroses and SCA values.

Each breeding programme needs to verify such results in the environment hosting the programme as different results are obtained at different environments and when different genotypes are used (Falconer and Mackay, 1996; Busboom and White, 2004).

The objective of this study was to determine the relationship of GD and heterosis and SCA in the mid-altitude maize inbred lines originating from two breeding programmes.

7.2 Materials and methods

7.2.1 Plant material

The experimental material described in section 4.3 and Table 4.1 composed of 12 inbred lines from southern Africa and 12 lines from central and West Africa and the 144 hybrids that were derived from a modified North Carolina Design II were used.

7.2.2 Environments

Apart from the three sites in Zimbabwe at which the ear rot study was conducted at RARS, SRC, and KRC as described in section 4.2.2, 2012/13 and 2013/14 seasons additional sites in West Africa, Sheda at Seed Co West Africa Research Centre (WARC) in Nigeria and at Kpeve in Ghana, were conducted in the 2013 and 2014 seasons. However, the Ghana site in 2013 and the 2014 sites for both Ghana and Nigeria were written off. This left the Nigerian WARC site only where the parent trial also was written off. The RARS trial was unique as it is the site where artificial inoculation and fumonisin contamination were derived while WARC was equally unique as it represented a completely different environment.

7.2.3 Field management

The normal field management practices described in section 4.2.3 were followed. Weed control was facilitated by application of herbicides and manual weeding as described in section 4.2.4.

7.2.4 Artificial inoculation

Details of the artificial inoculation process are provided in section 4.2.5.

7.2.5 DNA extraction and the genotyping

The DNA extraction and the genotyping processes are described in sections 6.2.3 and 6.2.4 respectively.

7.2.6 Trial data measurements

The determination of yield per hectare and incidence of *F. verticillioides* ear rot was the main focus, although several other agronomic traits were also evaluated. As explained in section 4.2.6, the plot grain weight was measured and translated into yield in t ha^{-1} after standardising moisture content to 125 g kg^{-1} H_2O. *F. verticillioides* ear rot incidence was determined by counting the ears that were visibly infected by the fungi and expressed as a proportion of the total number of ears harvested per plot while grain disease score (GDS) was a subjective score on the 1-9 scale where 1 = no visible infection on the kernels were found while 9 = all the kernels appeared infected by the fungus.

Foliar disease, flowering, lodging and height data were obtained as explained in section 4.2.6.

7.2.7 Experimental design and data analysis

The trials were planted following a 0.1 alpha-lattice (Patterson *et al*., 1978) design which is an incomplete block design that was analysed as a randomized complete block design due to limitation within the software used. Two rows were planted per plot spaced 0.75 m apart with in-row spacing of 0.5 m with two replications. Seed was planted at a seeding rate of four seeds per station that was subsequently thinned to two plants per station.

Individual sites were analysed separately before combining locations and years for the general ANOVA. The variation caused by the genotypes, environments and years was determined through the ANOVA. AGROBASE version II (2010) software was used to do ANOVA.

Line x tester analysis was done using SAS (SAS Institute, 2002).

The means derived from the ANOVA were used to calculate mid-parent heterosis (MPH) and high parent heterosis (HPH) as follows:

$MPH = F1 – [(P_1 + P_2)/2]/F1 \times 100$

Where: F1 is the mean value for the F1

P_1 and P_2 are parent one and parent two respectively

$(P_1$ and $P_2)/2$ is the mid-parent

$HPH = [(F1\text{-}HP)/HP] \times 100$

Where: HP = mean of the parent with the higher value

Effects of GCA and SCA were derived according to Dabhokar (1999).

The PowerMarker version 3.25 (Liu and Muse, 2005) was used to derive the genetic distances (GD) from the SNP data allele frequencies described in section 6.2.5. Pearson correlation coefficients between GD, ER, total fumonisins, grain yield of F1 hybrids, high-parent (HP), mid-parent (MP), MPH, HPH and SCA were calculated using Genstat (Genstat 16th edition, 2013).

7.3 Results

The data presented in tables within this chapter are for the best and poorest 10 entries while the entire data set is presented in Appendices 6 to 8. However, the statistics presented for each table represent the entire data set and not only for the presented 10 entries.

7.3.1 Genetic distance, specific combining ability, mid and high-parent heterosis across environments and years

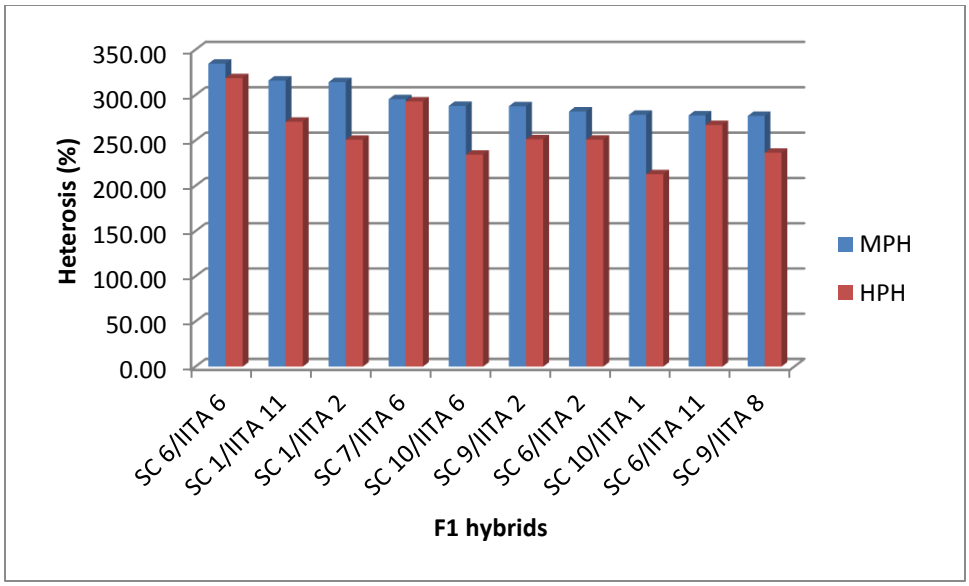
Across all the environments and years, the highest MPH was for hybrid SC 6/IITA 6 which also had the highest HPH (334.64% and 318.61% respectively). This was against the mean values of 199.08% and 163.03% respectively. It had a yield of 4.99 t ha^{-1}, a GD and a SCA of 0.34 and 0.441 respectively (Table 7.1, Figure 7.1 and Appendix 6). The highest GD value was for F1 hybrid SC 11/IITA 12 (0.38) which had a yield of 4.09 t ha^{-1}, a SCA value of 0.04 and MPH and HPH of

113.75% and 94.11% respectively. In terms of SCA, the highest value was obtained from the F1 hybrid SC 3/IITA 1 which had a value 0.74 while the mean grain yield was 4.44 t ha^{-1}, with MPH and HPH of 234.49% and 168.26% respectively, while its GD was 0.32. The lowest yielding hybrid was SC 12/IITA 5 that had a yield of 3.36 t ha^{-1} and MPH and HPH of 94.04% and 80.47% respectively. It had a GD value of 0.28 and SCA value of -0.58 which characterised all the poorest yielding F1 hybrids. The GD values of these poor hybrids were skewed as they ranged between 0.28 and 0.36 while the mean value was 0.32 (Table 7.1and Appendix 6).

Table 7.1 FI hybrid specific combining ability for the means of grain yield, mid- and high-parent heterosis and genetic distance across locations for the best and worst 10 entries in terms of mid-parent heterosis

Entry	F1 Hybrid Pedigree	GY t ha-1	SCA	MPH %	HPH %	GD
66	SC 6/IITA 6	4.994	0.41	334.64	318.61	0.33
11	SC 1/IITA 11	4.684	0.52	315.99	270.57	0.32
2	SC 1/IITA 2	4.997	0.26	314.17	250.67	0.30
78	SC 7/IITA 6	4.341	0.30	295.36	292.85	0.34
114	SC 10/IITA 6	5.108	0.36	288.00	234.29	0.32
98	SC 9/IITA 2	5.004	0.27	287.61	251.16	0.30
62	SC 6/IITA 2	4.999	0.16	281.89	250.81	0.32
109	SC 10/IITA 1	4.779	0.27	278.24	212.76	0.32
71	SC 6/IITA 11	4.639	-0.14	277.61	267.01	0.32
104	SC 9/IITA 8	4.961	0.71	276.98	236.34	0.30
72	SC 6/IITA 12	3.604	-0.52	118.49	71.13	0.35
60	SC 5/IITA 12	3.898	-0.62	115.00	85.09	0.34
132	SC 11/IITA 12	4.088	0.04	113.75	94.11	0.38
106	SC 9/IITA 10	3.463	-0.67	111.80	63.89	0.30
124	SC 11/IITA 4	4.593	-0.53	110.59	73.78	0.35
36	SC 3/IITA 12	3.815	-0.15	102.93	81.15	0.30
144	SC 12/IITA 12	4.021	0.26	102.82	90.93	0.31
123	SC 11/IITA 3	3.584	-1.06	98.95	90.23	0.36
88	SC 8/IITA 4	3.987	-0.47	95.49	50.85	0.33
137	SC 12/IITA 5	3.355	-0.58	94.04	80.47	0.28
Mean		**4.49**	**0.00**	**199.08**	**163.03**	**0.32**
Min		**3.36**	**-1.06**	**94.04**	**50.85**	**0.26**
Max		**5.94**	**0.74**	**334.64**	**318.61**	**0.38**

GY=grain yield; SCA=specific combing ability; MPH=mid parent heterosis; HPH=high parent heterosis; GD=genetic distance; Min=minimum; max=maximum



MPH=mid parent heterosis; HPH=high parent heterosis

Figure 7.1 The best F1 hybrids in terms of mid-parent heterosis across environments and their high – parent heterosis

7.3.2 F1 hybrid specific combining ability, means, mid and high – parent heterosis for grain yield, *F. verticillioides* ear rot, total mycotoxin at RARS in 2012/13 season and the genetic distances

The mean of the total amount of fumonisins at RARS where the trials were artificially inoculated and the grain analysed for fumonisins was 0.34 ppm and the F1 hybrid SC 5/IITA 11 had the least negative SCA for fumonisin of -1.16 with a mean fumonisin content of 0 ppm (Table 7.2 and Appendix 7). The SCA for the *F. verticillioides* was 0.06 while the mean incidence was 1.46%. Compared to the mean yield of 7.07 t ha^{1}, this top hybrid in terms of SCA for fumonisin had a yield of 6.77 t ha^{-1} and the GD between the parent lines for this hybrid was 0.30.

On the other hand, hybrid SC 5/IITA 12 had the highest SCA of 2.73 and mean total fumonisin content of 3.95 ppm as compared to the trial mean of 0.34 ppm (Table 7.2). Its yield SCA was -0.58 with a yield of 5.82 t ha^{-1} against the trial average yield of 7.07 t ha^{-1}. The 10 hybrids with the highest SCA for fumonisins had the highest fumonisin content. Their GD values were were skewed as they ranged between 0.30 (SC 3/IITA 12 and SC 4/IITA 11) and 0.36 (SC 11/IITA 1) while the mean GD was 0.32(Table 7.2).

Table 7.2 FI hybrid best and worst ten specific combining ability for the means of Fusarium ear rot, total mycotoxin and genetic distance at RARS in 2012/13 season

Entry	F1 Hybrid	Grain yield t ha-1				Ear rot				Fumonisin				GD
	Pedigree	SCA	mean	MPH	HPH	SCA	mean	MPH	HPH	SCA	Mean	MPH	HPH	
59	SC 5/IITA 11	-1.17	6.77	257.27	207.12	0.06	1.60	-89.19	-90.75	-1.16	0.00	-100.00	-100.00	0.30
71	SC 6/IITA 11	-0.26	7.69	235.31	222.79	-1.00	0.00	-100.00	-100.00	-0.90	0.00	-100.00	-100.00	0.32
52	SC 5/IITA 4	-0.28	7.90	174.36	89.35	-0.37	0.70	-95.33	-95.95	-0.84	0.00	-100.00	-100.00	0.33
50	SC 5/IITA 2	-0.07	7.59	276.30	210.05	-0.68	0.60	-94.85	-96.53	-0.82	0.05	-98.08	-98.08	0.32
72	SC 6/IITA 12	-0.84	5.57	117.25	103.03	-0.20	2.80	-84.18	-88.93	-0.81	0.15	-94.12	-94.23	0.35
55	SC 5/IITA 7	-0.03	7.17	210.95	136.93	-0.97	0.00	-100.00	-100.00	-0.76	0.05	-98.08	-98.08	0.34
49	SC 5/IITA 1	0.15	6.66	347.85	320.18	0.47	1.40	-93.79	-94.96	-0.69	0.10	-96.15	-96.15	0.34
84	SC 7/IITA 12	0.76	6.85	181.54	149.96	0.58	3.80	-73.61	-84.98	-0.57	0.00	-100.00	-100.00	0.34
38	SC 4/IITA 2	0.11	7.73	273.03	215.81	0.33	1.10	-92.44	-95.24	-0.56	0.00	-100.00	-100.00	0.30
51	SC 5/IITA 3	-0.43	6.47	176.86	109.59	1.08	2.70	-72.31	-84.39	-0.55	0.10	-96.36	-96.55	0.32
124	SC 11/IITA 4	-0.98	6.73	134.80	61.49	0.78	1.30	-89.72	-89.76	1.01	1.40	-45.10	-46.15	0.35
36	SC 3/IITA 12	-0.43	5.96	142.60	117.43	-0.57	2.50	-83.50	-90.12	1.07	1.75	-30.00	-30.00	0.30
121	SC 11/IITA 1	0.81	6.86	364.14	338.06	-0.28	0.00	-100.00	-100.00	1.11	1.45	-44.23	-44.23	0.36
47	SC 4/IITA 11	0.75	8.66	343.72	292.61	-0.38	0.70	-96.05	-96.97	1.30	2.15	-18.87	-20.37	0.30
7	SC 1/IITA 7	0.28	7.22	297.52	138.62	1.10	2.50	-89.29	-93.57	1.41	1.65	-37.74	-38.89	0.32
107	SC 9/IITA 11	0.38	8.09	287.59	266.94	-1.11	0.60	-93.48	-95.12	1.43	1.95	-30.36	-35.00	0.32
69	SC 6/IITA 9	-0.21	6.64	183.74	178.56	-0.29	0.70	-92.39	-93.07	1.71	2.45	-7.55	-9.26	0.34
54	SC 5/IITA 6	-0.19	7.19	315.66	283.72	-0.47	1.30	-89.68	-92.49	2.12	3.05	12.96	8.93	0.32
62	SC 6/IITA 2	0.33	8.00	231.15	226.76	-0.09	0.70	-91.30	-93.07	2.29	2.90	11.54	11.54	0.32
60	SC 5/IITA 12	-0.58	5.82	168.85	112.18	0.01	3.50	-83.57	-86.17	2.73	3.95	54.90	51.92	0.34
Mean		0.00	7.07	213.69	160.44	0.00	1.46	-84.00	-88.69	0.00	0.34	-86.9408	-87.21	0.32
Min		-2.30	3.63	38.56	-0.19	-3.10	0.00	-100.00	-100.00	-1.16	0.00	-100.00	-100.00	0.26
Max		1.77	9.06	532.57	338.06	3.95	7.60	-25.71	-40.00	2.73	3.95	54.90	51.92	0.38

GY=grain yield; SCA=specific combing ability; MPH=mid parent heterosis; HPH=high parent heterosis; GD=genetic distance; Min=minimum; Max=maximum, ppm=parts per million

7.3.3 F1 hybrid specific combining ability for grain yield and *Fusarium verticillioides* ear rot, their mid- and high-parent heterosis and genetic distance for the best 10 at WARC in the 2013 season

In terms of SCA for *F. verticillioides*, the best hybrid was SC 6/IITA 10 that had a SCA value of -1.39 but with a grain yield of 4.83 t ha^{-1} against a mean yield of 6.25 t ha^{-1} and a grain yield SCA of 0.30 compared with the minimum and maximum grain yield SCA of -3.36 and 2.23 respectively (Table 7.3 and Appendix 8). The best 10 hybrids had a nil incidence for *F. verticillioides* ear rot while the mean incidence was 0.44% with a range of 0.00 and 7.35%. The GD for the best entries were within the mean value of 0.32 with the exception of SC 11/IITA 9 (0.36) and SC 7/IITA 9 and SC 6/IITA 1 that both had a GD of 0. 0.34 while the best hybrid SC 6/IITA 10 had a GD of 0.33.

The highest SCA for *F. verticillioides* ear rot was recorded for F1 hybrid SC 6/IITA 9 that had a value of 5.8 and the *F. verticillioides* incidence of 7.5% with grain yield SCA of 1.35 while the GD was 0.34 (Table 7.3 and Appendix 8).

7.3.4 Means for various agronomic traits, F1 parents, mid- and high-parent heterosis across environments

For the flowering related traits DMP, DMS and ASI, the F1 hybrid means were 68.92, 70.23 and 1.28 days respectively compared with the respective parent means of 72.98, 74.50 and 1.53 days (Table 7.4). Their MPH values were -5.45%, -5.72% and -1.85% for DMP, DMS and ASI respectively while their respective HPH values were -4.49%, -7.12% and -35.07%. The height related traits PHT and CHT had means of 188.28 cm and 107.92 cm against the parents' respective mean heights of 124.27 cm and 69.55 cm. The PHT and CHT values for MPH were 51.86% and 55.51% while the mean values for HPH were 44.37% and 51.26% for these respective height related traits (Table 7.5). In terms of grain texture, the mean for the F1 hybrid, mean for the parents, MPH and HPH score were 1.16, 0.96, 23.35% and 7.44% respectively.

F. verticillioides ear rot incidence and the fumonisin content means for the F1 hybrids were 0.78% and 0.22 ppm respectively while parent respective means were 3.08% and 0.07ppm. MPH for the

ER and fumonisins were -68.79% and -46.06% while the HPH were -76.01% and -47.71% respectively (Table 7.4).

Table 7.3 FI hybrid specific combining ability for the grain yield and *F. verticillioides* ear rot, their mid- and high-parent heterosis and genetic distance at WARC in 2013 season

Entry	F1 Hybrid	Grain yield t ha-1		Ear rot		Genetic
	Pedigree	SCA	Mean	SCA	Mean	distance
70	SC 6/IITA 10	0.30	4.83	-1.39	0.00	0.33
62	SC 6/IITA 2	0.92	6.44	-1.24	0.00	0.32
93	SC 8/IITA 9	0.21	7.25	-1.22	0.00	0.33
117	SC 10/IITA 9	0.46	7.42	-1.08	0.00	0.32
81	SC 7/IITA 9	-0.77	5.85	-1.06	0.00	0.34
12	SC 1/IITA 12	-0.18	5.38	-0.93	0.00	0.33
33	SC 3/IITA 9	0.12	6.26	-0.88	0.00	0.31
129	SC 11/IITA 9	-0.10	6.71	-0.88	0.00	0.36
5	SC 1/IITA 5	-1.09	5.18	-0.86	0.00	0.32
61	SC 6/IITA 1	0.20	4.72	-0.85	0.00	0.34
86	SC 8/IITA 2	0.30	6.87	0.99	1.90	0.31
51	SC 5/IITA 3	-0.42	5.76	1.27	1.90	0.32
71	SC 6/IITA 11	-1.64	3.69	1.18	2.00	0.32
94	SC 8/IITA 10	0.83	6.41	1.04	2.10	0.32
54	SC 5/IITA 6	-1.17	6.02	1.56	2.20	0.32
115	SC 10/IITA 7	-2.22	4.78	2.02	2.25	0.33
110	SC 10/IITA 2	0.89	7.37	1.73	2.50	0.30
2	SC 1/IITA 2	0.07	6.14	2.18	3.35	0.30
9	SC 1/IITA 9	-0.42	6.11	2.22	3.70	0.32
69	SC 6/IITA 9	1.35	7.34	5.80	7.35	0.34
Mean		0.00	6.25	0.00	0.44	0.32
Min		-3.36	2.74	-1.39	0.00	0.26
Max		2.23	9.65	5.80	7.35	0.38

SCA=specific combing ability; Min=minimum; Max=maximum

Table 7.4 Means for various agronomic traits, F1 parents, mid- and high-parent heterosis across environments

	Hybrid			Parents			Mid-parent			High-parent		
	Mean	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**
DMP	68.92	66.00	72.00	72.98	69.25	75.50	-5.45	-9.59	-2.07	-4.49	-11.84	5.33
DMS	70.23	66.00	74.00	74.50	71.08	77.75	-5.72	-11.11	-1.99	-7.12	-12.82	-2.70
ASI	1.28	0.00	3.00	1.53	0.08	3.08	-1.85	-100.00	300.00	-35.07	-100.00	100.00
PHT	188.28	160.00	214.00	124.27	99.58	152.92	51.86	35.41	73.95	44.37	22.30	69.18
CHT	107.92	89.00	134.00	69.55	57.50	83.75	55.51	30.00	82.29	51.26	14.43	105.22
TEXT	1.16	0.50	2.33	0.96	0.58	2.92	23.35	-50.00	185.71	7.44	-68.97	185.71
ER	0.78	0.00	1.91	3.08	0.53	8.98	-68.79	-100.00	71.43	-76.01	-100.00	50.00
FUM	0.22	0.18	0.64	0.07	0.00	0.39	-46.06	-60.00	50.00	-47.71	-60.00	50.00

DMP=days to mid pollen shed; DMS=days to silking; ASI=anthesis to silking interval; PHT=plant height parent heterosis; CHT=cob height; TEXT=grain texture; ER=ear rot; FUM=fumonisin; Min=minimum; Max=maximum

7.3.5 Correlations among specific combining ability, F1 hybrid grain yield, heterosis (mid- and high-parent) and genetic distance

The correlation between GD and GY mean and grain yield MPH were positiveand low (0.02 and 0.04 respectively) while between grain yield HPH and GD was negative and low. These correlation coefficients were all not significant (P>0.05) across all the environments (Table 7.5).

The correlation between GD and SCA was negative, low and not significant (-0.02). Highly significant correlations were observed between F1 hybrid grain yields *per se* and HPH (P≤0.01), MPH and SCA (P≤0.001) with positive *r* values of 0.23, 0.31 and 0.61 respectively. Highly significant (P≤0.001) correlation coefficients between grain yield HPH and MPH, HPH and SCA, and MPH and SCA were observed (0.93, 0.33, and 0.42 respectively). Similar results were observed at RARS in the 2012/13 season (Table 7.5) where the correlations between the hybrid grain yield and HPH, MPH and SCA were positive and highly significant (P≤0.001). At RARS, the highest correlation coefficients were again between HPH and MPH (0.86) while that of HPH and SCA and between MPH and SCA were also highly significant and positive with *r* values of 0.30 and 0.32 respectively. At WARC where the parent trial was a write off, the correlation coefficients for GD and GY was negative and low(-0.12) and between GY and SCA (0.77) were not not significant (P>0.05) and highly significant (P≤0.001) respectively.

Table 7.5 Correlations coefficients among F1 hybrid grain yield, mid- and high-parent heterosis and specific combining ability across environments, and the average mid- and high-parent heterosis

	r(GD, HPH)	r(GD, GY)	r(GD, MPH)	r(GD, SCA)	r(GY,HPH)	r(GY,MPH)	r(GY,SCA)	r(HPH,MPH)	r(HPH,SCA)	r(MPH,SCA)
Across environments	-0.03	0.02	0.04	0.07	0.23**	0.31***	0.61***	0.93***	0.33***	0.42***
RARS 2012/13 season	0.02	-0.19*	0.05	0.03	0.42***	0.42***	0.54***	0.86***	0.30***	0.32***
WARC 2013 season	nr	-0.12	nr	0.04	nr	nr	0.77***	nr	nr	nr

*****P≤0.001; **P≤0.01; r=correlation coefficients; GY=grain yield; SCA=specific combing ability; MPH=mid parent heterosis; HPH=high parent heterosis; GD=genetic distance; RARS=Rattray Arnold Research Station; WARC=West Africa Research Centre; nr=not recorded**

Correlations for the *F. verticillioides* ear rot and the total fumonisin with various derived traits including GD, SCA, the mean, MPH and HPH of these traits, are presented in Table 7.6. From the fumonisin data, highly significant (P≤0.001) correlations were observed between fumonisin HPH against MPH (1.0), fumonisin mean (1.0) and SCA (0.89). Significant (P≤0.05) but low positive correlation was also observed on the correlations between grain yield HPH and both the fumonisin MPH and HPH (0.17). From the *F. verticillioides* ear rot data, highly significant (P≤0.001) correlation coefficients were observed between the ER HPH against ER mean (0.68), ER MPH (0.97) and ER SCA (0.62). The correlation between ER HPH and GY_MPH was negative but significant (P≤0.05) with *r* value of -0.21. Also highly significant (P≤0.001) were correlations between ear rot MPH and ear rot means and ear rot SCA that had respective coefficients of 0.67 and 0.60. The correlation coefficients between the ear rot MPH and MPH for GY and HPH for GY were highly significant (P≤0.01) and significant (P≤0.05) respectively with negative correlation coefficient values of -0.24 and -0.19.

Also highly significant (P≤0.001) was the correlation between the SCA of the ER and the ER incidence means (0.76).

Table 7.6 Correlation coefficients among F1 hybrid grain yield, mid- and high-parent heterosis, specific combining ability, *F. verticillioides* ear rot (ER) and fumonisin (FUM) at RARS in the 2012/13 season

Fumonisin			Ear rot		
x	*y*	*r*	*x*	*y*	*r*
FUM_HPH	FUM_SCA	0.89***	ER_HPH	ER_mean	0.68***
FUM_HPH	FUM_MPH	1.00***	ER_HPH	ER_MPH	0.97***
FUM_HPH	FUM_Mean	1.00***	ER_HPH	ER_SCA	0.62***
FUM_HPH	GD	0.10	ER_HPH	GD	-0.11
FUM_HPH	GY_MPH	0.13	ER_HPH	GY_HPH	-0.13
FUM_HPH	GY_HPH	0.17*	ER_HPH	GY_mean	-0.02
FUM_HPH	GY_SCA	0.04	ER_HPH	GY_MPH	-0.21**
FUM_HPH	GY_mean	0.06	ER_HPH	GY_SCA	-0.07
FUM_MPH	FUM_Mean	1.00***	ER_mean	GD	-0.04
FUM_MPH	GY_HPH	0.17*	ER_mean	GY_HPH	-0.15
FUM_MPH	FUM_SCA	0.89***	ER_mean	GY_mean	-0.10
FUM_MPH	GD	0.10	ER_mean	GY_MPH	-0.13
FUM_SCA	ER_mean	0.02	ER_mean	GY_SCA	-0.06
FUM_HPH	ER_SCA	0.02	ER_MPH	ER_mean	0.67***
FUM_SCA	ER_SCA	0.02	ER_MPH	ER_SCA	0.60***
FUM_MPH	ER_SCA	0.02	ER_MPH	GD	-0.10
FUM_Mean	ER_SCA	0.02	ER_MPH	GY_HPH	-0.19*
FUM_MPH	GY_SCA	0.03	ER_MPH	GY_mean	-0.04
FUM_MPH	GY_MPH	0.13	ER_MPH	GY_MPH	-0.24**
FUM_MPH	GY_mean	0.06	ER_MPH	GY_SCA	-0.07
FUM_SCA	GY_HPH	0.06	ER_SCA	ER_mean	0.76***
FUM_SCA	GD	0.01	ER_SCA	GD	-0.05
FUM_SCA	GY_MPH	0.04	ER_SCA	GY_HPH	-0.05
FUM_SCA	GY_SCA	0.05	ER_SCA	GY_mean	-0.04
FUM_SCA	GY_mean	0.05	ER_SCA	GY_MPH	-0.02
FUM_Mean	GD	0.10	ER_SCA	GY_SCA	-0.06
FUM_Mean	ER_mean	0.06	ER_MPH	FUM_SCA	0.02
FUM_HPH	ER_mean	0.06	ER_HPH	FUM_HPH	-0.05
FUM_MPH	ER_mean	0.06	ER_HPH	FUM_Mean	-0.05
FUM_HPH	ER_MPH	-0.08	ER_HPH	FUM_MPH	-0.05
FUM_Mean	ER_MPH	-0.08	ER_HPH	FUM_SCA	0.02
FUM_MPH	ER_MPH	-0.08			

GY=grain yield; SCA=specific combing ability; MPH=mid parent heterosis; HPH=high parent heterosis; GD=genetic distance; ***P≤0.001; **P≤0.01; r=correlation coefficients; GY=grain yield; SCA=specific combing ability; x=independent trait; y=dependent trait

7.4 Discussion

Maize, as a cross pollinating crop, has relied on population improvement techniques such as recurrent selection as well as pedigree breeding, particularly where inbred line development has become an important element of the breeding process. Hybrids and synthetics rely on the use of superior inbred lines that are developed with germplasm that is separated into heterotic groupings to ensure maximum heterosis (Xia *et al*., 2005). Heterosis can be expressed in various forms and yield heterosis is the most important. To enhance that, use of diverse germplasm plays an important part and this is partly achieved by use of introductions from different geographical areas which are often characterised by lack of adaptability. In this study that utilised southern African and central and West African mid-altitude lines, the range of the MPH and HPH was 94% and 334% for the former, and 51% and 319% for the latter across sites and years consistent with the results of Makumbi *et al*. (2011) who found a range of 74% to 1119% under drought, and a range of 17% to 448% under optimal conditions. Such results are not surprising as elite lines emanating from different breeding programmes were used. Wide diversity normally results in greater heterosis (Falconer, 1989). This is more so in this study where the parent lines used in each of the F1 hybrids, were from a different geographical location. The expressed heterosis might have been over expressed due to the failure of the parent material to adapt to the new environment resulting in the parents performing poorly when compared with the F1 hybrid performance, which, due to hybrid vigour, is less affected by the environmental effects as compared to the inbred lines. Makumbi *et al*. (2011) reported that the high heterosis they obtained could be attributed to poor performance of the inbred lines under drought stress environments where the inbred lines constituting the F1 hybrid would yield poorly while the F1 hybrid, due to hybrid vigour would give high yield. However, the best hybrid SC 5/IITA 4 had below meanMPH and HPH which is not surprising as inbred line IITA 4, had the highest yield across environments, suggesting its wide adaptability and it was involved in five of the best 10 hybrids, confirming its high inherent combining ability. Such a result reflects true heterosis since the inbred line equally had good yield. It can be postulated that non-additive gene effects such as dominance or over dominance can explain its ability to contribute to yield in the best F1 hybrids in combination with inbreds that gave across site yields that were below the average. Inbred lines SC 10, SC 5, IITA 4, IITA 2 and IITA 3 could be very useful where yield is the main target.

The range for the MPH was -100% to 55% while that for the HPH was -100% to 52% for fumonisin content, B_1, B_2 and B_3 added together, respectively, while the means were -86.94 and -87.21% respectively. The SCA effects for the best 10 hybrids for fumonisins for RARS in the 2012/13 season were all negative. The negative values observed for both the MPH and HPH indicate resistance to mycotoxins. Hybrid SC 5/IITA 11 had the least SCA for the ER while no fumonisins analogues were detected on it. Its SCA for ER was equally low as expected. It is however ironic that the same line used as the tester SC 5 when in combination with IITA 12 known to be susceptible to aflatoxins, the highest fumonisin content was realised. This is more so when the best F1 is considered which equally involved the same SC 5 and another susceptible tester IITA 11. Such a result demonstrates the importance of non-additive gene effects.

Across environments, the best yielding hybrid SC 5/IITA 4 was among the best 10 hybrids with inbred line SC 5 appearing in the top 10 hybrids that had low MPH and HPH for fomunisins. Most of the hybrids had a low ER incidence and negative SCA for yield, which is consistent with observations that sources of resistance tend to be poor agronomically (Hamblin and White, 1999; Busboom and White 2004; Warburton *et al*., 2009). Just as with ear rot and fumonisins, the flowering related traits, DMP, DMS and ASI had negative MPH, HPH and SCA. This is indicative of F1 hybrids flowering earlier than the parental inbred lines, which is desirable with the advent of global warming and recurrent drought. However, this may cause problems of nicking where a same day planting of the F1 and male plant to form a 3-way cross is desirable. Earliness also compromises yield as late hybrids have more prolonged time to intercept radiant energy that results in higher yield. MPH and HPH were medium with respective means of 52% and 44% for the PHT and 56% and 51% for the CHT respectively which is expected due to high expression of heterosis in vigour in the resulting F1 hybrids in maize. This is consistent with findings by Gissa (2008) who observed respective MPH and HPH of 57% and 47% for PHT and 63% and 50% for CHT. Grain texture had low but positive mean MPH and HPH of 23% and 7% respectively suggestive that the resultant hybrid becomes slightly more dent when two inbred lines are crossed.

Classification of germplasm on the basis of heterotic groups was achieved by SNP markers, particularly for the southern African inbred lines whose heterotic pattern is known. There were a few exceptions such as inbred line SC 3 that is normally regarded as a N3 that was closer to the

SC groups than the N3 while inbred line SC 1 commonly taken as a "P" was closer to the N3 group. This is not surprising as classification is generally arbitrarily done, particularly when new traits are introduced from exotic germplasm with new alleles (Lu *et al*., 2009) where the new line is classified according to the recurrent parental inbred line whose heterotic group is known. Inbred lines such as IITA 4 had good GCA with virtually all the heterotic groups (PO, OC, NN, PH, SC). Interestingly, four amongst the the best 10 hybrids (SC 10/IITA 3, SC 5/IITA 3, SC 12/IITA 2 and SC 10/IITA 2) had an inbred line with exotic germplasm denoted by an "O" which signifies an unknown heterotic grouping, while SC 1/IITA 1 and SC 8/IITA 1 within the poorest yielding hybrids, both had a common tester crossed to what SNP markers grouped together.

There has been a belief that the more diverse the maize inbred lines are, the higher the heterosis for yield when such lines are crossed (Falconer, 1989). Diversity as measured by GD has been reported to have positive correlation with *per se* mean yield and heterosis under drought conditions (Betran *et al*., 2003) and under both stressed and non-stressed environments (George *et al*., 2011). In this study, the correlation coefficients between GD and mean grain yield, MPH and HPH were not significant and low. These correlation values were positive for the mean and the MPH. Such results are consistant to other studies (Betran *et al*., 2003; Dhliwayo *et al*., 2009; George *et al*., 2011) who observed positive correlation coefficients. However, between the GD and HPH was negative. The contradiction could partly be attributed to the environments where the trials were conducted and different set of genetic materials used. The low level of genetic diversity as illustrated by the low range of GD (ranging from 0.26 to 0.38), the lines used could contribute to the decline in heterosis, consistent with the findings of George *et al*. (2011) that found heterosis increasing with an increase in GD up to a certain point and declines thereafter. The negative correlations could be attributed to randomly distributed molecular markers that are unlinked to the quantitative trait loci (QTL), (Bernado, 1992). It is also interesting to note that the highest GD values were broadly between lines used as males which were basically Seed Co lines which were not crossed against one another while determination of GD involved all the lines. This may suggest that those lines used were closely linked hence the negative and low values of correlations. George *et al*. (2011) did not observe any correlation between the SCA of lines that had a high range of GD. Dhliwayo *et al*. (2009) reported moderate MRD^2 whose range was small, which explained the observed non-significant results between GD and any other traits. The observation that the

correlation between GD and SCA was not significant and low is consistent with the findings by Dhliwayo *et al*. (2009). The correlations among the GY *per se*, MPH, HPH and SCA for grain yield were all highly significant across environments and at RARS in the 2012/13 season. The *r*(GY, SCA) was almost double that of *r*(GY, MPH) and *r*(GY, HPH) in conformity with Betran *et al*. (2003).

Significant but low correlation of Modified Rogers' Distance (MRD) with SCA for yield was found in temperate maize germplasm (Dudley *et al.*, 1991). In another study, significant and positive correlation between SCA and mid parent heterosis ($r = 0.47$), and SCA and high-parent heterosis where $r = 0.31$ (Betran *et al.*, 2003) while in a different study that combined MRD and heterosis, a significant ($P<0.01$) correlation between MRD and pelmitic mid parent heterosis (PMPH) of 0.63 was obtained (Reif *et al*., 2003). In sunflower, significant correlation between the hybrid performance and genetic distance (GD) was found while there was no correlation between diversity values and hybrid performance (Gutierrez *et al*., 2002).

The correlations betwwen GD and both *F. verticillioides* and GD were low and not significant ($P>0.05$). It therefore suggests that GD had no effects on the performance of the reaction of hybrids to both *F. verticillioides* ear rot and fumonisin accumulation. There are indications about the effectiveness of bringing resistance from diverse sources while closely related lines resulted in susceptibility of the hybrids when the small and negative correlation between GD and HPH are considered. The correlation between SCA of fumonisin content and the *per se* mean incidence, fumonisin MPH and fumonisin HPH were high, positive and significant, suggesting non-additive gene effects playing a major role in susceptibility. Consistent to that, ER incidences correlated highly, positively and were highly significant in terms of MPH, HPH and SCA for ER incidence itself. Such results are in consistent with findings by Robertson *et al*. (2006) who observed high genotypic and phenotypic correlations between ear rot and fumonisin concentration of 0.96 and 0.40 respectively. Desjardins *et al*. (1992) attributed resistance to fumonisin B_1 to a single gene or a group of closely linked genes which characterise non-additive inheritance estimated by SCA. Dominance was implicated in the conferring resistance to ER (Clements *et al*., 2004). The results in this study indicate that SCA can be used to select for resistance to *F. verticillioides* ER and

fumonisins, hence the need to select based on the reaction of the hybrid to compliment selection of lines that are inherently resistant, could contribute more in breeding for resistance.

It is, however, important to note that within the same heterotic group, a substantial amount of heterosis can be detected, resulting in weak correlation ($r<0.5$) between significant GD and SCA. Such observations precludes reliance soley on the determination of GD, hence needs to be complimented by evaluation of hybrids for SCA. This is as a result of several factors such as dominance, over-dominance, biochemical and molecular factors being implicated in heterosis.

7.5 Conclusions

Maize breeding relies on superior alleles for combination of traits with yield being of paramount importance as it is the ultimate product resilience of the germplasm to all the biotic and abiotic challenges that may exist. It is therefore imperative to identify suitable lines for either pedigree or population improvement. High levels of heterosis were seen within the set of lines used. The magnitude of heterosis obtained was high because the parental material used originated from different regions that failed to give high grain yields under stress conditions. This results in yield of inbred lines being significantly lower than the F1, hence exaggerating the heterosis. Lines contribution towards yield included SC 10, SC 12, SC 5, SC 7, and SC 6 and formed the best 10 hybrids in combination with IITA 8, IITA 4, IITA 2 and IITA 3. Inbred lines SC 5, SC 4, SC 7 and SC 6 in combination with testers from the central and West African mid-altitude mega environments, IITA 4, IITA 2, IITA 7, IITA 11, IITA 12 and IITA 1, had high utility value for resistance to *F. verticillioides* ear rot and the fumonisins. Inbred lines and testers SC 5 and IITA 4 respectively were superior for both grain yield, ER and fumonisin accumulation. GD was not effective in prediction of yield in hybrids but was effective in classification of the lines into groups. The highest GD values were predominately between Seed Co lines with a few exceptions. Since SCA had significant and positive correlations with MPH, HPH and *per se* grain yield, its use in the estimation of heterosis has been positively confirmed. This was also true for fumonisin SCA with fumonisin MPH and HPH, and for ER incidence SCA with MPH and HPH. The flowering traits DMP, DMS and ASI all had negative MPH and HPH which is desirable in bringing earliness to the F1 hybrids. When such hybrids are used as females in three-way hybrid formation, there is

a need to identify an earlier male to ensure synchronization where same day planting of parents are desirable.

7.6 References

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Chapter 8

General conclusions and recommendations

Maize remains an important crop in sub-Saharan Africa, including Zimbabwe, where it is a staple food. Most of the meals taken by the majority of people in Zimbabwe and other eastern, central and southern Africa, contains maize (*Zea mays* L.). In other countries such as central and West Africa, maize compliments other sources of food that include yam, cassava, plantain and rice. There are, however, some regions within such countries where maize remains important so much so that the total production from such areas could surpass the total production from countries that produce maize as a staple food. The total production from Nigeria that was expected in 2014 of 7.5 million metric tonnes (MT) of maize far exceeds the total requirement for Zimbabwe of 1.8 million MT per year. In countries such as Zimbabwe, the small holder farmers contribute the largest proportion to the national production, particularly the traditional communal areas. This is due to the fact that production at house-hold level endeavours to ensure food security while the excess is sold to the national buying authority for the national strategic reserves and distribution to industry and other non-maize producing regions. Being a small-holder crop, several production constraints affect its production. Among these are socio-economic where farmers lack the capacity to obtain the required inputs; abiotic stress such as drought and low fertility and biotic stresses. Within the biotic stresses are some fungi that affect the stalks, leaves and grain. The grain fungi give rise to mycotoxin production that is detrimental to the health of the direct consumers of maize or their livestock. The need to breed maize hybrids or varieties that have inherent resistance to both the fungi and the mycotoxins themselves, is of paramount importance. Breeding requires use of germplasm that contributes towards attainment of the required traits such as mycotoxin resistance. Such sources may not be available within the local gene pool hence making it necessary to introduce exotic sources. It is with this background that this study on mycotoxigenic fungi associated with ear-rots in Zimbabwe was done. Identification and inheritance of resistance in southern, central and West African maize inbred lines was conducted and determined. The objectives of the whole study were i) To study the strains of fungi causing ear rotting and subsequently producing mycotoxins in Zimbabwe, ii) to conduct a phylogenetic study on the Zimbabwe *Fusarium verticillioides* isolates, iii) assess the usability of the various sources of germplasm from the mid-altitude zones of southern Africa within the Seed Co breeding

programme and central and West Africa from the IITA maize breeding programme, iv) to determine combining ability and type of gene action controlling resistance to the most abundant mycotoxin producing fungi that was found to be *F. verticillioides* and its mycotoxin fumonisin, v) to determine the level of diversity between the maize inbred lines from southern, central and western Africa and vi) to determine correlations between *per se* performance in terms of means of traits of importance, their MPH and HPH, genetic distance and SCA of the lines used.

The literature reviewed reveals preceded risk as a result of non-availability of cultivars of maize that are resistant to mycotoxins. Various surveys conducted provide information indicative of the most occurring fungi and in some cases, the mycotoxins associated with them. The risk level is clearly elaborated from various studies conducted to such an extent that the FAO and the WHO found it fit to state the maximum allowable levels which many nations, particularly in sub-Saharan Africa like Zimbabwe, do not consider, despite the availability of legislation stating the limits (FAO, 2004). WHO regards aflatoxins as a class-1 carcinogen (Martinez *et al*., 2011) and fumonisin B1 as class-2. Most breeding institutions select maize lines or hybrids based on the incidences for ear rots based on a subjective score or by counting ears that have no visible symptoms of infection by various fungi. This does not take into consideration the possibility that at one stage, the fungus occurred and left these toxic metabolites that cannot be seen by the naked eye. This is the reason why mycotoxins are sometimes observed in asymptomatic samples. The occurrence of the mycotoxin causing fungi such as *F. verticillioides* is variable, therefore to ensure successful screening, there is need to artificially inoculate. This allows for breeding to take place as there is evidence of availability of sources of resistance which may be of temperate origin but can be introgressed into the local germplasm to transfer the resistance genes. Inheritance of resistance has been found to be both additive and non-additive as some studies found GCA and SCA being significant, hence exploitation of both additive gene action by use of resistant lines only and dominance by selecting specific combinations will go a long way in breeding for resistance to both the fungi and various mycotoxins. The general conclusions by various publications that the QTLs responsible for resistance of one species of fungi within either the same genus such as the Fusarium or across other genuses such as Aspergillus are the same, and simplifies the effort in developing resistant genotypes that cut across regions. This is more so where the various sources of resistance from one region such as central and West Africa are used in

conjunction with lines from southern Africa where mycotoxin causing fungi are different. The possibility of using such germplasm is further made possible with genetic diversity studies that culminate in the identification of lines that belong to the same grouping which is further confirmed by the line x tester analysis in the form of the North Carolina Design II. Apart from classification of the germplasm, such a mating design further elucidates the gene action pertaining to the germplasm in use, as the results obtained elsewhere with different germplasm may differ when another set of germplasm is used.

Questions raised on which fungi were predominant in grain storage in Zimbabwe was answered by a survey conducted from 23 grain storage facilities in maize producing areas in Zimbabwe in 2011 from the 2011 and 2010 delivered maize in some places. Thirty three fungal species were identified from the maize samples by using morphological characteristics in the pathology laboratory with the assistance of various fungal books. *F. verticillioides* was the most abundant field fungus while *Eurotium repens* had the highest incidence among the storage fungi whose incidences were clearly distinguished on appropriate medium. The genus with more species was Aspergillus with *A. flavus* that was only observed in the samples from the 2010 season correlating with a reduced incidence level of *F. verticillioides*. *F. verticillioides* did not show a specific pattern in terms of geographical distribution, while *A. flavus* was more prevalent in the northern parts of Zimbabwe. Since *F. verticillioides* was the most frequently occurring fungi in storage, the focus of the whole study turned to breeding for its resistance after confirmation of the morphological identity using DNA sequence comparisons. Due to the presence of cryptic species in Fusarium that can only be identified based on DNA sequence comparisons, the Translation Elongation Factor *1-*α gene sequences were used where the derived *Fusarium* species were compared to those of known species which confirmed the morphological data, with a few exceptions. The means of the subjective kernel rot score conducted were negatively correlated with the fumonisin contamination on the samples while positive but low correlations were observed between the incidence obtained from the morphological analysis and the fumonisin content. The need to breed for resistance was clearly observed.

The observation of the high incidences of *F. verticillioides* ear rots resulted in acquisition of 12 inbred lines from central and West Africa and 12 inbred lines from Seed Co in Zimbabwe, adapted to southern African mid-altitude regions with various levels of resistance to either the causing

fungi or the aflatoxins which literature indicates has the same pathway of resistance as fumonisins. The 144 derived F1 maize hybrids developed from 12 maize inbred lines from southern African used as female lines and 12 lines from central and West Africa used as male testers mated in a NCDII, were evaluated with six check varieties from these two respective regions. Alongside these hybrid trials were the parent trials with the 24 inbred lines. These trials included the artificially inoculated site at RARS in Zimbabwe in the 2012/13 season where samples were also analysed for fumonisin contamination. Low infection levels were observed with significant differences for ear rot incidence and fumonisin content for the F1 hybrids. The inbred lines showed no significant differences for *F. verticillioides* incidences, fumonisin contamination and the grain disease score (GDS). The lowest *F. verticillioides* ear rot incidences *per se* and the lowest fumonisin contamination were on F1 hybrids SC 2/IITA 7, SC 3/IITA 1, SC 3/IITA 10, SC 5/IITA 10 and SC 10/IITA 2. Within the best yielding hybrids, tester IITA 4 contributed more in terms of grain yield while it had the highest yield in the parent trial. This line, although coming from central and West Africa, exhibited high adaptability and can be recommended to be used in the southern African maize breeding programme as it also carries resistance to aflatoxins. Besides this line, several more lines and F1 hybrids with *per se* low infection levels for ear rot were identified and these lines and hybrids can be used in southern Africa to improve the local gene pool in terms of both reaction to ER causing fungi, fumonisins and even better agronomic performance.

The results obtained for the GCA and SCA effects for *F. verticillioides* incidence were variable across sites, for both the lines and testers. The GCA effects for grain yield, days to mid pollen, days to mid silk and anthesis to silking interval were significant across all sites. Since both the SCA and GCA were significant for *F. verticillioides* ear rots and fumonisin contamination, it can be concluded that additive and non-additive gene effects had a role in conferring resistance to these two traits, with additive gene effects playing a major role in the fumonisins, particularly for the southern African inbred lines. Additive gene effects were also observed to play a major role in agronomic traits such as grain yield and flowering related traits such as days to mid-pollen, days to mid-silking and anthesis to silking interval. The inbred lines from Seed Co, SC 2, SC 3, SC 4, SC 9, SC 11 and SC 12 had desirable GCA for *F. verticillioides* ear rot and can be used as a source for resistance. The lines SC 2, SC 3 and SC 12, besides having a negative and relatively high GCA for *F. verticillioides* ear rot incidences, had negative GCA for fumonisins. From the IITA testers,

those identified to be outstanding in terms of ear rots and fumonisins were IITA 4, IITA 8, IITA 3, IITA 5, IITA 7, IITA 6 and IITA 1 that had negative GCA for *F. verticillioides* ear rot and for fumonisins. In terms of agronomic performance, inbred lines SC 10, SC 9 and SC7 from Seed Co and testers IITA 4 and IITA 2 from IITA, were identified as lines that can play a significant role in the southern African maize breeding programmes.

SNP markers were used to study the level of genetic diversity among the Seed Co southern African lines and the IITA central and West African mid-altitude inbred lines. The genetic diversity level was low as the dissimilarity average was 0.29 The average genetic distance based on Rogers' dissimilarity coefficients was 0.30 which was equally low. The lines IITA 12 and SC 11 had the highest distance of 0.38. Most pairs with high genetic distances were from the southern African lines with SC 7 featuring the most frequently. Despite that, the markers successfully distinguished the Seed Co lines and the IITA lines which formed the main groups besides an additional group comprising of a single line with some temperate pedigree that was converted to white and quality protein maize. The main groups, particularly the group with mainly lines from Seed Co, had some sub-groups representing the four heterotic groups used, while the IITA group had two subgroups that could possibly represent two opposite heterotic groups. These resultsare consistent with the pedigree information, hence will assist in utilising the sources of mycotoxin resistance within the Seed Co breeding programme. Two lines from Seed Co were found to be closer to the IITA inbred lines, which can also be of potential use when it comes to developing hybrids for West Africa.

Correlations were determined on various traits including derived traits to determine their relationships. High heterosis for grain yield averaging 295% and 225% for the MPH and HPH, respectively was obtained across the three Zimbabwe sites in two years. Such results are encouraging as they indicate existence of high levels of heterosis between the central and West and the southern African lines. DMP, DMS and ASI had negative mean MPH of -5, -6% and -2% while the average HPH was -4%, -7% and -35% respectively. The ear rot and fumonisin accumulation had respective MPH of -9% and -46% and HPH of -76% and -48%. Negative heterosis with flowering related traits is desirable as earliness is induced by crossing these inbred lines. Inter crossing of the sub-regional lines also reduced the incidence of ear rots and the amount of fumonisins accumulated as illustrated by negative heteroses obtained.

The correlation between GD and MPH and HPH were significant, low and negative. The SCA for grain yield and the mean of GY *per se* when correlated with GD, their mean squares were significant and not significant respectively with positive and low correlation coefficients, while that of mean grain yield and grain yield SCA were significant. This could be due to use of lines from the same region as either males or females without allowing intra group mating where higher genetic distances were observed than across the sub-regions.

SCA for *F. verticillioides* ear rot and fumonisin had significant and positive correlation with MPH and HPH of the same traits, suggesting non-additive gene effects being important in the inheritance of these traits. The Seed Co inbred lines from southern Africa SC 10 and SC 5, and the IITA central and West Africa IITA 4, IITA 2 and IITA 3 were found to be superior in terms of yield with the best yielding hybrid SC 5/IITA 4 also showing resistance to both ER and fumonisin accumulation.

The GD was effective in classifying the lines into heterotic groups in line with the known pedigree information. Use of inbred lines in maize programmes have become important, especially in this era of exploitation of heterosis either in hybrids or where synthetics are regarded as alternatives to the traditional landraces still in use in some parts of Africa.

It can therefore be recommended that while the incidence of *F. verticillioides* were high, there is high probability of the presence of fumonisins, hence breeding programmes within Zimbabwe can take advantage of the availability of the aflatoxin resistant lines in central and West Africa in introgressing resistance into the local germplasm. Such sources have been identified, including some within the Seed Co breeding programme. Use of such lines contributed to earliness which is attractive besides high level of heterosis when crosses are made between these sub-regional inbred lines. Inversely, one line within Seed Co was identified to to be closer to IITA lines hence it could be utilised in the Seed Co breeding program for central and West Africa. The lines can be used in a way in which additive gene action can be exploited by ensuring that only resistant lines are used. Results from evaluation of F1 hybrids can also lead to faster progress as non-additive gene effects have also been identified where dominance and possibly epistasis or over dominance could also be playing a role in conferring resistance.

SUMMARY

Fumonisin, a mycotoxin produced by *Fusarium verticillioides,* is an intrinsic constraint in maize (*Zea mays* L.) that has received a low level of attention in Zimbabwe, despite existence of laws nationally and globally setting acceptable limits. Breeding for resistance to the causal fungus is important for the poor farmers that depend on this crop. A survey was conducted that highlighted the presence of *F. verticillioides* in 23 national storage facilities in the major maize growing areas of Zimbabwe. The morphological analysis identified 33 fungi in storage with *F. verticillioides* having the highest incidence among field fungi while *Eurotium repens* was the highest in storage. *Aspergillus flavus* was observed at significant levels in Bindura in the maize delivered in the previous year. The *Fusarium* species identified were confirmed by gene sequencing that clustered the derived isolates among the *F. verticillioides* sequences in the databases. Such high incidences motivated the study of inheritance of resistance where 12 mid-altitude lines from the Seed Co southern African breeding programme and 12 from IITA central and West Africa were mated in a NCDII. The lines had variable levels of resistance to the causal fungi and aflatoxins. F1 hybrids SC 2/IITA 7, SC 3/IITA 1, SC 3/IITA 10, SC 5/IITA 10 and SC 10/IITA 2 had the lowest *F. verticillioides* ear rot incidences *per se* and the lowest fumonisin contamination. Within the best yielding hybrids, tester line IITA 4 contributed most in terms of grain yield as it appeared more frequently in the best hybrids. In the parent trial planted alongside the F1 hybrid trials, tester IITA 4 had the highest yield besides also being one of the four lines classified as resistant to aflatoxins. The GCA effects for grain yield, days to mid pollen, days to mid silk and anthesis to silking interval were significant across all sites. Both GCA and SCA were significant for *F. verticillioides* ear rots and fumonisins contamination, it therefore can be concluded that additive and non-additive gene effects had a role in conferring resistance to these two traits, with additive gene effects playing a major role in the fumonisins, particularly for the southern African inbred lines. Seed Co inbred lines SC 2, SC 3, SC 4, SC 9, SC 11 and SC 12 had desirable GCA for *F. verticillioides* ear rot with lines SC 2, SC3 and SC12 also having negative GCA for fumonisins. The IITA tester lines with negative GCA for ear rots and fumonisins were IITA 4, IITA 8, IITA 3, IITA 5, IITA 7, IITA 6 and IITA 1. The inbred lines with the highest GCA for yield were SC 10, SC 5, SC 8 (Seed Co), and testers IITA 4 and IITA 2 (IITA) were identified as lines that can play a significant role in the

southern African maize breeding programmes. Besides these lines, several more lines and F1 hybrids with *per se* low infection levels for ear rot were identified and these lines and hybrids can be used in southern Africa to improve the local gene pool in terms of both reaction to ER causing fungi, fumonisins and agronomic performance. To study genetic diversity 1144 SNP markers were used on the 24 inbred lines. Rogers' dissimilarity coefficients successfully distinguished the Seed Co and the IITA lines which formed the main groups besides an additional group comprising of a single line. The lines IITA 12 and SC 11 had the highest distance of 0.38. There were some sub-groups with the Seed Co materials forming clusters that were consistent with the pedigree data except for one line (SC 3). The IITA material formed two subgroups that could possibly represent two opposite heterotic groups. High heterosis for grain yield averaging 295% and 225% for the MPH and HPH, respectively, was obtained across sites in two years. Negative MPH and HPH for flowering related traits were observed. The ear rot and fumonisin accumulation had negative MPH and HPH. The correlations between the GD and MPH and HPH were significant, low and negative. There was no significant correlation between the SCA for grain yield and GD while there was significant correlation between GD and mean of grain yield *per se*. Grain yield mean and grain yield SCA were significantly correlated. SCA for *F. verticillioides* ear rot and fumonisin was significantly positively correlated with MPH and HPH of the same traits. It can therefore be concluded that good genetic gain can be obtained from the use of exotic germplasm when targeting traits such as resistance to ear rot causing fungi and the fumonisins than yield with exceptional few cases.

Key words: *Fusarium verticillioides*, fumonisin, genetic diversity, combining ability, heterosis.

OPSOMMING

Fumonisien, ‘n mikotoksien wat deur *Fusarium verticillioides* geproduseer word, is ‘n intrinsieke beperking in die mieliebedryf, wat ‘n baie lae vlak van aandag in Zimbabwe geniet, ten spyte van die bestaande nasionale en internasionale wette wat aanvaarbare limiete stel. Teling van weerstand teen die mikotoksien produserende fungi is belangrik vir die arm boere wat afhanklik is van die gewas. ‘n Opname is gedoen wat die teenwoordigheid van *F. verticillioides* in 23 nasionale storingsfasiliteite in die hoof mielieproduserende streke van Zimbabwe getoon het. Die morfologiese analise het 33 fungi in storing getoon met die hoogste insidensie van *F. verticillioides* in die veld, en *Eurotium repens* was die hoogste in storing. *Aspergillus flavus* is gesien in betekenisvolle vlakke in Bindura in die mielies gelewer in die vorige jaar. Die *Fusarium* spesies wat geïdentifiseer is, is bevestig met geenvolgordebepaling wat die afgeleide isolate vanaf *F. verticillioides* gegroepeer het volgens volgordes in die databasisse. Hierdie hoë insidensies het die studie op die oorerwing van weerstand gemotiveer, waar 12 mid-hoogte lyne van die Seed Co suidelike Afrika teelprogramme en 12 van IITA sentrale en Wes Afrika programme gekruis is in ‘n NCDII. Die lyne het variënde vlakke van weerstand teen die veroorsakende fungi en aflatoksiene getoon. F1 basters SC 2/IITA 7, SC 3/IITA 1, SC 3/IITA 10, SC 5/IITA 10 en SC 10/IITA 2 het die laagste *F. verticillioides* kopvrot insidensie *per se* en die laagste fumonisien kontaminasie getoon. In die basters met die hoogste opbrengs, het toetser lyn IITA 4 die meeste bygedra in terme van graanopbrengs omdat dit die mees algemene ouer in die beste basters was. In die ouerproewe wat langs die F1 bastersproewe geplant is, het toetser IITA 4 die hoogste opbrengs getoon en dit is ook geïndentifiseer as een van die vier lyne met weerstand teen aflatoksiene. Die GCA effekte vir graanopbrengs, dae tot mid stuifmeel, dae tot mid baard en antese tot baard interval was betekenisvol oor al die omgewings. Beide GCA en SCA was betekenisvol vir Fusarium kopvrot en fumonisien kontaminasie. Daarom kan afgelei word dat additiewe en nie-additiewe geeneffekte ‘n rol gespeel het in weerstand teen hierdie twee eienskappe, met additiewe geeneffekte wat ‘n groot rol gespeel het in die fumonisiene, veral in die suidelike Afrika ingeteelde lyne. Seed Co ingeteelde lyne SC 2, SC 3, SC 4, SC 9, SC 11 en SC 12 het goeie GCA vir Fusarium kopvrot met lyne SC 2, SC3 en SC12 gehad met negatiewe GCA vir fumonisiene. Die IITA toetserlyne met negatiewe GCA vir kopvrot en fumonisiene was IITA 4, IITA 8, IITA 3, IITA 5, IITA 7, IITA 6 en IITA 1. Die ingeteelde lyne met die hoogste GCA

vir opbrengs was SC 10, SC 5, SC 8 (Seed Co), en toetsers IITA 4 en IITA 2 (IITA) is geïdentifiseer as lyne wat ‘n belangrike rol kan speel in suidelike Afrika mielieteelprogramme. Afgesien van hierdie lyne, is daar ‘n aantal ander lyne en F1 basters met *per se* lae infeksievlakke vir kopvrot geïdentifiseer en hierdie lyne en basters kan gebruik word in suidelike Afrika om die plaaslike geenpoel te verbeter in terme van reaksie vir kopvrot veroorsakende fungi, fumonisiene en agronomiese eienskappe. Om die genetiese diversiteit te bepaal is 1144 SNP merkers gebruik om die 24 ingeteelde lyne te karakteriseer. Rogers se koeffisiënt van verskille het die Seed Co en die IITA lyne suksesvol onderskei. Hulle het twee hoofgroepe gevorm afgesien van een lyn wat nog ‘n groep gevorm het. Die lyne IITA 12 en SC 11 het die grootste afstand van 0.38 gehad. Daar was sub-groepe in die Seed Co materiaal wat groepe gevorm het wat ooreengestem het met die stambome, behalwe vir een lyn (SC 3). Die IITA materiaal het twee sub-groepe gevorm wat moontlik twee heterotiese groepe verteenwoordig. Hoë heterose vir graanopbrengs met ‘n gemiddeld van 295% en 225% vir MPH en HPH, onderskeidelik, is gekry oor omgewings en jare. Negatiewe MPH en HPH vir blomverwante eienskappe is gesien. Kopvrot en fumonisien akkumulasie het negatiewe MPH en HPH getoon. Die korrelasies tussen die GD en MPH en HPH was betekenisvol, laag en negatief. Daar was geen betekenisvolle korrelasie tussen die SCA vir graanopbrengs en GD nie, maar daar was betekenisvolle korrelasie tussen GD en gemiddelde graanopbrengs *per se*. Graanopbrengs gemiddeld en graanopbrengs SCA was betekensivol gekorreleer. SCA vir Fusarium kopvrot en fumonisien was betekenisvol positief gekorreleer met MPH en HPH van die eienskappe. Dit kan dus gesê word dat goeie genetiese vooruitgang gemaak kan word deur die gebruik van eksotiese kiemplasma as daar gekyk word na eienskappe soos weerstand teen kopvrot veroorsakende fungi en die fumonisiene.

Sleutelwoorde: *Fusarium verticillioides*, fumonisien, genetiese diversiteit, kombineervermoë, heterose

Appendices

Appendix 1 Mean incidences of various fungi from the 2010 and 2011 grain samples sampled in 2011 at 23 locations in Zimbabwe

Location	**Year when grain was delivered**	***Fusarium verticilliodes***	***Aspergillus niger***	***Stenocarpella maydis***	***Penicillium* spp.**	***Epicoccum sorghinum***	***Rhizopus oryzae***	***Trichothecium roseum***	***Epicoccum nigram***	***Trichordema harzianum***
Murehwa	2011	66	0	26.00**	20	2	0	0	0	0
Hwedza	2011	24	98.00***	0	26	2	46	0	0	0
Mvurwi	2011	20	0	10	24	32	6	0	2.00***	4
Banket	2011	32	0	6	6	38	4	0	0	0
Chinhoyi	2011	26	0	8	2	16	2	0	0	0
Lions Den	2011	46	0	6	18	8	0	0	0	0
Mhangura	2011	40	2	2	12	20	2	0	0	0
Doma	2011	80	0	2	2	4	4	0	0	0
Karoi	2011	76	2	0	4	0	0	0	0	0
Magunje	2011	72	4	2	2	0	0	0	0	0
Mutare	2011	96	0	4	12	0	8	0	0	0
Marondera	2011	62	2	6	6	2	4	0	0	0
Marondera	2010	76	0	18	0	0	30	0	0	0
Macheke	2011	46	0	26	6	8	2	0	0	0
Macheke	2010	18	12	16	4	0	46	0	0	0

Appendix 1 (continued) Mean incidences of various fungi from the 2010 and 2011 grain samples sampled in 2011 at 23 locations in Zimbabwe

Location	**Year when grain was delivered**	***Fusarium verticilliodes***	***Aspergillus niger***	***Stenocarpella maydis***	***Penicillium* spp.**	***Epicoccum sorghinum***	***Rhizopus oryzae***	***Trichothecium roseum***	***Epicoccum nigram***	***Trichordema harzianum***
Rusape	2011	66	0	0	0	0	0	0	0	0
Rusape	2010	24	0	18	4	0	0	0	0	0
Norton	2011	62	10	20	68.00***	2	14	0	0	0
Norton	2010	10	0	8	4	84.00***	0	0	0	0
Chegutu	2011	38	40	2	14	18	4	0	0	0
Chegutu	2010	8	12	8	2	34	18	0	0	0
Kadoma	2011	26	10	4	12	2	0	0	0	0
Kadoma	2010	58	4	16	6	4	0	0	0	0
Centinery	2010	24	2	0	26	4	0	0	2	0
Bindura	2010	0	2	0	24	0	100.00**	0	0	0
Glendale	2010	2	0	0	4	2	76.00**	0	0	0
Concession	2010	6	0	8	6	6	64	0	0	0
Kwekwe	2010	28	0	4	0	4	2	0	0	0
Gweru	2010	94	8	6	2	2	4	0	0	0
Mean		**42.28**	**7.17**	**7.79**	**10.76**	**10.14**	**15.03**	**0**	**0.14**	**0.14**
SD		**28.17**	**19.19**	**7.83**	**13.77**	**17.86**	**26.16**	**0**	**0.52**	**0.74**

Appendix 1 (continued) Mean incidences of various fungi from the 2010 and 2011 grain samples sampled in 2011 at 23 locations in Zimbabwe

Location	Year when grain was delivered	*Trichordema viride*	*Mucor* spp.	*Fusarium graminearum*	*Fusarium* spp.	*Drechslera halodes*	*Aspergillus ochraceus*	*Fusarium oxysporium*	*Cunninghamella elegans*	*Aspergillus versicolor*
Murehwa	**2011**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Hwedza	**2011**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mvurwi	**2011**	0.0	6.0	6.0**	0.0	0.0	0.0	0.0	0.0	0.0
Banket	**2011**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Chinhoyi	**2011**	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Lions Den	**2011**	0.0	0.0	0.0	0.0	2.0***	10.0***	2.0	0.0	0.0
Mhangura	**2011**	2.0	4.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Doma	**2011**	0.0	0.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0
Karoi	**2011**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Magunje	**2011**	0.0	0.0	8.0***	0.0	0.0	2.0	0.0	0.0	0.0
Mutare	**2011**	6.0	2.0	2.0	0.0	0.0	0.0	2.0	0.0	0.0
Marondera	**2011**	10.0***	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Marondera	**2010**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Macheke	**2011**	4.0	0.0	2.0	0.0	0.0	0.0	0.0	2.0***	0.0
Macheke	**2010**	0.0	0.0	0.0	0.0	0.0	2.0	0.0	0.0	0.0

Appendix 1 (continued) Mean incidences of various fungi from the 2010 and 2011 grain samples sampled in 2011 at 23 locations in Zimbabwe

Location	Year when grain was delivered	*Trichordema viride*	*Mucor* spp.	*Fusarium graminearum*	*Fusarium* spp.	*Drechslera halodes*	*Aspergillus ochraceus*	*Fusarium oxysporium*	*Cunninghamella elegans*	*Aspergillus versicolor*
Rusape	**2011**	0.0	0.0	0.0	0.0	0.0	0.0	6.0***	0.0	0.0
Rusape	**2010**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Norton	**2011**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Norton	**2010**	0.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Chegutu	**2011**	0.0	0.0	0.0	0.0	0.0	2.0	0.0	0.0	0.0
Chegutu	**2010**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Kadoma	**2011**	0.0	0.0	0.0	0.0	0.0	2.0	0.0	0.0	0.0
Kadoma	**2010**	2.0	0.0	0.0	0.0	0.0	0.0	4.0**	0.0	0.0
Centinery	**2010**	0.0	0.0	0.0	0.0	0.0	2.0	0.0	0.0	0.0
Bindura	**2010**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Glendale	**2010**	2.0	0.0	0.0	0.0	0.0	4.0	0.0	0.0	0.0
Concession	**2010**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Kwekwe	**2010**	6.0	40.0***	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Gweru	**2010**	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mean		1.17	1.86	0.69	0	0.07	0.83	0.48	0.07	0
SD		2.42	7.46	1.87	0	0.37	2.04	1.38	0.37	0

Appendix 1 (continued) Mean incidences of various fungi from the 2010 and 2011 grain samples sampled in 2011 at 23 locations in Zimbabwe

Location	Year when grain was delivered	*Chaetomium globosum*	*Pestalotiopsisguepini*	*Aspergillus* spp.	*Drechslera hawaiiensis*	Unknown Fusarium	*Aspergillus clavatus*	*Streptomyces* spp.
Murehwa	**2011**	0	0	0	0	0	0	0
Hwedza	**2011**	0	0	0	0	0	0	0
Mvurwi	**2011**	0	0	0	0	0	0	0
Banket	**2011**	0	0	0	0	0	0	0
Chinhoyi	**2011**	0	0	0	0	0	0	0
Lions Den	**2011**	0	0	0	0	0	0	0
Mhangura	**2011**	0	0	0	0	0	0	0
Doma	**2011**	0	0	0	0	0	0	0
Karoi	**2011**	0	0	0	0	0	0	0
Magunje	**2011**	0	0	0	0	0	0	0
Mutare	**2011**	0	0	0	0	0	0	0
Marondera	**2011**	0	0	0	0	0	0	0
Marondera	**2010**	0	0	0	0	0	0	0
Macheke	**2011**	0	0	0	0	0	0	0
Macheke	**2010**	0	0	0	0	0	0	0
Rusape	**2011**	0	0	0	0	0	0	0
Rusape	**2010**	4	0	0	0	0	0	0
Norton	**2011**	0	4.00***	0	0	0	0	0
Norton	**2010**	2	0	0	0	0	0	0
Chegutu	**2011**	0	0	0	2.00***	0	0	0
Chegutu	**2010**	0	0	0	0	0	0	0
Kadoma	**2011**	4	0	0	0	0	0	0
Kadoma	**2010**	0	0	0	0	0	0	0
Centinery	**2010**	8.00**	0	0	0	2.00***	0	0
Bindura	**2010**	0	0	0	0	0	8.00***	0
Glendale	**2010**	12.00***	0	0	0	0	0	0
Concession	**2010**	0	0	0	0	0	0	0
Kwekwe	**2010**	0	0	0	0	0	0	0
Gweru	**2010**	0	0	0	0	0	0	0
Mean		**1.03**	**0.14**	**0**	**0.07**	**0.07**	**0.28**	**0**
SD		**2.76**	**0.74**	**0**	**0.37**	**0.37**	**1.49**	**0**

Appendix 2 Performance of the F1 hybrids in terms of grain disease score, fumonisins and Fusarium ear rot at Rattray Arnold Research Station in 2012/13 season

Entry	Pedigree	GDS	B1	B2	B3	Total Fumonisin	ER
		1-9	ppm	ppm	ppm	ppm	%
1	SC 1/IITA 1	1.1	-0.3	-0.3	-0.3	0.7	0.0
15	SC 2/IITA 3	1.1	-0.3	-0.3	-0.3	0.7	0.0
19	SC 2/IITA 7	1.1	-0.3	-0.3	-0.3	0.7	0.0
25	SC 3/IITA 1	1.1	-0.3	-0.3	-0.3	0.7	0.0
26	SC 3/IITA 2	1.1	-0.3	-0.3	-0.3	0.7	0.0
34	SC 3/IITA 10	1.5	-0.3	-0.3	-0.3	0.7	0.0
43	SC 4/IITA 7	1.1	-0.3	-0.3	-0.3	0.7	0.0
55	SC 5/IITA 7	1.1	-0.3	-0.3	-0.3	0.7	0.0
71	SC 6/IITA 11	1.1	-0.3	-0.3	-0.3	0.7	0.0
88	SC 8/IITA 4	1.1	-0.3	-0.3	-0.3	0.7	0.0
126	SC 11/IITA 6	1.1	-0.3	-0.3	-0.3	0.7	0.0
134	SC 12/IITA 2	1.1	-0.3	-0.3	-0.3	0.7	0.0
144	SC 12/IITA 12	1.1	-0.3	-0.3	-0.3	0.7	0.0
148	M0826-1	1.5	-0.3	-0.3	-0.3	0.7	0.0
22	SC 2/IITA 10	1.1	-0.3	-0.3	-0.3	0.9	0.0
28	SC 3/IITA 4	1.1	-0.3	-0.3	-0.3	-0.9	0.0
58	SC 5/IITA 10	1.1	-0.3	-0.3	-0.3	-0.9	0.0
81	SC 7/IITA 9	1.1	-0.3	-0.3	-0.3	-0.9	0.0
105	SC 9/IITA 9	1.1	-0.3	-0.3	-0.3	-0.9	0.0
114	SC 10/IITA 6	1.1	-0.3	-0.3	-0.3	-0.9	0.0
115	SC 10/IITA 7	1.1	-0.3	-0.3	-0.3	-0.9	0.0
123	SC 11/IITA 3	1.1	-0.3	-0.3	-0.3	-0.9	0.0
129	SC 11/IITA 9	1.1	-0.3	-0.3	-0.3	-0.9	0.0
6	SC 1/IITA 6	1.1	-0.1	-0.3	-0.3	-0.8	0.0
113	SC 10/IITA 5	1.1	-0.1	-0.3	-0.3	-0.8	0.0
13	SC 2/IITA 1	1.5	-0.1	-0.3	-0.3	-0.8	0.0
45	SC 4/IITA 9	1.1	-0.1	-0.3	-0.3	-0.7	0.0
111	SC 10/IITA 3	1.1	-0.1	-0.3	-0.3	-0.8	0.0
121	SC 11/IITA 1	1.1	0.7	-0.1	-0.3	0.3	0.0
147	SC719	1.1	2.4	0.1	-0.1	2.4	0.0
2	SC 1/IITA 2	1.1	-0.3	-0.3	-0.3	-1.0	0.6
39	SC 4/IITA 3	1.1	-0.3	-0.3	-0.3	-1.0	0.6
50	SC 5/IITA 2	1.1	-0.3	-0.3	-0.3	-1.0	0.6
68	SC 6/IITA 8	1.1	-0.3	-0.3	-0.3	-1.0	0.6
78	SC 7/IITA 6	1.1	-0.3	-0.3	-0.3	-1.0	0.6
90	SC 8/IITA 6	1.1	-0.3	-0.3	-0.3	-1.0	0.6
100	SC 9/IITA 4	1.1	-0.3	-0.3	-0.3	-1.0	0.6
128	SC 11/IITA 8	1.1	-0.3	-0.3	-0.3	-1.0	0.6
139	SC 12/IITA 7	1.1	-0.3	-0.3	-0.3	-1.0	0.6
142	SC 12/IITA 10	1.1	-0.3	-0.3	-0.3	-1.0	0.6

Appendix 2 (continued) Performance of the F1 hybrids in terms of grain disease score, fumonisins and Fusarium ear rot at Rattray Arnold Research Station in 2012/13 season

Entry	Pedigree	GDS	B1	B2	B3	Total Fumonisin	ER
		1-9	ppm	ppm	ppm	ppm	%
4	SC 1/IITA 4	1.1	-0.3	-0.3	-0.3	-0.9	0.6
79	SC 7/IITA 7	1.1	-0.3	-0.3	-0.3	-0.9	0.6
64	SC 6/IITA 4	1.1	-0.1	-0.3	-0.3	-0.8	0.6
11	SC 1/IITA 11	1.1	-0.1	-0.3	-0.3	-0.7	0.6
31	SC 3/IITA 7	1.5	0.5	-0.3	-0.3	-0.1	0.6
107	SC 9/IITA 11	1.1	1.2	0.0	-0.3	0.9	0.6
32	SC 3/IITA 8	1.1	-0.3	-0.3	-0.3	-1.0	0.7
37	SC 4/IITA 1	1.1	-0.3	-0.3	-0.3	-1.0	0.7
52	SC 5/IITA 4	1.1	-0.3	-0.3	-0.3	-1.0	0.7
67	SC 6/IITA 7	1.1	-0.3	-0.3	-0.3	-1.0	0.7
70	SC 6/IITA 10	1.1	-0.3	-0.3	-0.3	-1.0	0.7
74	SC 7/IITA 2	1.1	-0.3	-0.3	-0.3	-1.0	0.7
91	SC 8/IITA 7	1.1	-0.3	-0.3	-0.3	-1.0	0.7
106	SC 9/IITA 10	0.8	-0.3	-0.3	-0.3	-1.0	0.7
125	SC 11/IITA 5	1.1	-0.3	-0.3	-0.3	-1.0	0.7
135	SC 12/IITA 3	1.1	-0.3	-0.3	-0.3	-1.0	0.7
33	SC 3/IITA 9	1.1	-0.3	-0.3	-0.3	-0.9	0.7
73	SC 7/IITA 1	1.1	-0.3	-0.3	-0.3	-0.9	0.7
95	SC 8/IITA 11	1.1	-0.3	-0.3	-0.3	-0.9	0.7
76	SC 7/IITA 4	1.1	0.0	-0.3	-0.3	-0.6	0.7
137	SC 12/IITA 5	1.1	0.0	-0.3	-0.3	-0.6	0.7
47	SC 4/IITA 11	1.1	1.5	0.0	-0.3	1.2	0.7
69	SC 6/IITA 9	1.1	2.1	0.0	-0.1	1.9	0.7
62	SC 6/IITA 2	1.1	3.1	0.1	-0.3	3.0	0.7
38	SC 4/IITA 2	1.1	-0.3	-0.3	-0.3	-1.0	1.1
42	SC 4/IITA 6	1.1	-0.3	-0.3	-0.3	-1.0	1.1
150	M1124-29	1.1	-0.3	-0.3	-0.3	-0.9	1.1
80	SC 7/IITA 8	1.1	-0.3	-0.3	-0.3	-1.0	1.2
85	SC 8/IITA 1	1.1	-0.3	-0.3	-0.3	-1.0	1.2
110	SC 10/IITA 2	1.1	-0.3	-0.3	-0.3	-1.0	1.2
116	SC 10/IITA 8	1.1	-0.3	-0.3	-0.3	-1.0	1.2
130	SC 11/IITA 10	1.1	-0.3	-0.3	-0.3	-1.0	1.2
138	SC 12/IITA 6	1.1	-0.3	-0.3	-0.3	-1.0	1.2
143	SC 12/IITA 11	1.1	-0.3	-0.3	-0.3	-1.0	1.2
9	SC 1/IITA 9	1.1	-0.3	-0.3	-0.3	-0.9	1.2
46	SC 4/IITA 10	1.1	-0.3	-0.3	-0.3	-0.9	1.2
23	SC 2/IITA 11	1.1	-0.1	-0.3	-0.3	-0.8	1.2
133	SC 12/IITA 1	1.1	0.0	-0.3	-0.3	-0.6	1.2
131	SC 11/IITA 11	1.1	0.3	-0.1	-0.3	-0.2	1.2
40	SC 4/IITA 4	1.1	0.7	-0.3	-0.3	0.1	1.2

Appendix 2 (continued) Performance of the F1 hybrids in terms of grain disease score, fumonisins and Fusarium ear rot at Rattray Arnold Research Station in 2012/13 season

Entry	Pedigree	GDS	B1	B2	B3	Total Fumonisin	ER
		1-9	ppm	ppm	ppm	ppm	%
48	SC 4/IITA 12	1.1	0.5	-0.1	-0.3	0.1	1.2
10	SC 1/IITA 10	1.1	-0.3	-0.3	-0.3	-1.0	1.3
82	SC 7/IITA 10	1.1	-0.3	-0.3	-0.3	-1.0	1.3
98	SC 9/IITA 2	1.1	-0.3	-0.3	-0.3	-1.0	1.3
109	SC 10/IITA 1	1.1	-0.3	-0.3	-0.3	-1.0	1.3
112	SC 10/IITA 4	1.1	-0.3	-0.3	-0.3	-1.0	1.3
102	SC 9/IITA 6	1.1	-0.3	-0.3	-0.3	-0.9	1.3
63	SC 6/IITA 3	1.1	0.1	-0.3	-0.3	-0.5	1.3
44	SC 4/IITA 8	1.5	0.5	-0.1	-0.3	0.1	1.3
124	SC 11/IITA 4	1.1	0.7	-0.1	-0.3	0.3	1.3
54	SC 5/IITA 6	1.1	3.5	0.1	-0.3	3.4	1.3
97	SC 9/IITA 1	1.1	-0.3	-0.3	-0.3	-1.0	1.4
49	SC 5/IITA 1	1.1	-0.3	-0.3	-0.3	-0.9	1.4
61	SC 6/IITA 1	1.1	-0.1	-0.3	-0.3	-0.7	1.4
149	M0926-8	1.5	-0.3	-0.3	-0.3	-1.0	1.5
3	SC 1/IITA 3	1.1	-0.3	-0.3	-0.3	-0.9	1.5
59	SC 5/IITA 11	1.1	-0.3	-0.3	-0.3	-1.0	1.6
77	SC 7/IITA 5	1.5	-0.3	-0.3	-0.3	-0.9	1.7
17	SC 2/IITA 5	1.5	-0.3	-0.3	-0.3	-0.9	1.8
30	SC 3/IITA 6	1.1	0.0	-0.3	-0.3	-0.7	1.8
14	SC 2/IITA 2	1.1	0.1	-0.3	-0.3	-0.5	1.8
145	SC537	1.5	3.1	0.1	-0.1	3.1	1.8
27	SC 3/IITA 3	1.5	-0.3	-0.3	-0.3	-1.0	1.9
146	SC535	1.1	-0.3	-0.3	-0.3	-1.0	1.9
65	SC 6/IITA 5	1.1	-0.3	-0.3	-0.3	-0.9	1.9
93	SC 8/IITA 9	1.1	-0.3	-0.3	-0.3	-0.9	1.9
21	SC 2/IITA 9	1.5	-0.3	-0.3	-0.3	-1.0	2.0
94	SC 8/IITA 10	1.1	-0.3	-0.3	-0.3	-1.0	2.0
127	SC 11/IITA 7	1.5	-0.3	-0.3	-0.3	-0.9	2.0
29	SC 3/IITA 5	1.1	-0.3	-0.3	-0.3	-1.0	2.1
75	SC 7/IITA 3	1.1	-0.3	-0.3	-0.3	-1.0	2.1
66	SC 6/IITA 6	1.5	-0.3	-0.3	-0.3	-0.9	2.1
132	SC 11/IITA 12	1.1	-0.1	-0.3	-0.3	-0.7	2.1
16	SC 2/IITA 4	1.1	-0.3	-0.3	-0.3	-1.0	2.2
53	SC 5/IITA 5	1.1	0.3	-0.3	-0.3	-0.2	2.3
119	SC 10/IITA 11	1.1	-0.3	-0.3	-0.3	-1.0	2.4
20	SC 2/IITA 8	1.1	0.0	-0.3	-0.3	-0.5	2.4
8	SC 1/IITA 8	1.1	-0.3	-0.3	-0.3	-1.0	2.5
104	SC 9/IITA 8	1.1	-0.3	-0.3	-0.3	-0.9	2.5
57	SC 5/IITA 9	1.1	0.1	-0.1	-0.3	-0.3	2.5

Appendix 2 (continued) Performance of the F1 hybrids in terms of grain disease score, fumonisins and Fusarium ear rot at Rattray Arnold Research Station in 2012/13 season

Entry	Pedigree	GDS	B1	B2	B3	Total Fumonisin	ER
		1-9	ppm	ppm	ppm	ppm	%
7	SC 1/IITA 7	1.1	1.2	-0.1	-0.3	0.8	2.5
36	SC 3/IITA 12	1.5	0.9	-0.1	-0.3	0.6	2.5
101	SC 9/IITA 5	1.1	-0.3	-0.3	-0.3	-0.9	2.6
136	SC 12/IITA 4	1.1	-0.3	-0.3	-0.3	-0.9	2.6
140	SC 12/IITA 8	1.1	-0.3	-0.3	-0.3	-0.9	2.6
83	SC 7/IITA 11	1.1	0.5	-0.3	-0.3	0.0	2.6
86	SC 8/IITA 2	1.1	-0.3	-0.3	-0.3	-1.0	2.7
99	SC 9/IITA 3	1.1	-0.3	-0.3	-0.3	-1.0	2.7
118	SC 10/IITA 10	1.1	-0.3	-0.3	-0.3	-1.0	2.7
51	SC 5/IITA 3	1.1	-0.3	-0.3	-0.3	-0.9	2.7
5	SC 1/IITA 5	1.1	-0.1	-0.3	-0.3	-0.7	2.7
72	SC 6/IITA 12	1.1	-0.3	-0.3	-0.3	-0.9	2.8
96	SC 8/IITA 12	1.1	-0.3	-0.3	-0.3	-0.9	2.8
103	SC 9/IITA 7	1.5	-0.3	-0.3	-0.3	-0.9	2.8
56	SC 5/IITA 8	1.1	0.3	-0.3	-0.3	-0.3	2.8
122	SC 11/IITA 2	0.8	-0.1	-0.3	-0.3	-0.8	3.0
92	SC 8/IITA 8	1.1	-0.3	-0.3	-0.3	-1.0	3.1
60	SC 5/IITA 12	1.1	5.8	0.3	-0.1	5.9	3.5
141	SC 12/IITA 9	1.5	0.3	-0.3	-0.3	-0.3	3.7
84	SC 7/IITA 12	1.1	-0.3	-0.3	-0.3	-1.0	3.8
89	SC 8/IITA 5	1.1	-0.3	-0.3	-0.3	-1.0	3.9
117	SC 10/IITA 9	1.5	-0.3	-0.3	-0.3	-0.9	4.0
35	SC 3/IITA 11	1.5	-0.1	-0.3	-0.3	-0.7	4.0
24	SC 2/IITA 12	1.1	-0.3	-0.3	-0.3	-0.9	4.1
18	SC 2/IITA 6	1.1	-0.1	-0.3	-0.3	-0.8	4.4
41	SC 4/IITA 5	1.1	-0.3	-0.3	-0.3	-1.0	4.7
87	SC 8/IITA 3	1.1	-0.3	-0.3	-0.3	-1.0	4.7
108	SC 9/IITA 12	1.1	-0.3	-0.3	-0.3	-1.0	4.9
12	SC 1/IITA 12	1.1	-0.3	-0.3	-0.3	-0.9	4.9
120	SC 10/IITA 12	1.1	-0.3	-0.3	-0.3	-0.9	7.6
Mean		**1.1**	**0.6**	**0.4**	**0.4**	**1.3**	**1.4**
SE		**0.4**	**0.7**	**0.2**	**0.1**	**1.0**	**3.2**
LSD (5%)		**0.3**	**0.6**	**0.2**	**0.1**	**0.9**	**2.7**
P value		**ns**	*******	*******	*****	*******	*****

Appendix 3 Performance of the F1 hybrids in terms of grain yield and other agronomic traits from combined analysis of variance

Pedigree	GY	DMP	DMS	ASI	PHT	CHT	RL	SL	EASP	EPO	EPP	HC	TEXT	GLS	HT	MSV	PLS	RUST
	t ha^{-1}	days	days	days	m	m	%	%	1-9	ratio	no.	1-9	1-9	1-9	1-9	1-9	1-9	1-9
SC 5/IITA 4	5.94	69	70	2	1.9	1.1	0.0	20.0	2.2	0.5	0.7	1.3	1.8	2.1	1.6	1.0	0.2	1.2
SC 9/IITA 4	5.84	70	71	1	1.9	1.2	1.9	4.5	3.3	0.5	0.7	1.2	0.5	2.5	1.2	1.0	0.2	1.2
SC 9/IITA 2	5.56	70	72	1	1.8	1.0	0.0	1.4	3.0	0.5	0.8	1.5	0.8	2.6	1.3	1.2	0.2	1.2
SC 6/IITA 4	5.55	71	71	1	1.9	1.1	0.0	10.6	2.4	0.5	0.7	1.8	1.9	2.6	1.3	1.1	0.2	1.2
SC 1/IITA 4	5.52	68	70	2	2.0	1.2	0.0	6.6	3.0	0.5	0.7	1.4	1.0	2.3	0.9	1.0	0.2	1.2
SC 7/IITA 4	5.44	70	71	1	2.1	1.3	1.4	21.0	2.8	0.5	0.8	1.3	1.5	1.9	1.0	1.0	0.2	1.3
SC 9/IITA 3	5.42	69	70	1	1.8	1.0	0.2	6.7	3.1	0.5	0.7	1.2	0.7	2.8	0.9	1.0	0.2	1.2
SC719	5.36	71	72	1	2.0	1.2	0.0	22.5	2.6	0.5	0.7	1.5	1.9	2.1	1.2	1.0	0.2	1.2
SC 12/IITA 2	5.32	71	71	1	1.9	1.1	0.0	3.8	3.0	0.5	0.7	1.5	1.7	2.3	1.3	1.0	0.2	1.2
SC 5/IITA 3	5.29	67	69	1	1.8	0.9	1.0	10.8	2.7	0.4	0.8	1.6	1.7	2.2	1.3	1.0	0.2	1.2
SC 9/IITA 8	5.24	70	71	1	2.0	1.1	0.4	2.8	3.3	0.5	0.7	1.6	0.5	2.4	0.8	1.1	0.2	1.2
SC 8/IITA 10	5.23	68	69	1	1.9	1.0	0.2	14.5	3.1	0.5	0.7	1.4	0.7	2.1	0.9	1.0	0.2	1.2
SC 5/IITA 2	5.23	67	68	1	1.8	1.0	0.3	14.3	3.3	0.5	0.8	1.3	2.2	1.6	1.1	1.0	0.2	1.2
SC 8/IITA 8	5.22	70	70	0	2.0	1.1	0.0	12.2	3.8	0.4	0.8	1.7	0.9	3.0	0.8	1.0	0.2	1.1
SC 3/IITA 4	5.20	69	71	1	1.9	1.2	0.0	6.0	2.5	0.5	0.7	1.5	1.3	2.9	1.5	1.1	0.2	1.2
SC 8/IITA 2	5.20	70	70	0	1.9	1.0	0.0	9.9	3.3	0.5	0.7	1.3	1.2	2.1	0.8	1.0	0.2	1.1
SC 5/IITA 8	5.16	69	69	0	2.0	1.0	0.0	18.2	3.4	0.4	0.7	1.6	1.0	2.5	1.0	1.0	0.2	1.1
SC 2/IITA 4	5.14	68	70	2	2.0	1.1	0.2	12.6	2.3	0.5	0.7	1.3	1.7	2.5	1.2	1.0	0.2	1.2
SC 9/IITA 6	5.11	69	71	2	1.8	1.0	2.0	7.3	3.2	0.5	0.7	1.5	0.5	2.3	0.9	1.0	0.2	1.2
SC 2/IITA 5	5.10	69	70	1	1.9	1.1	0.3	18.7	3.3	0.5	0.8	1.3	1.8	2.1	1.0	1.0	0.2	1.3
SC 2/IITA 8	5.08	68	70	1	1.9	1.0	0.0	13.2	3.8	0.4	0.8	1.7	1.0	1.8	0.9	1.0	0.3	1.2
SC 4/IITA 4	5.08	70	72	2	2.0	1.2	0.5	18.9	2.7	0.5	0.7	1.6	1.0	2.2	0.9	1.0	0.2	1.3
SC 9/IITA 12	5.07	69	70	2	1.8	1.0	0.0	1.6	3.3	0.5	0.8	1.6	0.5	2.0	1.1	1.0	0.2	1.3
SC 2/IITA 9	5.05	69	71	2	1.8	1.1	0.4	13.8	3.5	0.5	0.7	1.8	1.4	2.4	1.0	1.0	0.2	1.2
SC 6/IITA 7	5.03	70	71	1	2.0	1.1	0.0	8.6	2.8	0.5	0.7	1.3	1.5	2.5	1.2	1.1	0.2	1.2

Appendix 3 (continued) Performance of the F1 hybrids in terms of grain yield and other agronomic traits from combined analysis of variance

Pedigree	GY	DMP	DMS	ASI	PHT	CHT	RL	SL	EASP	EPO	EPP	HC	TEXT	GLS	HT	MSV	PLS	RUST
	t ha^{-1}	days	days	days	m	m	%	%	1-9	ratio	no.	1-9	1-9	1-9	1-9	1-9	1-9	1-9
SC 8/IITA 3	5.02	68	69	0	1.8	1.0	0.8	11.2	3.8	0.5	0.8	1.4	1.8	2.1	1.2	1.1	0.2	1.2
SC 2/IITA 2	5.02	67	68	1	1.8	1.0	0.2	2.0	4.1	0.5	0.8	1.8	2.2	2.0	0.9	1.0	0.2	1.4
SC 8/IITA 7	5.01	70	71	1	2.0	1.2	0.0	6.6	3.0	0.5	0.7	1.4	0.9	2.4	1.0	1.1	0.2	1.3
SC 9/IITA 2	5.00	70	71	1	1.8	1.0	0.0	11.9	3.0	0.5	0.7	1.3	1.1	2.4	0.8	1.0	0.2	1.2
SC 6/IITA 2	5.00	70	72	1	1.8	1.0	0.2	1.1	3.1	0.5	0.7	1.3	1.5	2.4	1.3	1.1	0.3	1.2
SC 1/IITA 2	5.00	67	68	1	1.9	1.1	0.0	3.9	3.8	0.5	0.7	1.6	1.1	2.3	0.8	1.1	0.2	1.2
SC 6/IITA 6	4.99	68	70	2	1.7	1.0	0.0	23.1	3.1	0.5	0.8	1.3	2.2	2.4	0.8	1.0	0.2	1.3
SC 12/IITA 4	4.97	69	70	1	1.9	1.1	0.8	20.5	2.5	0.5	0.7	1.5	2.3	2.3	1.3	1.0	0.2	1.2
SC 9/IITA 8	4.96	70	71	1	2.0	1.1	0.5	10.6	3.3	0.5	0.7	1.5	0.5	2.1	0.8	1.0	0.2	1.3
SC 12/IITA 3	4.92	69	69	1	1.9	1.2	1.7	14.1	3.0	0.5	0.7	1.3	2.2	2.0	1.0	1.0	0.2	1.2
SC 5/IITA 7	4.91	68	69	1	2.0	1.1	0.8	13.2	2.7	0.5	0.8	1.5	1.5	1.8	1.2	1.0	0.2	1.2
SC 2/IITA 10	4.89	66	68	1	1.8	1.1	0.0	7.7	3.9	0.5	0.7	1.8	0.7	1.9	0.8	1.0	0.2	1.3
SC 8/IITA 5	4.85	70	72	1	1.9	1.2	0.0	25.3	3.5	0.5	0.7	1.5	1.0	1.9	0.8	1.0	0.2	1.2
SC 9/IITA 7	4.85	70	71	1	2.0	1.1	0.0	4.5	3.2	0.5	0.7	1.3	0.5	2.1	0.9	1.0	0.2	1.2
SC 2/IITA 6	4.84	66	68	2	1.7	1.0	0.5	13.0	3.3	0.5	0.8	1.5	2.1	1.8	0.8	1.1	0.2	1.3
SC 6/IITA 3	4.83	68	69	1	1.8	1.0	0.0	4.4	3.5	0.5	0.8	1.3	2.3	2.2	0.9	1.0	0.2	1.2
SC 5/IITA 11	4.82	66	67	1	1.8	1.0	0.9	6.4	2.9	0.5	0.7	2.1	0.7	2.3	0.9	1.0	0.4	1.2
SC 11/IITA 7	4.82	69	70	1	2.1	1.2	0.3	14.1	3.5	0.5	0.8	1.7	0.7	2.8	1.3	1.0	0.2	1.2
SC 9/IITA 4	4.82	69	71	2	1.8	1.1	0.0	21.6	3.2	0.5	0.7	1.3	1.6	2.3	1.0	1.1	0.2	1.2
SC 4/IITA 2	4.81	72	73	1	1.9	1.1	0.4	11.9	3.2	0.5	0.7	1.6	1.2	2.3	1.0	1.0	0.2	1.3
SC 6/IITA 10	4.80	69	69	1	1.8	1.0	2.2	17.1	3.3	0.5	0.7	1.3	1.6	2.2	0.9	1.2	0.2	1.2
SC 9/IITA 5	4.79	71	73	2	2.0	1.2	0.5	5.9	3.2	0.5	0.7	1.3	0.6	1.8	0.9	1.0	0.2	1.4
SC 9/IITA 1	4.78	70	72	2	1.9	1.1	0.0	10.4	2.8	0.5	0.6	1.2	0.5	2.0	1.5	1.0	0.2	1.2
SC 11/IITA 8	4.78	69	69	0	2.0	1.1	0.3	16.2	3.6	0.5	0.7	1.5	0.5	2.7	1.0	1.0	0.2	1.1
SC 11/IITA 5	4.77	68	69	1	2.1	1.1	0.8	8.4	3.5	0.5	0.8	1.4	1.1	2.1	1.3	1.0	0.2	1.2

Appendix 3 (continued) Performance of the F1 hybrids in terms of grain yield and other agronomic traits from combined analysis of variance

Pedigree	GY	DMP	DMS	ASI	PHT	CHT	RL	SL	EASP	EPO	EPP	HC	TEXT	GLS	HT	MSV	PLS	RUST
	t ha^{-1}	days	days	days	m	m	%	%	1-9	ratio	no.	1-9	1-9	1-9	1-9	1-9	1-9	1-9
SC 11/IITA 6	4.76	67	68	1	1.9	1.0	0.0	6.1	3.5	0.5	0.8	1.5	0.8	2.3	0.9	1.0	0.2	1.2
SC 2/IITA 1	4.75	68	70	2	1.9	1.1	0.0	8.3	3.5	0.5	0.8	1.6	1.1	1.8	1.3	1.0	0.2	1.3
SC 8/IITA 11	4.75	69	70	0	1.9	1.0	0.8	8.0	2.5	0.5	0.7	1.5	1.8	2.3	0.8	1.0	0.3	1.3
SC 11/IITA 10	4.74	68	69	1	1.9	1.1	0.0	12.4	3.1	0.5	0.7	1.3	0.5	2.3	0.9	1.0	0.2	1.3
SC 8/IITA 12	4.74	69	70	1	1.8	0.9	0.0	10.7	3.2	0.4	0.8	1.4	1.0	2.2	0.9	1.0	0.3	1.2
SC 9/IITA 10	4.72	69	70	2	1.8	1.0	0.8	8.0	3.1	0.5	0.7	1.6	0.5	2.0	0.8	1.0	0.2	1.3
SC 6/IITA 8	4.71	71	71	1	1.9	1.1	0.5	12.8	3.3	0.5	0.6	1.4	1.3	3.3	0.9	1.0	0.2	1.1
SC 3/IITA 11	4.69	66	68	1	1.9	1.1	1.1	3.1	2.9	0.5	0.7	1.3	1.0	2.2	0.8	1.0	0.3	1.2
SC 1/IITA 11	4.68	67	69	2	1.9	1.1	0.3	9.7	2.9	0.5	0.8	1.6	1.7	1.9	0.9	1.0	0.2	1.3
SC 4/IITA 8	4.68	70	71	1	2.0	1.2	0.0	22.2	3.1	0.5	0.7	1.6	0.5	2.2	0.9	1.1	0.2	1.2
SC 5/IITA 10	4.67	68	69	1	1.8	1.1	1.2	14.6	3.4	0.5	0.7	1.5	1.1	1.9	1.0	1.0	0.2	1.2
SC 1/IITA 7	4.67	69	70	1	2.0	1.1	0.0	10.5	3.1	0.5	0.8	1.8	0.7	2.3	0.8	1.0	0.2	1.3
SC 4/IITA 5	4.65	71	72	1	2.0	1.3	0.3	20.3	3.0	0.6	0.7	1.5	0.9	1.8	0.9	1.0	0.2	1.4
SC 6/IITA 11	4.64	68	69	1	1.9	1.1	0.0	11.5	2.8	0.5	0.7	1.3	2.2	2.1	1.2	1.0	0.2	1.2
SC 7/IITA 2	4.64	70	72	2	1.8	1.0	1.1	2.9	3.3	0.5	0.6	1.5	1.5	2.3	1.0	1.0	0.2	1.2
SC 1/IITA 5	4.62	69	71	2	2.0	1.1	0.3	14.0	3.2	0.5	0.7	1.3	1.2	2.1	1.3	1.0	0.2	1.3
SC 8/IITA 9	4.62	71	72	2	1.9	1.1	0.0	4.9	3.6	0.5	0.7	1.9	1.3	2.3	0.8	1.1	0.2	1.3
SC 4/IITA 3	4.62	69	70	1	1.9	1.2	2.4	19.0	3.2	0.5	0.7	1.8	1.3	2.0	0.8	1.0	0.2	1.2
SC 5/IITA 6	4.62	66	68	2	1.8	1.0	0.0	19.2	3.1	0.5	0.8	1.4	2.0	2.3	0.8	1.0	0.3	1.2
SC 12/IITA 10	4.61	68	69	1	1.9	1.1	0.4	12.2	3.2	0.5	0.7	1.3	1.4	2.3	0.9	1.0	0.2	1.2
SC 11/IITA 4	4.59	67	68	1	2.1	1.2	0.0	15.6	3.6	0.5	0.7	1.3	1.1	2.3	1.2	1.0	0.2	1.2
SC 2/IITA 3	4.58	68	69	1	1.8	1.0	0.0	19.4	3.4	0.5	0.8	1.7	1.4	2.2	1.0	1.0	0.2	1.2
SC 12/IITA 11	4.54	69	69	1	1.9	1.1	0.4	9.9	3.2	0.5	0.6	1.3	1.4	2.1	1.1	1.0	0.3	1.2
SC 9/IITA 11	4.52	68	69	1	1.9	1.1	0.0	13.4	2.8	0.5	0.7	1.6	1.3	1.8	0.9	1.0	0.2	1.5
SC 5/IITA 5	4.51	68	70	2	2.0	1.2	0.8	12.5	3.2	0.5	0.7	1.2	1.6	2.3	1.1	1.0	0.2	1.3

Appendix 3 (continued) Performance of the F1 hybrids in terms of grain yield and other agronomic traits from combined analysis of variance

Pedigree	GY	DMP	DMS	ASI	PHT	CHT	RL	SL	EASP	EPO	EPP	HC	TEXT	GLS	HT	MSV	PLS	RUST
	t ha^{-1}	days	days	days	m	m	%	%	1-9	ratio	no.	1-9	1-9	1-9	1-9	1-9	1-9	1-9
SC 3/IITA 10	4.49	68	69	1	1.8	1.1	0.0	13.8	2.8	0.5	0.7	1.2	0.5	2.6	1.0	1.0	0.2	1.1
SC 12/IITA 7	4.47	69	70	1	2.1	1.2	0.8	7.6	3.2	0.5	0.7	1.3	1.7	2.0	0.8	1.0	0.2	1.3
SC 3/IITA 7	4.46	70	71	1	2.0	1.1	0.3	2.1	2.8	0.5	0.7	1.5	0.7	1.9	0.9	1.1	0.2	1.2
SC 5/IITA 1	4.45	68	69	1	2.0	1.1	2.0	17.8	3.4	0.5	0.7	1.3	1.4	2.3	1.0	1.0	0.2	1.3
SC 7/IITA 10	4.45	69	72	3	2.0	1.2	0.2	10.6	2.8	0.5	0.7	1.4	0.6	2.2	0.8	1.0	0.3	1.2
SC 3/IITA 5	4.44	69	71	2	2.0	1.2	0.8	0.7	3.1	0.5	0.7	1.3	0.8	2.7	1.1	1.0	0.2	1.1
SC 3/IITA 1	4.44	71	72	2	1.8	1.0	0.4	1.5	2.6	0.5	0.7	1.2	0.9	2.3	0.9	1.0	0.2	1.2
SC 4/IITA 6	4.43	70	71	1	1.9	1.2	0.2	7.5	3.6	0.5	0.7	1.5	1.0	1.9	1.0	1.1	0.2	1.2
SC 11/IITA 11	4.38	67	68	1	1.9	1.0	0.9	10.8	3.0	0.4	0.6	1.2	0.7	2.3	0.9	1.0	0.2	1.2
SC 7/IITA 11	4.37	69	71	1	2.0	1.2	0.5	11.4	3.1	0.5	0.7	1.6	0.7	2.3	1.2	1.0	0.2	1.2
SC 7/IITA 8	4.36	70	72	2	2.0	1.1	0.0	9.3	3.7	0.5	0.6	1.4	0.8	2.6	0.9	1.0	0.2	1.2
SC 11/IITA 9	4.36	70	71	1	2.0	1.1	0.0	9.0	3.2	0.5	0.7	1.5	1.0	2.0	1.2	1.0	0.2	1.2
SC 2/IITA 12	4.35	66	68	2	1.7	1.0	0.0	11.5	3.6	0.5	0.7	1.8	1.2	2.3	1.6	1.0	0.2	1.2
SC 7/IITA 6	4.34	69	71	2	1.9	1.1	0.0	7.8	3.4	0.5	0.7	1.5	1.2	2.3	0.8	1.0	0.3	1.3
SC 7/IITA 7	4.33	69	70	1	2.1	1.2	1.2	10.3	3.0	0.5	0.7	1.6	1.0	2.3	0.8	1.0	0.2	1.2
SC535	4.33	67	68	1	1.8	0.9	0.7	12.1	3.6	0.4	0.6	1.2	1.4	1.9	0.9	1.0	0.2	1.2
SC 3/IITA 3	4.31	69	70	1	1.8	1.0	1.3	8.9	3.3	0.5	0.7	1.5	0.8	2.4	0.8	1.0	0.1	1.1
SC 1/IITA 3	4.30	68	69	1	1.9	1.0	0.0	18.4	3.7	0.5	0.7	2.1	0.9	2.7	0.8	1.0	0.2	1.2
SC 2/IITA 7	4.30	68	69	1	1.9	1.2	0.3	14.4	3.3	0.5	0.7	1.3	1.4	1.8	0.9	1.0	0.2	1.2
SC 6/IITA 5	4.27	71	72	1	2.0	1.1	0.3	18.3	3.3	0.5	0.7	1.1	2.0	2.5	0.9	1.1	0.2	1.2
SC 1/IITA 12	4.25	67	69	2	1.9	1.1	0.0	7.0	4.0	0.5	0.7	1.3	0.6	2.4	0.8	1.0	0.2	1.2
SC 3/IITA 6	4.23	69	70	1	1.6	1.0	0.4	8.7	3.5	0.5	0.8	1.3	0.8	2.2	0.8	1.0	0.2	1.2
SC537	4.23	68	70	2	1.9	1.0	1.5	15.3	3.4	0.5	0.7	1.5	0.6	2.5	0.8	1.1	0.2	1.2
SC 4/IITA 11	4.22	70	71	1	2.0	1.2	1.8	15.7	3.2	0.5	0.7	1.1	0.7	2.0	0.9	1.0	0.2	1.2
SC 9/IITA 9	4.22	70	73	2	1.8	1.1	0.0	2.4	3.3	0.5	0.7	1.4	0.7	2.1	1.0	1.1	0.2	1.2

Appendix 3 (continued) Performance of the F1 hybrids in terms of grain yield and other agronomic traits from combined analysis of variance

Pedigree	GY	DMP	DMS	ASI	PHT	CHT	RL	SL	EASP	EPO	EPP	HC	TEXT	GLS	HT	MSV	PLS	RUST
	t ha^{-1}	days	days	days	m	m	%	%	1-9	ratio	no.	1-9	1-9	1-9	1-9	1-9	1-9	1-9
SC 3/IITA 8	4.21	70	70	1	1.8	0.9	0.0	1.4	3.4	0.4	0.7	1.3	0.5	2.6	0.9	1.0	0.2	1.2
SC 7/IITA 12	4.21	69	71	2	1.9	1.0	0.8	9.8	3.3	0.5	0.7	1.6	1.3	2.2	0.8	1.0	0.3	1.2
SC 7/IITA 3	4.17	69	71	2	1.8	1.1	0.0	19.3	3.6	0.5	0.7	1.8	1.1	1.8	1.2	1.0	0.2	1.3
SC 9/IITA 11	4.17	69	71	2	1.9	1.0	1.0	0.4	2.4	0.4	0.8	1.2	0.7	2.2	1.2	1.1	0.2	1.2
SC 4/IITA 12	4.16	69	71	2	1.8	1.1	0.0	13.0	3.3	0.5	0.7	1.5	0.8	2.1	0.9	1.0	0.2	1.2
SC 7/IITA 5	4.16	70	72	2	2.1	1.3	0.0	22.7	2.9	0.5	0.6	1.5	1.7	2.5	1.0	1.0	0.2	1.3
M0926-8	4.14	68	69	1	1.9	1.1	3.2	4.8	3.6	0.5	0.7	1.5	0.7	2.3	1.0	1.0	0.2	1.2
SC 5/IITA 9	4.13	69	70	2	1.8	1.1	0.0	13.5	3.1	0.5	0.6	1.6	1.5	2.2	1.2	1.1	0.2	1.2
SC 11/IITA 12	4.09	67	68	1	1.8	1.0	0.4	2.6	3.8	0.5	0.8	1.2	0.8	2.6	1.8	1.0	0.2	1.2
SC 3/IITA 2	4.07	70	71	1	1.7	1.0	0.0	0.5	3.7	0.5	0.7	1.3	1.2	2.0	1.1	1.0	0.2	1.2
SC 9/IITA 3	4.06	68	69	2	1.8	1.0	0.0	17.4	3.2	0.5	0.7	1.7	2.2	1.9	0.8	1.0	0.2	1.2
SC 11/IITA 1	4.03	67	68	1	1.9	1.1	0.0	5.6	4.1	0.5	0.7	1.3	1.0	2.3	0.9	1.0	0.2	1.2
SC 12/IITA 12	4.02	69	69	1	1.9	1.1	0.0	13.0	3.4	0.5	0.6	1.5	1.4	2.3	1.3	1.0	0.2	1.2
SC 1/IITA 8	4.00	69	70	1	2.0	1.1	1.6	7.9	3.8	0.5	0.7	1.6	0.8	2.9	0.8	1.0	0.2	1.1
SC 4/IITA 9	4.00	72	74	3	1.8	1.2	0.2	5.4	3.4	0.6	0.6	1.3	1.0	2.3	1.0	1.0	0.2	1.2
SC 8/IITA 4	3.99	70	70	1	1.9	1.1	2.1	17.2	3.0	0.5	0.7	1.2	1.3	2.2	0.9	1.0	0.2	1.3
SC 3/IITA 9	3.94	71	73	2	1.8	1.1	0.0	0.6	3.8	0.5	0.6	1.3	0.8	1.8	0.9	1.0	0.2	1.2
SC 11/IITA 2	3.93	70	70	1	1.9	1.0	0.8	9.9	3.6	0.5	0.7	1.5	0.9	2.2	1.0	1.0	0.2	1.3
SC 4/IITA 7	3.92	70	71	1	1.9	1.2	0.9	10.3	3.0	0.5	0.7	1.6	0.8	1.9	0.9	1.0	0.2	1.2
SC 8/IITA 6	3.91	70	70	0	1.9	1.1	0.2	11.5	3.0	0.5	0.8	1.4	1.3	2.2	1.1	1.0	0.2	1.2
SC 7/IITA 1	3.90	71	72	1	2.0	1.2	0.0	18.6	3.5	0.5	0.6	1.7	0.9	2.4	1.1	1.0	0.2	1.3
SC 5/IITA 12	3.90	67	69	2	1.7	1.0	0.2	10.0	3.6	0.5	0.8	1.8	1.4	2.4	0.9	1.0	0.2	1.2
SC 12/IITA 1	3.90	70	71	1	1.9	1.1	0.0	25.4	3.2	0.5	0.7	1.3	2.2	1.8	0.9	1.0	0.2	1.2
SC 9/IITA 9	3.90	70	72	2	1.7	1.0	0.2	5.3	3.3	0.5	0.7	1.2	1.6	2.0	0.8	1.0	0.2	1.3
SC 12/IITA 8	3.88	70	71	0	2.0	1.1	0.0	18.2	3.3	0.5	0.7	1.5	0.9	2.0	1.0	1.0	0.2	1.2

Appendix 3 (continued) Performance of the F1 hybrids in terms of grain yield and other agronomic traits from combined analysis of variance

Pedigree	GY	DMP	DMS	ASI	PHT	CHT	RL	SL	EASP	EPO	EPP	HC	TEXT	GLS	HT	MSV	PLS	RUST
	t ha^{-1}	days	days	days	m	m	%	%	1-9	ratio	no.	1-9	1-9	1-9	1-9	1-9	1-9	1-9
SC 4/IITA 10	3.88	69	70	1	1.9	1.2	0.0	22.6	2.8	0.5	0.7	1.4	0.6	2.2	0.8	1.0	0.3	1.3
SC 9/IITA 7	3.88	70	71	2	1.9	1.2	1.4	11.8	3.3	0.5	0.7	1.3	0.9	2.1	1.0	1.0	0.2	1.2
M1124-29	3.85	69	70	1	1.8	1.1	0.0	9.3	3.8	0.5	0.7	1.3	0.8	2.1	1.0	1.0	0.2	1.3
SC 3/IITA 12	3.82	67	69	1	1.6	0.9	0.5	0.9	3.8	0.5	0.7	1.5	0.8	3.0	1.0	1.0	0.2	1.1
SC 1/IITA 10	3.80	67	69	2	1.9	1.0	0.8	10.3	3.8	0.4	0.7	1.5	0.5	2.0	0.9	1.0	0.2	1.2
SC 1/IITA 6	3.79	69	70	1	1.8	1.0	0.0	10.9	4.0	0.5	0.7	1.3	0.6	2.4	0.9	1.0	0.2	1.3
SC 12/IITA 6	3.79	69	70	1	1.8	1.1	0.7	9.6	3.6	0.5	0.6	1.8	2.0	2.3	0.8	1.1	0.2	1.2
SC 2/IITA 11	3.75	66	66	0	1.8	1.0	0.5	3.5	2.2	0.5	0.8	1.6	2.0	2.0	1.8	1.0	0.2	1.3
SC 4/IITA 1	3.70	71	72	1	2.0	1.3	0.0	15.1	3.4	0.6	0.7	1.5	1.0	2.3	1.1	1.0	0.2	1.2
SC 1/IITA 9	3.69	70	72	3	1.8	1.1	0.0	2.8	3.8	0.5	0.6	1.8	0.9	2.1	1.0	1.0	0.2	1.3
SC 6/IITA 9	3.69	70	72	2	1.9	1.1	0.2	6.1	3.3	0.5	0.6	1.3	1.6	2.6	1.1	1.0	0.2	1.2
SC 6/IITA 1	3.67	70	72	2	1.9	1.1	1.3	12.1	3.5	0.5	0.6	1.2	2.0	2.4	0.9	1.0	0.2	1.3
SC 9/IITA 1	3.66	70	71	1	1.9	1.1	0.0	24.9	3.7	0.5	0.7	1.6	0.5	2.2	0.8	1.0	0.2	1.2
SC 7/IITA 9	3.64	70	71	2	2.0	1.1	0.0	23.8	3.4	0.5	0.6	1.5	1.3	2.3	1.3	1.0	0.2	1.3
SC 9/IITA 12	3.61	69	70	1	1.6	0.9	0.2	6.1	4.0	0.5	0.7	1.6	1.0	2.1	1.0	1.0	0.3	1.2

Appendix 3 (continued) Performance of the F1 hybrids in terms of grain yield and other agronomic traits from combined analysis of variance

Pedigree	GY	DMP	DMS	ASI	PHT	CHT	RL	SL	EASP	EPO	EPP	HC	TEXT	GLS	HT	MSV	PLS	RUST
	t ha^{-1}	days	days	days	m	m	%	%	1-9	ratio	no.	1-9	1-9	1-9	1-9	1-9	1-9	1-9
SC 9/IITA 5	3.61	71	72	1	2.0	1.1	0.5	13.2	3.1	0.5	0.6	1.6	1.3	2.0	0.8	1.0	0.2	1.3
SC 9/IITA 6	3.61	68	69	1	1.7	1.0	0.4	22.5	3.4	0.5	0.7	1.8	0.8	2.3	0.9	1.0	0.2	1.2
SC 6/IITA 12	3.60	69	70	2	1.8	1.0	0.2	6.0	4.1	0.5	0.7	1.4	1.8	2.3	1.2	1.1	0.2	1.2
SC 11/IITA 3	3.58	67	69	1	1.9	1.1	0.5	6.9	3.8	0.5	0.7	1.3	1.2	1.9	1.0	1.0	0.2	1.2
SC 12/IITA 9	3.51	70	72	2	1.9	1.2	0.0	5.5	3.4	0.5	0.6	1.3	1.8	2.2	1.3	1.0	0.2	1.3
SC 9/IITA 10	3.46	69	71	3	2.0	1.2	0.0	18.2	3.2	0.5	0.7	1.5	1.1	2.5	0.8	1.0	0.2	1.3
M0826-1	3.39	70	71	1	2.0	1.1	0.0	13.1	4.1	0.4	0.6	1.3	1.3	2.0	1.4	1.0	0.2	1.2
SC 8/IITA 1	3.38	70	71	1	1.9	1.1	1.3	23.7	3.7	0.5	0.6	1.8	1.2	2.1	0.9	1.1	0.2	1.2
SC 1/IITA 1	3.36	69	71	2	2.0	1.1	0.8	16.9	3.9	0.5	0.7	1.3	0.6	2.3	0.9	1.0	0.2	1.3
SC 12/IITA 5	3.36	69	70	1	2.0	1.2	0.5	16.0	3.0	0.5	0.6	1.4	1.6	2.1	1.2	1.0	0.2	1.2
Mean	**4.5**	**68.9**	**70.2**	**1.3**	**1.9**	**1.1**	**0.4**	**11.3**	**3.3**	**0.5**	**0.7**	**1.5**	**1.2**	**2.2**	**1**	**1**	**0.2**	**1.2**
SE	**2.4**	**3.1**	**3.7**	**1.4**	**0.3**	**0.2**	**3.7**	**28.3**	**1.4**	**0.1**	**0.2**	**0**	**1**	**1.5**	**1**	**0.2**	**0.2**	**0.4**
LSD (5%)	**0.8**	**1**	**1.2**	**0.9**	**0.1**	**0.1**	**1.3**	**9.5**	**0.5**	**0**	**0.1**	**0.4**	**0.3**	**0.5**	**0.3**	**0.1**	**0.1**	**0.1**
P value	***	***	***	***	***	***	*	***	***	***	***	**	***	***	***	ns	ns	*

Appendix 4 Minor allele frequencies for various single nucleotide polymorphism markers

Frequencies for the minor alleles	SNP numbers
0.50	28
0.48	28
0.46	30
0.45	11
0.44	6
0.43	29
0.42	26
0.41	15
0.40	8
0.39	23
0.38	34
0.37	5
0.36	8
0.35	25
0.34	2
0.33	45
0.32	8
0.31	10
0.30	35
0.29	32
0.28	9
0.27	11
0.26	31
0.25	37

SNP=single nucleotide polymorphism

Appendix 5 Polymorphic information content frequencies for various single nucleotide polymorphism markers

PIC	No. of SNPs
0.10	1
0.23	1
0.12	3
0.11	4
0.18	4
0.04	5
0.09	7
0.16	7
0.22	7
0.26	9
0.17	11
0.27	11
0.21	15
0.29	21
0.32	23
0.34	24
0.20	28
0.38	28
0.25	31
0.31	31
0.15	34
0.24	40
0.30	40
0.14	44
0.19	48
0.28	57
0.33	64
0.35	67
0.36	75
0.08	111
0.00	146
0.37	147
Total	1144

PIC=polymorphic information content; SNP=single nucleotide polymorphism

Appendix 6 FI hybrid specific combining ability for the means of grain yield, mid- and high-parent heterosis and genetic distance across locations

Entry	F1 Hybrid	GY	SCA	MPH	HPH	GD
	Pedigree	t ha-1		%	%	
66	SC 6/IITA 6	4.994	0.41	334.64	318.61	0.33
11	SC 1/IITA 11	4.684	0.52	315.99	270.57	0.32
2	SC 1/IITA 2	4.997	0.26	314.17	250.67	0.30
78	SC 7/IITA 6	4.341	0.30	295.36	292.85	0.34
114	SC 10/IITA 6	5.108	0.36	288.00	234.29	0.32
98	SC 9/IITA 2	5.004	0.27	287.61	251.16	0.30
62	SC 6/IITA 2	4.999	0.16	281.89	250.81	0.32
109	SC 10/IITA 1	4.779	0.27	278.24	212.76	0.32
71	SC 6/IITA 11	4.639	-0.14	277.61	267.01	0.32
104	SC 9/IITA 8	4.961	0.71	276.98	236.34	0.30
110	SC 10/IITA 2	5.555	0.30	276.23	263.55	0.30
73	SC 7/IITA 1	3.902	0.04	273.40	257.65	0.34
107	SC 9/IITA 11	4.516	0.53	273.07	257.28	0.32
83	SC 7/IITA 11	4.371	0.07	271.21	245.81	0.34
74	SC 7/IITA 2	4.637	0.02	268.60	225.40	0.32
93	SC 8/IITA 9	4.623	0.31	268.51	221.94	0.33
42	SC 4/IITA 6	4.431	0.27	266.35	237.21	0.33
86	SC 8/IITA 2	5.198	0.10	263.37	261.98	0.31
6	SC 1/IITA 6	3.793	-0.20	262.45	243.26	0.32
92	SC 8/IITA 8	5.22	0.19	258.64	253.90	0.29
9	SC 1/IITA 9	3.691	-0.21	258.18	243.99	0.32
5	SC 1/IITA 5	4.623	0.18	257.40	189.12	0.32
50	SC 5/IITA 2	5.226	0.07	254.91	243.82	0.32
49	SC 5/IITA 1	4.45	0.07	253.31	192.76	0.34
68	SC 6/IITA 8	4.707	-0.38	252.85	219.12	0.30
95	SC 8/IITA 11	4.752	0.02	252.00	230.92	0.32
54	SC 5/IITA 6	4.615	-0.21	251.62	203.62	0.32
38	SC 4/IITA 2	4.812	0.09	251.37	237.68	0.30
105	SC 9/IITA 9	3.895	0.19	249.33	236.65	0.32
116	SC 10/IITA 8	5.239	0.16	248.92	242.87	0.33

Appendix 6 (continued) FI hybrid specific combining ability for the means of grain yield, mid- and high-parent heterosis and genetic distance across locations

Entry	F1 Hybrid	GY	SCA	MPH	HPH	GD
	Pedigree	t ha-1		%	%	
21	SC 2/IITA 9	5.047	0.60	247.23	175.19	0.32
59	SC 5/IITA 11	4.82	0.14	246.26	217.11	0.30
56	SC 5/IITA 8	5.157	0.19	244.37	239.28	0.34
80	SC 7/IITA 8	4.362	-0.10	239.98	195.73	0.32
97	SC 9/IITA 1	3.663	0.05	239.80	216.59	0.32
1	SC 1/IITA 1	3.356	-0.44	237.80	235.94	0.32
126	SC 11/IITA 6	4.755	0.44	236.76	176.61	0.36
81	SC 7/IITA 9	3.636	-0.33	236.04	233.27	0.34
13	SC 2/IITA 1	4.753	0.32	235.55	159.16	0.33
44	SC 4/IITA 8	4.676	-0.04	235.32	217.02	0.31
45	SC 4/IITA 9	4	-0.05	235.15	204.41	0.34
61	SC 6/IITA 1	3.672	-0.31	235.04	207.80	0.34
25	SC 3/IITA 1	4.437	0.74	234.49	168.26	0.32
18	SC 2/IITA 6	4.838	0.20	229.23	163.79	0.31
47	SC 4/IITA 11	4.223	-0.11	227.62	221.39	0.30
69	SC 6/IITA 9	3.687	-0.18	225.42	209.05	0.34
8	SC 1/IITA 8	4.002	-0.22	224.97	171.32	0.30
134	SC 12/IITA 2	5.324	0.08	224.24	186.39	0.29
117	SC 10/IITA 9	4.216	-0.19	224.18	175.92	0.32
35	SC 3/IITA 11	4.685	0.36	221.11	183.25	0.29
67	SC 6/IITA 7	5.03	0.52	220.59	158.61	0.34
37	SC 4/IITA 1	3.699	0.00	219.84	181.51	0.33
89	SC 8/IITA 5	4.852	-0.23	219.74	203.44	0.30
41	SC 4/IITA 5	4.653	0.14	219.46	190.99	0.31
102	SC 9/IITA 6	3.605	-0.52	218.74	211.58	0.31
57	SC 5/IITA 9	4.128	-0.17	218.40	171.58	0.32
7	SC 1/IITA 7	4.667	0.20	218.24	139.95	0.32
111	SC 10/IITA 3	5.419	0.35	217.64	187.63	0.33
63	SC 6/IITA 3	4.833	0.21	214.14	156.53	0.34
129	SC 11/IITA 9	4.361	0.33	212.39	153.69	0.36

Appendix 6 (continued) FI hybrid specific combining ability for the means of grain yield, mid- and high-parent heterosis and genetic distance across locations

Entry	F1 Hybrid	GY	SCA	MPH	HPH	GD
	Pedigree	t ha-1		%	%	
51	SC 5/IITA 3	5.292	0.30	210.93	180.89	0.32
77	SC 7/IITA 5	4.159	0.16	209.22	160.10	0.31
14	SC 2/IITA 2	5.015	-0.01	207.76	173.45	0.33
90	SC 8/IITA 6	3.91	-0.46	207.75	172.28	0.34
20	SC 2/IITA 8	5.078	0.19	206.92	176.88	0.34
30	SC 3/IITA 6	4.23	-0.23	206.63	155.74	0.30
113	SC 10/IITA 5	4.79	-0.01	206.36	199.56	0.30
65	SC 6/IITA 5	4.267	-0.24	205.66	166.85	0.33
4	SC 1/IITA 4	5.524	0.26	204.27	109.00	0.33
87	SC 8/IITA 3	5.021	0.25	202.47	166.51	0.33
3	SC 1/IITA 3	4.303	0.07	199.65	128.40	0.33
128	SC 11/IITA 8	4.778	0.24	199.19	177.95	0.34
119	SC 10/IITA 11	4.165	-0.90	198.35	172.58	0.31
17	SC 2/IITA 5	5.1	0.30	197.12	178.08	0.33
121	SC 11/IITA 1	4.032	0.17	196.69	134.55	0.36
91	SC 8/IITA 7	5.009	0.01	196.30	157.53	0.32
94	SC 8/IITA 10	5.228	0.49	194.62	147.42	0.32
131	SC 11/IITA 11	4.378	-0.05	193.53	154.68	0.36
76	SC 7/IITA 4	5.436	0.31	191.16	105.68	0.34
143	SC 12/IITA 11	4.544	0.26	191.00	144.43	0.27
70	SC 6/IITA 10	4.804	0.20	190.62	127.35	0.33
53	SC 5/IITA 5	4.512	-0.19	189.32	182.18	0.32
64	SC 6/IITA 4	5.549	0.28	189.31	109.95	0.34
33	SC 3/IITA 9	3.938	0.03	188.82	138.09	0.31
39	SC 4/IITA 3	4.618	-0.09	188.81	145.12	0.34
125	SC 11/IITA 5	4.773	0.42	187.70	177.66	0.35
79	SC 7/IITA 7	4.333	-0.38	185.44	122.78	0.32
52	SC 5/IITA 4	5.938	0.26	185.28	124.67	0.33
55	SC 5/IITA 7	4.914	-0.01	183.64	152.65	0.34
75	SC 7/IITA 3	4.173	-0.17	180.54	121.50	0.35

Appendix 6 (continued) FI hybrid specific combining ability for the means of grain yield, mid- and high-parent heterosis and genetic distance across locations

Entry	F1 Hybrid	GY	SCA	MPH	HPH	GD
	Pedigree	t ha-1		%	%	
112	SC 10/IITA 4	5.842	0.09	180.12	121.04	0.31
115	SC 10/IITA 7	4.852	-0.45	179.41	149.46	0.33
120	SC 10/IITA 12	5.066	0.25	178.81	140.55	0.33
85	SC 8/IITA 1	3.384	-0.69	177.95	135.65	0.34
82	SC 7/IITA 10	4.449	-0.05	177.72	110.55	0.34
12	SC 1/IITA 12	4.251	0.22	174.79	101.85	0.33
29	SC 3/IITA 5	4.443	0.17	173.16	168.62	0.27
133	SC 12/IITA 1	3.896	-0.22	172.64	109.58	0.31
32	SC 3/IITA 8	4.206	-0.43	168.84	154.29	0.26
96	SC 8/IITA 12	4.742	0.50	167.76	125.17	0.36
99	SC 9/IITA 3	4.058	-0.13	166.89	115.39	0.33
26	SC 3/IITA 2	4.069	-0.57	164.31	146.01	0.27
84	SC 7/IITA 12	4.206	0.11	163.12	99.72	0.34
127	SC 11/IITA 7	4.819	0.40	163.05	147.76	0.36
135	SC 12/IITA 3	4.916	0.70	162.68	160.93	0.30
101	SC 9/IITA 5	3.612	-0.12	162.12	125.89	0.28
118	SC 10/IITA 10	4.723	-0.23	159.43	123.52	0.33
58	SC 5/IITA 10	4.671	0.16	157.14	121.06	0.32
40	SC 4/IITA 4	5.077	-0.01	156.61	92.09	0.33
138	SC 12/IITA 6	3.788	-0.36	155.60	103.77	0.29
100	SC 9/IITA 4	4.816	-0.04	153.47	82.22	0.30
103	SC 9/IITA 7	3.876	-0.08	149.90	99.28	0.30
122	SC 11/IITA 2	3.925	-0.77	149.68	128.33	0.33
31	SC 3/IITA 7	4.461	0.10	147.90	129.36	0.28
22	SC 2/IITA 10	4.891	-0.04	147.83	131.47	0.33
130	SC 11/IITA 10	4.744	0.39	147.60	124.51	0.34
15	SC 2/IITA 3	4.579	-0.23	146.32	143.05	0.32
10	SC 1/IITA 10	3.801	-0.64	145.15	79.89	0.31
27	SC 3/IITA 3	4.314	-0.19	143.87	128.98	0.31
48	SC 4/IITA 12	4.16	0.13	143.27	97.53	0.33

Appendix 6 (continued) FI hybrid specific combining ability for the means of grain yield, mid- and high-parent heterosis and genetic distance across locations

Entry	F1 Hybrid Pedigree	GY t ha-1	SCA	MPH %	HPH %	GD
23	SC 2/IITA 11	3.751	-0.70	142.16	104.53	0.32
28	SC 3/IITA 4	5.202	0.28	142.12	96.82	0.29
43	SC 4/IITA 7	3.92	-0.41	140.56	101.54	0.31
141	SC 12/IITA 9	3.512	-0.31	139.56	88.92	0.30
34	SC 3/IITA 10	4.486	-0.12	138.17	112.30	0.30
139	SC 12/IITA 7	4.469	0.36	134.96	129.77	0.29
140	SC 12/IITA 8	3.884	-0.51	132.99	108.93	0.28
142	SC 12/IITA 10	4.614	0.43	132.33	118.36	0.29
16	SC 2/IITA 4	5.141	-0.34	129.66	94.51	0.34
19	SC 2/IITA 7	4.295	-0.25	127.31	120.82	0.34
46	SC 4/IITA 10	3.878	0.08	126.32	83.53	0.34
108	SC 9/IITA 12	3.613	-0.19	121.45	71.56	0.31
24	SC 2/IITA 12	4.354	-0.03	121.02	106.74	0.32
136	SC 12/IITA 4	4.969	-0.09	120.75	88.01	0.29
72	SC 6/IITA 12	3.604	-0.52	118.49	71.13	0.35
60	SC 5/IITA 12	3.898	-0.62	115.00	85.09	0.34
132	SC 11/IITA 12	4.088	0.04	113.75	94.11	0.38
106	SC 9/IITA 10	3.463	-0.67	111.80	63.89	0.30
124	SC 11/IITA 4	4.593	-0.53	110.59	73.78	0.35
36	SC 3/IITA 12	3.815	-0.15	102.93	81.15	0.30
144	SC 12/IITA 12	4.021	0.26	102.82	90.93	0.31
123	SC 11/IITA 3	3.584	-1.06	98.95	90.23	0.36
88	SC 8/IITA 4	3.987	-0.47	95.49	50.85	0.33
137	SC 12/IITA 5	3.355	-0.58	94.04	80.47	0.28
Mean		4.49	0.00	199.08	163.03	0.32
Min		3.36	-1.06	144.00	37.57	0.26
Max		5.94	0.74	468.35	453.40	0.38

GY=grain yield; SCA=specific combing ability; MPH=mid parent heterosis; HPH=high parent heterosis; GD=genetic distance; Min=minimum; max=maximum

Appendix 7 FI hybrid specific combining ability for the means of Fusarium ear rot, total mycotoxin and genetic distance at RARS in 2012/13 season

Entry	F1 Hybrid	Grain yield t ha-1				Ear rot				Fumonisin				GD
	Pedigree	SCA	mean	MPH	HPH	SCA	mean	MPH	HPH	SCA	Mean	MPH	HPH	
59	SC 5/IITA 11	-1.17	6.77	257.27	207.12	0.06	1.60	-89.19	-90.75	-1.16	0.00	-100.00	-100.00	0.30
71	SC 6/IITA 11	-0.26	7.69	235.31	222.79	-1.00	0.00	-100.00	-100.00	-0.90	0.00	-100.00	-100.00	0.32
52	SC 5/IITA 4	-0.28	7.90	174.36	89.35	-0.37	0.70	-95.33	-95.95	-0.84	0.00	-100.00	-100.00	0.33
50	SC 5/IITA 2	-0.07	7.59	276.30	210.05	-0.68	0.60	-94.85	-96.53	-0.82	0.05	-98.08	-98.08	0.32
72	SC 6/IITA 12	-0.84	5.57	117.25	103.03	-0.20	2.80	-84.18	-88.93	-0.81	0.15	-94.12	-94.23	0.35
55	SC 5/IITA 7	-0.03	7.17	210.95	136.93	-0.97	0.00	-100.00	-100.00	-0.76	0.05	-98.08	-98.08	0.34
49	SC 5/IITA 1	0.15	6.66	347.85	320.18	0.47	1.40	-93.79	-94.96	-0.69	0.10	-96.15	-96.15	0.34
84	SC 7/IITA 12	0.76	6.85	181.54	149.96	0.58	3.80	-73.61	-84.98	-0.57	0.00	-100.00	-100.00	0.34
38	SC 4/IITA 2	0.11	7.73	273.03	215.81	0.33	1.10	-92.44	-95.24	-0.56	0.00	-100.00	-100.00	0.30
51	SC 5/IITA 3	-0.43	6.47	176.86	109.59	1.08	2.70	-72.31	-84.39	-0.55	0.10	-96.36	-96.55	0.32
67	SC 6/IITA 7	0.87	8.09	199.00	167.20	0.17	0.70	-92.18	-93.07	-0.55	0.00	-100.00	-100.00	0.34
144	SC 12/IITA 12	1.05	7.51	185.86	173.92	-3.10	0.00	-100.00	-100.00	-0.54	0.00	-100.00	-100.00	0.31
68	SC 6/IITA 8	0.85	8.38	209.57	176.48	-0.80	0.60	-95.89	-96.86	-0.53	0.00	-100.00	-100.00	0.30
108	SC 9/IITA 12	0.46	6.61	180.73	141.21	1.24	4.90	-68.79	-80.63	-0.53	0.05	-98.18	-98.33	0.31
42	SC 4/IITA 6	0.26	7.59	325.32	305.23	-0.21	1.10	-92.90	-95.24	-0.52	0.10	-96.36	-96.43	0.33
37	SC 4/IITA 1	-2.15	4.32	179.82	154.51	0.23	0.70	-97.25	-97.48	-0.48	0.00	-100.00	-100.00	0.33
143	SC 12/IITA 11	0.32	8.33	253.16	231.52	0.05	1.20	-89.96	-90.24	-0.48	0.00	-100.00	-100.00	0.27
66	SC 6/IITA 6	0.34	7.73	263.07	224.30	0.77	2.10	-76.67	-79.21	-0.47	0.20	-92.59	-92.86	0.33
12	SC 1/IITA 12	0.48	6.61	294.80	141.10	1.08	4.90	-84.74	-87.40	-0.45	0.20	-92.31	-92.59	0.33
43	SC 4/IITA 7	-0.30	6.86	190.60	126.76	-0.51	0.00	-100.00	-100.00	-0.45	0.05	-98.11	-98.15	0.31
24	SC 2/IITA 12	-0.59	5.54	129.48	102.15	0.57	4.10	-73.72	-83.79	-0.45	0.15	-94.23	-94.44	0.32

Appendix 7 (continued) FI hybrid specific combining ability for the means of Fusarium ear rot, total mycotoxin and genetic distance at RARS in 2012/13 season

Entry	F1 Hybrid	Grain yield t ha-1				Ear rot				Fumonisin				GD
	Pedigree	SCA	mean	MPH	HPH	SCA	mean	MPH	HPH	SCA	Mean	MPH	HPH	
126	SC 11/IITA 6	0.89	7.81	353.90	316.60	-1.17	0.00	-100.00	-100.00	-0.43	0.05	-98.15	-98.21	0.36
58	SC 5/IITA 10	-0.07	7.20	180.42	102.90	-1.07	0.00	-100.00	-100.00	-0.43	0.15	-94.23	-94.23	0.32
65	SC 6/IITA 5	0.24	8.00	182.06	143.30	0.21	1.90	-66.96	-81.19	-0.41	0.10	-96.15	-96.15	0.33
119	SC 10/IITA 11	-0.60	7.62	194.64	156.92	0.65	2.40	-77.78	-80.49	-0.41	0.00	-100.00	-100.00	0.31
129	SC 11/IITA 9	0.01	6.39	230.81	178.22	-0.88	0.00	-100.00	-100.00	-0.40	0.15	-94.34	-94.44	0.36
21	SC 2/IITA 9	0.11	6.68	204.77	191.03	0.43	2.00	-71.83	-75.90	-0.37	0.00	-100.00	-100.00	0.32
33	SC 3/IITA 9	0.39	7.22	223.03	214.37	-0.41	0.70	-89.47	-91.57	-0.36	0.10	-96.15	-96.30	0.31
41	SC 4/IITA 5	0.91	8.62	245.90	162.26	2.98	4.70	-61.63	-79.65	-0.36	0.10	-96.23	-96.30	0.31
128	SC 11/IITA 8	1.13	8.19	256.28	170.17	-0.69	0.60	-96.21	-96.86	-0.35	0.00	-100.00	-100.00	0.34
39	SC 4/IITA 3	0.94	7.79	225.97	152.64	-0.60	0.60	-95.26	-97.40	-0.34	0.00	-100.00	-100.00	0.34
45	SC 4/IITA 9	-0.56	6.23	212.25	171.52	-1.02	0.00	-100.00	-100.00	-0.34	0.35	-87.04	-87.04	0.34
26	SC 3/IITA 2	-0.52	7.13	208.76	191.42	-0.81	0.00	-100.00	-100.00	-0.33	0.00	-100.00	-100.00	0.27
64	SC 6/IITA 4	-0.37	7.82	138.55	87.43	0.02	0.60	-94.74	-95.28	-0.32	0.25	-90.20	-90.38	0.34
125	SC 11/IITA 5	0.30	7.59	212.74	130.89	-0.93	0.70	-90.00	-94.44	-0.32	0.00	-100.00	-100.00	0.35
120	SC 10/IITA 12	0.17	6.84	139.61	130.63	3.95	7.60	-56.07	-69.96	-0.32	0.15	-94.23	-94.44	0.33
70	SC 6/IITA 10	-0.23	7.06	137.98	98.93	0.08	0.70	-86.14	-93.07	-0.32	0.00	-100.00	-100.00	0.33
9	SC 1/IITA 9	-0.77	5.80	299.59	152.61	-0.71	1.20	-94.92	-96.92	-0.28	0.15	-94.44	-94.44	0.32
23	SC 2/IITA 11	0.14	7.82	264.23	254.65	-0.38	1.20	-86.81	-90.24	-0.28	0.25	-90.57	-90.74	0.32
132	SC 11/IITA 12	-1.08	4.86	125.44	77.10	-0.74	2.10	-88.92	-91.70	-0.27	0.50	-80.39	-80.77	0.38
127	SC 11/IITA 7	0.27	7.01	205.29	131.62	1.58	2.00	-80.39	-84.13	-0.27	0.10	-96.15	-96.15	0.36
105	SC 9/IITA 9	-0.14	6.46	202.67	181.18	-1.65	0.00	-100.00	-100.00	-0.26	0.10	-96.49	-96.67	0.32

Appendix 7 (continued) FI hybrid specific combining ability for the means of Fusarium ear rot, total mycotoxin and genetic distance at RARS in 2012/13 season

Entry	F1 Hybrid	Grain yield t ha-1				Ear rot				Fumonisin				GD
	Pedigree	SCA	mean	MPH	HPH	SCA	mean	MPH	HPH	SCA	Mean	MPH	HPH	
25	SC 3/IITA 1	0.68	7.19	303.54	230.83	-0.46	0.00	-100.00	-100.00	-0.25	0.00	-100.00	-100.00	0.32
138	SC 12/IITA 6	0.00	7.45	239.41	196.26	-0.28	1.20	-87.69	-89.66	-0.25	0.00	-100.00	-100.00	0.29
96	SC 8/IITA 12	-0.01	6.48	208.82	136.18	-1.05	2.80	-85.57	-88.93	-0.25	0.15	-94.44	-94.83	0.36
29	SC 3/IITA 5	0.76	7.01	156.64	113.18	0.29	2.10	-34.38	-58.00	-0.23	0.00	-100.00	-100.00	0.27
98	SC 9/IITA 2	0.47	7.89	257.18	222.30	-0.15	1.30	-78.51	-78.69	-0.23	0.00	-100.00	-100.00	0.30
8	SC 1/IITA 8	-0.54	3.82	109.84	25.93	0.23	2.50	-91.38	-93.57	-0.23	0.00	-100.00	-100.00	0.30
35	SC 3/IITA 11	-0.53	7.41	238.56	236.10	2.93	4.00	-53.76	-67.48	-0.22	0.40	-84.31	-84.62	0.29
74	SC 7/IITA 2	-0.19	7.17	213.57	193.01	-0.31	0.70	-85.26	-88.33	-0.22	0.00	-100.00	-100.00	0.32
16	SC 2/IITA 4	-0.51	7.41	136.62	77.58	1.04	2.20	-76.34	-82.68	-0.21	0.00	-100.00	-100.00	0.34
32	SC 3/IITA 8	0.64	7.00	169.06	130.98	-0.82	0.70	-94.19	-96.34	-0.21	0.05	-98.04	-98.08	0.26
2	SC 1/IITA 2	0.38	7.78	409.00	217.61	-1.01	0.60	-97.33	-98.46	-0.20	0.10	-96.23	-96.30	0.30
100	SC 9/IITA 4	0.56	8.50	176.84	103.81	-0.69	0.60	-93.62	-95.28	-0.20	0.00	-100.00	-100.00	0.30
134	SC 12/IITA 2	-0.40	7.33	195.34	191.52	-0.89	0.00	-100.00	-100.00	-0.19	0.00	-100.00	-100.00	0.29
19	SC 2/IITA 7	-0.08	6.87	168.53	126.92	-1.01	0.00	-100.00	-100.00	-0.19	0.00	-100.00	-100.00	0.34
95	SC 8/IITA 11	0.70	8.74	377.82	296.24	-1.20	0.70	-94.57	-94.81	-0.18	0.15	-94.55	-94.83	0.32
78	SC 7/IITA 6	-0.01	7.07	253.61	232.58	-0.90	0.60	-89.47	-92.41	-0.18	0.10	-96.23	-96.43	0.34
122	SC 11/IITA 2	-0.09	3.96	97.51	61.93	2.32	3.00	-67.74	-76.19	-0.17	0.25	-90.38	-90.38	0.33
1	SC 1/IITA 1	-0.75	5.50	450.63	295.54	-1.25	0.00	-100.00	-100.00	-0.17	0.05	-98.11	-98.15	0.32
4	SC 1/IITA 4	0.18	8.09	238.54	93.91	-0.90	0.60	-97.67	-98.46	-0.17	0.10	-96.15	-96.30	0.33
28	SC 3/IITA 4	-0.51	8.93	181.48	114.08	-0.65	0.00	-100.00	-100.00	-0.15	0.15	-94.00	-94.00	0.29
81	SC 7/IITA 9	0.13	6.67	201.47	190.37	-1.21	0.00	-100.00	-100.00	-0.15	0.20	-92.31	-92.59	0.34

Appendix 7 (continued) FI hybrid specific combining ability for the means of Fusarium ear rot, total mycotoxin and genetic distance at RARS in 2012/13 season

Entry	F1 Hybrid	Grain yield t ha-1				Ear rot				Fumonisin				GD
	Pedigree	SCA	mean	MPH	HPH	SCA	mean	MPH	HPH	SCA	Mean	MPH	HPH	
97	SC 9/IITA 1	-0.19	6.09	262.44	209.09	0.35	1.40	-91.74	-94.96	-0.15	0.00	-100.00	-100.00	0.32
80	SC 7/IITA 8	-1.60	5.62	117.95	85.45	-0.42	1.20	-89.38	-93.72	-0.15	0.00	-100.00	-100.00	0.32
11	SC 1/IITA 11	-0.25	7.43	428.31	236.87	-1.32	0.60	-97.66	-98.46	-0.14	0.45	-83.02	-83.33	0.32
139	SC 12/IITA 7	0.47	7.74	179.39	155.67	-0.03	0.60	-93.81	-94.83	-0.13	0.00	-100.00	-100.00	0.29
27	SC 3/IITA 3	0.16	7.06	168.35	128.69	0.65	1.90	-47.22	-62.00	-0.11	0.00	-100.00	-100.00	0.31
90	SC 8/IITA 6	-0.78	6.69	302.41	257.10	-1.58	0.60	-94.39	-95.56	-0.10	0.00	-100.00	-100.00	0.34
123	SC 11/IITA 3	-2.30	4.14	77.94	34.13	-1.01	0.00	-100.00	-100.00	-0.10	0.10	-96.36	-96.55	0.36
130	SC 11/IITA 10	0.87	7.68	200.22	116.38	0.69	1.20	-80.95	-90.48	-0.08	0.05	-98.08	-98.08	0.34
114	SC 10/IITA 6	-0.59	7.06	191.65	138.02	-1.98	0.00	-100.00	-100.00	-0.08	0.10	-96.36	-96.43	0.32
61	SC 6/IITA 1	-0.46	6.07	221.55	154.55	0.96	1.40	-92.61	-94.96	-0.07	0.45	-82.69	-82.69	0.34
93	SC 8/IITA 9	0.98	7.90	321.72	244.21	-0.05	1.90	-82.57	-85.93	-0.07	0.10	-96.43	-96.55	0.33
103	SC 9/IITA 7	-0.28	6.69	167.64	120.91	1.61	2.80	-59.71	-64.10	-0.07	0.10	-96.43	-96.67	0.30
46	SC 4/IITA 10	0.11	3.63	38.56	2.42	0.55	1.20	-89.61	-94.81	-0.07	0.20	-92.45	-92.59	0.34
110	SC 10/IITA 2	-0.58	7.34	171.32	147.71	-0.24	1.20	-84.31	-87.10	-0.07	0.05	-98.11	-98.15	0.30
6	SC 1/IITA 6	0.74	7.85	532.57	318.73	3.40	0.00	-100.00	-100.00	-0.06	0.30	-89.09	-89.29	0.32
104	SC 9/IITA 8	0.32	7.60	203.74	150.58	0.44	2.50	-80.16	-86.91	-0.06	0.10	-96.43	-96.67	0.30
136	SC 12/IITA 4	-0.87	7.38	120.71	76.86	1.83	2.60	-78.60	-79.53	-0.06	0.10	-96.00	-96.00	0.29
117	SC 10/IITA 9	-0.86	6.25	137.49	110.73	2.31	4.00	-54.55	-56.99	-0.05	0.20	-92.59	-92.59	0.32
86	SC 8/IITA 2	0.52	8.27	324.21	237.91	1.05	2.70	-72.31	-80.00	-0.05	0.00	-100.00	-100.00	0.31
116	SC 10/IITA 8	-0.22	7.56	152.18	149.39	-0.95	1.20	-91.55	-93.72	-0.05	0.00	-100.00	-100.00	0.33
102	SC 9/IITA 6	-0.29	6.85	256.30	247.61	-0.64	1.30	-81.43	-83.54	-0.04	0.25	-91.38	-91.67	0.31

Appendix 7 (continued) FI hybrid specific combining ability for the means of Fusarium ear rot, total mycotoxin and genetic distance at RARS in 2012/13 season

Entry	F1 Hybrid	Grain yield t ha-1				Ear rot				Fumonisin				GD
	Pedigree	SCA	mean	MPH	HPH	SCA	mean	MPH	HPH	SCA	Mean	MPH	HPH	
73	SC 7/IITA 1	0.41	6.63	276.86	211.57	0.10	0.70	-95.53	-97.48	-0.04	0.10	-96.08	-96.15	0.34
109	SC 10/IITA 1	1.12	7.90	262.84	166.50	0.22	1.30	-92.99	-95.32	-0.04	0.00	-100.00	-100.00	0.32
112	SC 10/IITA 4	0.10	8.54	139.30	104.70	0.03	1.30	-88.18	-89.76	-0.04	0.05	-98.08	-98.15	0.31
15	SC 2/IITA 3	-0.67	5.97	130.77	93.52	-1.65	0.00	-100.00	-100.00	-0.02	0.00	-100.00	-100.00	0.32
140	SC 12/IITA 8	-0.89	6.70	141.63	120.98	1.05	2.60	-83.06	-86.39	-0.02	0.10	-96.08	-96.15	0.28
10	SC 1/IITA 10	0.10	7.11	242.22	100.39	-0.19	1.30	-93.32	-96.66	-0.01	0.00	-100.00	-100.00	0.31
88	SC 8/IITA 4	1.52	4.16	48.06	-0.19	-1.48	0.00	-100.00	-100.00	-0.01	0.00	-100.00	-100.00	0.33
99	SC 9/IITA 3	0.01	6.67	163.86	116.18	0.91	2.70	-34.94	-55.74	-0.01	0.00	-100.00	-100.00	0.33
18	SC 2/IITA 6	-0.31	6.81	243.68	225.99	2.59	4.40	-36.23	-44.30	0.00	0.30	-89.09	-89.29	0.31
75	SC 7/IITA 3	0.70	7.30	180.01	136.53	0.71	2.10	-26.32	-40.00	0.00	0.00	-100.00	-100.00	0.35
30	SC 3/IITA 6	-0.74	7.30	260.71	235.90	0.45	1.80	-72.09	-77.22	0.01	0.40	-84.91	-85.71	0.30
34	SC 3/IITA 10	0.18	7.44	160.17	109.78	-0.65	0.00	-100.00	-100.00	0.01	0.05	-98.04	-98.08	0.30
101	SC 9/IITA 5	-1.50	6.01	128.84	83.02	0.25	2.60	-30.67	-57.38	0.02	0.15	-94.64	-95.00	0.28
92	SC 8/IITA 8	-0.47	7.14	218.36	135.43	0.75	3.10	-80.98	-83.77	0.03	0.00	-100.00	-100.00	0.29
135	SC 12/IITA 3	1.34	8.30	196.64	169.14	-0.57	0.70	-89.86	-93.97	0.03	0.00	-100.00	-100.00	0.30
79	SC 7/IITA 7	-0.86	6.05	134.65	99.77	-0.10	0.60	-89.38	-92.31	0.04	0.20	-92.16	-92.31	0.32
85	SC 8/IITA 1	-0.38	6.22	338.00	328.65	-0.09	1.20	-94.19	-95.68	0.04	0.00	-100.00	-100.00	0.34
57	SC 5/IITA 9	0.41	7.25	273.52	215.77	0.97	2.50	-80.47	-85.55	0.05	1.05	-60.38	-61.11	0.32
17	SC 2/IITA 5	-0.23	7.26	169.99	120.82	-0.47	1.80	-50.68	-69.49	0.06	0.20	-92.45	-92.59	0.33
89	SC 8/IITA 5	-0.28	7.56	218.91	129.91	1.25	3.90	-47.65	-71.11	0.06	0.00	-100.00	-100.00	0.30
91	SC 8/IITA 7	-0.77	6.52	191.27	115.49	-0.73	0.70	-93.43	-94.81	0.06	0.05	-98.18	-98.28	0.32

Appendix 7 (continued) FI hybrid specific combining ability for the means of Fusarium ear rot, total mycotoxin and genetic distance at RARS in 2012/13 season

Entry	F1 Hybrid	Grain yield t ha-1				Ear rot				Fumonisin				GD
	Pedigree	SCA	mean	MPH	HPH	SCA	mean	MPH	HPH	SCA	Mean	MPH	HPH	
3	SC 1/IITA 3	-0.21	6.43	248.32	108.43	-0.54	1.50	-92.70	-96.14	0.07	0.15	-94.64	-94.83	0.33
82	SC 7/IITA 10	-0.24	6.73	137.36	89.85	0.51	1.30	-25.71	-62.86	0.07	0.00	-100.00	-100.00	0.34
77	SC 7/IITA 5	0.31	7.76	186.68	136.12	-0.21	1.70	-30.61	-51.43	0.08	0.20	-92.16	-92.31	0.31
142	SC 12/IITA 10	-0.71	6.62	118.58	86.72	-0.12	0.60	-89.66	-94.83	0.10	0.00	-100.00	-100.00	0.29
106	SC 9/IITA 10	0.21	3.88	40.47	9.25	-0.54	0.70	-77.05	-88.52	0.11	0.05	-98.21	-98.33	0.30
115	SC 10/IITA 7	-0.20	7.27	142.66	140.14	-1.18	0.00	-100.00	-100.00	0.14	0.20	-92.45	-92.59	0.33
22	SC 2/IITA 10	0.40	7.41	162.78	108.77	-1.10	0.00	-100.00	-100.00	0.15	0.10	-96.23	-96.30	0.33
56	SC 5/IITA 8	0.95	8.47	266.73	179.31	0.91	2.80	-84.62	-85.34	0.16	0.95	-63.46	-63.46	0.34
118	SC 10/IITA 10	-0.63	6.91	112.23	94.78	1.38	2.70	-41.94	-70.97	0.17	0.00	-100.00	-100.00	0.33
87	SC 8/IITA 3	-1.03	5.96	162.68	93.16	2.67	4.70	-40.13	-65.19	0.18	0.00	-100.00	-100.00	0.33
53	SC 5/IITA 5	1.31	9.06	271.72	175.56	0.07	2.30	-75.40	-86.71	0.18	0.95	-63.46	-63.46	0.32
13	SC 2/IITA 1	0.37	6.62	280.74	217.04	-0.92	0.00	-100.00	-100.00	0.19	0.35	-86.79	-87.04	0.33
113	SC 10/IITA 5	0.52	8.53	172.96	159.59	-2.39	0.00	-100.00	-100.00	0.23	0.25	-90.57	-90.74	0.30
5	SC 1/IITA 5	0.36	7.85	303.03	138.74	0.09	2.70	-86.60	-93.06	0.25	0.45	-83.02	-83.33	0.32
131	SC 11/IITA 11	0.18	7.66	306.21	247.35	0.31	1.20	-90.36	-90.48	0.29	1.00	-61.54	-61.54	0.36
94	SC 8/IITA 10	0.02	7.38	195.34	108.12	0.47	2.00	-70.37	-85.19	0.30	0.05	-98.18	-98.28	0.32
63	SC 6/IITA 3	-0.26	6.65	143.34	115.66	0.18	1.30	-78.86	-87.13	0.36	0.75	-72.73	-74.14	0.34
76	SC 7/IITA 4	0.25	8.13	158.22	94.96	-0.15	0.70	-91.36	-94.49	0.36	0.55	-78.00	-78.00	0.34
48	SC 4/IITA 12	0.63	6.98	214.49	154.56	-1.78	1.20	-95.04	-95.26	0.39	1.30	-50.00	-51.85	0.33
137	SC 12/IITA 5	-1.21	6.60	127.73	100.94	-1.14	0.70	-89.23	-93.97	0.46	0.55	-78.43	-78.85	0.28
111	SC 10/IITA 3	1.77	8.93	195.26	189.47	-1.82	0.00	-100.00	-100.00	0.50	0.40	-85.71	-86.21	0.33

Appendix 7 (continued) FI hybrid specific combining ability for the means of Fusarium ear rot, total mycotoxin and genetic distance at RARS in 2012/13 season

Entry	F1 Hybrid	Grain yield t ha-1				Ear rot				Fumonisin				GD
	Pedigree	SCA	mean	MPH	HPH	SCA	mean	MPH	HPH	SCA	Mean	MPH	HPH	
141	SC 12/IITA 9	0.52	7.42	208.46	195.15	2.51	3.70	-62.81	-68.10	0.53	0.85	-67.31	-68.52	0.30
133	SC 12/IITA 1	0.38	6.96	256.80	177.08	0.67	1.20	-93.91	-95.68	0.54	0.65	-74.51	-75.00	0.31
14	SC 2/IITA 2	0.05	7.45	228.37	204.29	0.48	1.80	-69.75	-70.00	0.56	0.80	-69.81	-70.37	0.33
20	SC 2/IITA 8	1.33	8.58	235.23	183.14	0.42	2.40	-80.80	-87.43	0.58	0.75	-71.70	-72.22	0.34
40	SC 4/IITA 4	-0.36	7.78	165.14	86.52	0.54	1.20	-93.30	-94.81	0.62	1.15	-55.77	-57.41	0.33
83	SC 7/IITA 11	0.34	7.99	268.70	262.18	1.38	2.60	-67.09	-78.86	0.74	1.25	-50.98	-51.92	0.34
31	SC 3/IITA 7	-0.07	7.84	201.62	159.07	0.05	0.60	-90.63	-92.31	0.78	1.05	-58.82	-59.62	0.28
44	SC 4/IITA 8	-0.34	7.14	201.90	135.47	-0.13	1.30	-93.84	-94.37	0.81	1.30	-50.94	-51.85	0.31
124	SC 11/IITA 4	-0.98	6.73	134.80	61.49	0.78	1.30	-89.72	-89.76	1.01	1.40	-45.10	-46.15	0.35
36	SC 3/IITA 12	-0.43	5.96	142.60	117.43	-0.57	2.50	-83.50	-90.12	1.07	1.75	-30.00	-30.00	0.30
121	SC 11/IITA 1	0.81	6.86	364.14	338.06	-0.28	0.00	-100.00	-100.00	1.11	1.45	-44.23	-44.23	0.36
47	SC 4/IITA 11	0.75	8.66	343.72	292.61	-0.38	0.70	-96.05	-96.97	1.30	2.15	-18.87	-20.37	0.30
7	SC 1/IITA 7	0.28	7.22	297.52	138.62	1.10	2.50	-89.29	-93.57	1.41	1.65	-37.74	-38.89	0.32
107	SC 9/IITA 11	0.38	8.09	287.59	266.94	-1.11	0.60	-93.48	-95.12	1.43	1.95	-30.36	-35.00	0.32
69	SC 6/IITA 9	-0.21	6.64	183.74	178.56	-0.29	0.70	-92.39	-93.07	1.71	2.45	-7.55	-9.26	0.34
54	SC 5/IITA 6	-0.19	7.19	315.66	283.72	-0.47	1.30	-89.68	-92.49	2.12	3.05	12.96	8.93	0.32
62	SC 6/IITA 2	0.33	8.00	231.15	226.76	-0.09	0.70	-91.30	-93.07	2.29	2.90	11.54	11.54	0.32
60	SC 5/IITA 12	-0.58	5.82	168.85	112.18	0.01	3.50	-83.57	-86.17	2.73	3.95	54.90	51.92	0.34
Mean		0.00	7.07	213.69	160.44	0.00	1.46	-84.00	-88.69	0.00	0.34	-86.94	-87.21	0.32
Min		-2.30	3.63	38.56	-0.19	-3.10	0.00	-100.00	-100.00	-1.16	0.00	-100.00	-100.00	0.26
Max		1.77	9.06	532.57	338.06	3.95	7.60	-25.71	-40.00	2.73	3.95	54.90	51.92	0.38

GY=grain yield; SCA=specific combing ability; MPH=mid parent heterosis; HPH=high parent heterosis; GD=genetic distance; Min=minimum; Max=maximum, ppm=parts per million

Appendix 8 FI hybrid specific combining ability for the grain yield and *F. verticillioides* ear rot, their mid- and high-parent heterosis and genetic distance at WARC in 2013 season

Entry	F1 Hybrid	Grain yield t ha-1		Ear rot		GD
	Pedigree	SCA	Mean	SCA	Mean	
70	SC 6/IITA 10	0.30	4.83	-1.39	0.00	0.33
62	SC 6/IITA 2	0.92	6.44	-1.24	0.00	0.32
93	SC 8/IITA 9	0.21	7.25	-1.22	0.00	0.33
117	SC 10/IITA 9	0.46	7.42	-1.08	0.00	0.32
81	SC 7/IITA 9	-0.77	5.85	-1.06	0.00	0.34
12	SC 1/IITA 12	-0.18	5.38	-0.93	0.00	0.33
33	SC 3/IITA 9	0.12	6.26	-0.88	0.00	0.31
129	SC 11/IITA 9	-0.10	6.71	-0.88	0.00	0.36
5	SC 1/IITA 5	-1.09	5.18	-0.86	0.00	0.32
61	SC 6/IITA 1	0.20	4.72	-0.85	0.00	0.34
141	SC 12/IITA 9	-0.23	7.73	-0.82	0.00	0.30
1	SC 1/IITA 1	-0.33	4.74	-0.78	0.00	0.32
66	SC 6/IITA 6	-0.52	5.70	-0.76	0.00	0.33
63	SC 6/IITA 3	0.26	5.47	-0.75	0.00	0.34
11	SC 1/IITA 11	1.15	7.02	-0.75	0.00	0.32
74	SC 7/IITA 2	0.74	6.89	-0.75	0.00	0.32
49	SC 5/IITA 1	-0.36	5.12	-0.73	0.00	0.34
34	SC 3/IITA 10	-1.30	3.39	-0.73	0.00	0.30
68	SC 6/IITA 8	-2.39	2.74	-0.70	0.00	0.30
59	SC 5/IITA 11	0.64	6.94	-0.70	0.00	0.30
67	SC 6/IITA 7	1.19	7.22	-0.70	0.00	0.34
64	SC 6/IITA 4	0.55	6.45	-0.68	0.00	0.34
8	SC 1/IITA 8	1.46	7.14	-0.63	0.00	0.30
7	SC 1/IITA 7	-0.45	6.13	-0.63	0.00	0.32
4	SC 1/IITA 4	-0.97	5.47	-0.60	0.00	0.33
21	SC 2/IITA 9	-0.26	5.69	-0.60	0.00	0.32
105	SC 9/IITA 9	-0.15	7.23	-0.60	0.00	0.32
89	SC 8/IITA 5	-2.70	4.06	-0.60	0.00	0.30
56	SC 5/IITA 8	0.36	6.45	-0.59	0.00	0.34
38	SC 4/IITA 2	0.71	7.74	-0.58	0.00	0.30
55	SC 5/IITA 7	-0.42	6.58	-0.58	0.00	0.34

Appendix 8 (continued) FI hybrid specific combining ability for the grain yield and *F. verticillioides* ear rot, their mid- and high-parent heterosis and genetic distance at WARC in 2013 season

Entry	F1 Hybrid	Grain yield t ha-1		Ear rot		GD
	Pedigree	SCA	Mean	SCA	Mean	
122	SC 11/IITA 2	-0.12	6.22	-0.57	0.00	0.33
52	SC 5/IITA 4	-0.45	6.41	-0.56	0.00	0.33
120	SC 10/IITA 12	-1.01	4.97	-0.53	0.00	0.33
85	SC 8/IITA 1	0.29	5.85	-0.52	0.00	0.34
134	SC 12/IITA 2	-3.36	4.13	-0.50	0.00	0.29
113	SC 10/IITA 5	0.47	7.15	-0.46	0.00	0.30
22	SC 2/IITA 10	-0.88	3.60	-0.45	0.00	0.33
106	SC 9/IITA 10	-0.59	5.34	-0.45	0.00	0.30
77	SC 7/IITA 5	1.77	8.12	-0.44	0.00	0.31
90	SC 8/IITA 6	0.99	8.25	-0.43	0.00	0.34
87	SC 8/IITA 3	0.43	6.69	-0.42	0.00	0.33
109	SC 10/IITA 1	-0.06	5.42	-0.38	0.00	0.32
92	SC 8/IITA 8	-0.43	5.75	-0.38	0.00	0.29
91	SC 8/IITA 7	-1.22	5.85	-0.37	0.00	0.32
119	SC 10/IITA 11	-1.79	4.50	-0.35	0.00	0.31
48	SC 4/IITA 12	0.38	6.90	-0.34	0.00	0.33
36	SC 3/IITA 12	0.13	5.30	-0.33	0.00	0.30
132	SC 11/IITA 12	0.07	5.91	-0.33	0.00	0.38
83	SC 7/IITA 11	0.07	6.03	-0.33	0.00	0.34
14	SC 2/IITA 2	0.62	6.09	-0.29	0.00	0.33
98	SC 9/IITA 2	-1.22	5.70	-0.29	0.00	0.30
114	SC 10/IITA 6	0.82	8.00	-0.29	0.00	0.32
111	SC 10/IITA 3	1.94	8.12	-0.28	0.00	0.33
41	SC 4/IITA 5	-0.85	6.37	-0.27	0.00	0.31
78	SC 7/IITA 6	1.31	8.15	-0.27	0.00	0.34
29	SC 3/IITA 5	0.29	6.15	-0.26	0.00	0.27
75	SC 7/IITA 3	0.17	6.01	-0.26	0.00	0.35
116	SC 10/IITA 8	0.57	6.66	-0.24	0.00	0.33
80	SC 7/IITA 8	0.26	6.02	-0.21	0.00	0.32
112	SC 10/IITA 4	-0.20	6.67	-0.21	0.00	0.31
79	SC 7/IITA 7	-2.31	4.35	-0.20	0.00	0.32

Appendix 8 (continued) FI hybrid specific combining ability for the grain yield and *F. verticillioides* ear rot, their mid- and high-parent heterosis and genetic distance at WARC in 2013 season

Entry	F1 Hybrid	Grain yield t ha-1		Ear rot		GD
	Pedigree	SCA	Mean	SCA	Mean	
121	SC 11/IITA 1	0.44	5.78	-0.18	0.00	0.36
47	SC 4/IITA 11	0.19	7.03	-0.16	0.00	0.30
35	SC 3/IITA 11	0.32	5.80	-0.15	0.00	0.29
131	SC 11/IITA 11	-0.45	5.70	-0.15	0.00	0.36
133	SC 12/IITA 1	-2.24	4.24	-0.11	0.00	0.31
42	SC 4/IITA 6	1.06	8.79	-0.10	0.00	0.33
30	SC 3/IITA 6	-1.61	4.76	-0.09	0.00	0.30
39	SC 4/IITA 3	-1.42	5.30	-0.09	0.00	0.34
126	SC 11/IITA 6	0.15	7.19	-0.09	0.00	0.36
27	SC 3/IITA 3	-0.64	4.72	-0.08	0.00	0.31
143	SC 12/IITA 11	-0.01	7.28	-0.08	0.00	0.27
123	SC 11/IITA 3	-2.11	3.92	-0.08	0.00	0.36
24	SC 2/IITA 12	0.00	4.97	-0.05	0.00	0.32
108	SC 9/IITA 12	-0.56	5.85	-0.05	0.00	0.31
43	SC 4/IITA 7	0.50	8.04	-0.04	0.00	0.31
128	SC 11/IITA 8	0.55	6.50	-0.03	0.00	0.34
31	SC 3/IITA 7	0.39	6.57	-0.03	0.00	0.28
127	SC 11/IITA 7	0.62	7.47	-0.03	0.00	0.36
138	SC 12/IITA 6	-0.42	7.76	-0.03	0.00	0.29
40	SC 4/IITA 4	0.50	7.90	-0.02	0.00	0.33
135	SC 12/IITA 3	1.42	8.60	-0.02	0.00	0.30
28	SC 3/IITA 4	1.40	7.45	-0.01	0.00	0.29
124	SC 11/IITA 4	-0.32	6.40	0.00	0.00	0.35
17	SC 2/IITA 5	-0.51	5.16	0.02	0.00	0.33
101	SC 9/IITA 5	1.64	8.74	0.02	0.00	0.28
140	SC 12/IITA 8	-0.12	6.98	0.03	0.00	0.28
139	SC 12/IITA 7	1.65	9.65	0.04	0.00	0.29
136	SC 12/IITA 4	-0.30	7.56	0.06	0.00	0.29
13	SC 2/IITA 1	-0.84	3.64	0.10	0.00	0.33
97	SC 9/IITA 1	-0.09	5.83	0.10	0.00	0.32
23	SC 2/IITA 11	0.81	6.09	0.13	0.00	0.32

Appendix 8 (continued) FI hybrid specific combining ability for the grain yield and *F. verticillioides* ear rot, their mid- and high-parent heterosis and genetic distance at WARC in 2013 season

Entry	F1 Hybrid	Grain yield t ha-1		Ear rot		GD
	Pedigree	SCA	Mean	SCA	Mean	
107	SC 9/IITA 11	0.90	7.62	0.13	0.00	0.32
18	SC 2/IITA 6	-0.01	6.17	0.19	0.00	0.31
102	SC 9/IITA 6	-1.50	6.11	0.19	0.00	0.31
15	SC 2/IITA 3	-0.29	4.88	0.20	0.00	0.32
99	SC 9/IITA 3	-0.29	6.31	0.20	0.00	0.33
20	SC 2/IITA 8	0.42	5.51	0.24	0.00	0.34
104	SC 9/IITA 8	0.91	7.44	0.24	0.00	0.30
19	SC 2/IITA 7	1.24	7.23	0.25	0.00	0.34
103	SC 9/IITA 7	1.03	8.46	0.25	0.00	0.30
16	SC 2/IITA 4	-0.30	5.55	0.27	0.00	0.34
100	SC 9/IITA 4	-0.09	7.20	0.27	0.00	0.30
50	SC 5/IITA 2	0.34	6.82	-0.62	0.50	0.32
45	SC 4/IITA 9	-0.44	7.06	-0.39	0.50	0.34
142	SC 12/IITA 10	1.32	7.81	-0.16	0.50	0.29
26	SC 3/IITA 2	0.11	5.78	-0.07	0.50	0.27
144	SC 12/IITA 12	1.26	8.24	0.23	0.50	0.31
44	SC 4/IITA 8	-0.68	5.97	0.46	0.50	0.31
96	SC 8/IITA 12	1.39	7.46	0.03	0.70	0.36
6	SC 1/IITA 6	0.90	7.66	0.11	0.80	0.32
57	SC 5/IITA 9	0.22	7.18	-0.48	0.95	0.32
72	SC 6/IITA 12	0.06	5.07	0.00	1.00	0.35
65	SC 6/IITA 5	-0.28	5.43	0.07	1.00	0.33
118	SC 10/IITA 10	0.14	5.63	0.08	1.00	0.33
82	SC 7/IITA 10	-0.67	4.49	0.10	1.00	0.34
53	SC 5/IITA 5	-0.01	6.68	0.19	1.00	0.32
46	SC 4/IITA 10	-1.31	4.74	0.27	1.00	0.34
3	SC 1/IITA 3	0.93	6.69	0.32	1.00	0.33
88	SC 8/IITA 4	0.09	7.04	0.66	1.00	0.33
76	SC 7/IITA 4	0.08	6.60	0.82	1.00	0.34
25	SC 3/IITA 1	1.72	6.40	0.82	1.00	0.32
37	SC 4/IITA 1	1.37	7.40	1.26	1.45	0.33

Appendix 8 (continued) FI hybrid specific combining ability for the grain yield and *F. verticillioides* ear rot, their mid- and high-parent heterosis and genetic distance at WARC in 2013 season

Entry	F1 Hybrid	Grain yield t ha-1		Ear rot		GD
	Pedigree	SCA	Mean	SCA	Mean	
125	SC 11/IITA 5	0.26	6.80	1.24	1.50	0.35
58	SC 5/IITA 10	2.23	7.73	0.28	1.55	0.32
137	SC 12/IITA 5	1.02	8.70	1.36	1.55	0.28
73	SC 7/IITA 1	-0.10	5.05	1.25	1.60	0.34
10	SC 1/IITA 10	-1.08	4.00	0.33	1.65	0.31
95	SC 8/IITA 11	-0.19	6.19	1.21	1.70	0.32
130	SC 11/IITA 10	0.99	6.34	1.08	1.80	0.34
60	SC 5/IITA 12	-0.98	5.00	0.97	1.85	0.34
84	SC 7/IITA 12	-0.56	5.08	1.34	1.85	0.34
32	SC 3/IITA 8	-0.93	4.35	1.81	1.85	0.26
86	SC 8/IITA 2	0.30	6.87	0.99	1.90	0.31
51	SC 5/IITA 3	-0.42	5.76	1.27	1.90	0.32
71	SC 6/IITA 11	-1.64	3.69	1.18	2.00	0.32
94	SC 8/IITA 10	0.83	6.41	1.04	2.10	0.32
54	SC 5/IITA 6	-1.17	6.02	1.56	2.20	0.32
115	SC 10/IITA 7	-2.22	4.78	2.02	2.25	0.33
110	SC 10/IITA 2	0.89	7.37	1.73	2.50	0.30
2	SC 1/IITA 2	0.07	6.14	2.18	3.35	0.30
9	SC 1/IITA 9	-0.42	6.11	2.22	3.70	0.32
69	SC 6/IITA 9	1.35	7.34	5.80	7.35	0.34
Mean		0.00	6.25	0.00	0.44	0.32
Min		-3.36	2.74	-1.39	0.00	0.26
Max		2.23	9.65	5.80	7.35	0.38

SCA=specific combing ability; Min=minimum; Max=maximum