Phenotypic characterization of mycotoxins resistant maize inbred families and regional hybrids under *Aspergillus flavus* and *Fusarium verticillioides* infestation

By

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A dissertation submitted in partial fulfilment of the requirements for the degree of

Master of Science (Plant Breeding)

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Pietermaritzburg, South Africa

December 2015

GENERAL ABSTRACT

Most South African households depend on maize as source of their staple food and daily calories intake, especially the rural communities which depend on the crop to maintain their livelihood. Despite the importance of maize, numerous factors either biotic or abiotic factors affect its production worldwide. Ear rot is one of the common diseases that affect maize production and productivity worldwide. *Aspergillus flavus* (Raper and Fennel) and *Fusarium verticillioides* (Sacc.) are two of the serious ear rot-causing maize fungi. These fungi secrete mycotoxins which are hazardous when consumed by humans or animals. The study was executed to characterize mycotoxins resistant maize inbred families at the phenotypic level and to determine the level of natural incidences of ear rot diseases which are associated with mycotoxins contamination. Understanding architecture of genetic of these resistant maize inbred families would greatly aid in breeding high yielding and stable ear rot and mycotoxins resistant hybrids.

Experimental trials were conducted at Ukulinga and Cedara Research Stations, during the 2014 to 2015 growing seasons. Further evaluation was conducted at the Makhathini Research Station during the winter season of 2015. The study was conducted using two experiments. The first experiment was assessment of natural ear rot incidences on regional maize hybrids. These hybrids represented a sample of varieties which are grown in the Southern African region. In the second experiment, $S_{3:4}$ families, which were derived from three way crosses among, *A. flavus* and *F. verticillioides* resistant maize families, were artificially inoculated with *A. flavus* and *F. verticillioides*. Grain yield and agronomic traits were measured in both

experiments. The grains were evaluated for ear rot infection at harvest. The analysis of variance and correlation analysis were conducted using Genstat 14th edition (Payne et al 2007) and Agronomix Generation II (2000), while the multivariate analyses were conducted using the NCSS (2004) statistical computer program.

The assessment of natural ear rot incidences on regional hybrids revealed that ear rot causing fungi is a challenge. The results revealed four fungi that were responsible for the natural incidences of ear rots. The fungi included *A. flavus* (Raper and Fennel), *Stenocarpella maydis* (Berk.), *Fusarium graminearum* (Schwein.) and *F. verticillioides* (Sacc.). Incidences of *F. verticillioides* were the highest during the two seasons. This might be due to hot dry weather conditions that occurred after flowering. Early maturing hybrids showed lower incidences of ear rots than hybrids that matured late. Although early maturing hybrids encountered less incidences of mycotoxin causing fungi, the results revealed early maturity period had a significant strong negative correlation with grain yield.

This trend was consistent with previous studies. Phenotypic characterization study revealed a significant variability among the mycotoxins resistant maize inbred families for resistance to Aspergillus ear rot, Fusarium ear rot and other selected secondary traits except husk cover, insect damage and days to mid maturity. Generally heritability (H^2) estimates were large for most traits, indicating an opportunity for selection of the best inbred families for advancement in the breeding programme. Plant height, ear height and primary tassel branches recorded higher heritability values (>80%) compared to the other traits. This was followed by Fusarium ear rot and Aspergillus ear rot resistance scores (≥77%) and grain yield (73%). The results revealed five principal components contributing more than 69% of

the total variation and the traits responsible to this variation are Fusarium ear rot, Aspergillus ear rot, plant height, ear height, days to mid maturity, husk cover, insect damage and primary tassel branches. The inbred families were grouped into five principal component groups based on their phenotypic characteristics. Lines to be derived from these grouped families would be exploited to make heterotic combinations by crossing lines from the different phenotypic clusters.

DECLARATION

I, Masemola Bogaleng Milcah, declare that:

1. The research reported in this dissertation, except where otherwise indicated is my original research.

2. This dissertation has not been submitted for any degree or examination at any other university.

3. This dissertation does not contain other person's data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

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   a. Their words have been re-written but the general information attributed to them has been referenced.

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5. This dissertation does not contain text, graphics or tables copied and pasted from the internet, unless specifically acknowledged, and the source being detailed in the dissertation and in the references sections.

Signed 

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Bogaleng Milcah Masemola

As the candidate's supervisors, we agree to the submission of this thesis:

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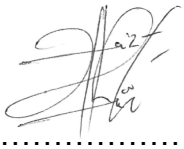
Prof. John Derera (Supervisor)



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Dr. Kwasi S.Yobo (Co-Supervisor)

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Dr. Abe S Gerrano (Co-Supervisor)

ACKNOWLEDGEMENTS

I thank my heavenly father, the Almighty God for his plan and purpose for my life, it is by his guidance I have made it this far. I will forever be thankful to the following individuals who brought me in and took me through the pursuit of my studies:

- My supervisors Prof John Derera, Dr Abe S. Gerrano and Dr Kwasi S.Yobo for the academic wisdom they have imparted on me, their guidance, encouragement, patience and support kept me focused.
- Agricultural Research Council and National Research Foundation for supporting me financially throughout the study.
- Ukulinga research Station support team for the support they gave me during my field work.
- Irene and Isaac for helping with the transport.
- Nyashadzashe Chiuraise for the advices and information he gave me.
- Mr Richard Burgdoff, Cleopus and Zandile for assistance in the laboratory.
- Dr Cosmos Magorokosho (CIMMYT, Zimbabwe) for providing germplasm for the survey on natural incidence of ear rots.
- My family and Mophuting Boikanyo for their moral support; love and prayers though out my studies.

DEDICATION

To my Family:

For their endless love and fervent prayers.

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LIST OF ABBREVIATIONS

PCA	Principal Component Analysis
PC1	First Principal Component
PC2	Second Principal Component
A	Aspergillus
F	Fusarium
SA	South Africa
SSA	Sub Saharan Africa
SACU	South African Customs Union
UKZN	University of KwaZulu- Natal
AF	Aflatoxins
ELEM	Equine leukoencephalomalacia
FB	Fumonisins
PCR	Polymerase chain reaction
VG	Genetic variance
VP	Phenotypic variance
GxE	Genotype by Environment Interaction
CIMMYT	International Maize and Wheat Improvement Centre
DA	Days to anthesis
DS	Days to silking
ASI	Anthesis silking interval
LSD	Least significant differences
CA	Cluster analysis
UPGMA	Unweighted Pair-Group Method with Arithmetic Averages
DMF	Days to mid flowering
FWR	Fusarium ear rot
AER	Aspergillus ear rot

EH	Ear height
PH	Plant height
NL	Number of leaves above the first ear
NP	Number of plants/row
PTB	Primary tassel branching
GT	Grain texture
HC	Husks cover
ID	Insect damage
GY	Grain yield

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CHAPTER ONE

General Introduction

1.1. Introduction

This chapter outlines the importance of maize, its production and the problem of ear rot and mycotoxins in sub-Saharan Africa, thus the justification for further breeding programmes in South Africa.

1.2. Maize production and its significance in South Africa

Maize is one of the principal staple food crops in South Africa (SA) and sub-Saharan Africa (SSA) as a whole. It is also the third most important cereal crop after rice and wheat (Shiferaw et al., 2011) making it a significant crop for research, not only in South Africa but worldwide. In South Africa, the Free State, Mpumalanga, North West and Gauteng Provinces are the primary maize producing regions. Collaboratively, these four regions have the capacity to provide over 85% of the national maize output and the highest average yield per hectare.

South Africa can attain yields of 4.96 t ha^{-1} and 1.1 t ha^{-1} on commercial and small scale farms, respectively under good climatic conditions. However, under drought conditions, yield can drop to as low as 2.67 t ha^{-1} on commercial farms and 0.5 t ha^{-1} on small scale farms. Roughly 60% of maize produced in South Africa is white kernel maize and the other 40% is yellow maize (South African Government, 2009). South Africa is reported to be the main maize producer in the South African Customs Union (SACU) producing about 9.0 million tons per annum (South African Government, 2009). Consequently maize influences the economy and the food security of the country and the region. Most households in the country depend on

maize for their daily calories intake, especially the rural communities. They depend on the crop to maintain their livelihood. White maize is commonly consumed directly as food and to a smaller extend for other uses such as animal feed and industrial processing. It is consumed at various developmental stages, from baby corn to mature grain (FAO, 1997). Most of the yellow maize is consumed by animals as green chop, dry forage, silage or grain feed and for industrial processing. Despite the importance of maize in the region, numerous factors either biotic or abiotic factors, affect its production. Out of these factors ear rot disease is one of the common diseases that substantially affect maize production throughout SSA.

Common prevalent maize ear rot causing fungi are *Fusarium moniliforme,* which causes Fusarium ear rot, Fusarium *graminearum,* which causes Gibberella ear rot, *Stenocarpella maydis,* which causes Stenocarpella ear rot, *Stenocarpella macrospora* which is caused by *Diplodia macrospora* and Aspergillus ear rot which is caused by *Aspergillus flavus* (Smith and White, 1988). These rots cause prevalent damage in humid areas especially when rainfall is above normal at the silking to harvest stages. The fungi produce mycotoxins, which are hazardous when consumed by humans or animals. Therefore, identification, evaluation and characterization of resistant materials remains most important in order to combat the problem of ear rots and their mycotoxins.

1.3. Research justification

Maize is frequently infected with fungi which produces mycotoxins that affect the quality and safety of food and animal feeds. *A. flavus* and *F. verticillioides* are two of the serious sources of mycotoxins contamination in maize. The maize crop is

principal in SSA, hence the problem of ear rot and contamination with mycotoxins is of major concern for both human safety and viability of the livestock industry. In recent years, aflatoxin contamination of maize products has led to outbreaks of acute aflatoxicosis (Lewis et al., 2005). In April 2004, an outbreak of hepatotoxicity was identified in Kenya, and this was the most severe outbreaks of acute aflatoxicosis documented worldwide (CDC, 2004). Human oesophageal cancer was found to correlate with fumonisin B_1 contaminations of maize, in the Transkei regions of the Eastern Cape Province, South Africa (Rheeder et al., 1992). Rheeder et al.(1992) reported more than 117 parts per million (ppm) of fumonisin B_1 in maize from the Butterworth and Centane district in Transkei. The development of maize varieties with resistance to aflatoxin and fumonism contamination could, therefore, serve as a valuable tool in addressing the mycotoxins challenge, reducing economic losses and health hazards which are associated with mycotoxin contaminations (Holbrook et al., 2008).

The management of mycotoxin contamination requires preventive as well as remedial approaches, starting from sowing, harvesting to processing and storage. Resistant maize cultivars should serve as an effective low-cost part of an integrated mycotoxin management programme and the most viable economical solution to the problem (Narasimhulu, 2007). Maize ear rot resistance and grain quality improvement has to remain one of major objectives in maize improvement programmes.

Prior to selection for adaptive traits there is a need to assess the genetic variation for these traits in the breeding base population and to identify sources of resistance that have agronomically sound characteristics. The breeding programme at the University

of KwaZulu-Natal (UKZN) had previously generated maize inbred families that showed resistance to ear rot diseases and mycotoxins (Aflatoxin and Fumonisin) contamination (Chiuraise, 2014). However it's not known whether the genotypes vary genetically and phenotypically. It is also not known whether there is sufficient variability to be exploited to select appropriate materials to develop new maize cultivars with high ear rot resistance levels and desirable agronomic attributes. There is also no evaluation and characterization studies which have been conducted on these breeding materials, which justifies this study. Limited knowledge on the diversity among the different inbred families has led to slow progress in developing ear rot resistant cultivars. Therefore, the current study of phenotyping the ear rot resistant inbred lines will go a long way towards speeding up breeding progress for ear rot resistance. The information will aid development of resistance lines from the identified diversity groups for use in the development of resistant hybrids.

1.4. Research objectives

The overall objective of this study was to characterize mycotoxins resistant maize inbred families at the phenotypic level. The specific objectives of the study were to:

i. Determine the natural incidences of ear rot disease associated with mycotoxins contamination in regional maize hybrids.
ii. Estimate the level of agro-morphological variability and genetic distances among the mycotoxins resistant maize inbred families.
iii. Determine the relationship between *A. flavus*, *F. verticillioides* infections and secondary traits of resistant maize inbred families.

iv. To estimate heritability of, resistance to Aspergillus ear rot, Fusarium ear rot and secondary traits of $S_{3:4}$ maize inbred families.

1.5. Research Questions

The following research questions were pursued in the study:

i. Are there any natural incidences of ear rot diseases associated with mycotoxins contamination on regional maize hybrids?
ii. Is there large agro-morphological variability and genetic distances among the mycotoxins resistant maize families which can be exploited in breeding?
iii. Is there a relationship between *A. flavus*, *F. verticillioides* infections and secondary traits of resistant maize inbreds families?
iv. What is the extent of heritability of, resistance to Aspergillus ear rot, Fusarium ear rot and secondary traits of $S_{3:4}$ families?

1.6. Study Hypothesis

The following hypotheses were tested in the study:

i. There are high natural incidences of ear rot diseases, which are associated with mycotoxins contamination on regional hybrids.
ii. There is large agro-morphological variability among the mycotoxins resistant maize inbred families.
iii. There a relationship between *A. flavus*, *F. verticillioides* infections and secondary traits of resistant maize inbreds families.

iv. Large heritability is present for, resistance to Aspergillus ear rot, Fusarium ear rot and secondary traits in the population of mycotoxins resistant inbred families.

1.7. Scope of the Dissertation

The structure of the dissertation is as follows:

Chapter One- General introduction: This chapter outlines the importance of maize, its production in sub-Sahara Africa, the problem of ear rot and mycotoxins, and provides justification for further breeding programmes that emphasise ear rot resistance in maize.

Chapter Two- Literature review: The chapter summarizes important findings on the biology of maize, its developmental stages, mycotoxins and the ear rot diseases associated with maize. It also reviews the importance of evaluation and characterization of maize for the important traits in conventional breeding to reduce fumonisins and aflatoxins in maize.

Chapter Three- Research design and methodology: This chapter describes the designs, materials and methods which were employed to pursue and answer the research questions. This includes field preparation, planting, isolation and inoculation of pathogens, data collection, and harvesting and data analysis.

Chapter Four- Results: This chapter presents the results observed from the experiments, including the level of agro-morphological variability and genetic distances, relationship between *A. flavus, F. verticillioides* infections and secondary traits, heritability of the mycotoxins resistant maize inbred families for grain yield, ear rots and secondary traits. The results on natural incidences of ear rot disease on Southern African maize hybrids are also presented.

Chapter Five- General discussion: This chapter discusses the results obtained from the experiments and it outlines the outcome of the findings.

Chapter Six- Conclusion and recommendations: The chapter concludes the research findings and present recommendations for future work.

1.8. Summing up rationale for the study

Mycotoxins contamination in food and feed poses a serious hazard for animal and human health. Aflatoxin and fumonism are two of the major classes of mycotoxins which gained a considerable attention, however research and regulatory efforts to combat the impact of these mycotoxins have not resulted in much reduction of the carcinogen in food supply. Grain quality remain low yet food requirements increases tremendously leading to a huge gap between population growth and food production.

Research studies on phenotypic diversity for maize resistance to ear rots and overall production improvement are vital in breeding programmes that seek to develop new maize varieties, which are adapted to South African growing conditions. In order to achieve this the inbred families which have been developed at the UKZN should be evaluated for variation of ear rots resistance and desired secondary traits. The information will assist in developing hybrids with high resistance to ear rots fungi which cause mycotoxins (aflatoxin and fumonisin) contamination in grain. Furthermore, the study will contribute to an increase in quality maize production in South Africa.

1.9. Conclusion

The foregoing has indicated the problem mycotoxins contamination poses in food and feed. Research studies on phenotypic diversity of mycotoxin resistant maize and overall production improvement are vital in breeding programms to establish new varieties adapted to South African growing conditions and SSA as a whole. The following chapter reviews the literature in line with objectives of the study.

CHAPTER TWO

Literature Review

2.1. Introduction

This chapter reviews literature on the biology of maize, maize plant developmental stages, mycotoxins and the ear rot diseases associated with maize. The importance of evaluation and characterization of maize for the important traits in conventional breeding to reduce fumonisins and aflatoxins in maize.

2.2. Biology and origin of maize

Maize is a monoecious, cross pollinated grain crop belonging to the tribe Andropogoneae in the subfamily Panicoideae and the family Poaceae. There are 86 recognized genera within the Andropogoneae tribe (USDA, 2003) and five species included in genus Zea that have been identified largely by having chromosome number of 2n= 20 except for Zea perennies (perennial teosinte) with 2n =40. Maize (*Zea mays* L.) is said to have originated from its wild species ancestor, teosinte (*Zea mays* L. ssp. parviglumis), about 9000 years ago. The domestication had taken place in the mid-elevation of South Central Mexico, and started with the teosinte race Balsas (Matsuoka et al., 2002, Abbassian, 2006). It was then introduced to different continents, including the North and South Americas, Europe, Africa and Asia (Rebourg et al., 2003). After the introductions farmers selected maize landraces with better adaptability to the new environmental conditions, leading to several new derivatives in the processe. For example, maize was introduced in Africa nearly five centuries ago (McCann, 2005). Since then, the crop expanded in its range from the lowlands to the highlands, and has become the most important crop in the continent in terms of cultivated area and total grain producing crop in the continent (FAOSTAT,

2010). The typical maize plant is 1- 4 m tall annual grass,which forms a seasonal root system bearing a single erect stalk made up of nodes where leaves develops. The stalk has staminate spike-like racemes that form large, spreading, terminal panicles (tassels) and pistillate inflorescences in the leaf axils.

Maize is predominately cross pollinated by wind, but both self and cross pollination are possible. The pollen grain has a relatively thin outer membrane that gives little environmental protection; consequently, viability may be lost in few minutes because of desiccation. Shed pollen usually remains viable for 10 to 30 minutes, but can be preserved under favourable conditions (Simmond and Smartt, 1999). The silk normally emerges at the top ear node one to three days after anthesis. Tassel development controls ear shoot development, and this dominance is greatest for genotypes that produce only one ear per plant in any environment. Some genotypes may have no dominance for the tassel, and their silks frequently emerge before the tassel begins to shed pollen (Hitchcook and Chase, 1971).

2.3. Global importance of maize

Maize is the most vital cereal crop in sub-Saharan Africa (SSA) and an important staple food for more than 1.2 billion people in SSA and Latin America. Almost every part of the maize plant has an economic value, including the grain, stalks, leaves, silks, husks and the tassel (Pingali, 2001). In industrialized countries, maize is mainly used as livestock feed and as a raw material for industrial products. In South Africa, maize adds up to 60% of all land covered with cereals and about 40% of total calories consumed (McCann, 2005). Figure 2.1 shows the top three African maize

consuming countries on the list which exceed even Guatemala and Mexico, maize's homeland. In East Africa as a whole, maize accounts for 30% of all calories.

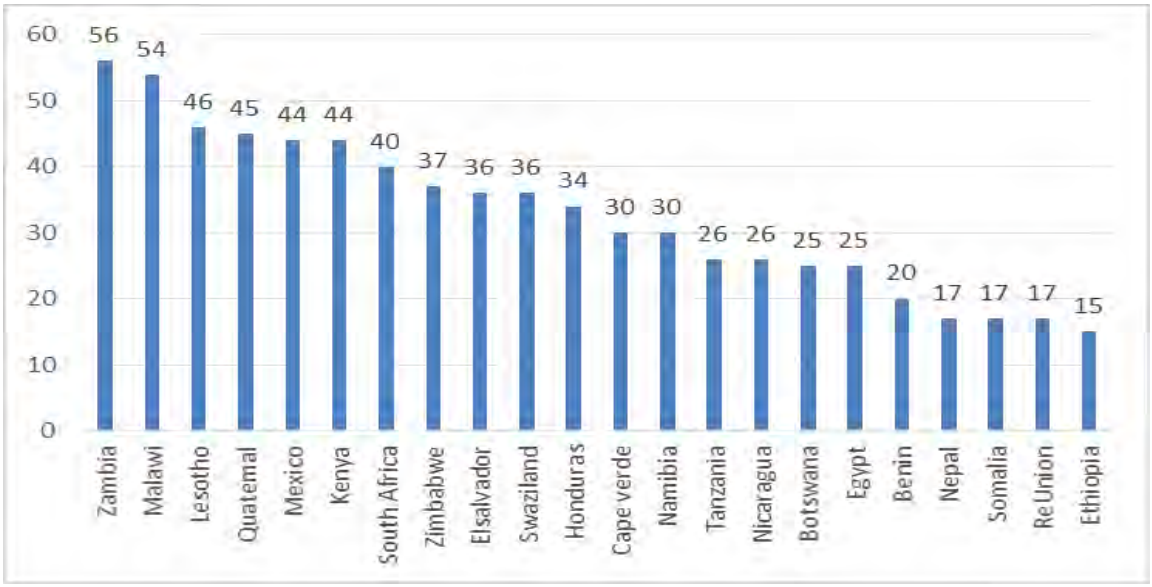


Figure 2.1: Maize calorie consumption as percentage of total diet (McCann, 2005).

2.4. Types of maize

Maize can be classified into different types based on kernel morphology, texture, usage, functionality and other characteristics. There are different types of maize based on their functionality including waxy maize, high protein maize, high oil maize, flour maize, sweet-corn and popcorn (Johnson, 2000). Flint type maize has kernels consisting of hard endosperm, smooth rounded glassy appearance and popping ability. Kernels of dent maize are characterized by the presence of a small proportion of hard endosperm (Figure 2.2) at the side and back of the kernel (Johnson, 2000). Their inner core consists of soft floury endosperm, extending to the crown of the endosperm. Dent maize kernels have less popping due to large amounts of soft endosperm. Maize kernels, with soft dent endosperm are more vulnerable to fungal attacks compared to flint (Johnson, 2000).

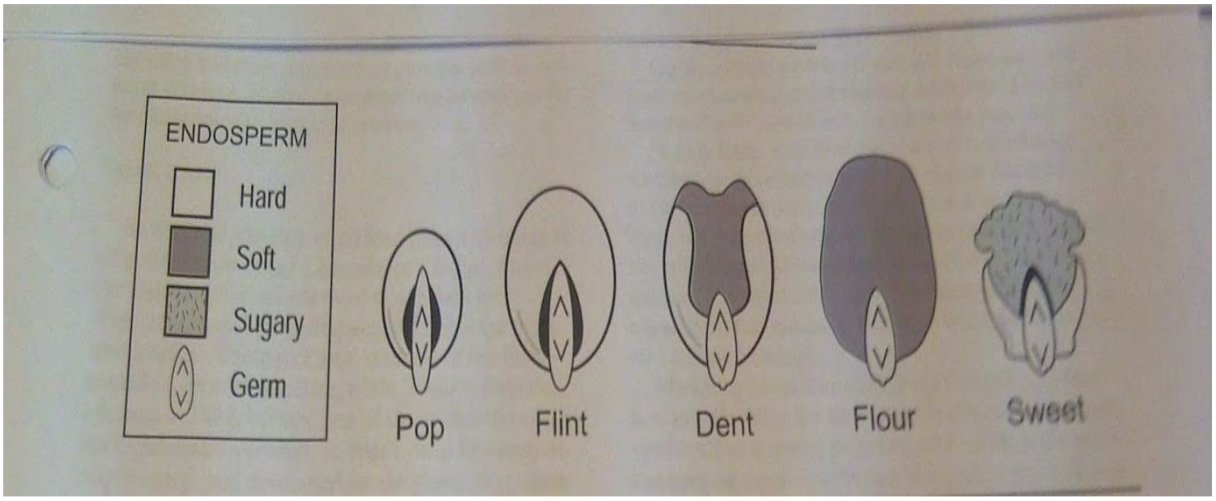


Figure 2.2: Morphology and endosperm kernel type for different maize groups (Dickerson, 2003).

2.5. Growth stages of maize

The growth cycle of maize is described differently by several authors, as a result, assigned different numerical designations as representative of different growth phases. The first and last vegetative phases are labelled as VE (emergence) and VT (tasselling) respectively. The relative maturity of most maize cultivars is achieved when the plant has produced from 16 to 20 leaves depending on cultivar, season, location and planting date (Vorst, 1990).

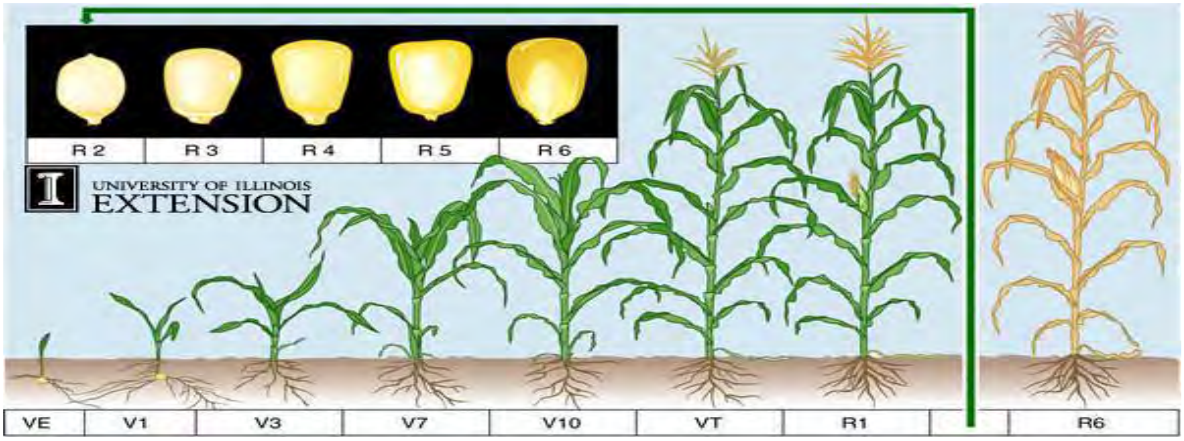


Figure 2.3: Growth stages of maize (Source: University of Illinois Extension, 1992).

The vegetative phase begins when the seedling emerges and ends by initiation of tassels (Figure 2.3 and Table 2.1). However, leaves still emerge from the whorl even though the primordia are now on reproductive stage (Ritchie et al.,1992). Reproductive phase begins when the pollen starts to shed (anthesis) and it ends at physiological maturity which is recognized by the black layer at the base of the grain.

Ear shoots develop at the top most axillary bud and ear continues to develop as the last few leaves enlarge before the tassel appears (VT). Any form of stress during this phase may affect yield as it inhibits ear development than tassel development (Vorst,1990). Tasselling is the stage where the last branch of the tassel is completely visible and silking (R1) begins when silks are visibly outside the husks. Most ear rot fungi attacks maize at this stage, asthe fungi enters the husk and invade the ears. Most artificial inoculation methods including spraying of the silks with a suspension containing the pathogen conidia and silk channel injection are done at this stage.

Table 2.1: Developmental stages of maize based on the Leaf Collar Method (Ritchie et al., 1992).

Vegetative Stages	Reproductive Stages
VE (emergence)	R1 (silking)
V1 (first leaf)	R2 (blister)
V2 (second leaf)	R3 (milk)
V3 (third leaf)	R4 (dough)
V (n) (nth leaf)	R5 (dent)
VT (tasselling)	R6 (physiological maturity)

Blister stage (R2) is identified by the blister like shape white kernels and silks begin to dry. Milk (R3) phase is characterized by yellow colour on the outside of the kernel. Dough (R4) stage occurs when the inner fluid thickens as the starch accumulates inside the endosperm to form a dough (Ritchie et al., 1992). Dent stage (R5) starts

when the top of the kernel dries and sag to form a ridge around the endosperm. As the kernel dries down, a hard white layer is formed and it advances towards the base of the kernel. The line between milky layer and hard starch line is called milk line. When the milk line is 50 percent, the kernel has a moisture content of about 40-45 percent and potential final dry weight of 95 percent (Ritchie et al., 1992). Physiological maturity (R6) is reached when a black abscission layer has developed at the base of the kernel. Black layer starts at the tip of the cob and progresses to the base of the cob (Ritchie et al., 1992).

2.6. Ear rot diseases of maize

The Maize crop usually get infected by various ear rot fungi. The most common and serious maize ear rots are Fusarium ear rot caused by *Fusarium verticillioides*, Gibberella ear rot caused by *Fusarium graminearum*, Diplodia ear rot caused by *Stenocarpella maydis*, and Aspergillus ear rot caused by *Aspergillus flavus* (Smith and White, 1988). These fungi cause a lot of damage in areas which are humid, particularly where rainfall is above normal during silking to harvest. The fungi produce mycotoxins which get potentially hazardous when consumed by humans or animals.

The prevalence of rots is increased by insects and bird damage to the ear and by lodging where ears touch the ground. The fungus *Aspergillus flavus* (the fungus of Aspergillus ear rot) infects and grows on the maize cob/ears and the fungi usually appears as yellow-green symptoms on the maize kernels. *Aspergillus* moulds are found throughout the world and favours drought and high temperature conditions. The fungus *Fusarium verticillioides* (the fungus of Fusarium ear rot) appears as a

white to pale pink or pale purple coloured growth on the maize kernels. Other prevalent ear rot of maize include Gibberella ear rot which is common throughout different maize production areas. Gibberella ear rot symptoms are pink to reddish colored mould, starting near the tip of the ear and progressing down toward the base of the ear. *Gibberella* produces vomitoxin and zearalenone and these toxins are harmful to many kinds of livestock (Agrios, 1988).

2.7. Occurrence of mycotoxins on maize

Mycotoxins are secondary natural poisons, exhibiting a toxic effect termed mycotoxicoses in both humans and animals. The toxic effects are normally as a result of exposure through ingestion of contaminated food or feed, by inhalation of airborne mycotoxin producing fungus or by direct skin absorption (Pitt, 1996). The fungi attacks the maize throughout its growing stages and a range of climatic conditions. Accumulation of mycotoxins in food and feed threaten human and animal lives by causing serious health problems (FAO, 2006). Research over the past had contributed significantly to the global understanding of mycotoxins and their effects on both humans and animals.

Holzapfel et al. (1966) isolated and identified aflatoxin M1 and M2 produced by *Aspergillus flavus*. Marasas et al. (1976) discovered leukoencephalomalacia (LEM) disease in a horse, which is caused by mycotoxin produced by *Fusarium verticillioides.* Marasas et al. (1977) reported the occurrence of Zearalenone and deoxynivaltrichoend, a 12, 13–epoxytrichotechene in South Africa. Aucock et al. (1980) demonstrated the effect of maize contaminated with Zearalenone on the health of pigs. Rabie et al. (1987) identified the mycotoxin, Rhizonin produced by

zygomycetous fungi. Bezuidenhout et al. (1988) discovered fumonisins which are regarded as a very important group of mycotoxins of maize worldwide.

The percentage to date of maize grains contaminated with mycotoxins and their levels are very high, especially some important toxins such as aflatoxins, deoxynivalenol, zearalenone and fumonisins (Biomin Newsletter, 2008; Solovey et al., 1999). The presence of mycotoxins in food and feeds has had a profound effect on the trading of Maize and many developing countries have been unable to export their grain (Waliyar and Reddy, 2009) due to unacceptable contamination levels.

2.7.1. Aflatoxins (AF)

Aflatoxins are destructive, carcinogenic secondary metabolites produced by the species of the fungal genus *Aspergillus* including *flavus* (Raper and Fennell), *parasiticus* (Speare.), and *niger (*ASPENI) moulds. This mycotoxin group has difurocoumarolactone compounds possessing furan, coumarin and lactone rings (Brown et al., 2001). The major aflatoxins are classed as B1, B2, G1, G2, and M1 (Holcomb et al., 1992), the letters G and B designate the colour of the fluorescent emissions from the two categories of aflatoxins, the letter G represents the yellow-green spectacles, and the letter B indicate the spectacles of blue fluorescence under ultraviolet light (Phillips, 1999), and M indicates that the traces of the B and G aflatoxins are found in milk. There are two strains of *Aspergillus flavus*, the S and L strains; the S strain produces more aflatoxin and many tiny sclerotia, while the L strain produces few bigger sclerotia and small amount of afflation (Cotty and Cardwell, 1999; Varga et al., 2003). Chemically, these mycotoxins are crystalline substances, and freely soluble in moderately polar organic solvents such as acetone, isopropanol and methanol. They also dissolve in water up to the size of 10-20

mg/liter (Leatherhead Food Research Association, 2004). Consumption of diets contaminated with aflatoxin may cause serious long term chronic effects in humans and animals resulting in a carcinogenic or immunosuppressive impact (Huwig et al., 2001). Aflatoxins have been associated with various diseases including aflatoxicosis, in livestock, domestic animals and humans throughout the world (Reddy et al., 2011). Aflatoxin was found to be associated with the Turkey X disease in England (Wannop, 1961). The threshold level of aflatoxins that was established worldwide to combat the potential health hazards for humans ranges from 4 and 50 µg/kg (FAO,2004).

2.7.2. Fumonisins (FB)

Fumonisins are carcinogenic mycotoxins produced by the species of the genus *Fusarium*. There are more than ten species producing these toxins, but *F. verticillioides* and *F. proliferatum (*Matsush.) are the only two species capable of producing a significant amount of fumonisins. *Fusarium verticilliodes (moniliforme)* causes stalk andear rot of maize worldwide (Joint FAO/WHO Expert Committee on Food Additives, 2001). Fumonisin was first isolated in South Africa in 1988 by Gelderblom and colleagues, and then Bezuidenhout et al. (1988) identified and characterized FB into seven analogous. Yazar and Omurtag (2008) reported that 28 FB analogues do exist. Fumonisins formation happens only before harvest or during the early stage of drying, but never in the storage stage (Arora and Khachatourians, 2004). In animals, fumonisins are known to cause the equine leukoencephalomalacia (ELEM) in the horse and porcine pulmonary edema (CAST, 2003; Piva et al., 2005).

They are poorly absorbed in the digestive tract and are quickly removed from the body of animals. However, they mainly remain in liver and kidney (Joint FAO/WHO Expert Committee on Food Additives, 2001). Human oesophageal cancer was found to correlate with fumonisin B_1 contaminations of maize, in the Transkei regions of the Eastern Cape Province, South Africa (Rheeder et al., 1992). Rheeder et al. (1992) reported more than 117 parts per million (ppm) of fumonisin B_1 in maize from the Butterworth and Centane district in Transkei. Levels of fumonisins legislated by the countries ranges from 1000 and 3000 µg/kg. The current regulation in South Africa indicates that no food commodities meant for human consumptions may contain more than 10 µg/kg aflatoxin (Governent gazette no. 26849,2009). A total of 50n ppm is set for horses and pets regarding fumonisn B_1 (Governent gazette no. 26849,2009).

2.8. Ear rots disease control

Diseases in plants are controlled by different measures including eradication, exclusion, avoidance and resistance (Day, 1974). The methods used to control ear rots on maize include crop rotation, improved tillage practices, fertilisation practices, planting date alteration, supplementary irrigation, and correct harvesting times. The use of cultural and chemical control measures has given little control over ear rots, hence Nankam and Pataky (1996) proposed the use of genetically resistant varieties. However, there are few commercial varieties with adequate levels of resistance to be used for such a purpose. Inherent resistance to ear rot fungi has been shown to exist in maize, but with poor agronomic performance.

2.9. Germplasm evaluation and Characterization

Characterization is a cautious depiction of remarkable characteristics that are heritable and expressible accurately in all environments (Day-Rubenstein and Heisey, 2003). Heritable traits of plant germplasm are studied consistently through characterization and evaluation. Two techniques are used in characterization, first is morpho-agronomic evaluation of germplasm by morphological markers and molecular characterization of the germplasm at the DNA/molecular level using molecular markers. In cases where characterization data and morpho-agronomic evaluation are insufficient in establishing distinctiveness between species or germplasm, genome characteristics such as the karyotype, chromosome number and ploidy level may be studied utilizing molecular markers (Kiran Patro and Ravisankar, 2004; Ariyo, 1990). Since most of the traits recorded during characterization are the visible ones, the person in charge of managing the germplasm material is systematically responsible for documenting these characteristics (De Vicente et al., 2005). The characteristics that are documented on individual accessions can be used as diagnostic descriptors for germplasm. Descriptors' lists are serving as an important tool in ensuring same language and standards during characteristics documentation of conserved plant species (De Vicente et al., 2005). One vital objective of germplasm characterization is to spot the germplasm collection so that they can be clearly distinguished or individualized (CIAT, 2007).

2.9.1. Morphological characterization

Morphological characterization has been used in several studies to identify reliable and useful information in various crops. For instance, Stoilova and Berova (2009)

successfully evaluated 15 common beans and three accessions of *Vigna unguiculata* (L.) in Bulgaria. Moukoumbi et al (2011) employed morphological markers to investigate the morphological diversity in 78 *Oryza sativa* materials in Benin Republic. Morphological characterization and evaluation has also been used to study qualitative and quantitative traits of thirty seven sorghum landraces collected mainly from Tanzania (Bucheyeki et al., 2008). Several maize breeders have made use of morphological markers to study genetic relationships among different germplasm. As useful as morphological markers are in characterization and evaluation, they have low polymorphism, late expression and are highly influenced by environmental conditions which calls for area specific characterization (Cadee, 2000).

2.10. Conventional Breeding to Reduce Fumonisins and Aflatoxins

2.10.1. Breeding Options

The development of a successful breeding program for ear rots and mycotoxins resistant maize germplasm requires a detailed understanding of the gene action involved in the inheritance of the traits and the breeding gain. Breeders have to consider whether breeding for mycotoxins and ear rot resistance can be attained without compromising the grain yield potential of the hybrids (Frankham et al., 2002). When breeding for pathogen resistance, the exposure to the disease must be such that escapes are prevented, yet those with resistance or tolerance can still be recognized. In some situations, natural inoculum in the field is enough to screen for resistance (Schumann, 1991), while in other instances artificial inoculum is needed for precise screening. In the current study, both natural and artificial inoculation were

applied at the two sites. This is because the natural inoculum is high at Cedara research Station but very low at Ukulinga Research Station.

2.10.2. Heritability

Heritability is a quantitative measure which gives information about the proportion of phenotypic variance attributable to genetic variance (Dabholkar, 1999). This term is further divided into broad sense and narrow sense heritability. Heritability in the broad sense is determinedas the ratio of genetic variance to phenotypic variance (VG/VP) (Nyquist, 1991). It exhibits the extent to which individual phenotypes are dictated by the genotypes. A huge percentage of heritability for a character is regarded as highly heritable and in contrast if it is smaller, it is considered as less heritable (Dabholkar, 1999). The ratio of additive variance to phenotypic variance (VA/VP) is called heritability in the narrow sense (Gebre, 2005). Several authors reported on heritability for resiatance to Aspergillus ear rots, Fusarium ear rots, yield and other secondary traits.

Falconer et al. (1996) reported polygenic and low heritability for Resistance to Fusarium ear rot. Robertson-Hoyt et al. (2006) found high heritabilities for Fusarium ear rots and Menkir et al. (2008) found moderate to high heritability for, resistance to Aspergillus ear rot. Walker and White (2001) found broad sense heritability values 0.26 and0.48 for resistance to Aspergillus ear rot. Khoza (2012) reported heritability (h^2) of 0.86 to 0.94 for grain yield and plant height heritability of 0.87. Mahmood et al. (2004) also reported 0.99 heritability (h^2). Ali et al. (2011) found a heritability of 0.67(h^2) for grain yield.

2.10.3. Genetic variation

Genetic variation is defined as “the variety of alleles and genotypes present in a population, reflected in morphological, physiological, biochemical and behavioral differences between individuals and populations” (Frankham et al., 2002). Knowledge and understanding of genetic diversity enables maintenance and broadening of the genetic base of the elite germplasm, selection of appropriate parental lines for hybrid combinations, and generation of segregating progenies with maximum genetic variability for further selection (Prasanna et al, 2002). This is due to the fact that selection of improved genotypes depends heavily on the presence of genetic variability. Genetic variation has been reported to exist for resistance to ear rots among both tropical and temperate maize inbred lines and hybrids (Naidoo et al., 2002). Significant progress has been made in North America and Europe in understanding the genetics of resistance to maize ear rots (Munkvold, 2003). However, the amount of resistance realized has been limited due to complicated genetics and/or allelic-linkage to undesirable agronomic traits (Duvick, 2001), such as low yields, small hard kernels and small stout ears with long husks.

2.10.4. Correlation

Correlation is a technique used to measure association or relationship among traits. Several studies have reported association among resistatance to Fusarium eat rot, Aspergillus ear rot, yield and other secondary traits. For instance, Robertson-Hoyt et al. (2007) reported high correlations between, resistance to Fusarium and Aspergillus ear rot, r = 0.81. Rossouw et al. (2002) found a significant correlation between husk cover and ear rot infection. Ako et al. (2003) reported that ear rot infected maize ears had higher insect damage than uninfected maize. Loose husk

cover was reported to highly correlates with insect damage. Ma et al. (2013) and Eller et al. (2008) reported that the kernel moisture content influences the degree of ear rot. Breeders are therefore posed with a challenge of ensuring that the breeding strategies employed achieve optimal response to selection for both ear rot resistance and improved maize quality.

2.10.5. Effect of Genotype by Environment interaction on mycotoxin accumulation in maize

Though some traits are expressed completely under genetic control, other traits are influenced by environmental factors. In breeding programmes, environmental effects must be accounted for and removed in order to accurately assess genetic differences and select superior genotypes for the traits of interest. When genotypes are similarly affected by the environment, the effect does not account for genotypic differences or selection. When the environment affects some genotypes differently compared to others, genotype by environment interaction is significant (Fehr, 1991). This interaction complicates breeding efforts, and requires more extensive evaluation over multiple years and environments in replicated trials. GxE interactions have been significant in several studies on the genetics of mycotoxins production in maize (Payne, 1992). Menkir et al. (2008) reported that maize hybrids planted outside their adapted areas are more likely to be susceptible to ear rot infections, hence it is important to evaluate potential genotypes across a range of environmental conditions.

2.11. Inoculation Methods

Inoculation is a technique that is commonly used to enable screening precision by minimizing fungi infection escapes, thus ensuring high selection power for breeding. There are several inoculation methods that have been used to find out the response of maize genotypes to *A. flavus* and *F. verticillioides.* These techniques are classified as either wounding or non-wounding. Wounding inoculation techniques include the knife (Widstrom et al., 1996), pin bar (Tubajika and Damann, 2001;Tubajika et al., 2000), pin board (Naidoo et al., 2002), side needle(Windham and Williams, 2002), toothpick (Zhang et al., 1998), and punch drill/pipe cleaner methods (Wicklow et al., 1994). Non-wounding techniques include spraying of the silks with a suspension containing the pathogen spores (Cardwell et al., 2000), and silk channel injection (Zummo and Scott, 1989). Time and the point of inoculation has been found to be critical for effective artificial inoculation and for, the current study inoculation was done 17 days after silking and toothpick method was used. Reid and Hamilton (1996) reported a decrease in severity as the kernel or silk aged and they recommended that inoculation should be done 15 days after the silk has emerged.

CHAPTER THREE

Materials and methods

3.1. Introduction

This chapter presents the designs, materials and methods which were employed to pursue the studies. This includes field preparation, planting, isolation and inoculation with pathogens, data collection, harvesting and data analysis.

3.2. Research Structure

The study was conducted using two methods, the first method was the assessment of natural ear rot incidences on regional hybrids associated with mycotoxins contamination. On second method, $S_{3:4}$ families were artificially inoculated with *Aspergillus flavus* and *Fusarium verticillioides*. At harvest the cobs were evaluated for ear rot infection and phenotypic variation.

3.3. Assessments for ear rot incidences in southern African maize hybrids

3.3.1. Germplasm

Regional hybrids from four countries (Zimbabwe, Zambia, Malawi and South Africa) were screened for ear rot incidences. The hybrids had different physiological maturities, some hybrids were known to be early, medium or late in maturity. The hybrids were provided by the International Maize and Wheat Improvement Centre (CIMMYT) in three sets. A set of 60 early, 60 medium and 40 late maturing hybrids were evaluated in 2013/14 (Chiuraise, 2014). A set of 55 early, 60 medium and 42

late maturing hybrids were evaluated in the 2014/15 summer season. Hybrids 11C1579 and 11C1774 were used as local checks in the study.

3.3.2. Experimental design and management

The study was conducted during summer seasons of 2013/14 and 2014/15 at Cedara Research Station (29°54`S, 30°26`E, altitude of 1066m above sea level). The experiment was arranged in an alpha lattice incomplete block design with three replicates. Planting was done by hand at two seeds per hole and three weeks after germination the seedlings were thinned out to one plant. Plots of $9m^2$ size were arranged in two rows of 5m per hybrid, consisting of spacing of 0.9m between the rows and 0.3m within the rows. Each row had 17 plants resulting in 34 plants per plot. Basal fertilizers (NPK) were applied (75 kg N, 50 kg P, 25 kg K per hectare) before planting and four weeks after the seedlings had emerged, top dressing of 120 kg per hectare in the form of Limestone Ammonium Nitrate, LAN (28% N) was applied. The experiment was rain fed and was subjected to natural fungal infection. The field was kept clean of weeds using hand weeding. The trial was manually harvested during June 2015.

3.3.3. Collection of Meteorological records from, Cedara,Ukulinga and Makhathini Research Stations.

Climatic data (temperature, rainfall and relative humidity) was collected at the nearby station for Cedara, Makhathini and Ukulinga trials. The data was used to identify whether the incidences of ear rots is associated with the weather conditions in the growing areas.

3.3.4. Data collection on natural incidences of ear rots.

Data was recorded on plot to plot basis for each hybrid. Flowering date was recorded as days to 50% tasselling (days to anthesis, DA) and 50% silking (days to silking) .Anthesis silking interval was calculated by subtracting silking date from anthesis date. At harvest diseased ears per plot were counted and categorized from the scale of 1 (no rots) to 7 (severe rots). The symptoms were classified as Aspergillus (yellow-green mycelia growth), Fusarium (cottony, whitish-pink growth scattered randomly on ear), Diplodia (dense whitish fungal growth matted between the kernels) and Gibberella ear rot (Pink to reddish mould usually starting at the tip). Grain texture was recorded using a rating scale of from 1= hard, completely rounded flint kernel to 5= soft, distinct dent (CIMMYT, 1985). The grain moisture content was noted using the grain moisture meter, MC-7825G (ONMI instruments, UK). Grain yield was measured as plot weight and transformed to t ha^{-1}. Data on insect damage was taken were 1= no damage and 9= heavy damage (Badu-Apraku et al., 2012).

3.4. Isolation and morphological identification of cultures

Maize kernels suspiciously infected with *Fusarium* spp, *Diplodia*, *Gibberella* and *Aspergillus* spp were sterilized with 2% jik (sodium hypochlorite) for one minute, then washed three times with distilled water. The kernels were then cultured on plates containing selective media Synthetic Nutrient Deficient Agar (consisting of glucose 0.2 g, sucrose 0.2 g, KH_2PO_4 1 g, KNO_3 1 g, $MgSO_4$ 0.25 g, KCL 0.5 g, agar 14 g/L). Pieces of sterile filter paper were placed on the SNA media to ensure quick sporulation, the plates were incubated for 14 days at 25°C under UV light. Formation of macro conidia chains (Leslie and Summerell, 2006) were used as an indicator to confirm the *F. verticillioides* isolate at UKZN laboratory under a light microscope.

Aspergillus flavus selective Media (AFPA – dichloran 0.002 g, ferric ammonium citrate 0.5 g, peptone 10 g, KH_2PO_4, $MgSO_4$.7H20 0.5 g, chloramphenicol 0.2 g, agar 15 g/L) was used to culture the kernels suspected to be infected with *A. flavus.* The plates were incubated for seven days at 28°C. Spore-bearing structure and yellow-green moulds on media were used as an indicator to confirm *A. flavus* in the laboratory.

3.5. Evaluation of $S_{3:4}$ families for agro-morphological variability and resistance to mycotoxins contaminations/ ear rots

3.5.1. Germplasm

The germplasm were developed by first stacking aflatoxin and fumonisin resistant genes in three- way crosses (Chiuraise, 2014). The three way-crosses were advanced in to $S_{2:3}$ and then $S_{3:4}$ families. Aflatoxin resistant lines, TZAR102 and TZAR103 were obtained from the International Institute of Tropical Agriculture (IITA) maize breeding programme at Ibadan, Nigeria. The two inbred lines are known to have a combination of tropical and temperate genomes in the background and they also have sufficient genes for resistance to prevalent foliar diseases (Menkir et al., 2008). Fumonisin resistant inbred lines, CML444 and CML390 were obtained from the International Maize and Wheat Improvement Centre (CIMMYT) regional research station in Harare, Zimbabwe. These lines have been reported to be resistant to Fusarium ear rot infection which is associated with contamination of grain with fumonisins and other mycotoxins (Small et al., 2012). They have white grain with high yield potential and they are also adapted to the medium altitude environments in eastern and southern Africa. Adapted lines from the University of Kwa Zulu-Natal (UKZN) maize breeding programme and additional 28 experimental lines were used

as recipients of genes for resistance to contamination by aflatoxin and fumonisin (Chiuraise, 2014). During the summer of the year 2012 at Makhathini Research Station (27°39`S; 32°10`E; altitude 72 m above sea level), crosses were made between 41 adapted maize inbred lines and Fumonisin resistant lines (CML444 & CML 390) to stack the resistant genes into the recipient lines. 82 F1 single crosses were developed; the single crosses were then crossed with two aflatoxin resistant inbred lines: TZAR102 and TZAR103 in the greenhouse during the 2012/13 summer season to develop 44 three- way hybrids (S_1) stacked with aflatoxin and fumonisin resistant genes. The S_1 (three- way crosses) were then self-pollinated at Makhathini Research Station, during the 2013 winter season to produce the $S_{1:2}$ generation seeds. The sum of 146 $S_{1:2}$ families were then advanced to $S_{2:3}$ during the summer season of 2013/14 at Cedara Research Station. The $S_{3:4}$ families were advanced at Ukulinga Research Station during 2014/15 cropping season and Makhathini Research Station during winter season of 2015 (Figure 3.1).

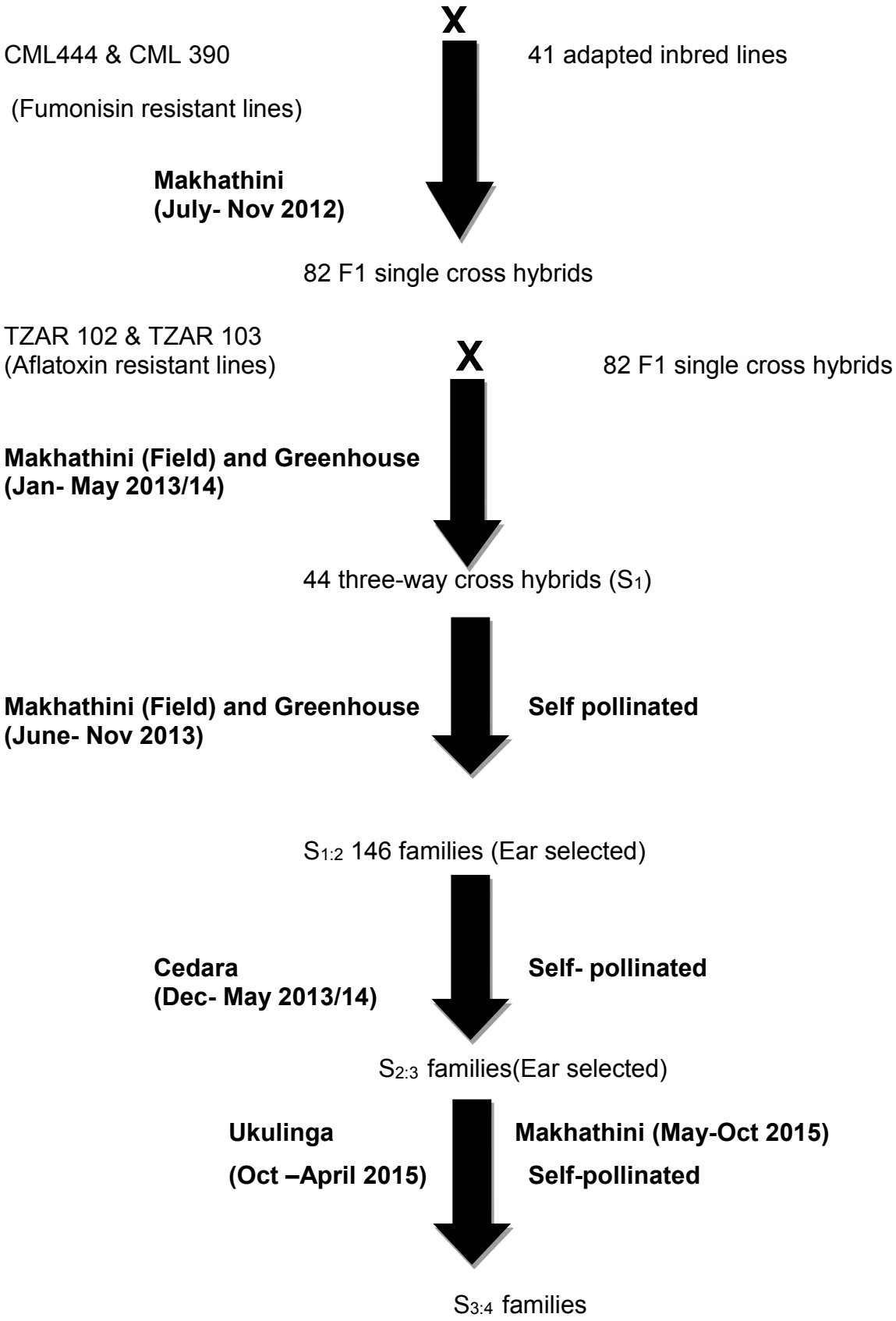


Figure 3.1: Flow diagram of the introgression of resistant genes into adapted lines over five seasons (Chiuraise, 2014).

3.5.2. Experimental design and management

The $S_{3:4}$ families [208 mycotoxins (aflatoxin and fumonisin) resistant inbred families] were planted at Ukulinga Research Farm (population 1) (altitude 812 m above sea level, latitude 29.660S; longitude 30.400E) during 2014/15 summer season (October- April) and at Makhathini Research Station (population 2) (latitude 27°39`S; longitude 32°10`E; altitude 72m above sea level) during May- October 2015. Inbred lines CML390 and CML444 were used as positive controls for fumonisin contamination resistance and TZAR102 and TZAR103 were used as positive controls for aflatoxin contamination resistance. DTA inbred line was used as negative controls or susceptible controls for both aflatoxin and fumonisin contamination.

The experiment was arranged in an augmented alpha lattice incomplete block design. Planting was done by hand at two seeds per hole and three weeks after germination the seedlings were thinned out to one plant. Plots of 4.5m^2 size were arranged in one row of 5m per family, consisting of spacing of 0.9 m between the rows and 0.3m within the rows. Each row had 17 plants resulting in 17 plants per plot. In each plot, five plants were self-pollinated to advance to the $S_{4:5}$ generation, and the remaining 12 plants were subdivided by marking six plants with red and the other six with blue spray paint. Six plants marked red were artificially inoculated with *F. verticillioides* and another six plants marked blue with *A. flavus*. Inoculation was not done at Makhathini Research Station due to some logistical reasons.Basal fertilizers (NPK) were applied (75 kg N, 50 kg P, 25 kg K per hectare) before planting and four weeks after the seedlings had emerged, top dressing of 120 kg per hectare in the form of Limestone Ammonium Nitrate, LAN (28% N) was done. The

experiment was rain fed at both sites. The nursery was manually harvested on the 20th of May 2015.

3.5.3. Isolation, inoculum preparation and inoculation

3.5.3.1. Isolation of cultures

Naturally infected maize ears were obtained at Cedara Research Station as a source of isolates of *F. verticillioides* and *A. flavus* for inoculation. The fungal isolates were isolated and identified as described in Section 3.4 and fungal isolates were kept in 15% glycerol at -80 ºC.

3.5.3.2. Preparation of inoculum and inoculation process

The fungal isolate of *verticillioides* and *flavus* were cultured on plates containing selective media Synthetic Nutrient Deficient Agar and *A. flavus* Selective Media, respectively. Toothpicks were placed in 800 ml beaker and 300 ml of distilled water was added as described by Nordby et al. (2007). The beaker was then covered with Petri dish lids to keep the toothpicks tight during sterilization. Toothpicks were autoclaved for 15 minutes at 121°C and 1.5 cm^2. After cooling, toothpicks were rinsed five times repeatedly with distilled water and autoclaved again. The toothpicks were then seeded with cultured *F. verticillioides* and *A. flavus* as described by Nordby et al. (2007).

To ensure uniform disease infestation, artificial inoculation was conducted. Ears were inoculated 10 days after 50% silking by inserting a single contaminated

toothpick through the husk methodperpendicular to the ear axis and midway between the butt and ear. The toothpicks remained on the ears until harvest.

3.5.4. Assessment of Disease

Physiologically matured ears were harvested on the same day. Ears of the same plot were hand-picked, dehusked and evaluated for ear rot symptoms. The severity of the diseases were assessed by determining the percentage of each ear covered by symptoms using a 7- class rating scale, as described by Afolabi et al. (2007). The symptoms of infection by Fusarium ear rot (FER) are characterized by pinkish white mycelia growth on the grains and yellow-green mycelia for Aspergillus ear rot (AER).

3.5.5. Data collection on agronomic traits

Data was recorded on plot basis at Ukulinga and Makhathini Research Stations for the following traits using the descriptors for maize (IBPGR, 1991).

- Days to mid flowering: number of days from planting until the day when 50% of the plants showing silks.
- Grain texture: visual rating of kernel on a scale of 1 to 5 where 1= flinty endosperm and 5 = soft endosperm.
- Percentage of rotten ears: measured as percentage of the number of diseased ears as described by Afolabi et al. (2007).
- Husk cover was rated using a scale from 1 = long husk covering the entire length of the ear to 9 = short husk with ear protruding and kernels exposed.

- Insect damage ratings were recorded from 1 = no damage to 9 = heavy damage (Badu-Apraku et al., 2012).
- Plant height: recorded from the ground to the point of insertion of the flag leaf.
- Ear height: taken from the ground level to the insertion of the highest ear in the stem.
- Number of primary tassel branches (PTB): total number of primary tassel branches counted per plant/plot.
- Number of plants (NP): total number of plants counted for each row.
- Number of leaves (NL): the number of leaves above the ears.
- Grain yield (kg ha^{-1}) = (Field weight x10/plot area)* (100-GM) x Shelling percent. Where GM = Grain moisture percentage, shelling percentage (weight shelled/weight unshelled)*100.

3.6. Statistical analysis

3.6.1. Analysis of collected data

The data was analysed using GenStat 14th edition (2011), Agrobase Generation II (Agronomix 2008) and Number Cruncher Statistical System (NCSS, 2004) computer programs. Differences between inbred families were determined using Fisher's unprotected least significant differences (LSD) test. Average data was standardised and subjected to multivariate analysis in GenStat 14th edition (Payne et.al., 2007) and NCSS (2004) statistical computer programs. Data was subjected to analysis of variance and multivariate analysis to study and analyse genetic relationships among the mycotoxins resistant maize families. Principal component analysis (PCA) and cluster analysis (CA) were used to discriminate and group genotypes respectively.

Principal Component Analysis was used to determine the phenotypic traits that contributed to variation among the families. Cluster analysis, based on Euclidean distances as dissimilarity measures and the Unweighted Pair-Group Method with Arithmetic Averages (UPGMA), was employed to determine the genetic relationships among and between them. Linear Pearson's correlation coefficients were used to decide on the relationship between selected traits.

3.6.2. Estimation of heritability

The constituents of variance were estimated using REML tool, the replications were regarded as fixed effects and the genotype effects were considered a random. Heritability for single environment on an entry mean basis was then calculated using the following formula suggested by Nyquist (1991): $H^2 = \sigma^2_G / \{\sigma^2_G + (\sigma^2_e / r)\}$.

Where H^2 is the broad sense heritability, σ^2_e is the overall error variance, σ^2_G is the genotypic variance and r is the number of replications for the experiment.

CHAPTER FOUR

Results

4.1. Introduction

This chapter presents the findings observed from the completed experiments, including the results on natural incidences of ear rot disease on Southern African maize hybrids, the level of agro-morphological variability and genetic distances, relationship between *A. flavus*, *F. verticillioides* infections and secondary traits. The results on heritability among the mycotoxins resistant maize inbreds families for grain yield, ear rots and secondary traits are also presented.

4.2. Meteorological data

Weather conditions influence the incidence and severity of fungal diseases. Therefore weather data was collected at all stations where the study was conducted. The average temperature at Ukulinga Research Station from October 2014 to July 2015 was 19.45°C and average rainfall of 57 mm. The temperature reached a maximum of 29.4°C in March, minimum temperature of 7.5°C in July (Figure 4.1). Makhathini Research Station reached a maximum temperature of 21°C in October and minimum temperature of 13 °C in July (Figure 4.2). The average temperature at Makhathini Research Station from October 2014 to July 2015 was 19.45°C and average rainfall of 24 mm. The average relative humidity (%) for Cedara Research Station from October 2013 to July 2015 was 77.81%, the maximum relative humidity was 90.2% in Jan 2014 and the minimum relative humidity of 51.7% was reached in July 2014. The average temperature at Cedara Research Station from October 2013 to July 2015 was 19.45 °C. The temperature reached a maximum of 36.9°C in

January 2015 and minimum temperature of 6.2°C in July 2015. The average rainfall at Cedara Research Station from October 2013 to July 2015 was 58.06 mm, with maximum of 229.4 mm of rainfall accumulated in March 2015 and minimum rainfall of 1.8 mm in July 2014 .

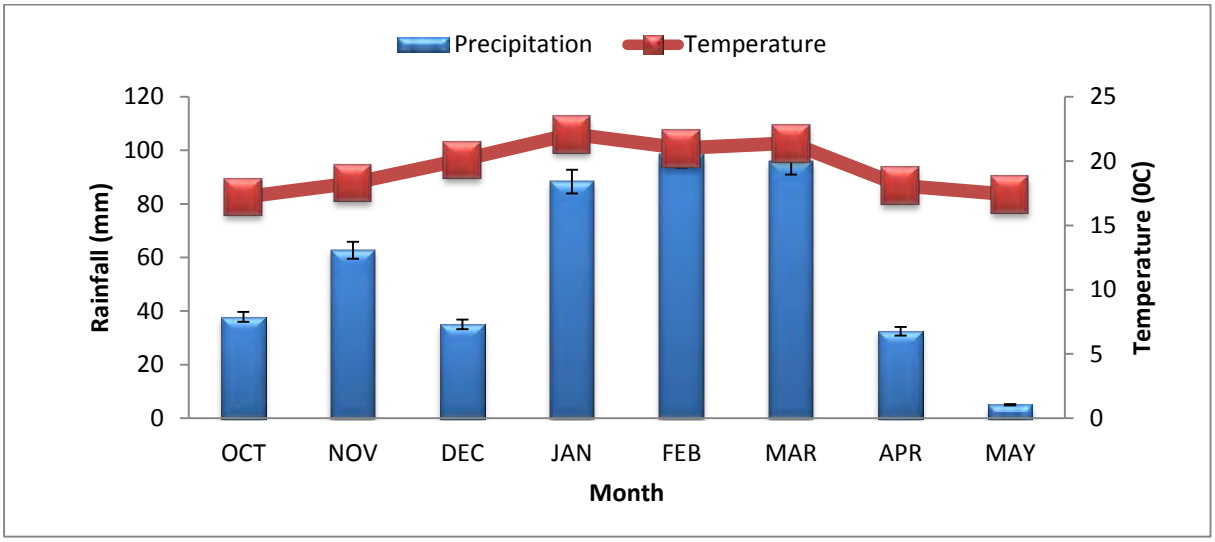


Figure 4.1: Rainfall (mm) and temperature (°C) records from October 2014 to July 2015 at Ukulinga research Station.

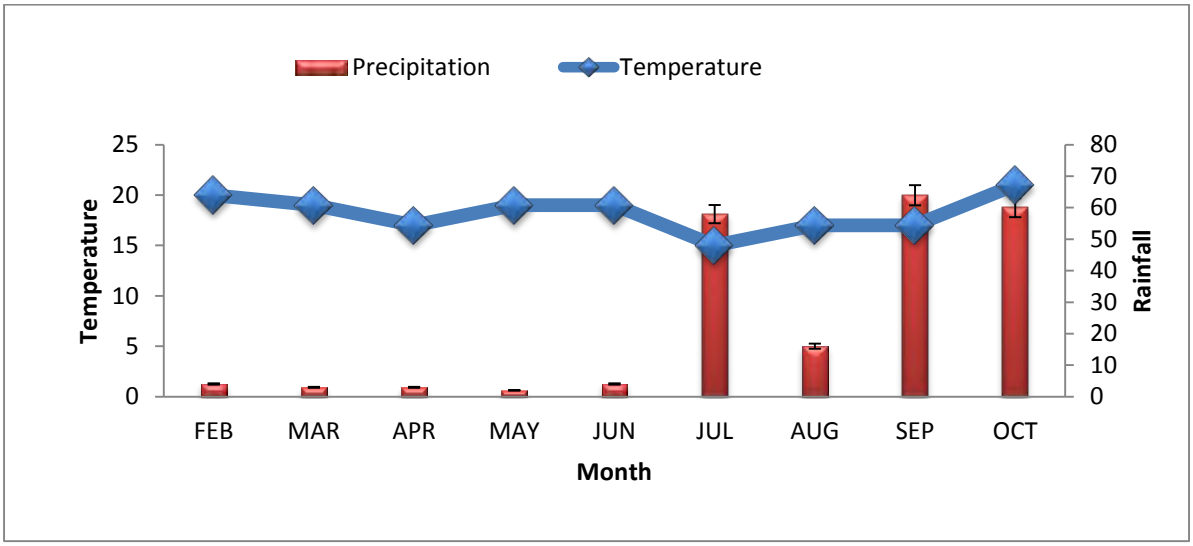


Figure 4.2: Rainfall (mm) and temperature (°C) records from February 2015 to October 2015 at Makhathini Research Station.

Table 4.1: Rainfall (mm), temperature (°C) and relative humidity records from October 2013 to July 2015 at Cedara research Station.

Date	Temperature	Maximum temperature	Minimum temperature	Humidity	Precipitation
Oct-13	16.4	35.3	6.8	68.2	12.2
Nov-13	16.8	36.8	8	77.9	94
Dec-13	18.4	36.6	10.4	83.8	10.2
Jan-14	18.2	31.3	12.5	90.2	89.7
Feb-14	20.9	34.2	14.8	87.5	57.9
Mar-14	21.4	34.2	13.9	84.6	75.2
Apr-14	20.4	32.6	11.6	84.4	134.6
May-14	17.8	30.3	7.7	75.1	10.2
Jun-14	17.5	31.2	7.9	66	1.8
Jul-14	15.6	29	5.3	51.7	3.8
Oct-14	18.4	35.7	7.7	67	19.3
Nov-14	16	34.7	8.3	82	65.5
Dec-14	17.2	31.4	9.2	85.9	90.2
Jan-15	19.2	36.9	13.3	86.8	52.8
Feb-15	20.5	34.8	13	85.2	76.5
Mar-15	20	33.9	11.5	87.6	229.4
Apr-15	20.3	33.8	11.8	82.8	90.9
May-15	17.2	29.6	8.8	82.8	40.9
Jun-15	18.2	30.2	10.3	66.6	3.3
Jul-15	15	26.2	6.2	60	2.8

TM-maximum temperature (^{0}C), Tm-minimum temperature (^{0}C), H- relative humidity (%).

4.3. The levels of ear rot disease incidence on regional experimental maize hybrids

4.3.1. Incidences of ear rots

Significant variation was observed (Table 4.2 and 4.3) among regional maize hybrids for incidences of ear rot over two growing seasons. The mean squares for early, intermediate and late maturing hybrids showed highly significant differences. *Aspergillus flavus* was the least prevalent ear rot causing fungus (Figure 4.3), followed by *S. maydis and F. graminearum* and the most prevalent fungus was *F. verticillioides* in the 2013/14 season. For the 2014/15 growing season, *F.*

verticillioides was the most prevalent fungi followed by *S. maydis, F. graminearum,* and then *A. flavus* .The overall incidences of ear rots were higher during the 2013/14 growing season compared to the 2014/15 season.

Table 4.2: Analysis of variance for ear rots incidence for early, medium and late maturity maize hybrids during the 2013/14 season at Cedara (adapted from Chiuraise, 2014).

	Early maturity		Medium maturity		Late maturity	
Source	**df**	**Ms**	**df**	**ms**	**df**	**ms**
Rep.	2	10.26	2	8.94	2	0.24
Entry	59	2.23***	59	3.20***	39	3.72***
Error	118	1.13	118	1.13	78	1.30
Total	179		179		119	
LSD		1.72		1.72		1.85
Cv (%)		20.4		19.3		19.2

*** **, * significant at P < 0.05, P < 0.01, P < 0.001 probability level, respectively.

Table 4.3: Analysis of variances of natural ear rot incidences of regional hybrids, for summer growing season of 2014/15 at Cedara Research Station.

	Early maturity		Medium maturity		Late maturity	
Source	**df**	**Ms**	**df**	**ms**	**Df**	**ms**
Rep.	2	0.66	2	21.77	2	2.60
Entry	54	10.09**	59	22.44*	41	59.78**
Error	108	0.86	118	15.88	82	4.90
Total	164		179		125	
LSD		1.499		2.394		1.597
Cv (%)		7.2		33.8		9.3

*, **, represents the term is significant at P≤0.01, P≤0.05, not significant respectively.

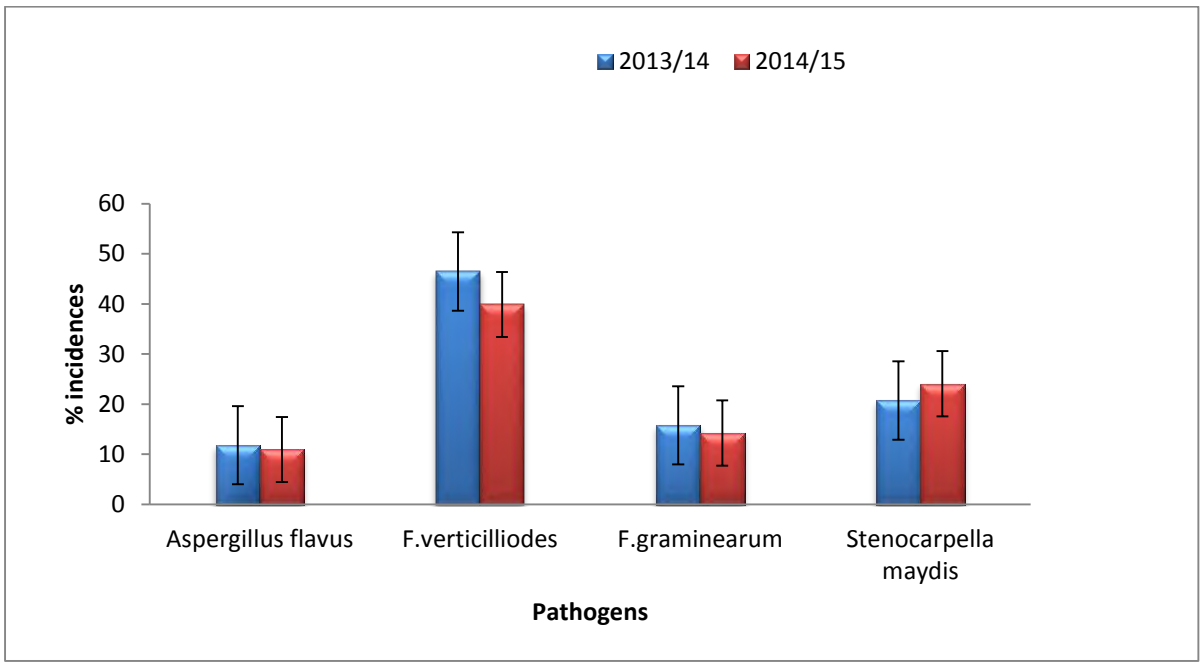


Figure 4.3: Incidences of ear rots causing fungi on regional maize hybrids evaluated at Cedara Research Station in summer season of 2013/14 and 2014/15.

More incidences of ear rots were recorded on late maturing hybrids for both growing seasons (37% and 35%, respectively), intermediate maturing hybrids recorded 34.9% of ear rot for 2013/14 and 32% of ear rot incidences for year 2014/15 (Figure 4.4). Hybrids that matured early were noticed to have low incidences of ear rots, in 2013/14 cropping season, 29% infections were recorded, while 26% of infections were recorded in 2014/15 cropping season.

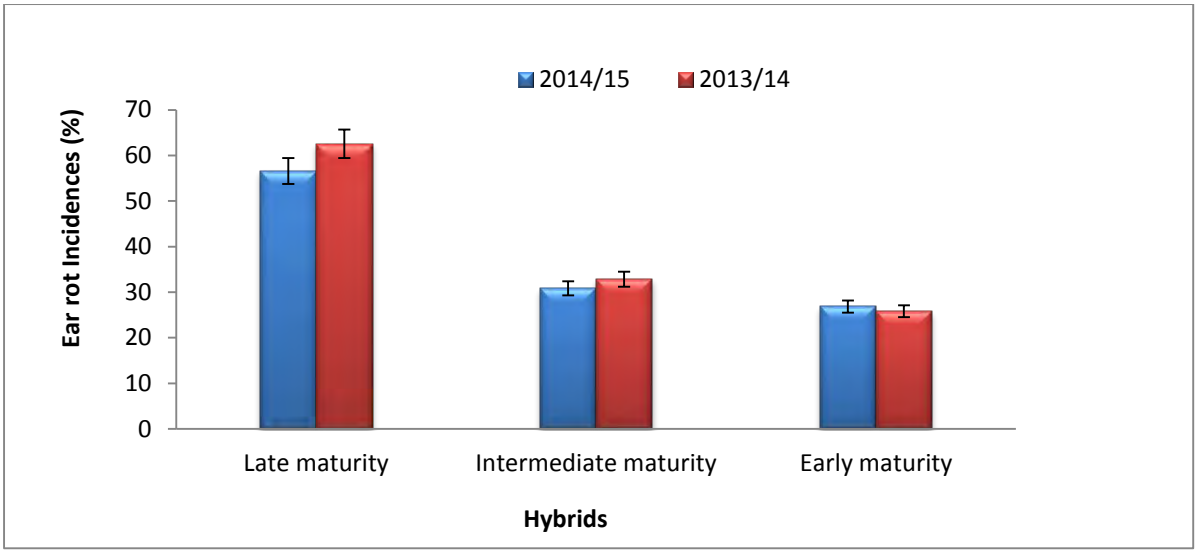


Figure 4.4: The percentage mean incidences of infected ears for the early, intermediate and late maturing maize hybrids evaluated at Cedara Research Station in summer season of 2013/14 and 2014/15.

4.3.2. Correlation between, ear rots and selected agronomic traits

Correlation between cob rot incidences and selected agronomic traits were obtained from two cropping seasons on early, medium and late maturing hybrids to prove if there is a relationship among them (Table 4.4). Cob rots had a significant negative correlation with grain yield in both seasons for the different hybrids maturity groups. Significant positive correlations were observed for days to anthesis, silking and grain texture for early and late maturing hybrids in 2014/15 growing season. The relationship between cob rots and ASI was not significant for all hybrids. Cob rots showed a strong and positive association with insects damage. The results also showed no correlations between grain moisture content and cob rots incidences.

Table 4.4: Correlation coefficients of ear rot incidence and selected agronomic traits for 317 regional maize hybrids, planted at Cedara research station.

Hybrids	Early maturity		Average maturity		Late maturity	
	2013/14	2014/15	2013/14	2014/15	2013/14	2014/15
Traits						
Days to Anthesis	0.17**	0.38***	0.21**	0.35ns	0.12ns	0.86***
Days to silking	0.16*	0.23**	0.22*	0.82ns	0.13ns	0.36**
Anthesis silking interval	-0.03ns	-0.05ns	0.09ns	-0.15ns	0.14ns	-0.17ns
Grain moisture	-0.04ns	0.17ns	-0.07ns	0.03ns	0.03ns	-0.04ns
Grain yield	-0.52***	-0.55***	-0.48***	-0.45***	-0.43**	-0.35***
Grain texture	0.27***	0.31**	0.30***	0.31***	0.24***	0.27**
Insects damage	0.70***	0.54***	0.74***	0.63***	0.70***	0.65*

***, **, *, ns represent significance at P < 0.05, P < 0.01, P<0.001, ns respectively.

4.4. Agro-morphological characterization among mycotoxins resistant maize inbred families

4.4.1. Sample for Characterization of $S_{3:4}$ families

Thirty two mycotoxins resistant maize inbred families were randomly selected from the 208 inbred families which were evaluated at Ukulinga and Makhathini Research

Station. The sampled families (as described in Table 4.5) were used for phenotypic evaluation.

Table 4.5: List of thirty two randomly selected mycotoxins resistant inbred families.

Name	Pedigree	Origin
DMTX-356	DMTX-356:((CML390/12MAKCB4-123//TZAR103)-2)-5	CEDARA-14CED1-90-5
DMTX-142	DMTX-142:((CML444/08CED6-7//TZAR102)-2)-2	CEDARA-14CED1-36-2
DMTX-610	DMTX-610:((TZAR103/TZAR102)-2)-4	CEDARA-14CED1-160-4
DMTX-256	DMTX-256:((CML390/12MAKCB4-3//TZAR103)-2)-4	CEDARA-14CED1-63-4
DMTX-613	DMTX-613:((TZAR103/TZAR102)-3)-2	CEDARA-14CED1-161-2
DMTX-358	DMTX-358:((CML390/12MAKCB4-123//TZAR103)-3)-2	CEDARA-14CED1-91-2
DMTX-605	DMTX-605:((TZAR103/TZAR102)-1)-4	CEDARA-14CED1-159-4
DMTX-176	DMTX-176:((CML444X12MAK9-136//TZAR102)-2)-2	CEDARA-14CED1-44-2
DMTX-546	DMTX-546:((CML444X12MAKCB4-9//TZAR103)-2)-3	CEDARA-14CED1-139-3
DMTX-16	DMTX-16:((CML390/12MAKCB4-3//TZAR102)-1)-2	CEDARA-14CED1-4-2
DMTX-96	DMTX-96:((CML444/12MAKCB4-120//TZAR102)-4)-1	CEDARA-14CED1-25-1
DMTX-387	DMTX-387:((CML390/PAN6611//TZAR103)-4)-2	CEDARA-14CED1-99-2
DMTX-409	DMTX-409:((CML444/12MAKCB4-123//TZAR103)-1)-1	CEDARA-14CED1-106-1
DMTX-531	DMTX-531:((CML444X12MAK9-112//TZAR103)-1)-4	CEDARA-14CED1-135-4
DMTX-296	DMTX-296:((CML390/12MAKCB4-44//TZAR103)-2)-1	CEDARA-14CED1-74-1
DMTX-363	DMTX-363:((CML390/12MAKCB4-123//TZAR103)-5)-1	CEDARA-14CED1-93-1
DMTX-421	DMTX-421:((CML444/12MAKCB4-123//TZAR103)-4)-2	CEDARA-14CED1-109-2
DMTX-422	DMTX-422:((CML444/12MAKCB4-123//TZAR103)-4)-3	CEDARA-14CED1-109-3
DMTX-382	DMTX-382:((CML390/PAN6611//TZAR103)-3)-1	CEDARA-14CED1-98-1
DMTX-367	DMTX-367:((CML390/PAN6227F2-8//TZAR103)-1)-1	CEDARA-14CED1-94-1
DMTX-533	DMTX-533:((CML444X12MAK9-112//TZAR103)-2)-1	CEDARA-14CED1-136-1
DMTX-450	DMTX-450:((CML444/PL720//TZAR103)-4)-3	CEDARA-14CED1-116-3
DMTX-452	DMTX-452:((CML444/PL720//TZAR103)-4)-5	CEDARA-14CED1-116-5
DMTX-342	DMTX-342:((CML390/12MAKCB4-104//TZAR103)-1)-2	CEDARA-14CED1-87-2
DMTX-223	DMTX-223:((CML390/12MAKCB3-1//TZAR103)-5)-2	CEDARA-14CED1-56-2
DMTX-225	DMTX-225:((CML390/12MAKCB3-1//TZAR103)-5)-4	CEDARA-14CED1-56-4
DMTX-493	DMTX-493:((CML444/08CED6-7//TZAR103)-4)-1	CEDARA-14CED1-128-1
MTX-113	DMTX-113:((CML444/12MAKCB4-123//TZAR102)-3)-5	CEDARA-14CED1-28-5
MTX-284	DMTX-284:((CML390/12MAKCB4-42//TZAR103)-3)-2	CEDARA-14CED1-71-2
MTX-642	DMTX-642:((CML390)-1)-4	CEDARA-14CED1-171-4
MTX-599	DMTX-599:((TZAR102)-5)-1	CEDARA-14CED1-158-1
DTA	DTA	

4.4.2. Phenotypic variation among mycotoxins resistant inbred families

The inbred families ($S_{3:4}$ families) showed significant differences in general ear rot infections by both *Fusarium verticillioides* and *Aspergillus flavus*. Infection by *F. verticillioides* ranged from 1 (0% infections) to 5.33 (50% infections) and from 1 (0% infections) to 4.67 (40% infections) for *A. flavus.* The negative control (DTA) showed severe infections by both fungi, followed by inbred line DMTX-256 (Table 4.6). Inbred lines DMTX-96 and DMTX-387 had no signs of infection. Fusarium ear rot was more prevalent (Figure 4.5) than Aspergillus ear rot. Table 4.6 shows the top ten most resistant inbred familes, the bottom five susceptible lines and controls (positive and negative).

Significant variability was observed among the mycotoxins resistant inbred families for all secondary traits except for husk cover, insect damage and days to mid maturity (Table 4.7 and 4.8). DMTX-96 registered the highest plant and ear height, and it also recorded the highest yield (Table 4.10). An association among the traits was also observed. Plant height and ear height showed a positive correlation. Fusarium and Aspergillus ear rot correlated positively with insect damage. Grain yield was negatively correlated with the ear rots (Table 4.11).

Table 4.6: Evaluation of selected mycotoxin resistant inbred familes for Fusarium and Aspergillus ear rot during 2014/15 season.

Inbred lines	Grain texture	Fusarium ear rot (score)	Aspergillus ear rot (score)
Top 10			
DMTX-96	Flint	1.00a	1.00a
DMTX-605	Flint	1.00a	1.00a
DMTX-409	Flint	1.00a	1.67abc
DMTX-422	Flint	1.17ab	2.33cd
DMTX-225	Flint	1.17ab	1.00a
DMTX-610	Flint	1.33abc	2.00bc
DMTX-387	Flint	1.33abc	1.00a
DMTX-533	Flint	1.33abc	1.00a
DMTX-356	Flint	1.67abcd	1.67abc
DMTX-452	Flint	1.67abcd	1.00a
Bottom 5			
DMTX-367	Flint	3.00efg	3.07de
DMTX-284	Flint	3.00efg	3.00de
DMTX-176	Flint	3.07fg	2.50cde
DMTX-113	Flint	3.83gh	3.30ef
DMTX-256	Dent	4.67hi	3.33ef
Controls			
DMTX-599 (Resistant control)	Flint	1.50abcd	1.00a
DMTX-642 (Resistant control)	Dent	1.00a	1.33ab
DTA (negative control)	Flint	5.33i	4.67g
Mean		2.10	1.99
LSD		1.18	0.90
%CV		28.0	31.4

Means of the same letter do not differ significantly according to Fischer's least significant difference test (P≤ 0.05).

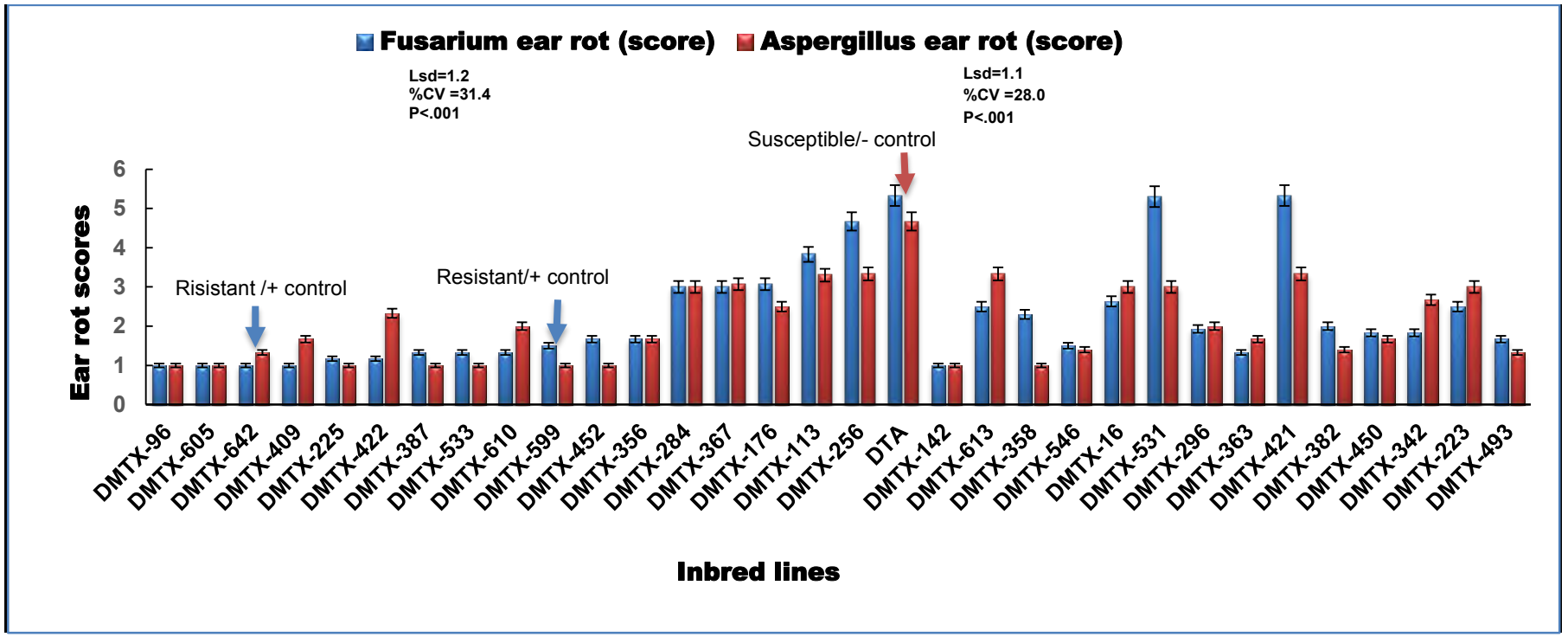


Figure 4.5: The severity of ear rot among the thirty two selected resistant inbred lines including positive and negative control.

Table 4.7: Analysis of variance for the yield, ear rots and other selected secondary traits of the $S_{3:4}$ maize families evaluated at Ukulinga Research Station.

Traits		Mean Squares											
Source	**df**	**FER**	**AER**	**PH**	**EH**	**HC**	**ISD**	**GT**	**GY**	**DMF**	**NL**	**PTB**	**NP**
Rep	2	0.6	0.1	46.3	38.2	2.9	2.2	0.4	408	27.4	0.1	2.9	1.1
Entry	31	6.1**	3.4**	731.8**	702.2**	1.7ns	1.9ns	1.6*	2206.7**	36.4ns	1.5**	68.0**	5.9**
Error	62	0.5	0.3	97.8	58.2	0.3	0.3	0.3	238	6.3	0.3	4.2	1.3
Total	95												
LSD		1.1	1.2	16.1	12.5	0.9	0.9	0.9	29	4.1	0.9	3.3	1.9
Cv %		28	31.4	4.1	6.8	29.2	31	29.3	16	2.6	8.8	13.6	7.1

FER- Fusarium ear rot, AER-Aspergillus ear rot, GY-Grain yield (kg ha^{-1}), DMF-days to mid flowering, EH-ear height (cm), PH-plant height (cm), NL-number of leaves above the first ear, NP- number of plants/row, PTB-primary tassel branching,GT-grain texture, HC-huskcover, ISD-insect damage.
Ns, **, * significant at $P < 0.05$, $P < 0.01$ respectively.

Table 4.8: Analysis of variance for the selected secondary traits of the sampled $S_{3:4}$ maize families evaluated at Makhathini Research Stations.

Traits		Mean squares									
Source	**df**	**PH**	**EH**	**HC**	**ISD**	**GT**	**GY**	**DMF**	**NL**	**PTB**	**NP**
Rep	2	796.9	26.3	0.1	0.1	0.6	200.04	20.5	0.1	8.2	0.1
Entry	31	464.0**	697.9**	9.9*	4.4*	2.3*	1017.03**	52.5**	1.5**	71.2**	9.6**
Error	62	281.1	54.1	0.1	0.1	0.8	352.86	7.7	0.3	4.4	0.1
Total	95										
LSD		27.4	12	0.2	0.2	1.23	30.7	4.1	4.5	3.4	0.3
Cv %		7.4	6.4	8.9	11.5	24.7	17.1	2.6	3.2	12.3	1.4

EH-ear height (cm), PH-plant height (cm), DMF-days to mid flowering, NL-number of leaves above the first ear, NP- number of plants/row, PTB-primary tassel branching, GT-grain texture, HC- husk cover, ISD-insect damage, GY-Grain yield (kg ha^{-1}).
Ns, **, * significant at $P < 0.05$, $P < 0.01$ respectively.

Table 4.9: Analysis of variance for selected secondary traits of the Sampled $S_{3:4}$ maize families evaluated at Ukulinga and Makhathini Research Stations.

Source		Mean square									
Traits	**DF**	**PH**	**EH**	**HC**	**ISD**	**GT**	**GY**	**DMF**	**NL**	**PTB**	**NP**
LOC	1	605.5	145.9	12.3	4.8	0.3	12.5	29.1	0.4	99.2	111
REP(LOC)	4	451.1	58.5	0.1	0.1	0.4	131.6	20.4	0.1	5.0	2.8
ENTERY	31	997.4**	755.8**	1.6ns	1.1ns	2.6	1867.2**	39.5**	1.3	59.4**	3.9ns
LOC* ENTRY	31	544.2**	389.5*	1.2ns	0.7ns	0.9	1356.5**	42.3**	1.0**	51.0**	3.6ns
ERROR	124	190.1	155.8	0.1	0.2	0.3	295.3	12.3	0.4**	11.2	2.9
TOTAL	191										
LSD		15.8	14.3	0.4	0.5	1.26	19.6	4	0.7	3.8	1.9
Cv %		5.9	10.9	23.2	31.3	20.4	15.6	6.2	9.7	20.4	10.8

GY-Grain yield (kg ha^{-1}), DMF-days to mid flowering, EH-ear height (cm), PH-plant height (cm), NL-number of leaves above the first ear, NP- number of plants/row, PTB-primary tassel branching,GT-grain texture, HC-husk cover, ISD-insect damage. Ns. **, * significant at $P < 0.05$, $P < 0.01$ respectively.

Table 4.10: Means of 10 selected secondary traits for the thirty two randomly selected $S_{3:4}$ families.

Inbred Lines	DMF	EH	PH	NL	NP	PTB	GT	HC	ISD	GY
DMTX-356	101	109.3	216.0	6	16	16	1	1.7	1.0	83.3
DMTX-142	97	123.3	238.3	6	17	16	2	1.0	1.7	130.7
DMTX-610	95	131.0	261.3	6	15	18	2	1.0	1.0	149.7
DMTX-256	95	118.3	240.3	5	18	20	2	1.7	1.7	49.0
DMTX-613	101	120.0	259.3	5	17	14	2	1.7	1.7	87.7
DMTX-358	95	128.7	248.7	6	18	7	1	1.0	1,3	109.3
DMTX-605	96	101.0	233.7	5	16	8	1	1.0	1.0	106.0
DMTX-176	89	96.3	246.0	5	17	9	2	1.0	1.3	128.3
DMTX-546	99	107.0	241.0	5	14	20	2	1.0	1.0	103.3
DMTX-16	101	126.0	246.3	6	18	15	3	1.0	1.3	126.7
DMTX-96	93	135.3	266.7	7	17	13	1	1.0	1.0	154.0
DMTX-387	93	126.7	248.3	6	16	15	2	1.0	1.0	119.3
DMTX-409	93	104.7	231.3	6	17	17	2	1.0	1.0	128.0
DMTX-531	95	136.7	262.7	6	15	13	2	1.0	1.3	120.0
DMTX-296	97	135.0	257.7	6	17	9	2	1.0	1.7	122.0
DMTX-363	100	115.7	251.0	6	16	22	2	1.0	1.3	146.7
DMTX-421	93	122.0	232.7	5	15	17	2	1.0	1.0	113.3
DMTX-422	97	106.7	228.3	5	16	17	2	2.7	2.0	95.0
DMTX-382	93	112.3	237.0	6	18	17	2	2.0	2.0	110.7
DMTX-367	89	114.3	241.0	6	18	25	2	1.0	1.0	93.0
DMTX-533	91	123.7	236.7	6	18	27	2	1.0	1.0	96.0
DMTX-450	94	107.0	227.0	6	16	21	2	1.0	1.0	107.7
DMTX-452	99	127.7	237.0	6	17	9	2	1.0	1.0	102.0
DMTX-342	98	95.7	223.7	7	17	9	2	1.0	1.3	118.7
DMTX-223	101	89.3	229.3	7	16	13	2	1.7	1.7	111.7
DMTX-225	102	109.3	233.0	5	16	13	1	1.0	1.0	120.3
DMTX-493	99	94.0	212.0	6	18	15	1	1.0	1.0	89.3
DMTX-113	97	110.3	235.7	7	15	13	2	1.0	1.0	58.7
DMTX-284	90	107.7	230.7	7	16	19	2	1.0	1.0	107.0
DMTX-642(+ control)	95	107.3	256.0	7	16	15	3	1.7	1.7	133.0
DMTX-599(+ control)	95	138.0	265.7	8	16	17	2	1.7	1.7	150.0
DTA(- control)	92	57.3	152.0	4	16	10	4	1.0	1.3	38.7
Overall Mean	96	114	238	6	16	15	2	1	1	110
Mean Square	36.4ns	702.2**	731.8**	1.8**	3.3**	36.4**	1.6**	1.7ns	1.9ns	2365.5**
LSD	4.16	12.67	15.70	0.83	2.84	3.43	0.94	1.01	1.04	25.16
Cv (%)	2.70	6.80	4	8.40	10.60	13.70	30.10	41.40	39.80	14.10

The symbols (ns, **) represent the term is non-significant and significant at P <0.05 respectively. CV = coefficient of variation, LSD = least significant difference. GY-Grain yield (kg ha^{-1}), DMF-days to mid flowering, EH-ear height (cm), PH-plant height (cm), NL-number of leaves above the first ear, NP- number of plants/row, PTB-primary tassel branching, GT-grain texture, HC- husk cover, ISD-insect damage.

Table 4.11: Phenotypic association among ten secondary traits for the mycotoxin resistant $S_{3:4}$ maize families.

Traits	PH	EH	NL	FER	AER	GT	GY	HC	ISD	PTB	NP	DMF
PH	1	0.75*	0.09	-0.02	0.03	-0.11	0.29*	0.03	0.03	-0.09	0.1	0.49
EH		1	0.12	0.02	-0.04	-0.13	0.21*	0.01	0.04	0.02	0.06	0.41
NL			1	0.01	0.01	0.04	0.17*	0.02	-0.01	0.08	-0.17	0.07
FER				1	0.20**	0.15**	-0.10**	0.18*	0.27**	0.05	-0.15	-0.1
AER					1	0.03*	-0.11**	0.20*	0.25*	-0.08	0.06	-0.11
GT						1	0.11*	0.12	0.03	0.03	0.09	-0.11
GY							1	0.01	0.11	0.01	0.14	0.14*
HC								1	0.67**	0.02	0.06	0.02
ISD									1	0.02	0.1	0.08
PTB										1	0.09	0.05
NP											1	0.02
DMF												1

**,*, indicates the term is significant at $P < 0.05$, $P < 0.01$ respectively. FER- Fusarium ear rot, AER-Aspergillus ear rot, GY-Grain yield (kg ha^{-1}), DMF-days to mid flowering, EH-ear height (cm), PH-plant height (cm), NL-number of leaves above the first ear, NP- number of plants/row, PTB-primary tassel branching, GT-grain texture, HC- husk cover, ISD-insect damage.

4.4.3. Heritability estimates of phenotypic traits for mycotoxin resistant inbred families

Plant height, ear height and primary tassel branches recorded higher heritability compared to all the other traits, followed by grain yield, Fusarium ear rot and Aspergillus ear rot resistance. Traits with moderate heritability estimates were are days to mid flowering, number of leaves and grain texture. The traits with low heritability were number of plants, husk cover and insects damage (Table 4.12).

Table 4.12: Means, mean squares, variance components and heritability estimates of phenotypic traits for mycotoxin resistant inbred lines evaluated at Ukulinga Research Station.

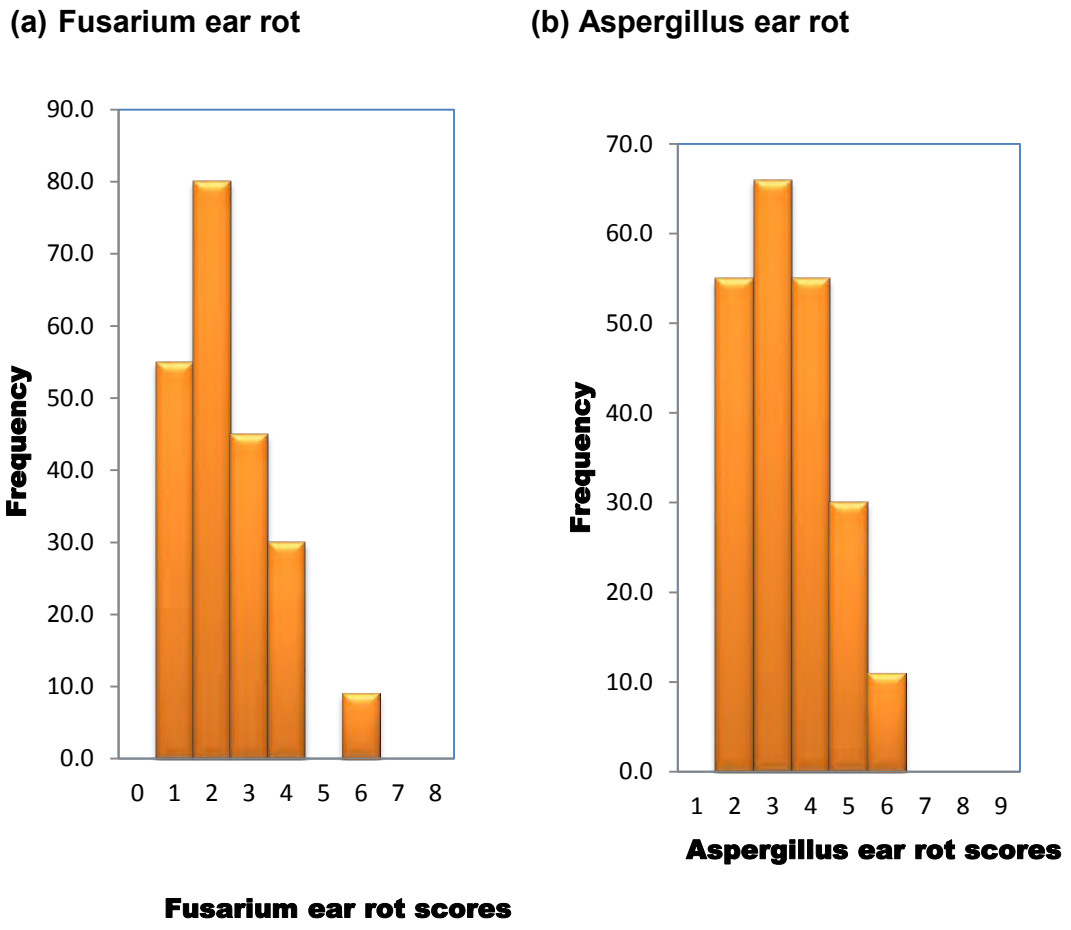
Traits	FER	AER	PH	EH	DMF	NL	NP	PTB	GT	HC	ISD	GY
Mean	2.3	2.0	238.3	113.7	95.5	6.0	16.0	15.0	1.9	1.2	1.3	109.7
MSg	6.1	3.4	1323.9	842.0	37.5	1.8	3.3	68.3	0.9	0.5	0.3	2206.7
Mse	0.5	0.3	92.5	60.2	6.5	0.3	3.0	4.4	0.3	0.4	0.4	237.7
σ^2g	1.9	1.0	410.5	260.6	10.3	0.5	0.1	21.3	0.2	0.0	0.0	656.3
σ^2p	2.4	1.3	502.9	320.8	16.8	0.8	3.1	25.7	0.5	0.4	0.4	894.0
H^2 (%)	78.2	77.2	81.6	81.2	61.3	67.2	3.2	82.8	36.0	8.2	7.3	73.4

MSg-mean square of genotypes, Mse-mean square of error, FER- Fusarium ear rot, AER-Aspergillus ear rot, GY-Grain yield (kg ha^{-1}), DMF-days to mid flowering, EH-ear height (cm), PH-plant height (cm), NL-number of leaves above the first ear, NP- number of plants/row, PTB-primary tassel branching, GT-grain texture, HC-husk cover, ISD-insect damage, σ^2_e - overall error variance, σ^2_G - genotypic variance.

4.4.4. Frequency distribution of the secondary traits

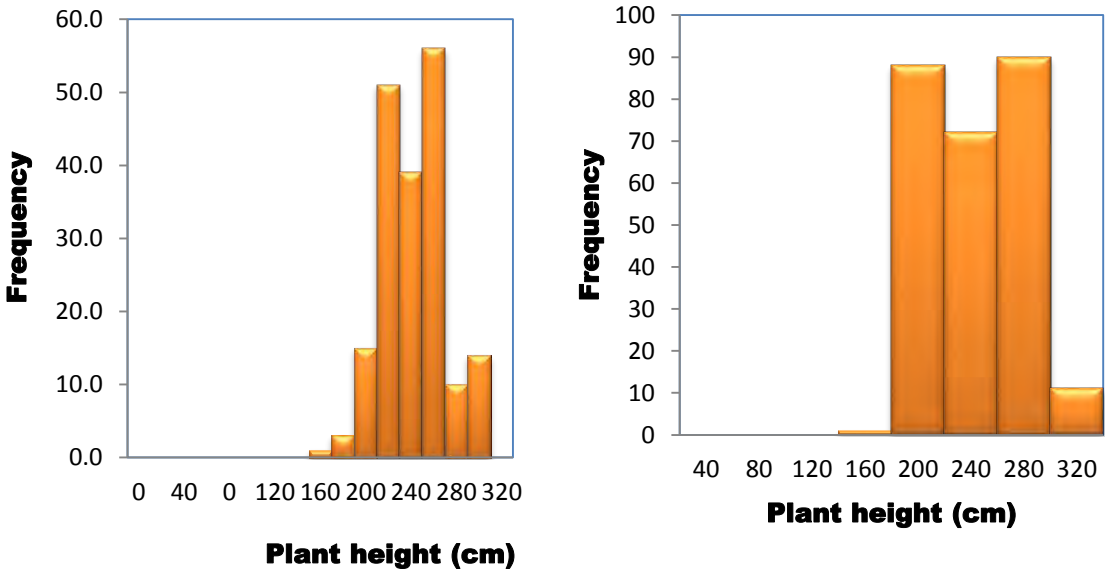
The frequency distribution of phenotypic traits for the mycotoxins resistant inbred families are shown from Figure 4.6 to Figure 4.23 (population 1 refers to inbred families at Ukukinga Research Station and, population 2 refers to families evaluated

at Makhathini Research Station). The frequency distributions indicates large variation among the $S_{3:4}$ families based on the phenotypic traits.



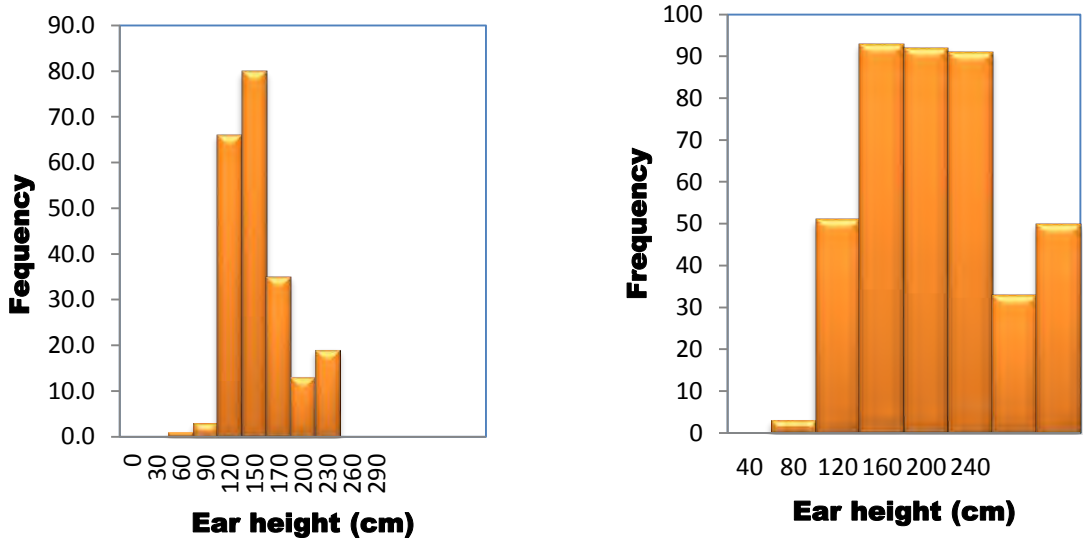
Figures 4.6 and 4.7: Fusarium and Aspergillus ear rot distribution among the maize $S_{3:4}$ families.

(c) Plant height



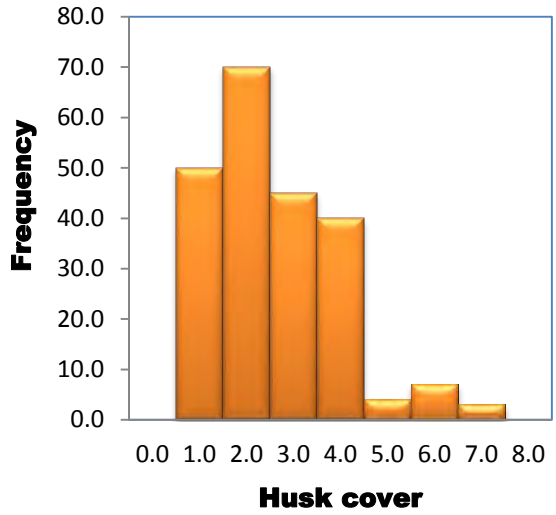
Figures 4.8 and 4.9: The frequency distribution of plant height data for Population 1 and Population 2.

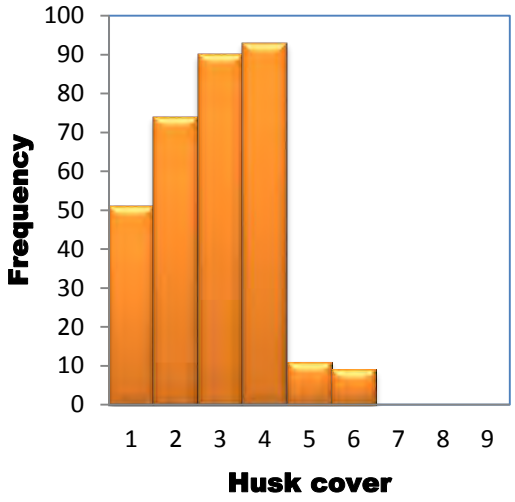
(d) Ear height



Figures 4.10 and 4.11: The frequency distribution of ear height for Population 1 and Population 2.

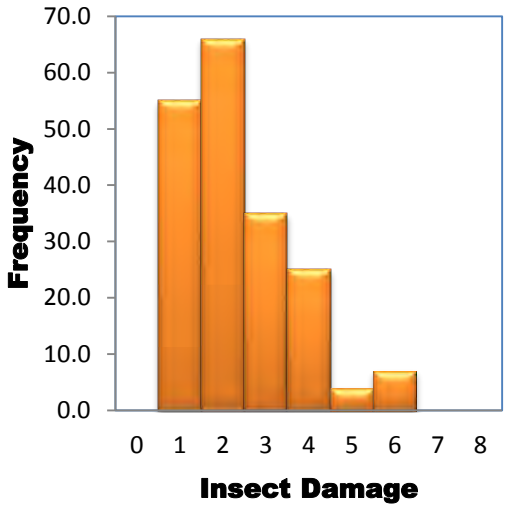
(e) Husk cover

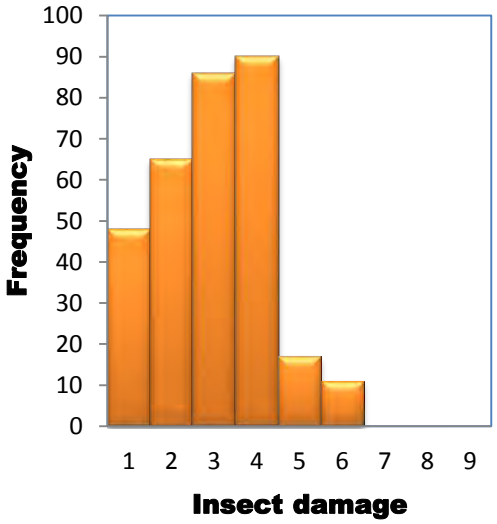




Figures 4.12 and4.13: The frequency distributions of husk cover for Population 1 and Population 2.

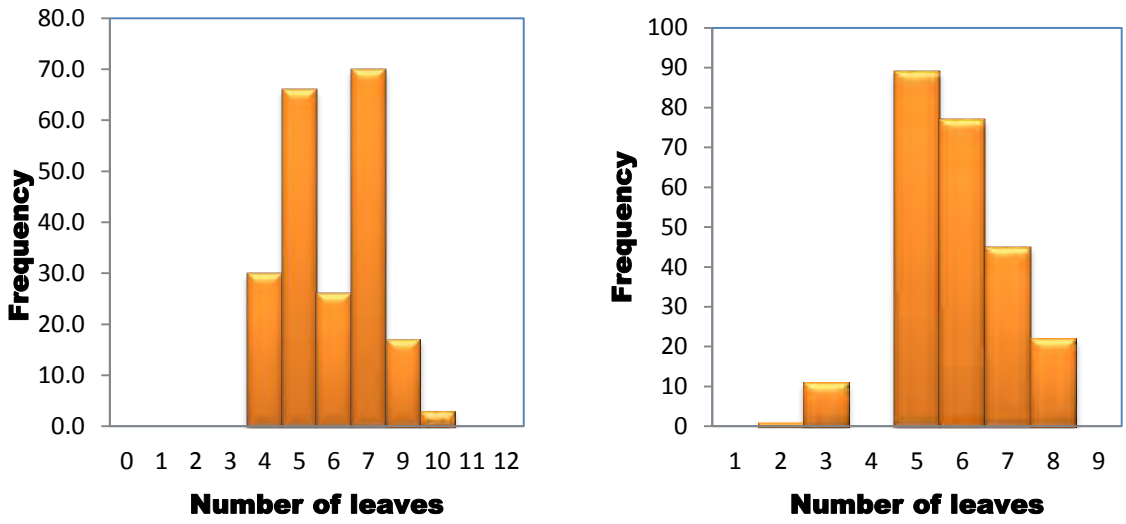
(f) Insects damage





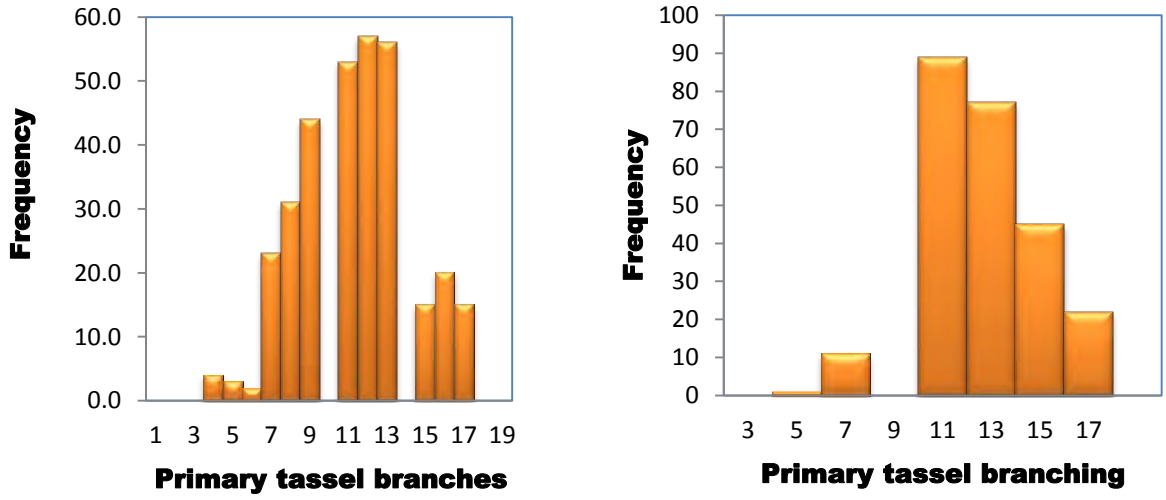
Figures 4.14 and 4.15: The frequency distribution of insects damage for Population 1 and Population 2.

(g) Number of leaves



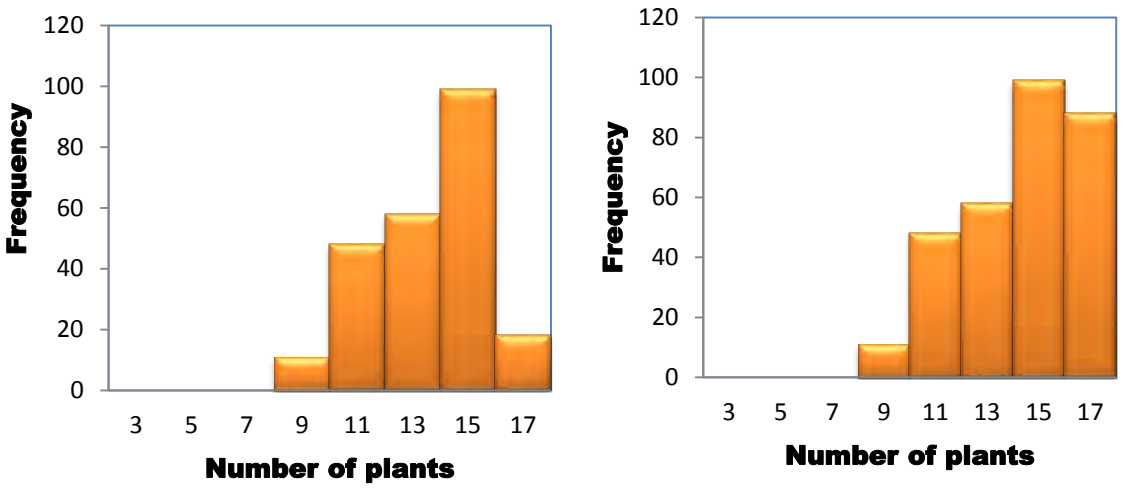
Figures 4.16 and 4.17: The frequency distribution of number of leaves above the ears for Population 1 and Population 2.

(h) Primary tassel branching



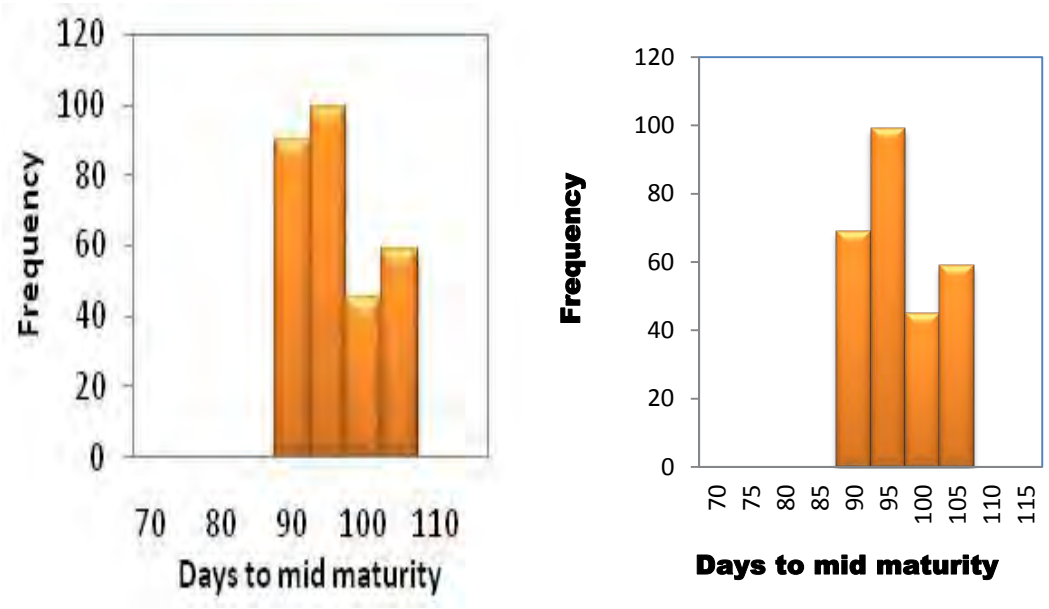
Figures 4.18 and 4.19: The frequency distribution of primary tassel branches for Population 1 and Population 2.

(i) Number of plants



Figures 4.20 and 4.21: The frequency distribution of number of plantsfor Population 1 and Population 2.

(j) Days to mid pollination



Figures 4.22 and 4.23: The frequency distribution of days to mid pollination Population 1 and Population 2.

4.4.5. Principal component analysis

Table 4.13 shows that five principal components contributed more than 69% of the total variation. The first principal component accounted for 22.2% of the total variability and the traits responsible for the variation were plant height, Fusarium ear rot, Aspergillus ear rot, plant height, ear height and insects damage. Traits associated with the second principal component were plant height, grain yield and primary tassel branches, and number of plants which contributed 16.0% of the total variation. Primary tassel branching and number of plants contributed equal variation with negative loading. The highest loading was contributed by plant height in this principal component. Ear height, grain texture, number of leaves above the first ear, Fusarium ear rot and number of leaves contributed 11.6% of the total variation for the third principal component with the eigenvalue of 1.6. Fourth principal component was highly associated with number of plants and Aspergillus ear rot and it contributed 10.3% of the total variation. On the other hand, number of primary tassel branches, Fusarium ear rot contributed 9.6% of total variation for the last principal component. The principal component analysis further clustered the phenotypic traits among the mycotoxins resistant inbred families into different several groups over the four quadrants (Figure 4:24). The PCA grouped the maize inbred lines into clusters over the quadrants based on thier phenotypic characteristics and the inbred lines remained scattered in the quadrants (Figure 4.25).

Table 4.13: Principal component analysis of 12 phenotypic traits for mycotoxin resistant $S_{3:4}$ maize families.

PC	Eigenvalue	Total variance		Eigenvector (loading) for phenotypic traits											
		Individual (%)	Cumulative (%)	PH	EH	GT	FER	AER	GY	HUS	ISD	DMF	NL	PTB	NP
1	3.1	22.2	22.2	0.52	-0.09	-0.18	0.5	0.5	0	-0.05	0.18	-0.02	0.08	0.16	0.02
2	2.2	16	38.2	-0.53	-0.26	-0.16	0.24	-0.02	-0.49	-0.1	0.05	-0.03	-0.03	-0.3	-0.3
3	1.6	11.6	49.8	-0.18	-0.57	0.35	-0.44	-0.24	-0.28	-0	0.09	-0.08	0.38	0.14	0.12
4	1.4	10.3	60.1	0.28	-0.06	0.03	-0.06	-0.57	0.18	-0.1	-0.1	-0.12	-0.2	0.01	-0.6
5	1.3	9,6	69.7	0.02	0.28	-0.09	-0.4	-0.28	-0.05	-0.2	-0	-0.11	0.06	-0.4	0.01

DMF-days to mid flowering, EH-ear height (cm), PH-plant height(cm), FER- Fusarium ear rot, AER-Aspergillus ear rot, NL-number of leaves above the first ear, NP- number of plants/row, PTB-primary tassel branching, GT-grain texture, HC- husk cover, ISD-insect damage, GY-Grain yield (kg ha^{-1}).

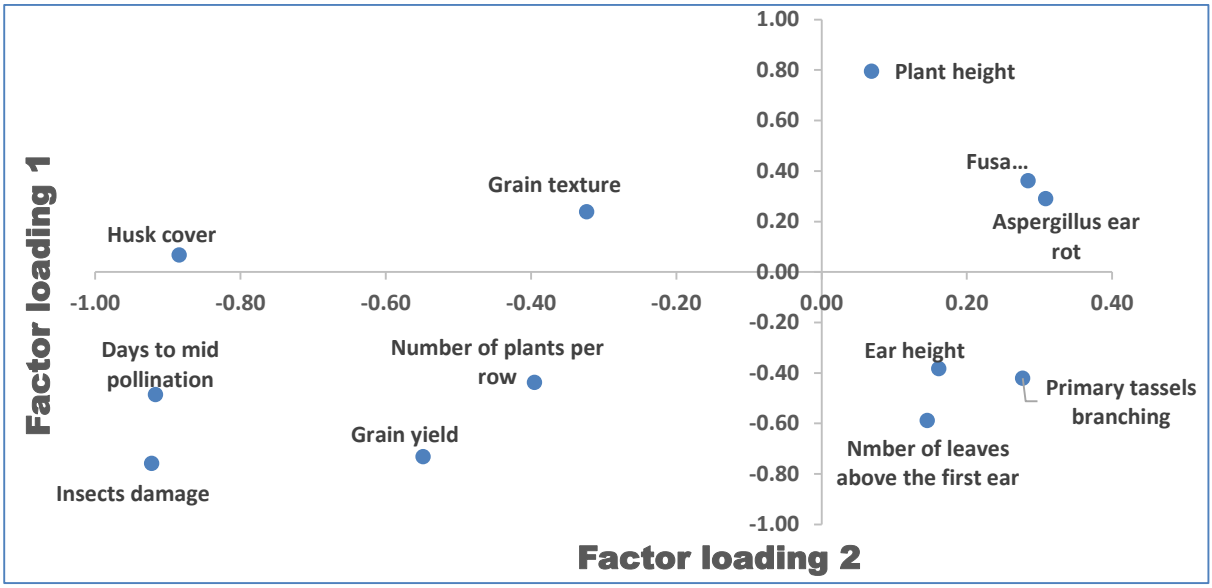


Figure 4.24: Principal component analysis loading plot for the 14 phenotypic traits of the selected $S_{3:4}$ maize families.

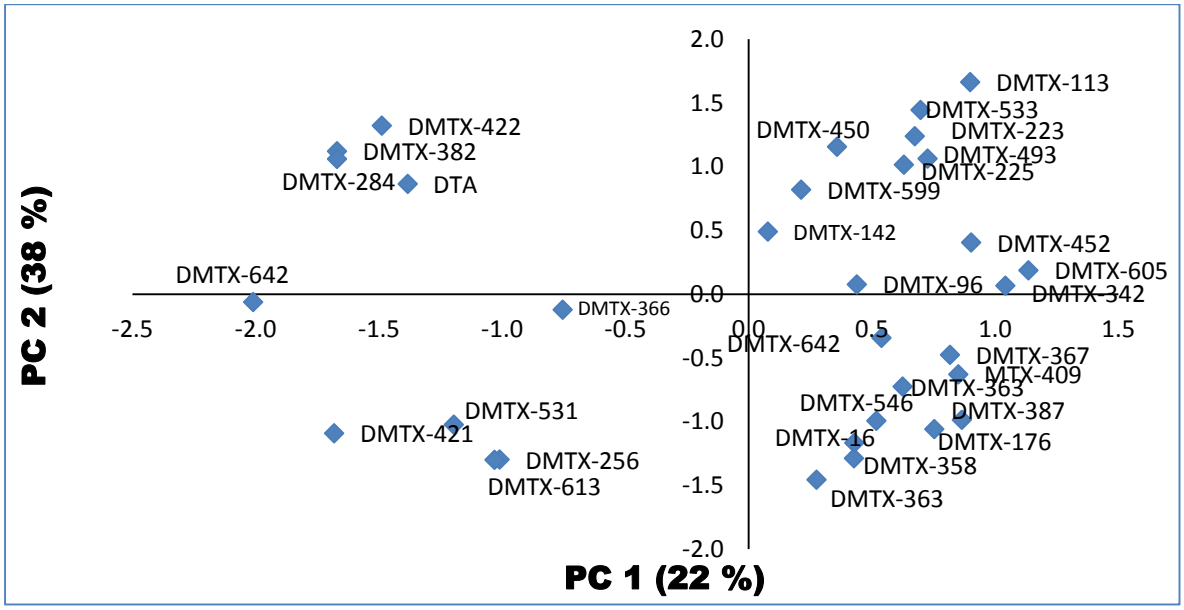


Figure 4.25: Principal component analysis score plot of the randomly selected $S_{3:4}$ maize families.

4.4.6. Cluster analysis

i. Cluster analysis based on grain yield

Figure 4.26 shows a dendrogram which clustered thirty two mycotoxins resistant inbred families based on yield, six main groups cutting at 0.99 and three sub groups cutting at 0.987 were observed and lines within a cluster are closely related in relation to their yielding ability. Similarity matrix in Table 4.14 shows euclidean distance (0-100) among resistant inbred families.

ii. Cluster analysis based on incidences of, Fussarium ear rot

Cluster analysis based on, Fusarium ear rot revelead the dendrogram shown in Figure 4.27. Four main clusters (cut off at 0.99) and two sub-clusters (cut off at 0.985) were revealed. Similarity matrix in Table 4.15 shows euclidean distance (0-100) among resistant inbred families.

iii. Cluster analysis based on incidences of, Aspergillus ear rot

Figure 4.28 shows a dendrogram which clustered 32 mycotoxins resistant inbred families based on, Aspergillus ear rot, seven main clusters (cut off at 0.9925) and three sub-clusters (cut off at 0.9825) were revealed and lines within a cluster are closely associated in relation to their resistance to Aspegillus ear rots. Similarity matrix in Table 4.16 shows euclidean distance (0-100) among resistant inbred familes .

iv. Cluster analysis based on traits with heritability >50%

Cluster analysis based on traits with heritabity estimates greater than 50% revelead the dendrogram in Figure 4.29. Six main clusers (cut off at 0.990) and three sub-

clusters (cut off at 0.980) were revealed. Similarity matrix in Table 4.17 shows euclidean distance (0-100) among resistant inbred families.

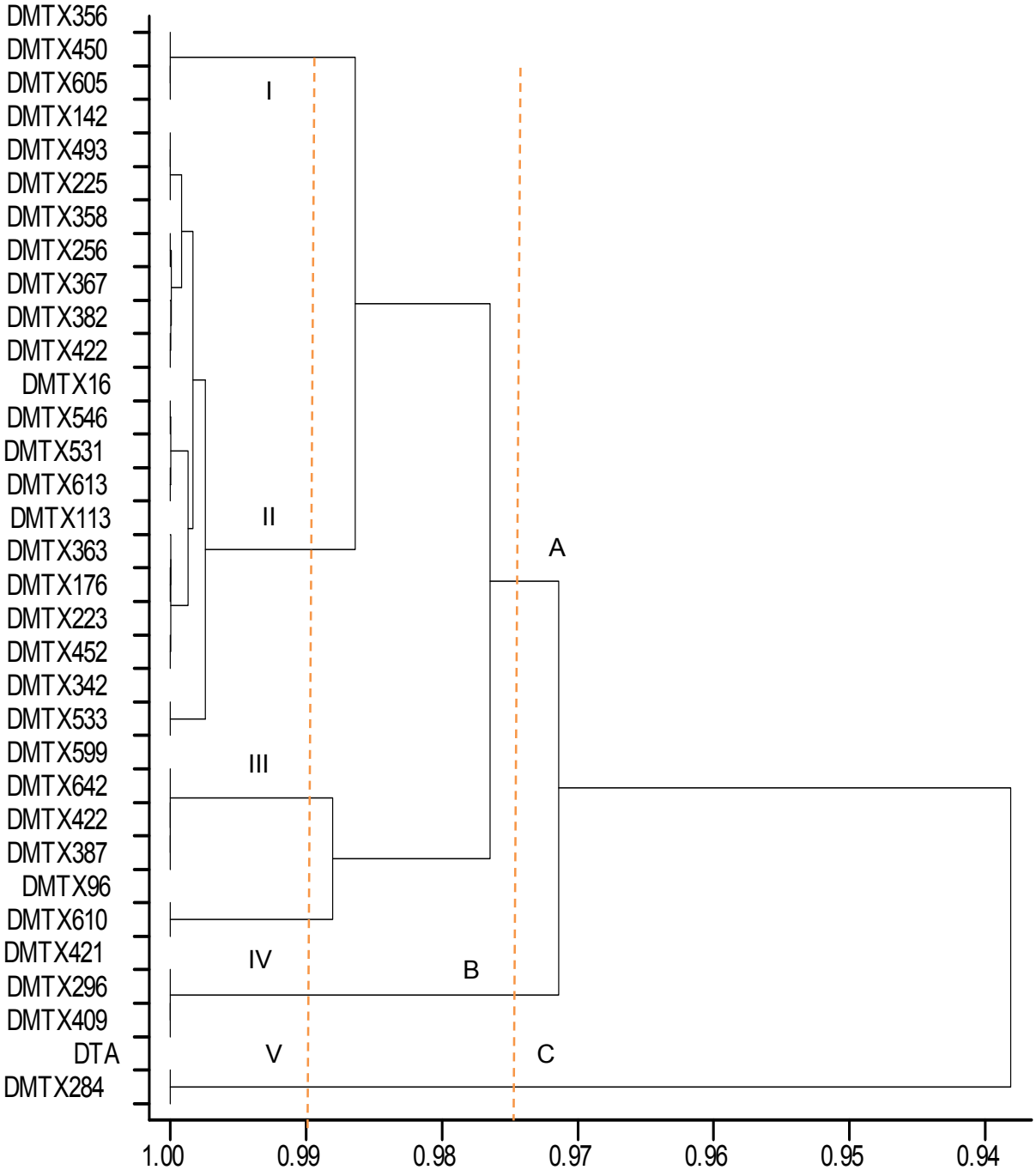


Figure 4.26: Dendrogram for the $S_{3:4}$ families based on grain yield of the mycotoxins resistant inbred lines.

Table 4.14: Similarity matrix table of euclidean genetic distance for the sampled $S_{3:4}$ families based on grain yield.

1																																
2	100																															
3	88	87																														
4	99	99	93																													
5	94	94	98	98																												
6	99	99	93	100	98																											
7	100	100	92	100	97	100																										
8	88	88	100	93	99	93	92																									
9	95	95	98	98	100	98	98	99																								
10	95	95	98	98	100	98	98	99	100																							
11	77	76	98	84	94	84	83	98	93	93																						
12	86	86	100	91	98	91	90	100	98	98	99																					
13	99	99	92	100	97	100	100	93	98	98	84	91																				
14	94	94	99	98	100	98	97	99	100	100	94	98	97																			
15	98	98	95	100	99	100	100	96	99	99	88	94	100	99																		
16	88	88	100	93	99	93	92	100	99	99	98	100	93	99	96																	
17	95	95	98	98	100	98	98	98	100	100	93	97	98	100	99	98																
18	99	98	95	100	99	100	100	95	99	99	87	94	100	99	100	95	99															
19	99	98	95	100	99	100	100	95	99	99	87	94	100	99	100	95	99	100														
20	99	98	95	100	99	100	100	95	99	99	87	94	100	99	100	95	99	100	100													
21	99	99	93	100	98	100	100	94	99	99	86	93	100	98	100	94	99	100	100	100												
22	77	76	898	84	94	84	83	98	93	93	100	99	84	94	88	98	93	87	87	87	86											
23	82	87	100	923	99	92	91	100	98	98	99	100	92	99	95	100	98	94	94	94	94	99										
24	77	76	98	84	94	84	83	98	93	93	100	87	94	94	88	98	93	87	87	87	86	100	99									
25	87	87	100	92	99	92	99	100	98	98	99	86	92	99	95	100	98	94	94	94	94	99	100	99								
26	100	100	88	100	95	100	100	89	96	96	78	100	100	95	99	89	96	99	99	99	99	78	99	100	88							
27	100	100	88	99	94	99	100	88	95	95	77	92	99	94	98	88	95	99	99	99	99	77	78	88	87	100						
28	89	89	100	94	99	94	93	100	99	99	98	100	94	99	96	100	99	96	96	96	95	98	77	87	100	90	90					
29	99	99	93	100	98	100	100	94	98	98	85	92	100	98	100	94	98	100	100	100	100	85	98	100	93	99	99	94				
30	87	87	100	92	99	92	91	100	98	98	99	100	92	99	95	100	98	94	94	94	94	99	85	93	100	88	87	100	81			
31	85	84	100	90	98	90	89	100	97	97	99	100	90	98	93	100	97	93	93	93	92	99	99	100	100	86	85	100	99	91		
32	73	84	24	64	43	64	65	26	45	45	0	20	65	43	57	26	46	59	59	59	61	0	0	23	23	72	73	29	83	63	23	
	1	2	3	4	5	6	9	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32

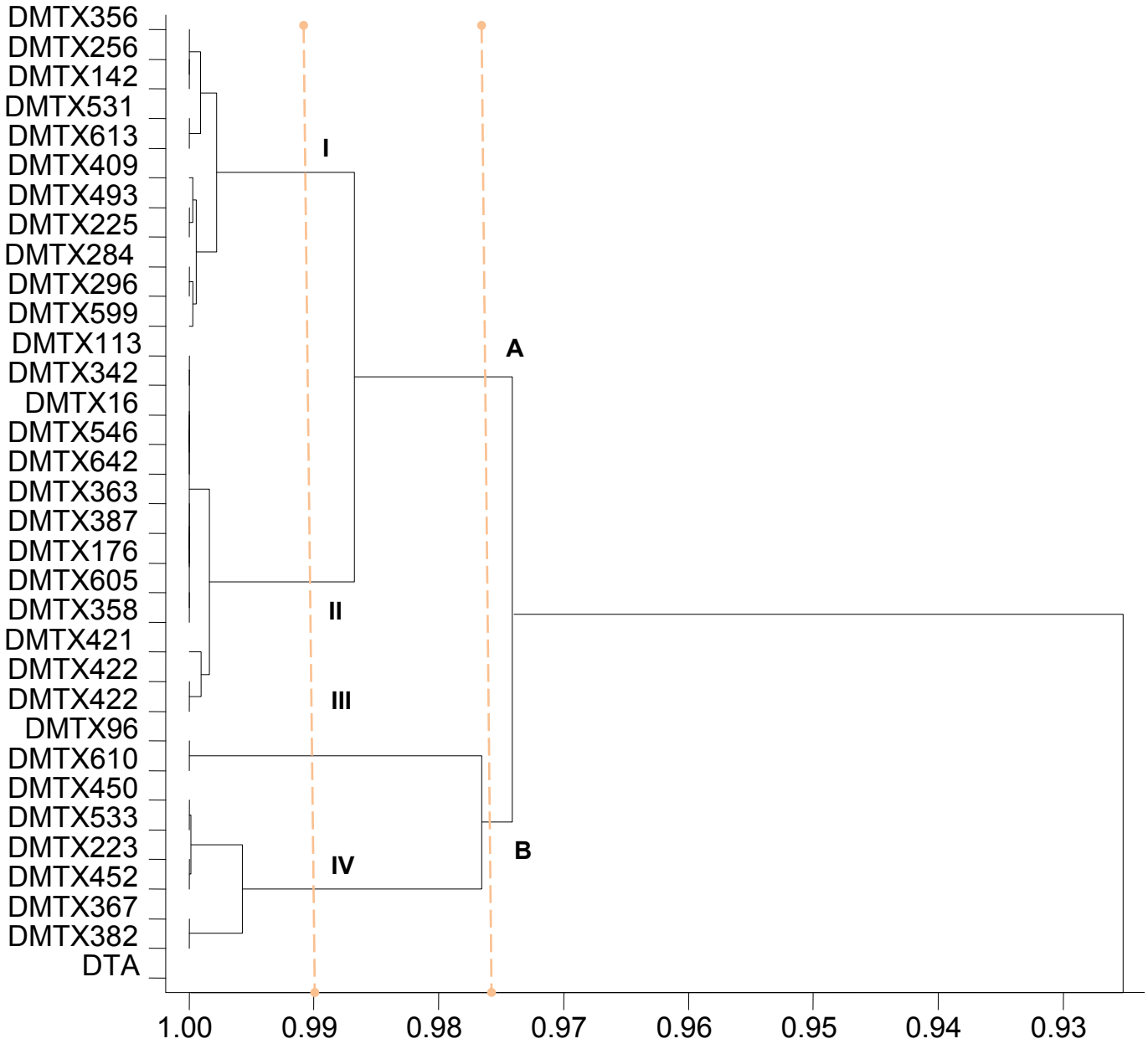


Figure 4.27: Dendrogram for the $S_{3:4}$ families based on, Fusarium ear rots among the mycotoxins resistant inbred lines.

Table 4.15: Similarity matrix table for euclidean genetic distance among sampled $S_{3:4}$ families based on, Fusarium ear rots.

1	___																														
2	100	____																													
3	93	93	___																												
4	100	100	93	___																											
5	100	100	91	100	___																										
6	98	98	82	98	98	___																									
7	98	98	82	98	98	100	___																								
8	98	98	82	98	98	100	100	___																							
9	98	98	83	98	99	100	100	100	___																						
10	98	93	83	98	99	100	100	100	100	___																					
11	93	98	100	93	91	82	82	82	82	82	___																				
12	98	100	82	98	98	100	100	100	100	100	82	___																			
13	100	100	95	100	99	96	96	96	96	96	95	96	___																		
14	100	99	91	100	100	98	98	98	99	99	91	98	99	___																	
15	99	98	97	99	99	94	94	94	94	94	97	94	100	99	___																
16	98	96	82	98	98	100	100	100	100	100	82	100	96	98	94	___															
17	96	95	79	96	97	100	100	100	100	100	79	100	94	97	92	100	___														
18	95	95	76	95	96	100	100	100	99	99	76	100	92	96	90	100	100	___													
19	95	100	76	95	96	100	100	100	99	99	76	100	92	96	90	100	100	100	___												
20	100	99	96	100	99	96	96	96	96	96	95	96	100	99	100	96	94	92	92	___											
21	99	99	97	99	98	93	93	94	94	94	97	94	100	98	100	94	91	89	89	100	___										
22	99	99	97	99	98	93	93	94	94	94	97	94	100	98	100	94	91	89	89	100	100	___									
23	99	99	97	99	98	93	93	94	94	94	97	94	100	98	100	94	91	89	89	100	100	100	___								
24	98	98	83	98	99	100	100	100	100	100	83	100	96	99	95	100	100	89	99	96	94	94	94	___							
25	100	100	94	100	100	97	97	97	97	97	94	97	100	10	99	97	96	94	94	100	9	99	99	98	___						
26	100	100	96	100	99	95	95	95	95	95	96	95	100	99	100	95	93	91	91	100	100	100	100	96	100	___					
27	100	100	96	100	99	95	95	95	95	95	96	95	100	99	100	95	93	91	91	100	100	100	100	96	100	100	___				
28	98	98	83	98	99	100	100	100	100	100	83	100	96	99	941	100	100	99	99	96	94	94	94	100	98	96	96	___			
29	99	99	97	99	99	94	94	94	94	94	87	94	100	99	100	94	92	90	90	100	100	100	100	95	99	100	100	95	___		
30	98	98	83	98	99	100	100	100	100	100	83	100	96	99	94	100	100	99	99	96	94	94	94	100	97	95	95	100	94	___	
31	99	99	97	99	98	93	93	93	94	94	97	93	100	98	100	93	91	89	89	100	100	100	100	94	99	100	100	94	100	93	___
32	41	41	75	41	35	14	14	14	15	15	75	14	48	36	53	14	6	0	0	48	55	55	55	16	42	50	50	16	53	15	56
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	55	24	25	26	27	28	29	30	31	32

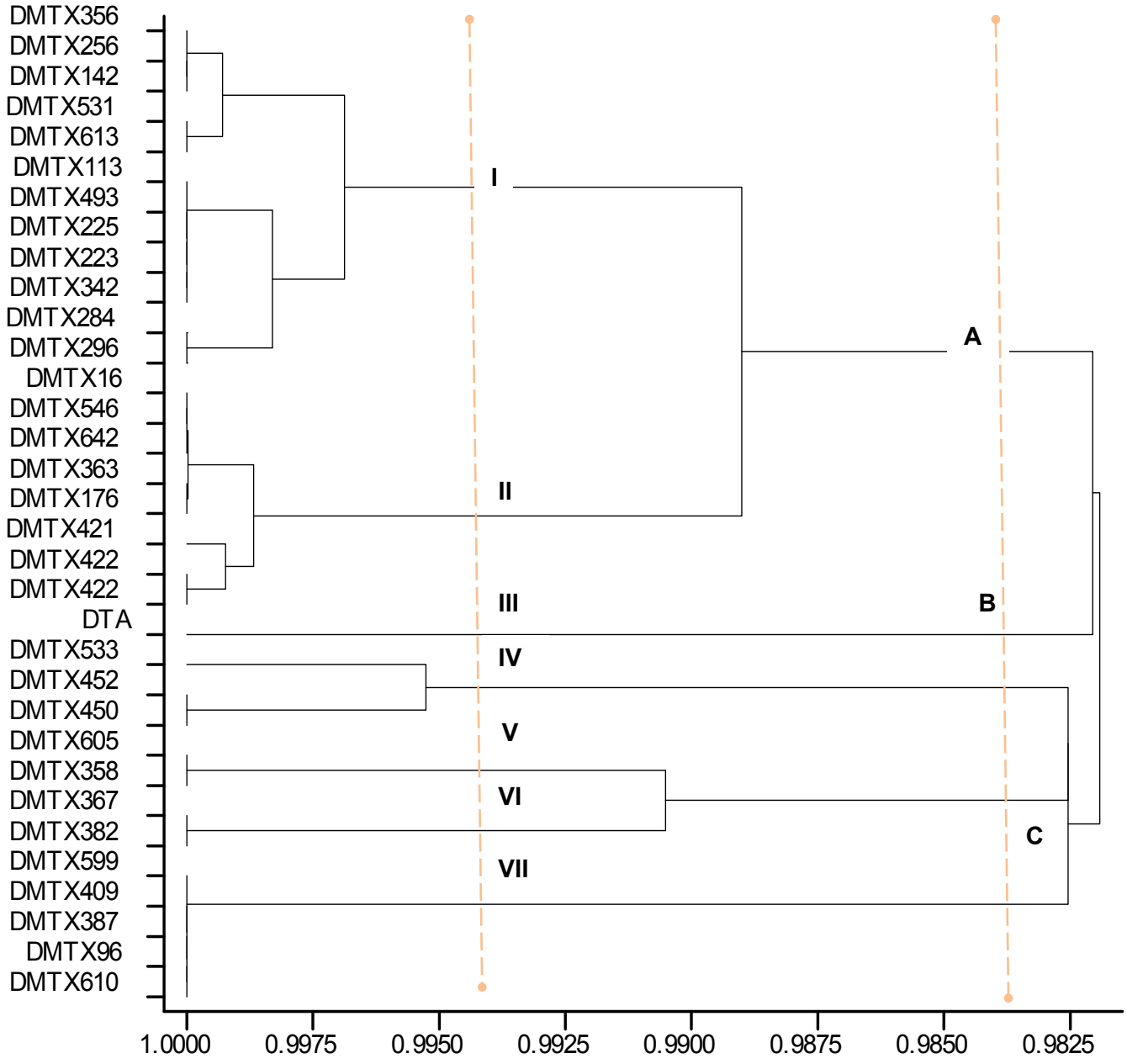


Figure 4.28: Dendrogram for the $S_{3:4}$ families based on, incidences of Aspergillus ear rot among the mycotoxins resistant inbred lines.

Table 4.16: Similarity matrix table of euclidean genetic distance for the sampled $S_{3:4}$ families based on, incidences of Aspergillus ear rot.

1	___																																
2	100	____																															
3	100	100	___																														
4	100	100	100	___																													
5	100	100	100	100	___																												
6	90	90	90	90	93	___																											
7	90	90	90	90	93	100	___																										
8	90	90	90	90	94	100	100	___																									
9	91	91	91	91	94	100	100	100	___																								
10	91	91	91	91	94	100	100	100	100	___																							
11	91	91	91	91	94	100	100	100	100	100	___																						
12	90	90	90	90	94	100	100	100	100	100	100	___																					
13	93	93	93	93	96	100	100	100	94	100	100	100	___																				
14	100	100	100	100	100	93	93	94	72	94	94	94	96	___																			
15	95	95	95	95	92	70	70	70	100	72	72	70	75	92	___																		
16	90	90	90	90	94	100	100	100	99	100	100	100	100	94	70	___																	
17	84	84	84	84	89	99	99	99	97	99	99	99	97	89	61	99	___																
18	78	78	78	78	84	98	98	98	97	97	97	98	97	84	52	98	99	___															
19	79	79	79	79	84	98	98	98	100	97	97	98	97	84	52	98	98	100	___														
20	93	93	93	93	96	100	100	100	100	100	100	100	100	96	75	100	98	100	100	___													
21	93	93	93	93	96	100	100	100	100	100	100	100	100	96	75	100	100	96	96	100	___												
22	93	93	93	93	96	100	100	100	100	100	100	100	100	96	75	100	100	97	97	100	100	___											
23	93	93	93	93	96	100	100	100	81	100	100	100	100	96	75	100	100	97	97	100	100	100	___										
24	98	98	98	98	96	100	100	100	81	81	81	100	84	96	99	80	80	65	65	84	84	84	100	___									
25	98	98	98	98	96	80	80	80	81	81	81	80	84	96	99	80	80	65	65	84	84	84	84	84	___								
26	98	98	98	98	96	80	80	80	81	81	81	80	84	96	99	80	80	65	65	84	84	84	84	84	100	___							
27	98	98	98	98	96	80	80	80	81	81	81	80	84	96	99	80	80	65	65	84	84	84	84	84	100	100	___						
28	98	98	98	98	96	80	80	80	81	81	81	80	84	96	99	80	80	65	65	84	84	84	84	84	100	100	100	___					
29	95	95	95	95	96	80	80	80	72	81	81	80	84	92	100	71	71	53	53	84	84	84	75	75	99	99	99	99	___				
30	91	91	91	91	92	71	71	71	100	72	72	71	75	94	72	100	100	98	98	75	75	75	100	100	81	81	81	81	81	___			
31	96	96	96	96	94	100	100	100	74	74	74	100	100	93	100	72	72	54	54	100	100	100	76	76	99	99	99	99	99	72	___		
32	71	71	71	71	64	27	27	27	30	40	40	27	34	64	91	27	27	0	0	34	34	34	34	34	83	83	83	83	83	90	89	___	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	

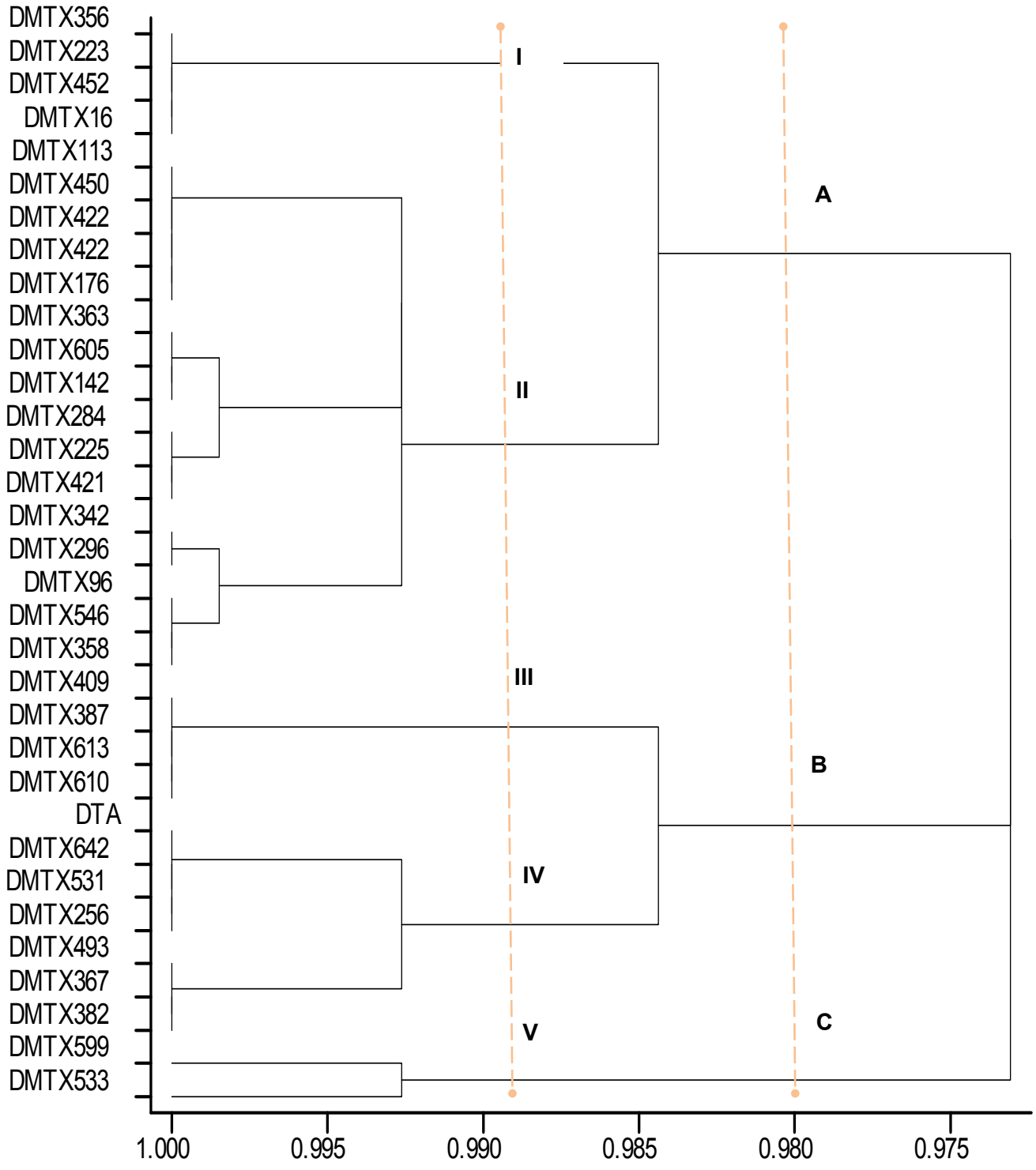


Figure 4.29: Dendrogram for the $S_{3:4}$ families based traits with heritability (%) greater than 50.

Table 4.17: Similarity matrix table of euclidean genetic distance for the sampled $S_{3:4}$ families based on H^2>50%.

1	___																														
2	97	____																													
3	71	73	___																												
4	60	60	99	___																											
5	98	91	64	53	___																										
6	93	97	52	36	85	___																									
7	98	99	60	45	93	99	___																								
8	89	93	92	84	80	83	86	___																							
9	92	96	49	32	86	100	99	81	___																						
10	100	97	68	57	98	93	98	87	93	___																					
11	93	97	52	35	85	100	99	83	100	93	___																				
12	98	93	82	74	96	84	91	91	82	97	83	___																			
13	96	92	88	81	94	80	88	94	79	95	80	99	___																		
14	95	88	79	73	97	76	86	85	76	95	76	99	98	___																	
15	94	98	57	41	87	100	99	86	100	94	100	86	84	80	___																
16	98	100	68	54	92	98	100	91	98	98	98	92	91	87	99	___															
17	97	99	61	46	91	99	100	87	99	97	99	90	87	84	100	100	___														
18	99	98	63	49	96	97	100	86	97	99	97	94	91	90	98	99	99	___													
19	99	98	63	49	96	97	100	86	97	99	97	94	91	90	98	99	99	100	___												
20	92	83	78	72	95	70	81	81	97	92	70	98	97	100	74	83	79	86	86	___											
21	93	83	75	69	96	71	82	80	70	92	71	98	96	100	74	83	80	87	87	100	___										
22	80	64	56	52	89	50	65	58	50	80	50	87	84	93	54	65	62	72	72	95	96	___									
23	97	97	87	78	92	88	93	97	87	96	88	98	99	95	90	95	93	94	94	92	91	75	___								
24	94	98	54	38	87	100	99	84	100	94	100	95	82	79	100	99	100	98	98	73	74	54	94	___							
25	100	97	71	60	98	93	98	89	92	100	93	98	96	95	94	98	97	99	99	92	93	80	98	94	___						
26	97	99	64	49	91	99	100	88	99	97	99	91	88	95	100	100	100	99	99	80	80	61	99	99	97	___					
27	92	83	78	72	95	70	81	81	670	92	70	98	97	100	74	83	979	86	86	100	100	95	99	73	92	80	___				
28	99	98	62	49	96	97	100	86	97	99	97	94	91	90	98	99	99	100	100	86	86	72	86	98	99	99	86	___			
29	97	100	66	52	90	99	99	90	98	97	99	91	89	85	99	100	100	99	99	80	80	61	100	99	97	100	80	99	___		
30	90	84	92	88	88	69	78	91	67	88	69	97	99	97	73	82	78	82	82	97	96	85	99	71	90	79	97	82	80	___	
31	85	72	75	72	90	56	70	73	55	84	56	93	92	97	60	71	67	76	76	99	98	91	82	59	85	68	99	76	68	95	___
32	56	57	98	100	49	32	41	82	28	53	31	71	79	69	38	51	42	43	43	69	66	48	45	34	56	46	69	45	49	86	69
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31

4.5. Conclusion

This chapter presented the findings observed from the experiments, including the results on natural incidences of ear rot disease on Southern African hybrids, the level of agro-morphological variability and genetic distances, relationship between *A. flavus*, *F. verticillioides* infections and secondary traits. Large variation was observed for all traits and significant differences between families and hyrbids were observed. The inbred families were divided into many clusters based on individual traits and a group of traits with heritability greater than 50%. These findings are interpreted and discussed in the following chapter.

CHAPTER FIVE

General discussion

5.1. Introduction

The main objective of this study was to characterize mycotoxins resistant maize inbred families at the phenotypic level. The specific objectives of the study were toto determine the natural incidences of ear rot disease associated with mycotoxins contamination, estimate the level of agro-morphological variability and genetic distances among the mycotoxins resistant maize inbreds families, to determine the relationship between *A. flavus*, *F. verticillioides* infections and secondary traits and to estimate heritability between ear rots severity and secondary traits among resistant maize inbred families. In this chapter the results are interpretated and discussed.

5.2. The levels of ear rot disease incidence on regional experimental maize hybrids

The study revealed four fungi that were responsible for the natural incidences of ear rots. The fungi included *Aspergillus flavus, Stenocarpella maydis,* and *Fusarium graminearum* and *Fusarium verticillioides*. The four fungi have a potential to secrete mycotoxins in maize grain which are potentially hazardous to the health of animals and humans. The presence of these fungi on the Southern African hybrids implies that consumers might be exposed to the mycotoxins and this poses a serious risk to their health (Pitt, 1996). Incidences of *F. verticillioides* were higher during the two seasons, and this might be because of hot and dry weather conditions that occurred after flowering (Payne, 1998). Munkvold (2003) reported that dry conditions at the

silking stage favours the spread of *F. verticillioides*. The weather conditions in both seasons had favoured *F. verticillioides* compared to the other fungi. Mukanga et al. (2010) and Ncube et al. (2011) also reported the prevalence of *F. verticillioides* and fumonisins in maize and maize based food.

Early maturing hybrids showed lower incidences of ear rots whereas hybrids that matured late had the highest incidences. Flint textured hybrids showed better resistance to ear rots than dent textured hybrids which were susceptible. Czembor and Ochodzki (2009) agreed with the findings of these results by reporting increased resistance in flint than dent textures. Robutti et al. (2000) reported that endosperm component and kernel structure contributed to resistance or susceptibility of genotypes to fungal infection. Flint type maize has kernels consisting of hard endosperm, whereas the kernels of dent maize are characterized by the presence of a small proportion of hard endosperm, hence flint maize are more resistant to ear rot infections compared to dent maize. Insects damage showed high correlation with ear rot incidences, due to the fact that when the kernels get wonded by the insects, the kernels get more exposed to fungal infections. Early maturing hybrids showed a strong negative correlation with grain yield compared to medium and late maturing hybrids. This confirms reports by Gasura et al. (2010) and Chiuraise (2014) that early maturing hybrids produces less yield compared to late maturing hybrids.

5.3. Genetic variability of mycotoxins/ ear rot resistant maize inbred families

5.3.1. Phenotypic variability of mycotoxin resistant maize inbred families

The existence of significant variation among inbred families for *Aspergillus flavus,*

Fusarium verticillioides infections and agronomic performance offers an opportunity for genetic improvement. The results showed that the $S_{3:4}$ families had significant differences in general ear rot infections by both *F. verticillioides* and *A. flavus,* therefore there is opportunity for selection. The negative control (DTA) showed severe infections by both fungi, indicating that inoculation was effective in causiing the disease. This was followed by inbred line DMTX-256 which can be classified as susceptible because it was worse than the negative control. In contrast, inbred lines DMTX-96 and DMTX-387 had no signs of infection, indicating that significant progress can be achieved in selecting for ear rot resistance in maize.

Significant variability was observed among the mycotoxins resistant inbred families for all secondary traits implying that breeders can select for these traits. However, selection would not be effective for husk cover, insect damage and days to mid maturity, which did not show any significant variation. Line DMTX-96 registered the highest plant and ear height, and it recorded the highest grain yield, qualifying as the target family for selction of lines with high yield potential. Fusarium and Aspergillus ear rot, correlated positively with insect damage and grain texture. This results are in agreement with previous studies which showed that flint textured kernels are less vulnerable to insect damage thus fungal infections as compared to dent textured maize (Wit et al., 2011). Abbas et al. (2006) also reported a significant correlation for Aspergillus and Fusarium ear rot, and insect damage. Warburton and Williams (2014) reported that tight long husk cover protects the kernels from fungal invation and the finding was confirmed by the significant correlation between Fusarium, Aspergillus ear rot and husk cover. Grain yield exhibited positive association with plant height, ear height, grain texture and number of leaves above the ears. This implies that increasing expression of these traits can positively influence grain yield

and findings are in agreement with Selvaraj and Nagarajan (2011) who found significant positive association between plant height, ear height and grain yield. Agronomic performance of a line/hybrid is very important when evaluating for resistance, meaning commercially acceptable hybrids should be the ultimate goal. The studies (both current and previous) had found it difficult to get genotypes that satisfactorily combine these traits of resistance with desirable agronomic traits (Brown et al., 1999).

5.3.2. Heritability of secondary traits

The heritability of resistace to Fusarium ear rot and Aspergillus ear rot, were high for this study indicating the selection of this trait would be effective and this is in agreement with Chiuraise (2014) who reported high heritability values for, resistance to Fusarium and Aspergillus ear rot. Robertson-Hoyt et al. (2006) also found high heritabilities for, resistance to Fusarium ear rots and Menkir et al. (2008) found moderate to high heritability for, resistance Aspergillus ear rot. The results are in contrast with Falconer et al. (1996) whom reported polygenic low heritability for, resistance to Fusarium ear rot. Plant height, ear height, primary tassel branches and grain yield exhibited high heritabilities, which is in accordance with findings by Khoza (2012) who reported high heritability for grain yield, grain moisture and plant height. Mahmood et al. (2004); Nadagoud (2008) and Ali et al. (2011) also reported high heritability for grain yield. Husk cover and insect damage exhibited low heritabilities, indicating that selection would not be effective for these traits.

5.3.3. Frequency distribution of the secondary traits

The frequency distributions revealed a variation among the $S_{3:4}$ families based on the phenotypic traits. Evidence of continuous distribution on most of the traits indicated the presence of large genetic variation among the mycotoxin resistant inbred families.

5.3.4. Principal component and cluster analysis

Principal components and cluster analysis further revealed variation among maize inbred families. The principal component analysis clustered the mycotoxin resistant inbred families into groups over the quadrants based on their phenotypic characteristics. This results are in accordance with the findings by Bucheyeki (2012) who reported five principal components contributing 71.98% of the total variations among maize landraces. The currect study showed that Fusarium ear rot, Aspergillus ear rot, plant height and insects damage contributed most of the total variation. The inbred familes which were close to each other were genetically similar in regards to the traits, while lines that are scattered on the biplot are said to be different (Gerrano et al., 2015). Phenotypic traits that discriminated most inbred families on cluster analysis were plant height, ear height, Fusarium ear rot and Aspergillus ear rot. This showed that there were a wide genetic variability among inbred families based on the phenotypic traits evaluated, this would help in effective selection of parents for the breeding programme. Taking into account the findings of the study, the variation on $S_{3:4}$ families gives an opportunity for selection. Lines to be derived from the grouped families would be exploited to make heterotic combinations by crossing lines from the different phenotypic clusters for the traits of interest.

5.4. Conclusions

The study's overall objective of characterizing mycotoxins resistant maize inbred families at the phenotypic level was achieved to certain extent because:

i. Flint textured hybrids revealed more resistance to ear rots, while dent textured hybrids were more susceptible, which is consistent with findings in the literature. Early maturing hybrids showed lower incidences of ear rots whereas hybrids that mature late had most incidences, indicating that the early hybrids escaped the infection which occured late in the season. Although early maturing hybrids got less infections, the results also revealed it had a significant strong negative correlation with grain yield, implying that farmers who choose to grow early hybrids on the basis of ear rot resistance would get less yield .

ii. Variation among resistant maize inbred familes based on, agro-morphological traits were observed, therefore selection would be effective for these traits. The results revealed five principal components contributed more than 69% of the total variation.

iii. Resistant maize inbred families were further clustered into different groups based on important agronomic traits evaluated indicating that there is diversity.

iv. Five derversity groups where observed based on ear rot incidences.

v. Heritability was large for most traits, indicating opportunity for selection of the best inbred families for advancement in the programme.

CHAPTER SIX

Overview of findings and recommendations

6.1. Introduction

The research study focus was to characterize mycotoxins resistant maize inbred families at the phenotypic level and evaluate the natural incidences of ear rot disease associated with mycotoxins contamination. The objectives were addressed by determining the level of agro-morphological variability among the mycotoxins resistant maize inbreds families, by estimating the relationship between resistance to *A. flavus*, *F. verticillioides* infections and secondary traits.

6.2. Overview of findings

6.2.1. The levels of ear rot disease incidence on regional experimental maize hybrids.

The results revealed that:

i. Four fungi that were responsible for the natural incidences of ear rots including *Aspergillus flavus, Stenocarpella maydis, Fusarium graminearum* and *Fusarium verticillioides.*

ii. Early maturing hybrids showed fewer incidences of ear rots where as hybrids that mature late had most incidences. Though, early maturing hybrids got less infections, the results also revealed that early maturity had a significant strong negative correlation with grain yield.

iii. Cob rots had a significant negative correlation with grain yield in both seasons for the different hybrids maturity. Significant positive correlation was noticed

on insects damage, days to anthesis and silking for early and late maturing hybrids. Late flowering hybrids showed a stronger positive correlation whereas the earlier flowering hybrids revealed a significant negative correlation with grain yield.

6.2.2. Phenotypic variability among mycotoxins resistant maize inbred families.

The study revealed phenotypic variability among the mycotoxins resistant maize inbred lines.

i. $S_{3:4}$ families showed significant differences in general ear rot infections caused by both *Fusarium verticillioides* and *Aspergillus flavus*.

ii. Significant variability was observedamong the mycotoxins resistant inbred families for all secondary traits indicating that there was a wide genetic variability among the inbred families tested, except for husk cover, insect damage and days to mid maturity.

iii. Plant height and ear height showed a highly significant positive correlation among the traits evaluated. Fusarium and Aspergillus ear rots, correlated positively with insects damage. The positive correlation among the traits would help the breeder for the selection and improvement of traits of interest simultaneously. Grain yield are negatively correlated with the ear rots, meaning an increase of ear rots will negatively affect the yield.

iv. Principal components and cluster analysis further revealed variation among maize inbred families.

6.3. Recommendations

Molecular characterization of the resistant inbred lines should be employed for further validation of the present phenotypic results. Evaluations should be done at multi-locations for several seasons for further confirmation and selection of stable resistant inbred lines for future breeding activities. Agronomic performance of a line/hybrid is very substantial when evaluating for resistance, meaning commercially acceptable lines/hybrids should be the ultimate goal.

6.4. Conclusions

The present findings show considerable variations among resistant inbred families for important agronomic traits, and general ear rot infections by both *Fusarium verticillioides* and *Aspergillus flavus*. The information generated from this study will assist in developing local hybrids with high resistance to ear rots and mycotoxin contaminations, that attribute better agronomic performance. Furthermore, the study will contribute to an increase in South African quality maize production for food and nutritional security for the communities.

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