ASPERGILLUS FLAVUS EAR ROT AND AFLATOXIN IN UGANDA AND GENETICS OF RESISTANCE IN TROPICAL MAIZE GERMPLASM

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This thesis is my original work and has not been presented for the award of a degree in any other University.

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To the memories of my late father Mr Kasoma Jones Mayega and late sister Sophia Tekityamazzi who did not live to see my progress in life as I had vowed. May their souls rest in eternal peace.

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ABBREVIATIONS AND ACRONYMS

AEZ	Agro-ecology Zone
AFLP	Amplified Fragment Length Polymorphism
AFs	Aflatoxins
ANOVA	Analysis of Variance
ATAAS	Agricultural Technology and Agribusiness Advisory Services
AUDPC	Area Under Disease Progress Curve
BGYF	Bright Greenish-Yellow Fluorescence
CFU	Colony Forming Unit
CIMMYT	International Maize and Wheat Improvement Center
CO	Carbonmoxide
DAI	Days After Inoculation
DNA	Deoxyribonucleic acid
DSIP	Development Strategy and Investment Plan
DSIP	Development Strategy and investment Plan
EU	European Union
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
FISH	Florescent In Situ Hybridization
GC	Gas Chromatography
GCA	General Combining Ability
GLM	General Linear Model
GLS	Gray Leaf Spot

GxE	Genotype Environmental Interaction
HPLC	High-Performance Liquid Chromatography
IAC	Immuno-Affinity Column
IITA	International Institute of Tropical Agriculture
ISSR	Inter-Simple Sequence Repeats
ITS	Internal Transcribed Spacer
KAFACI	Korean-African Food and Agriculture Cooperation Initiative
KIR	Kernel Infection Rate
LIRS	Laboratory-based Infection Resistance Screening
LSD	Least Significant Differences
MAAIF	Ministry of Animal Industry and Fisheries
Masl	Meters above sea level
MEGA	Molecular Evolutionary Genetics Analysis
MFPED	Ministry of Finance, Planning and Economic Development
MIKI	Media-free and Isolated Kernel Incubation
MS	Mass Spectroscopy
MSV	Maize Streak Virus
MT	Metric Tonnes
NaCRRI	National Crops Resources Research Institute
NARO	National Agriculture Research Organization
NCII	North Carolina Design II
NOR	Norsolorinic Acid
O_2	Oxygen

PCA	Principle Component Analysis
PCR	Polymerase Chain Reaction
PIC	Polymorphism Information Content
RAPD	Random Amplified Polymorphic DNA
RDA	Rural Development Administration
rRNA	Ribosomal ribonucleic acid
RFLP	Restriction Fragment Length Polymorphism
RH	Relative Humidity
RT-PCR	Reverse Transcription-Polymerase Chain Reaction
SCA	Specific Combining Ability
SNP	Single Nucleotide Polymorphims
SSA	Sub-Saharan Africa
SSCP	Single Strand Conformation Polymorphism
SSLPs	Simple Sequence Length Polymorphism
STRs	Short Tandem Repeats
TLB	Turcicum Leaf Blight
TLC	Thin Layer Chromatography
UBOS	Uganda Bureau of Statistics
UNDP	United Nations Development Program
USA	United States of America
USAID	United States Agency for International Development
USDA-ARS	United States Department of Agriculture - Agricultural Research Service
WEMA	Water Efficient Maize for Africa

GENERAL ABSTRACT

The risk of exposure to aflatoxin in tropical agricultural systems is high due to factors that favour fungal growth and toxin production. Maize is among the staple crops with high aflatoxin contamination in Uganda. An understanding of host, pathogen and resistance to infection facilitates the development of germplasm with elevated resistance to infection. The objective of the study was to contribute to maize ear rots management and the associated mycotoxins through host resistance. A various levelled testing method was utilized to haphazardly collect 247 diseased maize cob samples in maize fields in 16 districts for aflatoxin quantification and *Aspergillus flavus* isolations and characterization. Also, 25 SSR markers were used to fingerprint 41 inbred lines to determine genetic diversity and their relationship, 35 lines were used to evaluate aflatoxin accumulation using High-Performance Liquid Chromatography and 19 inbred lines generated 90 F1 using North Carolina Design II mating scheme to understand the gene action controlling kernel infection resistance by *Aspergillus flavus* and also to estimate additive and dominance genetic variances. Results showed significant differences among areas of study for *A. flavus* incidence and severity. Utmost incidence and severity of *Aspergillus flavus* was recorded in Pallisa (74.2% and 4.8, respectively). Among agro-ecological zones, the utmost incidence and severity of *Aspergillus flavus* were in the eastern region at 62.4% and 4.6% respectively. At all the loci, 184 alleles were recorded with an average of 7.36 and a range: 2 - 19. The gene diversity average was 0.65 and ranged from 0.18 to 0.92. Percentage heterozygosity was 4% on average and ranged from 0.0% to 2.0%. Average polymorphism information content recorded was 0.61. In understanding the inheritance of resistance to kernel infection: The

environment plus, Year x Environmental interaction was significantly high ($P < 0.001$) for percent kernel infection at 7 days after inoculation, percent kernel infection at 14 days after inoculation, percent kernel infection at 21 days after inoculation, mean percent kernel infection and Kernel texture. Inbred parents WL 118-3, WL 429-18, WL 429-14, CL-RCW35, CML495, CML264, and WL 118-11 emerged as the best general combiners for *Aspergillus* ear rot while inbred CML247 and WL118-10 emerged as best combiners for aflatoxin accumulation. The non-additive effects were slightly greater than additive genetic effects for percent kernel infection and aflatoxin accumulation. Maize Inbred lines with good general combining ability effects for resistance to Aspergillus flavus kernel infection and aflatoxin accumulation save as good candidates to use in breeding resistant varieties. These results provide information on *Aspergillus flavus* strain prevalence and distribution in maize growing agro-ecological zones. It's prudent standard bureau to observe levels of aflatoxin in grain to set up legitimate aflatoxin administration rules. Results from kernel screening of material show that significant reduction in grain damage can be enhanced through host plant resistance by hybridization with resistant parents; therefore the materials eye marked in the study provides candidates for breeding for resistance to *Aspergillus flavus.*

Key words: Ear rots, *Aspergillus flavus*, Aflatoxin, Maize, heterozygosity

CHAPTER ONE

GENERAL INTRODUCTION

1.1. Background information

In most parts of Sub-Saharan, Africa maize serves as the prime crop, cultivated on 27 Million hectares (Cairns et al., 2013). Maize accounts for 30 % of the add up to range beneath cereal generation in this locale: 19 percent in West Africa, 61 percent in Central Africa, 29 percent in Eastern Africa and 65 percent in Southern Africa (FAO, 2010). In East Africa, maize is widely used as a foremost staple. In the last ten years, total maize production was 236.65 million metric tons harvested from 147.17 million hectares (FAOSTAT, 2014). The east African economy earns more from maize since more than half of what is produced is for commercial purposes.

In Uganda, Maize is positioned among the most imperative cereals in terms of generation, utilization, and wage era. Total production in 2012 was 2,734,000 metric tons (MT) harvested on a total area of 1,094.000Ha (FAO, 2014). The crop is grown in about 50 districts located in the West, East, North and Southeastern Uganda. In terms of export, maize is ranked third after fish and fish product and floriculture of the non-traditional export in Uganda (Kaaya and Kyamuhangire, 2006; FAO, 2014). In 2011, Uganda exported 54978 MT of maize valued at US $ 17,096,000 (FAO, 2014). Up to 1930, the government of Uganda advanced maize generation, however, this arrangement was afterwards switched owing to the maize crop's overwhelming take-up of soil supplements, helplessness to sheet disintegration beneath destitute administration and competition with cotton, the major trade edit at the time (Jameson

1970). A regional research station was opened at Namulonge by the Cotton Research Corporation in 1949, however, the emphasis was on coffee and cotton since they were principal cash crop (Laker-Ojok, 1994). Maize got to be a vital subsistence and non-traditional cash trim in the 1970s and 1980s as promoting frameworks for cotton collapsed. Considering maize as a non- traditional export, the exports were up to 4.71 million metric tons of maize equivalent to the US $1.72 million in the last 10 years (FAOSTAT, 2014).

1.2. Problem statement

Maize production in Uganda is constrained by many factors among which include biotic stresses, such as pests, leaf and ear diseases, and Striga, and abiotic stresses mainly low fertility in soils and erratic drought. Among ear diseases in maize, important are cob rots caused by various fungi that result in discolouration of grain, and accumulation of mycotoxins (Chandrashekar et al., 2000). Fungal pre- and post-harvest infection results into amassing of different sorts of toxins, with aflatoxins being common that is produced by *Aspergillus flavus* (Agag, 2004; Bandyopadhyay et al., 2016).

In maize grain, elevated levels of aflatoxin are as often as possibly related with high temperatures and dry spell; the issue is assist aggravated by creepy crawlies bolstering on the ears. Aflatoxins are toxic and carcinogenic and hence if ingested from contaminated grains has health implications to livestock and humans (Brown et al., 1999; Bandyopadhyay et al., 2016). Aflatoxin B1, B2, G1, and G2 occurs in a wide range of crops: maize, peanut butter and cocoa (Diener et al., 1987; Bandyopadhyay et

al., 2016). Maize (*Zea mays* L.) that succumbs to infection and aflatoxin accumulation by *Aspergillus flavus* and *Aspergillus parasiticus* has caused tremendous economic losses to farmers in Sub-Saharan countries.

Strict regulations and monitoring in food and feed according to Codex Alimentarius standards serve as a robust proactive measure to consumers and hence restrict the sale of products with levels above 10 ng/kg (European Union Commission regulation, 2010). Conversely, this isn't the case in most vulnerable African nations. This was reported in studies conducted in Uganda on the occurrences and exposure of aflatoxin to humans (Simyung et al., 2013), and also during the acute aflatoxicosis outbreak in Kenya due consumption of contaminated maize resulted into death (Probst et al., 2007; Mutiga et al., 2014).

1.3. Justification

Differences in reaction to infection and aflatoxin accumulation have been observed in maize hybrids, inbred lines and open-pollinated varieties Campbell and white, 1995; Garrido-Bazan et al., 2018). Although developing hybrids that are resistant to *Aspergillus flavus* contamination and aflatoxin aggregation is for the most part considered a vital methodology in lessening aflatoxin defilement of maize with satisfactory resistance levels for elevated stress situations are not accessible. However, materials developed with resistance to aflatoxin accumulation are not stable in different environments (Clements and white, 2004; Wahl et al., 2017; Williams et al., 2018). The inconstancy related to environments and significant genotype × environment

interactions is major obstacles to recognizing and creating unused sources of resistance to *Aspergillus flavus* infection and aflatoxin contamination (Anderson et al., 1995; Wahl et al., 2017). Even in zones where aflatoxin contamination is an incessant issue, depending on natural infection is not satisfactory for compelling germplasm screening. Inoculating developing ears with *Aspergillus spp* has been utilized to facilitate germplasm screening and diminish variation within experiments across locations and years (Zummo and Scott, 1989; Windham et al., 2018). Damage from insects that feed on ears such as southwestern corn borer; fall armyworm; which is a new challenge in Africa; and other insect damages are regularly related with elevated levels of aflatoxin accumulation (Williams et al., 2002; Yao et al., 2015).

Although a number of aflatoxin resistant inbred lines have been developed and released elsewhere (Betrán et al., 2002; Wahl et al., 2017; Williams et al., 2018), these materials may not be adapted to Ugandan agro-ecologies and also the pathogenic races of the pathogen may not be the same. It is, therefore, worthwhile to combine these materials with local lines, study the prevailing genetics of resistance and use resistant crosses to generate new lines which are adapted to Ugandan conditions. To achieve effective screening, it is vital to characterize the prevailing strains in the country and determine their potential to produce aflatoxins. This will help to identify strains suitable for resistance screening; the information obtained may also be used to estimate the risk of consumer exposure to aflatoxins.

1.4. Research objectives

The overall objective of this research was to contribute to the management of maize ear rots and associated mycotoxins through host plant resistance.

The specific study objectives were:

i. To determine the distribution of maize ear rots, *Aspergillus flavus* and aflatoxin in the major maize growing areas of Uganda
ii. To determine aflatoxin accumulation among maize inbred lines and their genetic variability.
iii. To determine the inheritance of resistance to *Aspergillus flavus* kernel infection and aflatoxin accumulation in tropical maize.

1.5. Hypotheses

i. There is a wider distribution of *Aspergillus* ear rots, aflatoxins and vast variability within the population of *Aspergillus* section *flavus* due to conducive environmental conditions in the major maize growing areas of Uganda
ii. There is significant variation in aflatoxin accumulation in maize inbred lines due to high genetic diversity among the tropical maize germplasm because of wide genetic base.
iii. Resistance to *Aspergillus flavus* kernel infection and aflatoxin accumulation is conditioned additively by multiple genes.

CHAPTER TWO

LITERATURE REVIEW

2.1. Constraints to maize production in Uganda

Despite the importance of maize in Uganda, a wide extent of biotic and abiotic imperatives, including destitute soils, drought, pests and diseases are known to decrease crop output, and eventually diminished nourishment security (Strange and Scott, 2005, Knox et al., 2012). Concerns that biotic and abiotic constraints have been exacerbated by farming practices both in the broad maize crop production system and intensive systems in sub-Saharan Africa respectively which have had a negative impact on the environment (Poppy et al., 2014, Dogliotti et al., 2014, Chartres and Noble, 2015). Common illustrations, disease and pests and diseases recurrent imperatives, adequately destroying crops plants that are rarely produced.

Resistance within some pests has been as a result of pesticides applications and alterations to pest and disease-resistant germplasm, hence resulting in epidemics (Oerke, 2006). In other cases, endeavours to address crop production imperatives have accidentally presented new and emerging diseases and pest problems for illustration, improvement coupled with utilization of early-maturing varieties of millet, maize and sorghum to combat dry spell limitations has uncovered grains to fungus and moulds that presently demolish harvests in a few locales (Haussmann et al., 2012, Sserumaga et al., 2016). These fungi are the number one producers of secondary metabolites which have an impact on both human and animal health. A key fungus called *Aspergillus flavus* is known to produce aflatoxin in most of the cereals.

2.2. *Aspergillus* ear rots in maize

In Africa, *Aspergillus* ear rots are one of the most important diseases as they reduce grain quality and yield. The most predominate fungi that cause ear rots is *Aspergillus flavus* Link: Fr. In addition to the grain damage, the fungi produced toxins which reduce the quality of the grain. In most parts of the world, especially Africa, maize and its product contamination is the source for several human and animal (Bandyopadhyay et al., 2007; Udomkun et al., 2017).

There are two modes in which the fungus again access to the ear at silking; Germination of the spores down the silk to the kernel and through wounds due to hails, insects and birds (Hesseltine and Bothast, 1977; Reid et al., 1996; Xiang et al., 2012). There are many factors enable fungal infection; insect damage, open husk cover but drought plays a lot in influencing fungal infection in the field. Suppression of bio-competitive antifungal proteins, phytoalexins or protective compounds (phenols) is attributed to drought (Bhatnagar-Mathur et al., 2015) by preventing proper seed maturation but also influence the growth of *A. flavus* and aflatoxin synthesis (Kambiranda et al., 2011; Bhatnagar-Mathur et al., 2015).

This hinders the seeds' capacity to produce phytoalexins due to reduced moisture content during drought. This enhances fungal colonization and hence compromises

defence and also increases aflatoxin formation thus resulting in devastating economical loses (Bhatnagar-Mathur et al., 2015; Guo et al., 2005).

Although Aflatoxin contamination is exacerbated by drought intensity, drought tolerance does not seem to be sufficient in itself to reduce aflatoxin contamination in all crops (Bhatnagar-Mathur et al., 2015; Hamidou et al., 2014). Ear rots show up as mouldy growth on ears and kernels. Aspergillus ear rot can be identified as a greenish-yellow mould on and between kernels. The spores of the fungus look like a powder and may spread in the air when the corn is husked. Most commonly it can be seen on the top of the corn ear, but it can also infect other parts of it too, all the way to the bottom. For *Aspergillus* to survive in the soils for an extended period, it produces specialized survival structures.

2.3. The genus *Aspergillus*

There are approximately 250 species with *Aspergillus* species being ubiquitous environmental moulds frequently isolated from soil, air, water, and vegetation (Geiser, 2009; Nouripour-Sisakht et al., 2015). They are known to cause aspergillosis, which is one of the most important causes of mortality and morbidity monge patients with health status which is immunocompromised (Bernardeschi et al., 2015; Tsang et al., 2016). Among the known *Aspergillus* spp., *Aspergillus oryzae, Aspergillus fumig*atus, *Aspergillus flavus, Aspergillus niger,* and *Aspergillus terreus* are most commonly associated with *Aspergillus* infections, including *Aspergillus* onychomycosis (Fernández et al., 2013; Tsang et al., 2016).

However, they are also involved in many industrial processes including production of enzymes, commodity chemicals which is produced by *Aspergillus niger* and food stuff like soy sauce produced by *Aspergillus oryzae* (Gomi and Abe, 2007). *Aspergillus nidulans* has been used to illustrate the para-sexual cycle, basic concepts in the regulation of metabolic pathways, the cell cycle, intron splicing and hypha polarity (Bentley and Bennett, 2008). Several species of *Aspergillus* contaminate grains and other foods with harmful metabolites that are a threat to the health of both humans and livestock (Wild and Gong, 2010).

2.4. *Aspergillus flavus* ecology and biology

Aspergillus flavus is one of the ubiquitous fungi belonging to Aspergillus genus that are therapeutically and pathologically important (Vadlapudi et al., 2017). *Aspergillus* is a large genus that is comprised of 184 accepted anamorphic and 70 associated teleomorphic names (Pitt et al., 2000; Samson et al., 2014). The genus is divided into subgenera and sections, of which we have 4 subgenera and 20 sections (Houbraken et al., 2014). The genus is characterized by conidiophore though species identification and differentiation is traditionally based on morphological features (Rodrigues et al., 2007; Vadlapudi et al., 2017). These include conidial and mycelial colour, colony diameter, colony reverse colour, production of soluble pigments and exudates, sclerotia and cleistothecia among the macro-morphology (Rodrigues et al., 2007; Calderari et al., 2013; Gonçalves et al., 2012; Luo et al., 2014). *Aspergillus* Section *Flavi* has attracted worldwide attention due to its industrial use and toxigenic potential (Norlia et al., 2018). It is divided into aflatoxigenic species mainly *Aspergillus flavus*, *Aspergillus parasiticus* and *A. nomius*, and the atoxigenic species which includes: *A. oryzae*, *A. sojae* and *A.*

tamarii, known for its use in the production of fermented foods in Asia (Kumeda and Asao, 2001; Norlia et al., 2018).

Aspergillus flavus is mainly competent saprophyte that lives in soil and organic plant materials as mycelium (hypha) or sclerotia (Jaime-Garcia and Cotty, 2004; Abbas et al., 2008; Ehrlich, 2014) that in turn serve as the source of new conidia (Abbas et al., 2009). *Aspergillus flavus* propagules overwinter in plant debris and soils hence serve as a primary inoculum for an infestation of below ground plant parts. Soil populations of *Aspergillus flavus* under maize cultivation have been reported to range from 200 to >300,000 colony forming units (CFU) g^{-1} soil (Abbas et al., 2004, Zablotowicz et al., 2007) and can constitute from 0.2% to 8% of the culturable soil fungi population. Soil organic matter is the major soil property associated with maintaining soil populations of *Aspergillus flavus* (Zablotowicz et al., 2007, Abbas et al., 2009).

Aflatoxigenic isolates occurrence in any environment can range from 50% to 80% (Abbas et al., 2004). The distribution of non-aflatoxigenic and aflatoxigenic isolates is influenced but a number of factors which include: temperature, rainfall, plant species, soil composition, cropping history, and crop management (Zablotowicz et al., 2007). These factors can either reduce or elevate the levels of *Aspergillus flavus*, for example, noncultivated fields near cultivated land are observed to have very low populations of *Aspergillus flavus* and also the frequency of drought reduces soil populations of *Aspergillus flavus* (Horn, 2007; Yao et al., 2015).

2.5. Aflatoxin synthesis

Extensive research has been conducted to understand the mechanism for aflatoxin biosynthesis and it has revealed that about 30 genes clusters with more than 23 steps of enzymatic reactions are involved in the pathway (Georgianna and Payne, 2009; Yu, 2012; Yang et al., 2015). This makes it so sophisticated (Klich, 2007; Yang et al., 2015) however, for most aflatoxin producers *Aspergillus flavus* and *Aspergillus parasiticus* all these genes are high homologous and 25 genes have been shown to be identical (Yu et al., 1995; Šimončicová et al., 2017). Within the gene cluster, 29 genes have been identified to date (Cleveland et al., 2009; Šimončicová et al., 2017) and their functions elucidated.

Biosynthesis process of aflatoxin is stimulated by several environmental factors, these are transduced via complex signaling cascades that control the expression of both global-acting and aflatoxin pathway-specific transcription factors and orchestrated by a complex regulatory network of genes and enzymatic steps (Yu et al., 2004; Chanda et al., 2009; Yin and Keller, 2011; Brakhage, 2013; Kenne et al., 2018;). This network operation is governed by the interaction of a set of global transcription factors, including LaeA and VeA (Calvo et al., 2004; Duran et al., 2006; Bayram et al., 2008; Calvo, 2008; Kale et al., 2008; Brakhage, 2013). Once the signal is received from the cell surface receptors, communication with pathway-specific transcription factors by the global transcription factors like is AflR (Cary et al., 2006; Kenne et al., 2018) and GliZ (Bok et al., 2006; Scharf et al., 2012) to activate specific aflatoxin biosynthesis genes. Two known aflatoxin pathway-specific regulator genes, aflR and aftJ, localized to the

aflatoxin biosynthetic gene cluster in *Aspergillus flavus*. The enzymes involved in the synthesis are localized in toxisomes (Chanda et al., 2010; Roze et al., 2011; Lim and Keller, 2014) these provide a platform for the completion of the biosynthesis, and exportation of aflatoxin to the environment (Menke et al., 2013; Lim and Keller, 2014).

2.6. Aflatoxin production and accumulation

There are several factors that influence the development of *Aspergillus flavus* and production of aflatoxins, these include physical, chemical and biological factors (Kosegarten et al., 2017). Among these factors may either be: strain of the fungus, substrate nature, moisture and relative humidity, temperature, time of incubation, aeration, damage, growth and maturity of the host, and irradiation (Frisaved, 1995; Marai and Asker, 2008; Schmidt-Heydt et al., 2010; Gallo et al., 2016).

Various strains have different abilities to produce aflatoxins. There are several species which can produce one or more aflatoxins and these include *Aspergillus flavus, Aspergillus parasiticus, Aspergillus oryzae, Aspergillus tamari, Aspergillus flavus Var. columnaris, Aspergillus parasiticus Var. globosus, Aspergillus niger, Aspergillus wentii, Aspergillus ruber, Penicillium puberulum*, *P. variable* and *P. frequentans* but also some isolates of actinomycetes were found to be aflatoxin producers (Marai and Asker, 2008; Niessen et al., 2018).

Aspergillus flavus is capable of produces aflatoxins on various foods although each supports production differently (Marai and Asker, 2008; Garcia et al., 2011; Niessen et

al., 2018). In general, structure and composition of the substrate as well as the presence of antifungal agents and ability of microbial interaction contribute to aflatoxigenicity (Gougouli et al., 2011; Kosegarten et al., 2017). Among the elements, Zinc plays an important role in the biosynthesis of aflatoxins (Marai and Asker, 2008; Wee et al., 2016).

Aspergillus flavus has a wide range of growth temperatures with a minimum range of 6-8 °C, the optimum range of 36-38 °C and maximum range of 44-46 °C (Pitt and Hocking 2009; Mousa et al., 2011). Maximum growth and aflatoxin production can occur at 25 °C and 0.95 water activity respectively (Peromingo et al., 2016). However, temperature (30/37 °C) and water stress (0.99/0.91) affects aflatoxin B1 production (Medina et al., 2017).

Oxygen requirement for aerobic fungal organism for their sporulation, spore germination and vegetative growth is highly variable (Littlefield et al., 1966; Walker and White, 2017). In vitro, aflatoxin productions are exacerbated by oxidative stress in vitro (Fountain et al., 2016).

Earworm and fall armyworm caterpillars are a common cause of damage to Kernels as they feed (Siebert et al. 2012; Flanders et al. 2014). *Aspergillus* Section *Flavi* species invade during maturation in the field or after harvest (Nesci et al., 2016) hence gain entry through the damaged kernels.

Ultraviolet (UV) irradiation is proven to be an effective method to reduce or detoxify aflatoxins (Tripathi and Mishra, 2010; Mao et al., 2016). However, the degradation efficiency of these toxins varies with the differences of irradiation conditions (Tripathi and Mishra, 2010; Liu et al., 2011; Mao et al., 2016).

2.7. Mechanisms of resistance to *Aspergillus flavus*

2.7.1. Structural resistance of the plant

Structural resistance is a collection of traits that physically impair entry and infection by *Aspergillus flavus.* They include the extent of cob husk coverage and tightness and the physical structure of the kernel (Cary et al., 2011). A number of studies have shown a strongly positive correlation of husk cover with *Aspergillus flavus* infection (Betran et al., 2002; Betran and Isakeit, 2004; Melanie, 2006). Husk cover, however, is influenced by the length of time to maturity of a given genotype. Full season genotypes have been shown to have better husk cover than early maturing genotypes. In areas where grain moisture content at harvest generally exceeds 20%, and therefore additional costs of post-harvest drying are incurred. In contrast, where the grain dries in the field to a moisture content less than 15%, good husks are desired. It is, therefore, difficult to separate the effects of maturity, husk cover and weather on the degree of infection by *Aspergillus flavus* (Betran and Isakeit, 2004). For this reason, there are variations in correlations between husk cover and *Aspergillus flavus* among experimental locations and seasons (Melanie, 2006).

Kernel-based resistance represents host resistance since it is a major target of aflatoxigenic fungi (Cary et al., 2011). Kernel features that impend entry of the fungus into the plant tissues and cells include a thick cuticle and cell wall and, kernel texture. From Kernel Screen Assay studies revealed that two levels of resistance, at the pericarp and at the sub pericarp level exits (Cary et al., 2011).

2.7.2. Biochemical composition of the kernel

Virulence proteins of *Aspergillus flavus* by several constitutively expressed and inducible proteins of the host have been described in the literature (Xie et al., 2010). Antifungal and avirulence proteins accumulation has shown to aid resistance in several maize lines (Fountain et al., 2015). Studies have identified proteins and the genes encoding them in maize kernels which have an effect on *Aspergillus flavus* growth (Chen et al., 2010). In addition to proteins, also phytohormones play an integral role in the defence mechanism (Robert-Seilaniantz et al., 2011).

2.7.3. Response mechanisms of the plant

Plants have different components in their system which they switch on when they are challenged. This is based on the gene for gene perspective, were an invading pathogen that produces virulent gene is counteracted by a single avirulence gene (Fountain et al., 2015). Studies have been conducted to study the defence mechanism but information about signal transduction pathways that link receptors to response pathways has not been elucidated especially in maize (Moreno and Kang, 1999; Chandrashekar et al., 2000; Pechanova et al., 2013).

2.8. Resistance maize breeding to *Aspergillus flavus* ear rot

The interest of most breeders is to identify traits that confer resistance to *Aspergillus flavus* infection which have been reported to be genetically controlled (Brown et al., 1999; Brown et al., 2010; Brown et al., 2016). The genetic component of tolerance to aflatoxin production is quantitatively inherited, with low broad-sense heritability, and hampered by the effects of genotype x environment interaction. Additive gene effects have been shown to be more significant than dominance effects (Zuber et al.,1978; Hamblin and White, 2000; Fountain et al., 2015). Several other studies have indicated that variances due to general combining ability effects are more than variance due specific combining ability effects (Naidoo et al., 2002; Cary et al., 2011).

Many control measures used to suppress aflatoxin accumulation in maize appear to be economically unfeasible, but developing resistance in the host to avoid pre-harvest infection appears to be a coherent and prudent way to fathom this issue in maize ((Widstrom, 1996; Brown et al., 1999; Bhatnagar-Mathur et al., 2015). Growing understanding of the mechanisms of resistance and the genetic control of the traits related to it has facilitated attempts to develop resistant varieties (Brown et al., 1999; Naidoo et al., 2002; Cary et al., 2011; Fountain et al., 2015). It is also exceptionally troublesome to anticipate the reaction of a genotype to *Aspergillus* spp infection and aflatoxin accumulation in any specific location. This large genotype by environment interactions makes it necessary to test genotypes over several years and locations (Melanie, 2006; Hamidou et al., 2014). Breeding for resistance may be facilitated by the

identification of antifungal proteins as biochemical markers, or by the development of molecular markers from the encoding genes for these proteins.

2.8.1. Methods of testing maize for resistance to *Aspergillus flavus*

Screening of maize in the field based on natural inoculum of *Aspergillus flavus* can lead to misleading data in evaluating aflatoxin resistance since inoculum in the field is random every year therefore artificial inoculation is used (Windham and Williams, 1998; Williams et al., 2008). Two major techniques for inoculation are non-wounding and wounding. The effectiveness of these techniques relies on proper timing of inoculation during the stage of the cob development (Widstrom, 1996; Windham and Williams, 1998; Naidoo et al., 2002; Williams et al., 2008; Henry et al., 2012). To asses resistance to natural fungal infection, non-wounding methods may be used (Gorman and Kang, 1991, Li, 2004; Williams et al., 2008; Henry et al., 2012). These include soil inoculation, silk inoculation, toothpick-under-husk inoculation and bag inoculation (Zhang et al., 1998; Henry et al., 2010).

2.8.2. Assessment of kernel infection

Bright greenish-yellow fluorescence (BGYF) is a visual marker to identify maize or other grains that may be contaminated with *Aspergillus flavus* and aflatoxin. The presence of BGYF is used as a presumptive test in grain elevators to identify corn samples that should be examined with a determinative test for aflatoxin (Maupin et al., 2003; Lunadei et al., 2013). Breeders can develop maize with resistance to kernel infection through selection based on a reduced percentage of kernel infection (Zhang et al.,

1997; Alunga et al., 2016). This involves a random selection of kernels from all ears in each plot that was inoculated.

Determination of Percent-Kernel-Infection can be done with or without the presence of culture media. Two methods of media-free assays include media-free and isolated-kernel incubation and laboratory-based infection resistance screening (Li, 2004; Alunga et al 2016). They substantially improve the accuracy and reduce the cost of determining kernel infection rate (KIR) (Ruming et al., 2002; Alunga et al., 2016). Both methods utilize an exceptionally basic and reasonable device which include bioassay trays, petri dishes, and Whatman paper to hold moisture. The kernels are kept in 100% relative humidity at a favourable temperature of 31^{o}C, and incubated for 7 days (Brown et al., 1999; Alunga et al., 2016). Kernels are placed in micro-cups in such a way that *Aspergillus flavus* can be induced to grow out with an equal chance from every infection site. This should prevent underestimation of kernel infection rate. Kernel isolation with micro-cups also results in significantly less cross-infection among kernels than among the non-isolated ones, suggesting that kernel isolation is necessary for kernel plating (Brown et al., 1999; Alunga et al., 2016).

The micro-environment inside a micro-cup favourably induces the development of *Aspergillus flavus* in an incubator with minimal bio-competition (Li, 2004; Alunga et al., 2016). The difference between media-free and kernel isolated-kernel incubation and laboratory-based infection resistance screening is that the former is used to evaluate

kernels from cobs inoculated in the field, while the latter, involves laboratory inoculation of kernels from cobs that were not inoculated in the field.

2.9. Testing and quantification of aflatoxin in grain

In order to obtain reliable results, proper sampling procedures are pre-requisite since aflatoxin is always heterogeneous distributed because fungus normally colonizes isolated pockets, therefore a tendency of the uneven distribution of infection and aflatoxin accumulation. Also, the detection method of aflatoxin should be both specific and sensitive to quantify the trace levels (Babu and Muriana, 2014). Therefore, development of a strategy that ensures that samples taken for analysis represent any consignment is vital (Zheng et al., 2006, Melanie, 2006; Babu and Muriana, 2014). A number of direct and indirect assays are available for quantification of aflatoxin content. There are different methods of quantifying aflatoxin accumulation and these are either rapid methods or conventional analytical methods.

Quantitative methods of aflatoxin detection include high-performance liquid chromatography and thin-layer chromatography, recently liquid chromatography-tandem mass spectrometry, suitable for use in regulatory laboratories (Shephard, 2009; Yao et al., 2015) and a real-time immunoquantitative polymerase chain reaction method (Babu and Muriana, 2014). Also, several qualitative and semi-quantitative methods based on immunology including immunoaffinity column assays and enzyme-linked immunosorbent assays (Pittet, 2005 Yao et al., 2015).

Other detection systems for screening and for rapid detection in the field and laboratory include dip-stick kits (Pittet, 2005, Yao et al., 2015), optical-based sensing methods (Stark, 2010; Yao et al., 2010; Harvey et al., 2013; Yao et al., 2015), and g biosensors (Pittet, 2005; Pascale, 2009; Tothill, 2011; Vidal et al., 2013 Malhotra et al., 2014).

2.10. Mating designs used to develop ear rot resistant hybrids

There are several mating designs that are widely used when developing new hybrids and carrying out recurrent selection (National Research Council, 1963; Hallauer et al., 2010; Hinkelmann, 2011; Wen et al., 2015). These mating designs include North Carolina I, II and III design (Comstock and Robinson, 1952; Kusterer et al., 2007; Wen et al., 2015) and Diallel (Griffing, 1956; Hallauer et al., 2010). In order to achieve heterosis, potentially suitable parents and superior combinations must be identified. Also, appropriate mating designs like North Carolina and diallel are useful when determining the best combination of female and male which will produce a desirable hybrid. They also provide information on genetic components and enable the breeder to choose appropriate breeding methods for hybrid variety or cultivar development programs.

A diallel mating design can be referred to as the analysis of all possible combination among a different group of parents. The method as defined by Griffing (1956) and it has been used extensively in plant breeding (Griffing, 1956; Hallauer et al., 2010). Inferences from data generated from a diallel trials can only be valid if assumptions about parental materials are true: gene frequencies are equal to one-half at all

segregating loci, diploid, homozygous parents, genes are independently distributed between parents, segregation, and no non-allelic interaction occur (Sokol and Baker, 1977; Hallauer et al., 2010).

North Carolina designs are commonly used in estimating genetic variance and the degree of dominance (Wolf et al., 2000; Yu and Bernardo, 2004; Hinkelmann, 2011; Wen et al., 2015). In these designs, inbred lines are usually crossed with one another and their general combining abilities and specific general combining abilities evaluated (Meseka et al., 2006; Hinkelmann, 2011; Wen et al., 2015). A good line to use should be simple to use, with high genetic gain and high yielder. However, it is difficult to identify an inbred line with these characteristics (Meseka et al., 2006; Hinkelmann, 2011; Wen et al., 2015). It has been reported that inbred lines show general deterioration in yield and vigor while hybrids developed from these lines produce yields that exceed them (Mhike et al., 2011). This is attributed to hybrid vigor (heterosis) that results when two inbred lines are combined (Echarte and Tollenaar, 2006; Li et al., 2007; Birchler et al., 2010; Qu et al., 2012; Wen et al., 2015). Therefore, commercial production hybrids are more preferred than inbred lines since they also produce desirable uniformity.

CHAPTER THREE

DISTRIBUTION OF MAIZE EAR ROTS, *ASPERGILLUS FLAVUS* AND AFLATOXIN IN THE MAJOR MAIZE GROWING AREAS OF UGANDA

3.1. Abstract

Although aflatoxin in Uganda has been reported in maize, scanty comprehensive evidence on the distribution of maize ear rots, *Aspergillus flavus* strains, and aflatoxins levels from maize growing areas has been reported. This study determined the distribution of maize ear rots, *Aspergillus flavus* strains, and aflatoxin levels in the major maize growing areas of Uganda. A hierarchical method was utilized to collect 257 infected grain samples from major maize growing areas of Uganda. These were evaluated for *Aspergillus flavus* ear rots, and *Aspergillus flavus* strains on their incidence and severity plus aflatoxin occurrence and contamination. Results showed significant differences ($P<0.001$) within and among agro-ecological zones and districts for *Aspergillus spp,* maize ear rots and aflatoxins levels in terms of incidence and severity. Very high levels of aflatoxin accumulation were detected with one sample had over 3760 ng/kg, which is 376 times higher than the legal limit. These results reveal the presence of *Aspergillus flavus* ear rots, diverse *Aspergillus flavus* populations plus aflatoxin accumulation and their wider distribution in most of the agro-ecological zones in Uganda. These calls for routine stop checks are needed by the Uganda National Bureau of standards to monitor aflatoxin levels in different agricultural commodities in order to enforce management guidelines.

3.2. Introduction

Aspergillus section *Flavi* is a group that has saprophytic fungus and among these include *Aspergillus flavus* which produces secondary metabolites referred to as mycotoxins in particular aflatoxins. This may results in aspergillosis and aflatoxicosis in both animals and humans (Khrishnan et al., 2009; Yunus et al., 2011; Salem et al., 2018). Infection by *Aspergillus flavus* in various crops can occur either before or after harvest thereby resulting into aflatoxin accumulation (Cotty et al., 1994; Scully et al., 2009; Probst et al., 2014; Fountain et al., 2015). The impact of aflatoxins could result in even greater crop losses in maize with predicted changes in global climate, (Gilbert et al., 2016; Gilbert et al., 2018), with estimates as high as $1.68 billion/year (Mitchell et al., 2016; Gilbert et al., 2018). Effects of aflatoxin in animals can result into reduced growth, immune system suppression, and death or reduced feed use efficiency (Sisson, 1987; Yunus et al., 2011).

There are various studies conducted in Uganda to access aflatoxin levels in different agricultural commodities (Kaaya and Warren, 2005; Simyung et al., 2013; Agol et al., 2017; Muzoora et al., 2017) however, scanty comprehensive information on distribution and diversity of *Aspergillus flavus* strains, ear rots and aflatoxins levels in different parts of the country where maize is highly produced. Uganda is characterized with diverse climatic conditions, hence the interaction of these conditions with the type of cropping system plus the fungal community influences the aetiology of contamination (Cotty et al., 2008, Probst et al., 2010). This study was conducted to determine the distribution

and occurrence of maize ear rots, *Aspergillus flavus* strains, and aflatoxin levels in Uganda.

3.3. Materials and Methods

3.3.1. Description of study regions

The survey covered the major maize producing agro-ecological zones of Uganda, taking into consideration at least one main maize producing District from each agroecological zone; Lake Victoria crescent with districts; Wakiso, Luwero, Mityana and Mubende is characterized by rainfall range 1,200-1,450 mm, at 1,000-1,800 masl., with wetland and forests plus hilly and flat areas. The soils are good to moderate. Lake Albert crescent with districts; Hoima, Masindi, Kiryandongo, Kyenjojo and Kabarole is characterized by Rainfall ranging from 800-1,400 mm, 620-1,585 masl., with undulating flat hills. Soils are good to moderate. Eastern savannah with districts; Kumi, Pallisa and Soroti are characterized by rainfall ranging from 800-1500 mm, 1,200-1,340 masl., generally flat with undulating hills, moderate to good soils. Busoga farming system with districts; Iganga and Bugiri is east of river Nile and north of Lake Victoria, rainfall ranging from 1,000-1350 mm; 1,215-1,320 M, flat and swampy in places, soils poor to moderate and Northen farming system is characterized by average rainfall 1200 mm, 975-1,520masl., with fairly heavy fertile soils on isolated hills.

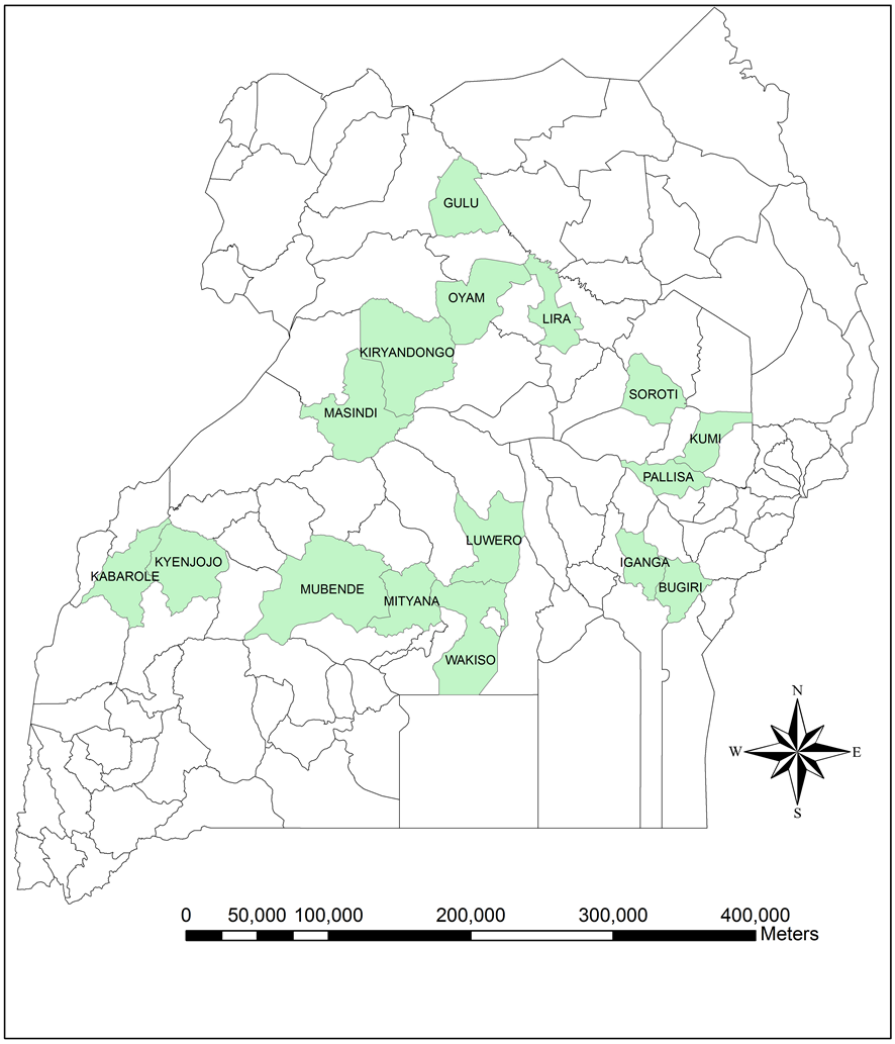

Figure 3. 1 Districts in Uganda where sampling for infected maize was done developed using QGIS software.

3.3.2. Sample collection and assessment of *Aspergillus flavus* infection in maize kernels

Sampling was done using a three-level hierarchical sampling method to collect infected maize cobs. Maize sample was obtained from the farmer's field where 10 pre-harvested maize cobs were randomly selected from 5 x 5 quadrate for aflatoxin testing. The samples were assayed for levels of aflatoxin as described below. Climate data during the sampling period was accessed from the website (AwhereAp, 2017) (Table 3.1).

Sampled maize cobs were kept in bags to absorb at the moisture and later sun dried for seven days. Using direct plating techniques, 100 kernels per sample was assayed for fungal colonization (Zhang et al., 1997; Moreno and Kang, 1999; Brown et al., 2016; Alunga et al., 2016). Prior to assessment, kernels were sterilized in 2.5% Sodium hypo-chloride, later washed in distilled water 3 times and plated 20 kernels on Potato Dextrose Agar amended with 2 ml/l of lactic acid in a 90 mm petri dish in two replicates and stored at 31 ^{o}C for 7 days. *Aspergillus flavus* cultures were identified based on the macromorphological features such as conidial and mycelial colour, colony diameter, reverse colony colour, and the presence of sclerotia (Klich, 2002; Calderari et al., 2013; Gonçalves et al., 2012; Luo et al., 2014). Using percentage kernel infection method by Alunga et al., (2016) *Aspergillus* ear rot disease incidence was assessed:

$$\text{Incidence} = \frac{(\text{Number of kernels infected with } A.\ flavus) \times 100}{\text{Total number of kernels incubated}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\text{(i)}$$

Table 3.1 Weather conditions prevailing in sampled districts and samples collected.

Districts	Annual rainfall (mm)	Temperature (^{o}C)		Relative humidity (%)	Number of samples collected
		Min	Max		
Hoima	608.0	19.6	27.7	71.4	15
Masindi	560.3	19.2	27.4	70.7	29
Kabarole	598.4	18.0	26.2	72.0	13
Kyenjojo	610.0	18.5	26.7	72.2	18
Kiryandongo	615.8	20.1	28.3	70.4	19
Wakiso	356.3	18.8	27.4	73.3	5
Luwero	484.8	19.4	27.9	73.1	17
Mityana	399.1	18.9	27.4	73.2	15
Mubende	804.6	18.2	26.6	72.5	18
Lira	687.6	20.0	28.6	68.6	23
Oyam	1120.6	20.3	28.7	68.6	14
Iganga	709.6	19.0	28.1	73.0	21
Bugiri	776.9	18.9	28.1	72.9	18
Kumi	527.5	19.2	28.4	70.2	13
Pallisa	535.7	19.1	28.3	71.2	12
Soroti	792.5	19.5	28.5	69.7	7

Source: (AwhereAp, 2017)

3.3.3. Determination of *Aspergillus flavus* strains in maize samples

3.3.3.1. Preparation of culture media

Isolation, identification, and characterization of members of *Aspergillus flavus* strains were done on Modified Rose Bengal agar and 5/2 medium. The Modified Rose Bengal agar medium contains dichloran which restricts the growth of *Aspergillus niger* and other fast-growing fungi. It also contains rose Bengal, chloramphenicol, and streptomycin which restricts bacterial growth (Garber et al., 2012). One media bottle was prepared for every 500 ml of the medium. A stir bar and 10 g (2%) of Bacto^{TM} agar were added to each bottle. The beaker was placed on a stir plate. A stir bar was added and for each litre of purified water, 3 g sucrose, 3 g $NaNO_3$, 0.75 g KH_2PO_4, 0.25 g K_2HPO_4, 0.5 g $MgSO_4.7H_2O$, 0.5 g KCl, 10 g NaCl, 1 ml A and M micronutrients and

5ml rose Bengal stock solution was added. After all the ingredients dissolved, the final volume was brought to 1 litre and the pH adjusted to 6.5 (by adding KOH or HCL) while the solution is stirring. Thereafter, 500 ml of the medium was measured and added to individual media bottles. The bottles were loosely capped and placed on a stir plate to disperse agar. The bottles were placed in a microwave and heated for 15 minutes until agar melted. Then 5 ml of chloramphenicol stock was added (2.5 ml/bottle). The bottles were removed, placed on a stir plate to mix for a few minutes and then placed in the autoclave basket. The media bottles were autoclaved for 20 minutes at 121ºC, at 15 mPa then removed and let to cool to 55-60 ºC. In the biosafety cabinet, 5 ml dichloran stock solution/bottle and 2.5 ml streptomycin stock solution/bottle were added, placed on a stir plate at 70 ºC until pouring (Garber et al., 2012).

3.3.4. Isolation of *Aspergillus* spp from maize samples

Isolation of *Aspergillus* spp was done on Modified Rose Bengal agar following the method by Garber *et al*. (2012). One gram of ground maize samples was weighed and emptied into 10 ml of sterile distilled water in a 40 ml glass vial. The vials were placed on a vortex mixer (Velp Scientifica, Europe) for three minutes at 1750 revolution per minute rpm. Using a pipette and tip, 10, 20 and 40 µl aliquots were dispensed into Modified Rose Bengal agar (three plates per sample which were labelled with the date of inoculation, sample code and amount of inoculum) inside a biological safety cabinet. The suspension was evenly distributed across the surface of Modified Rose Bengal agar plate using a cell spreader starting from lowest to highest dilution. Once all isolation plates were inoculated, they were incubated for three days at 31ºC under no light. Later colonies of *Aspergillus flavus* strains were counted; plates with 8-10 colonies

were selected and the colonies with distinct greenish yellow colour were marked for transfer to 5-2 agar (50 ml of V8 juice and 10 g of agar in 1 L of distilled water) plates (Probst et al., 2007). The number of colony forming unit per gram (CFU/gm) of *Aspergillus flavus* L and S strains were determined using the formula (Olsen et al., 1996):

$$\text{CFU/ml} = \frac{\text{Number of colonies x dilution factor}}{\text{Volume Plated}} \quad \text{……(ii)}$$

The relative isolation frequency of each genus was calculated as follows (González et al., 1996)

$$\text{Frequency (\%)} = \frac{\text{No. of samples with occurrence of species of colonies}}{\text{Total no. of samples}} \times 100 \quad \text{…… (iii)}$$

The modified Rose Bengal agar plates were examined taking note of colour and texture of colonies, size and number of sclerotia. Using a permanent marker, each colony was circled on the bottom of the plate and the total number of colonies written on the plate.

For any plate with colonies exceeding 10, the colonies were not picked because of the possibility of cross-contamination and thus they were autoclaved and disposed of. To pick up colonies, one conidiophore was lightly touched on one colony with the pointed tip of an applicator stick. The 5-2 agar plate was opened in the biological safety cabinet and the stick was stabbed into the centre of the agar and this process was repeated for each colony. Using a label, each 5-2 agar plate was labelled with the correct sample

identification and the date. The inoculated plates were placed in the incubator at 31ºC for five days (Garber et al., 2012).

3.3.5. Identification of *Aspergillus flavus* strains

After 5 days of incubation at 31ºC, identification of *Aspergillus flavus* strains isolates was done on 5-2 plates based on microscopic and macroscopic characteristics of each species. Differentiation of members of *Aspergillus flavus* strains was centred on cultural and macro-morphological features, namely; colony diameter, colour, size and texture of conidia and conidiophore structure (Klich, 2002, Garber et al., 2012). Ridell slides were prepared for confirmation of morphological characteristics where *Aspergillus* colonies were sub-cultured on 5-2 agar and incubated for three days at 31 ºC without light. Slide cultures of *Aspergillus* species were made by placing 5-2 agar squares on a microscope slide raised with a V-shaped glass rod in a sterile glass petri dish plate covered with a sterile paper at the bottom. Some of the *Aspergillus* mycelia and spores were transferred from the isolate to the four edges of the 5-2 agar block using a sterile toothpick. A sterile coverslip was placed on the surface of the 5-2 agar block, the filter paper wetted with distilled water and the plate incubated for three days at 31ºC in the dark.

Slides for light microscopy were prepared by removing coverslips with grown colonies of *Aspergillus flavus* strains and placing them carefully on a microscope slide with a drop of water. The slide was viewed under a light microscope (Leica DM 500, Leica Microsystems, Wetzler, Germany) fitted with a camera (LEICA ICC 50, Leica Microsystems, Wetzler, Germany) and images were taken at ×1000 magnification.

Members of *Aspergillus flavus* producing numerous small sclerotia and scanty conidia were identified as S- strains while those with few large sclerotia and numerous conidia were regarded as L-strain.

3.3.6. Determination of aflatoxin levels in maize grains at harvest

Aflatoxin levels in maize samples was quantified using Enzyme-Linked Immuno-Sorbent Assay Accuscan Pro reader based technique. The ground maize sample in zip-lock bag was homogenized by shaking for 1 minute and five grams of the sample was weighed into 100 ml media bottle and mixed for three minutes with 25 ml of 65% ethanol using an orbital shaker (HS501 IKA-WERKE, Germany); the mixture was filtered through Whatmann filter paper and filtrate obtained in a Tripor beaker. Dilution cups were placed in the sample cup rack and labelled. To each dilution cup, 500 µl of sample diluents was added. A hundred microliters of sample extract was added to the dilution cup with sample diluents and mixed well by pipetting up and down several times. A hundred microliters of the sample extract was transferred into a new clean sample cup. A new reveal Q+ strip was placed into the clear sample cup and left for 6 minutes and the test strip read within 1 minute using the Accuscan Pro reader (Neogen Corporation, 2013). Aflatoxins levels were read in parts per billion (ng/kg) with a lower limit of 2 ng/kg and a high limit of 150ng/kg. Samples with more than 150ng/kg were further diluted in 65% ethanol in the ratio of 1:9. For every 100µl of sample 900µl of 65% ethanol was added. Then the sample was mixed with diluent as explained above.

3.3.7. Data analysis.

Data was subjected to nested analysis of variance using MINITAB release 15 version 15.0.0.1, 2007 (Minitab.Inc, Pennsylvania, USA). The general linear model option was used to ascertain the influence of Agroecologies, district, and field on incidence and severity of *Aspergillus flavus* ear rots. Also, analysis of variance (ANOVA) using PROC ANOVA procedure version 15 and pair-wise treatment mean differences were determined by Tukey's least significant difference test at 95% confidence limit was computed from data obtained from isolations. Data that was not normally distributed was transformed to arcsine before analysis.

3.4. Results

3.4.1: Prevalence, incidence, and severity of *Aspergillus flavus* ear rots

The prevalence of *Aspergillus flavus* ear rots in Uganda was 100%. The mean incidence and severity of *Aspergillus flavus* ear rots across different Agroecologies of Uganda were 48.7% and 4.47%, respectively (Table 3.2). The disease incidence and severity varied among farms and among agro-ecological zones (Table 3.2). The incidence of *Aspergillus flavus* ear rots varied between 0% and 100% while severity varied from 1 to 5. There were highly significant ($p = 0.05$) differences in *Aspergillus flavus* ear rots severity among the three agro-ecological zones as well as incidence. The highest *Aspergillus flavus* ear rots incidence and severity were registered in the Busoga farming system zone and the lowest incidence and severity was registered in Northern Farming system (Table 3.2).

Table 3.2 Incidence and severity *Aspergillus flavus* ear rots on maize samples from five Agroecological zones of Uganda

Agroecologies	Incidence	severity
Northern Farming System	28.92	3.98
Lake Albert Crescent	45.27	4.39
Lake Victoria Crescent	52.43	4.54
Eastern Savannah	56.56	4.61
Busoga Farming System	67.18	4.78
Mean	48.70	4.47
$LSD_{0.05}$	14.36	0.41

Results indicate that there is significant evidence for agroecologies and district effects at a = 0.05 (F-test p-values < 0.05) on the incidence of *Aspergillus flavus*. The variance component estimates indicate that the variability attributable to agroecological zones, districts within agroecological zones, and fields within districts (Table 3.3) was 2.72, 20.13, and 77.15 percent, respectively, of the total variability. The fully nested analysis variance was also similar for severity except that the variability component attributable to agroecological zones, districts within agroecological zones, and fields within districts (Table 3.3) was 6.55, 19.54, and 73.90 percent, respectively, of the total variability.

Table 3.3 Nested analysis of variance for agro-ecologies, district, and field for maize ear rots incidence and severity in four agro-ecological zones in Uganda

Source	DF	Mean squares		Percentage Variance of Total (%)	
		Incidence	Severity	Incidence	Severity
Agro-ecologies	4	7902.19***	17.94***	2.72	6.55
District	11	5705.14***	9.59***	20.13	19.54
Field	241	1123.80	1.87	77.15	73.9
Total	256	365200.19	627.81	38.17	1.59

* Significant at P < 0.05; ** Significant at P < 0.01; *** Significant at P < 0.001

3.4.2. The incidence of *Aspergillus flavus* ear rots from different districts in Uganda

Aspergillus flavus ear rots were presented and isolated from all the maize kernels sampled from different districts of Uganda (Table 3.4). The mean incidence and severity of maize kernels with *Aspergillus flavus* ear rots were 48.70% and 3.83% respectively. Maize ear rots' incidence and severity of the *Aspergillus flavus* ear rots were significantly different ($p \leq 0.05$) among districts. The highest incidence was recorded in Pallisa (74.2%) followed by Bugiri (73.9%) among the districts surveyed. This was the same for severity values of *Aspergillus flavus* ear rots (Table 3.4). Overall and in the central region, Wakiso district registered the lowest incidence (7%) and least severity (2.0) of *Aspergillus flavus* ear rots.

3.4.3 Incidence and diversity of strains of *Aspergillus* spp

The two isolated members of *Aspergillus* spp from maize samples were *Aspergillus flavus* L strain and *Aspergillus flavus* S strain. The S strain produced many small sclerotia (<400 µm in diameter), relatively few conidia and consistently high levels of aflatoxin. The L strain produced fewer, larger sclerotia (>400 µm in diameter), more conidia and, on average, less aflatoxin than the S strain. The cultural and morphological characteristics of *Aspergillus* spp isolated from maize samples are shown in Figures 3.2 and Figure 3.3.

Table 3.4 Incidence and severity *Aspergillus flavus* ear rots from 16 districts of Uganda

District	Incidence	Severity
Pallisa	74.17	4.83
Bugiri	73.89	4.83
Kiryandongo	63.95	4.47
Luweero	62.94	4.24
Iganga	61.43	4.62
Kabarole	60.00	4.15
Mubende	59.72	4.11
Kyenjojo	58.06	4.44
Mityana	55.67	3.60
Kumi	48.46	4.15
Soroti	41.43	4.43
Hoima	31.33	3.20
Oyam	30.00	3.29
Lira	28.26	2.87
Masindi	16.72	2.30
Wakiso	7.00	2.00
Mean	48.7	3.83
LSD 0.05	31.98	1.30

The incidence of *Aspergillus flavus* strains varied in different agro-ecologies, with *Aspergillus flavus* L strain being the most abundant species isolated (96.9%) and S-strain at 3.1%. The incidence of L-strain was significantly ($p<0.05$) different among the groecologies but not significantly different for the S-strain (Table 3.5). Among the agroecologies, the incidence of L-strain was highest in Busoga Farming System with the mean population of 9.2×10^3 CFU/g and lowest in Eastern highland with the mean population of 2.9×10^3 CFU/g. S-strain was highest in Lake Albert Crescent and lowest in

eastern savannah with their mean population of 1.3×10^3 CFU/g and 2.9×10^3 CFU/g respectively (Table 3.5).

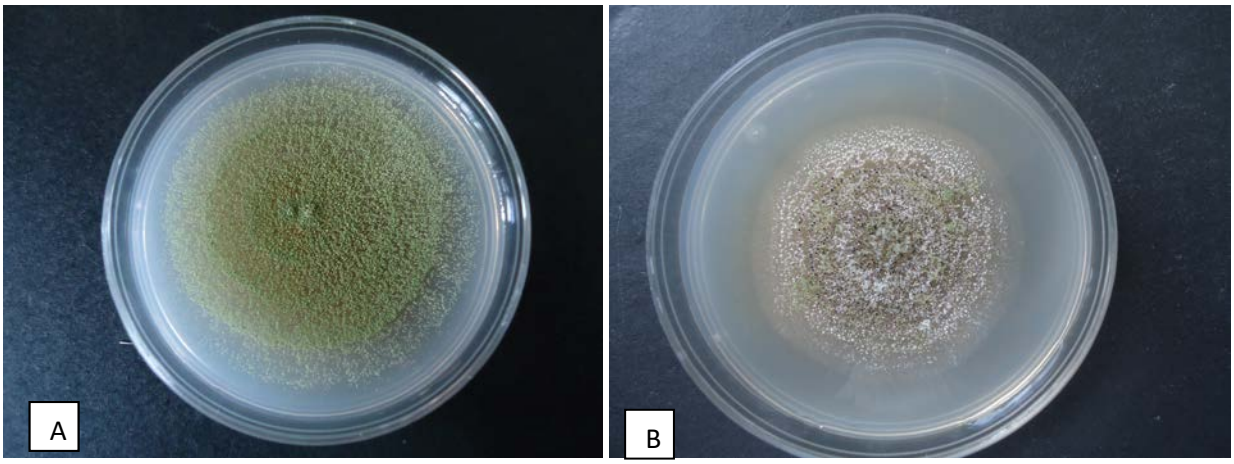

Figure 3. 2 Cultures of *Aspergillus flavus* L strain (A) and S strain (B) growing on 5/2 agar.

Table 3.5 Frequency (%) of *Aspergillus flavus* strains isolated from maize from different agroecologies

Agroecologies	*Aspergillus flavus* L strain	*Aspergillus flavus* S-strain	CFU/g
Eastern Highlands	9.20	0.2	5696
Eastern Savannah	9.95	0.05	2951
Lake Victoria Crescent	10.14	0.17	1341
Lake Albert Crescent	10.56	0.67	950
Northern Farming System	11.08	0.15	4683
Busoga Farming System	13.64	0.09	9234
Mean	10.78	0.341	2998
$LSD_{0.05}$	1.14	NS	4842

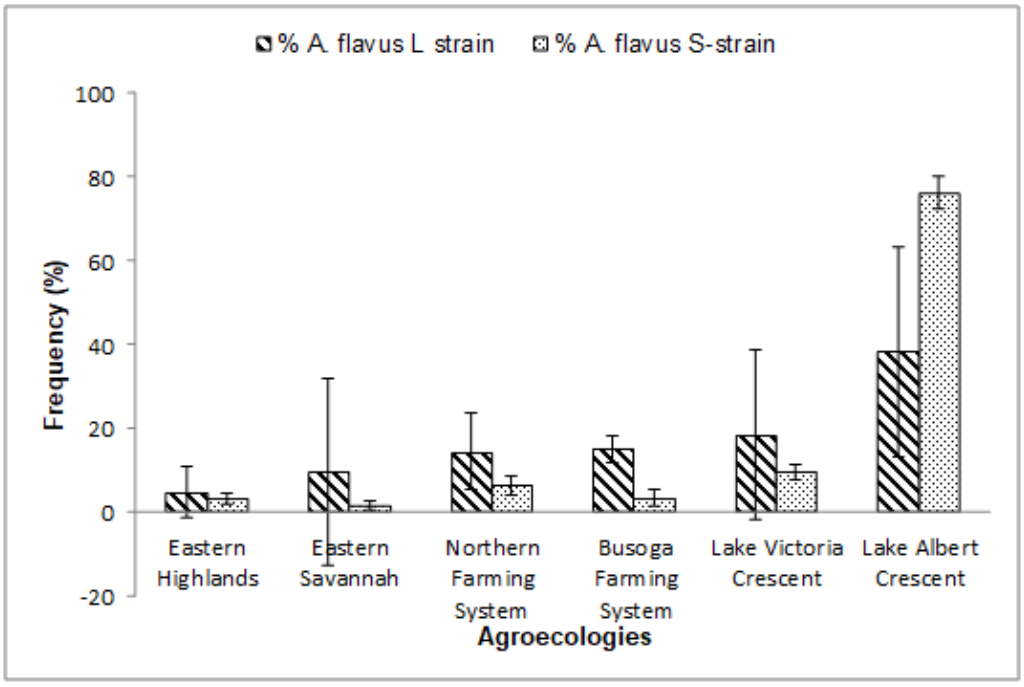

Figure 3. 3 Mean isolation frequency (%) distribution of *Aspergillus flavus* L and S strains from maize sampled from different Agro-ecological zones of Uganda. Error bars represent standard error of the means.

3.4.4 Incidence and distribution of strains of *Aspergillus flavus* across districts

The levels of incidences varied across the districts, with *Aspergillus flavus* L strain being the most prevalent. The incidence of both L-strain and S-strains were significantly different ($p<0.05$) among the different districts (Table 3.6). Among the districts, the incidence of L-strain was highest in Bugiri district and lowest in Kumi district with their mean population of 2.7×10^3 CFU/g and 5.7×10^3 CFU/g respectively. The s-strain population was also highest in Kabarole district and lowest in Mubende with their mean population of 2.5×10^3 CFU/g and 2.1×10^2 CFU/g respectively but absent in Iganga, Pallisa, Kiryandogo, Wakiso and Lira districts (Table 3.6).

Table 3.6 Frequency (%) of fungi isolated from maize kernels from different districts

Districts	*Aspergillus flavus* L strain	*Aspergillus flavus* S-strain	CFU/g
Bugiri	14.8	0.15	2703
Iganga	11.89	0.00	188667
Lira	11.54	0.00	6644
Kiryadongo	11.40	0.00	203
Kyenjojo	11.30	0.50	185
Mityana	10.90	0.30	1437
Masindi	10.69	0.61	252
Hoima	10.62	0.69	2043
Oyam	10.62	0.31	2722
Soroti	10.33	0.11	4604
Kabarole	10.31	1.92	2453
Luwero	10.00	0.23	698
Wakiso	9.69	0.00	1912
Pallisa	9.60	0.00	1464
Mubende	9.38	0.08	213
Kumi	9.20	0.20	5696
Mean	10.78	0.34	2998
$LSD_{0.05}$	2.92	0.91	5846.8

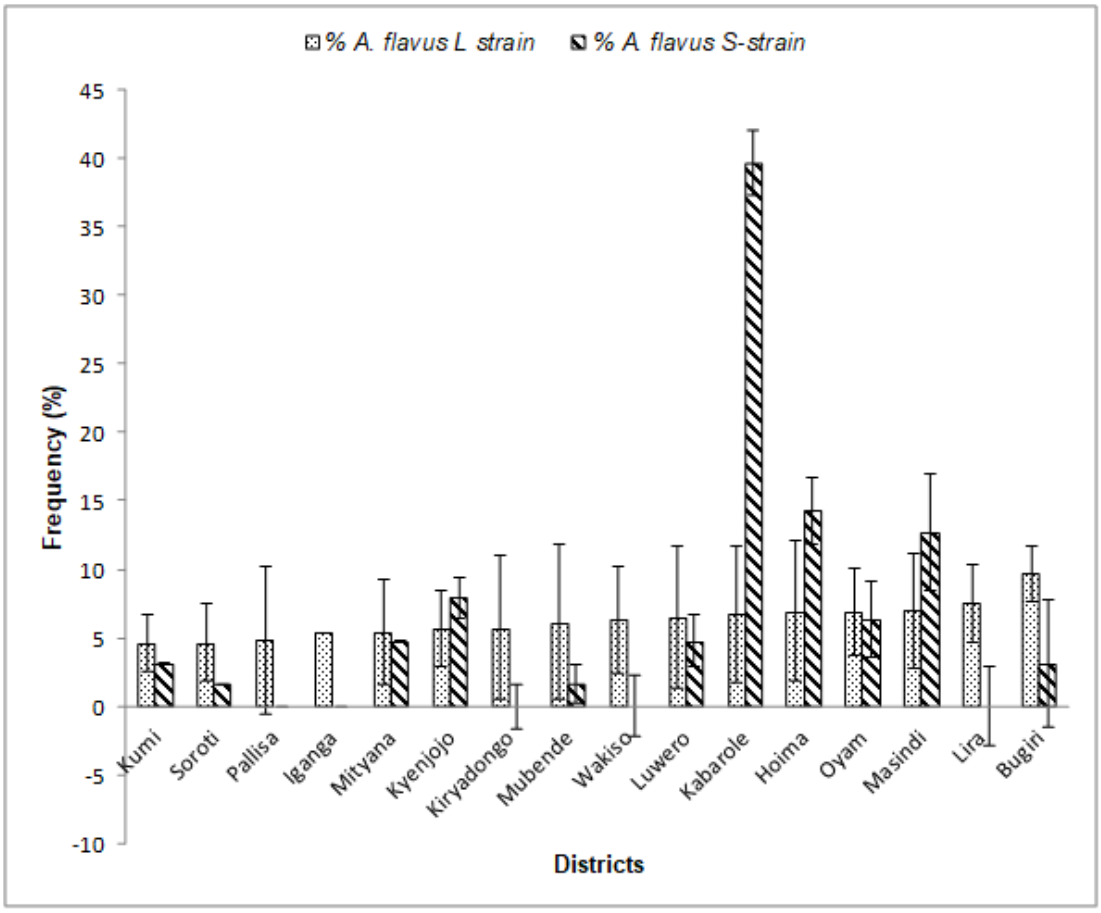

Figure 3. 4 Mean isolation frequency (%) of *Aspergillus flavus* L and S strain from maize sampled from different districts of Uganda. Error bars represent standard error of the means.

3.4.5. Aflatoxin levels in maize grains from surveyed agro-ecological zones

Among the samples of maize collected for different locations, 25% had detectable levels of aflatoxin and 5% were contaminated above the regulatory limit of >10 ng/kg (Table 3.7). The highest percentage of aflatoxin-contaminated maize was recorded in Eastern Savannah Agro-ecological zone and Kumi district. (Table 3.7 and 3.8). Mean levels of aflatoxin were not significantly different between agro-ecological zones in the samples collected. Mean levels of aflatoxin were highly significantly different among districts where the samples collected ($P < 0.001$).

Table 3.7 Aflatoxin level in maize sampled from different agroecologies

Agroecology	Range (ng/kg)	Arithmetic mean (ng/kg)	Geometric mean (ng/kg)	Percent exceeding >2ng/kg	Percent exceeding 10ng/kg
Busoga Farming System	<2 - 41.90	4.43	2.67	18.18	13.64
Eastern Savannah	<2 - 174.43	9.73	2.91	40.00	10.00
Lake Albert Crescent	<2 - 3760.00	53.89	2.48	20.83	1.39
Lake Victoria Crescent	<2 - 7.67	1.79	2.31	27.78	0.00
Northern Farming System	<2 - 181.33	10.58	3.10	23.08	11.54
total				25.27	5.38

Table 3.8 Aflatoxin level in maize sampled from different districts

Districts	Range (ng/kg)	Arithmetic mean (ng/kg)	Geometric mean (ng/kg)	Percent exceeding >2 ng/kg	Percent exceeding >10 ng/kg
Bugiri	<2 - 41.90	5.80	2.83	23.08	15.38
Iganga	<2 - 13.13	2.44	2.47	11.11	11.11
Kumi	<2 - 60.73	7.85	3.09	80.00	10.00
Pallisa	<2 - 2.20	1.04	2.03	30.00	0.00
Soroti	<2 - 174.43	20.30	3.95	30.00	20.00
Mubende	NA	0.66	2.00	0.00	0.00
Hoima	<2 - 3760.00	290.65	3.86	15.38	7.69
Kiryadongo	<2 - 2.50	1.71	2.08	30.00	0.00
Kyenjojo	<2 - 4.13	1.65	2.15	20.00	0.00
Masindi	<2 - 7.97	2.92	2.85	50.00	0.00
Luwero	<2 - 2.30	1.68	2.19	15.38	0.00
Mityana	<2 - 4.97	1.34	2.03	50.00	0.00
Wakiso	<2 - 7.67	2.03	2.41	30.77	0.00
Lira	<2 - 181.33	1.90	2.43	46.15	23.08
Oyam	<2 - 2.57	20.06	4.70	7.69	0.00
Kabarole	<2 - 2.63	1.10	2.04	46.15	0.00

3.5. Discussion

3.5.1 Prevalence of aflatoxin contamination

Although 5% of maize samples were contaminated with Aflatoxin was above the regulatory limits, concentrations varied among and within districts and agroecologies. Our results indicate that the crop is at a high risk for aflatoxin accumulations once harvested and stored in an environment conducive for fungal growth. These results are consistent with the Simyung et al (2013) who reported variability in aflatoxin concentration from the samples collected in Uganda. The arithmetic mean for aflatoxin level was had 3760 ng/kg, which is 376 times higher than the Uganda national bureau of standards threshold and it's also greater than that in previous studies conducted in Uganda (Simyung et al. 2013; Osuret et al., 2016; Tibagonzeka et al., 2018). Similar high levels of aflatoxins contaminations have been found in Kenya (Lewis et al., 2005; Probst et al 2007; Sirma et al., 2016; Nduti et al., 2017) however higher than other studies (Maina et al., 2016). Distribution of ear rots was found to be paramount in all areas meaning most maize varieties produced are susceptible to *Aspergillus flavus* hence high aflatoxin accumulation. From these results, it is highly probable that maize from household represents a substantial source of aflatoxin exposure in Uganda. The high levels of aflatoxin reported was attributed to the strategic mode of sampling adopted from earlier studies which were aimed (Simyung et al. 2013). Results demonstrate that aflatoxin levels unacceptable for both livestock and human consumption are prevalent at all sampled levels in Uganda. It is paramount to examine aflatoxin levels at different nodes of the value chain in order to formulate guiding principle of managing aflatoxin in Uganda.

3.5.2. Prevalence and distribution of *Aspergillus flavus*

Aspergillus flavus communities consist of a complex assemblage of individuals that vary widely in their phenotypic and genotypic characteristics (Agbetiameh et al., 2018; Cotty et al., 1994; Mehl and Cotty, 2010) and represent an important factor in incidences and severities of contamination (Probst et al., 2010). In this study, two morphotypes of *Aspergillus flavus* were identified across agroecologies and districts of Uganda using morphological (colony characteristics and spore ornamentation) and physiological (aflatoxin-producing profile) criteria (Klich and Pitt 1988; Thathana et al., 2017). This follows the same trend as other earlier studies conducted in other countries (Maina et al., 2016; Thathana et al., 2017).

Fungal quantities (CFU g^{-1}) in maize were in different all agroecologies and districts. In these agroecologies and districts, the mean monthly temperatures and humidity usually exceed 25^0C and 70% respectively (Sserumaga et al., 2015). Thus the population of the *Aspergillus* Section *flavus* was expected to be high in crop samples generally. Variation in a population of the *Aspergillus flavus* from different agroecologies and districts, this is could be attributed different climatic condition (Cotty and Jaime-Garcia, 2007; Kachapulula et al., 2017a), type of varieties grown, and method of storage (Sétamou et al., 1997; Kachapulula et al., 2017b) and the farming system in different region/districts in Uganda (Sserumaga et al 2013).

These temperatures have been reported before to be conducive for infection and growth of *A. flavus* (Diener et al., 1987; Pitt and Hocking 2009; Mousa et al., 2011). The host and fungus physiology is normally impacted by high temperatures and drought stress. In the plants, the composition of the kernel has a direct effect on the development of Aspergillus spp hence results into a low or high severity (Atehnkeng et al., 2008; Chanda et al., 2009). In most districts the mean temperature was 28°C and it has been reported that *Aspergillus spp* can survive 19-35 °C (Northolt and Van Egmond, 1981; Pitt and Hocking 2009; Mousa et al., 2011) and produce aflatoxins conducively at 28 °C (Sanchis and Magan, 2004; Peromingo et al., 2016) this explains the high prevalence in Pallisa district with the mean temperatures of 28 °C.

Also, *Aspergillus flavus* growth and aflatoxin accumulation is affected by different farming practices therefore, this can explain as to why high incidences of *Aspergillus flavus* in samples from Bugiri, Iganga, Kiryandongo and Pallisa was registered since maize is left in the field after physiological maturity for more than 3 weeks which leads to increased incidence of *Aspergillus flavus* (Kaaya et al., 2005). Fungal communities tend to be influenced by the farming practices (Sserumaga et al., 2013; Akwero et al., 2016). Eastern region farming system grow most of the crops which are alternative hosts to the fungus and the southern region alternate maize growing with other crops which breaks the cycle of fungal growth. Differences in climatic patterns in the different districts are the key factors that contributed to prevalence variability of *Aspergillus flavus*.

This study provides comprehensive information on potential sources of aflatoxin exposure maize following the recommendation from Sserumaga et al., (2013) study in Uganda. These findings show that significant levels of the aflatoxin exists in maize, therefore, the existence of high aflatoxin-producing strains. Hotspot regions for aflatoxin contamination where these crops have been identified based on both environmental conditions favourable for aflatoxin contamination and high frequencies of *Aspergillus flavus* fungi, suggesting areas for preferential aflatoxin management efforts. Hence this study contributes towards identifying native, widely distributed, and *Aspergillus flavus* associated with crops of target agroecologies. Implementing aflatoxin managements strategies would result in improved health, enhanced trade, increased income, and the welfare of farmers and consumers.

In Uganda, the majority of the population consumes maize on a daily basis with an average of 415 Kcal person^{-1} yr^{-1} derived from Maize and its products (FAO, 2013). It's has been a global concern of home-stored subsistence crops like maize to be contaminated with fungus and its secondary metabolites. In Uganda has been reported in maize, scanty comprehensive evidence on the distribution of maize ear rots, *Aspergillus flavus* strains, and aflatoxins levels from maize growing areas has been reported. Therefore, unacceptable levels found in the current study provides a greater risk to the population whose primary staple crops is maize compared to regions with higher incidences and concentrations but with reduced rates of consumption and diverse diets.

CHAPTER FOUR

AFLATOXIN ACCUMULATION AMONG MAIZE INBRED LINES AND THEIR GENETIC VARIABILITY

4.1. Abstract

Increasing cases of aflatoxicosis and its effect in agricultural systems have resulted in intensification on the use of diverse resources to discover sources of resistance. Little information is known about the genetic diversity and resistance to tropical adapted germplasm. Insight on resistance to aflatoxin accumulations with inbred lines and their genetic diversity is imperative for crop improvement in any breeding program. The study was conducted to assess levels of genetic differences and relationships patterns among inbred lines and evaluate aflatoxin accumulation in inbred lines. Twenty-five Simple sequence repeats (SSR) markers were used to fingerprint 41 inbred lines to asses levels of genetic differences and relationships patterns among inbred lines, from which, 35 inbred lines were used to evaluate aflatoxin accumulation. At all loci, 184 alleles were identified that ranged 2.0 to 9.0 with an average of 7.4 alleles per locus. The minor alleles ranged from 0.10 to 0.8 with an average of 0.5 and major alleles ranged from 0.2 to 0.9 with an average of 0.5. Gene diversity ranged from 0.2 to 0.9 with an average of 0.7. Average heterozygosity percentage of the varieties was 4.0%, ranging from 0.0% to 2.0%, indicating the low level of heterozygosity within the inbred lines. The average polymorphism information content was 0.6. A dendrogram formed three distinct groups, thus suggesting three heterotic patterns. Results have demonstrated that we can form genetically diverse populations and also exploit heterosis in different hybrid combinations using these inbred lines. The comparison of aflatoxin accumulation levels

in different genotypes observed that 20% of the entire samples exceeded Codex standard of 10 ng/kg. The Aflatoxin varied widely from as low as 0 to as high as 142.36 ng/kg and average 8.75. Genotype “CML444” showed significantly high levels of accumulation of Aflatoxin. Hence genotype with low aflatoxin accumulation should be used in hybrid formation.

4.2. Introduction

Aflatoxins produced by *Aspergillus* genus are the most thoroughly studied mycotoxins (Baranyi et al., 2013). Their discovery was a result of the death of 100,00 turkeys after they were fed on contaminated peanut meal which resulted into acute necrosis of the lives in the sixties (Van der Zijden et al., 1962; Varga et al., 2015). Aflatoxin is capable of being toxic and carcinogenic thus possess a negative impact on the heath of both animal and human health (Bennett and Klich, 2003, Baranyi et al., 2013). In international public health concern for a high risk for human hepatocellular carcinoma due to aflatoxins has been proved (Wogan, 1992; Groopman et al., 2005; Blonski et al., 2010; Bray et al., 2013; Chitapanarux and Phornphutkul, 2015; Vartanian et al., 2017). Numerous deaths incidences were also attributed to acute aflatoxicosis (Nyikal et al., 2004; Chitapanarux and Phornphutkul, 2015). Due to the toxic potential, over 100 countries restrict the content of aflatoxin in the food and feed supplies (van Egmond et al., 2007; Bui-Klimke et al., 2014), which has impacted trade across boarders most especially in Africa.

The majority of *Aspergillus flavus* strains produce only aflatoxin B1 and B2, while the majority of *Aspergillus parasiticus* strains (or isolates) produce aflatoxins G1 and G2 in

addition to B1 and B2. These fungi survive in soil, in plant and animal remains and in grains which they produce aflatoxin as their secondary metabolite (Pitt, 2000; Wu, 2015; Bandyopadhyay et al., 2016). Although host resistance is a highly desirable strategy to control infection and aflatoxin accumulation, most commercial are susceptible to *Aspergillus flavus* in Uganda. Identifying germplasm with resistance to a pest or pathogen requires a source of germplasm and reliable techniques for evaluating the germplasm.

In order to start a successful breeding program to address any challenge, prior knowledge on diversity and relationship germplasm have a significant contribution towards the identification of relevant hybrid combinations from an inbred line. Previously best combinations were identified using different mating designs and pedigree information but with the advance in science, the use of molecular markers has proven to be more useful to study diversity first in plant species (Melchinger and Gumber, 1998; Grover and Sharma, 2016). Advantages of molecular makers, they are not affected by environmental factors like morphological makers hence they reveal the actual level of genetic difference that exist in different germplasm (Westman and Kresovich, 1997; Grover and Sharma, 2016).

Characterization of greater number lines by grouping them in heterotic groups can be achieved using molecular markers hence this allows increase in efficiency of breeding program because it avoids the development and evaluation of crosses that will be discarded (Terron et al., 1997, Reif et al., 2003b, Choukan et al., 2006). Several

approaches used to investigate relationships and heterotic groupings among inbred lines included the use of biochemical Assay and DNA molecular makers (Labate et al., 1997, Carena and Hallauer, 2001; Barata and Carena, 2006; Grover and Sharma, 2016). Also, comparative studies to estimate the effectiveness of different makers to discriminate genetic relationships in maize and SSR makers have shown to detect high levels of polymorphism per locus this is attributed to their codominant nature and number of alleles per locus (Pejic et al., 1998; Cömertpay et al., 2012; Frascaroli et al., 2013; Akinwale et al., 2014). The objectives of this study were to; (i) investigated the extent of genetic differences and patterns of relationships. (ii) Evaluate aflatoxin accumulation in inbred lines.

4.3. Materials and methods

4.3.1. Determination of genetic diversity of maize inbred lines

4.3.1.1. Plant materials for DNA isolation

Forty-one maize inbred lines (Table 4.1) comprising 24 weevil resistant lines from National Agricultural Research Organization (NARO) in Uganda, 13 lines with Aspergillus ear rot resistance, three inbred line with drought-tolerant from CIMMYT Kenya and one inbred line also with Aspergillus ear rot resistance from IITA Nigeria were studied. The 24 maize inbred lines developed from the cross between elite drought tolerant CIMMYT maize inbred lines that had been nominated based on resistance to diseases mainly maize streak virus, gray leaf sport, and turcicum leaf blight and the best weevil NARO lines. The lines from CIMMYT and IITA are resistant or tolerant to the fungal pathogen that causes mycotoxins in maize. Since aflatoxin

accumulation is attributed to drought and insect damage, the aim of the study was to exploit the three traits to develop novel inbred lines and hybrids from these known sources.

Table 4. 1 Maize inbred lines used in the study

No	Identification	Original Pedigree	Origin
1	WL 118-1-1	[WEEVIL/CML197]-B-13-B-B-B-B	NARO
2	WL 118-3	[WEEVIL/387]-B-19-B-B-B-B-B-B	NARO
3	WL 118-6	[WEEVIL/CML390]-B-19-B-B-B-B	NARO
4	WL 118-9	[WEEVIL/COMPE20]-B-26-B-B-B	NARO
5	WL 118-10	[WEEVIL/CML202]-B-7-B-B-B-B	NARO
6	WL 118-11	[WEEVIL/CML205]-B-24-B-B-B-B	NARO
7	WL 118-16	SZSYNA99-F2-79-2-3-B-B-B	NARO
8	WL 118-17	SZSYNA99-F2-81-4-2-B-B-B-B	NARO
9	WL 429-8	[CML312/MAS[MSR/312]-117-2]-B-50-B-1-B-B	NARO
10	WL 429-12	[CML312/MAS[MSR/312]-117-2]-B-91-B-B-B-B	NARO
11	WL 429-16	[WEEVIL/CML197]-B-9-B-B-B-B	NARO
12	WL 429-18	[WEEVIL/CML197]-B-12-B-B-B-B	NARO
13	WL 429-19	[WEEVIL/CML197]-B-18-B-B-B-B	NARO
14	WL 429-14	[WEEVIL/CML444]-B-22-B-B-B-B	NARO
15	WL 429-24	[WEEVIL/CML312]-B-1-B-B-B-B	NARO
16	WL 429-26	[WEEVIL/CML312]-B-23-B-B-B-B	NARO
17	WL 429-27	[WEEVIL/CML312]-B-32-B-B-B-B	NARO
18	WL 429-30	[WEEVIL/CML387]-B-8-B-B-B-B	NARO
19	TZAR504	(GT-MAS:gk/*2/ KU1414SR)-8-1-2-3-B*7	IITA
20	WL 429-33	[WEEVIL/CML389]-B-5-B-B-B-B	NARO
21	WL 429-35	[WEEVIL/CML389]-B-11-B-B-B-B	NARO
22	WL 429-36	[WEEVIL/CML389]-B-15-B-B-B-B	NARO
23	WL 429-38	[WEEVIL/CML389]-B-17-B-B-B-B	NARO
24	WL 429-39	[WEEVIL/CML389]-B-18-B-B-B-B	NARO
25	WL 429-43	[WEEVIL/CML389]-B-24-B-B-B-B	NARO
26	CML247	(G24F119*G24F54)-6-4-1-1-BB-f	CIMMYT
27	CML495	[P NVA. BCO.(S/D)xNPH-28]F32-B-1-B-1-2-BBBBBB	CIMMYT
28	CML264	Pob21C5F219-3-1-B-##-8-1-3-BBB-f	CIMMYT
29	POB.501	POB.501c3 F2 20-3-1-2-B-B-B-B	CIMMYT
30	MIRTC5	MIRTC5 Bco F78-2-2-1-1-1xDERRc2 15-3-7-1-1-B-B-B	CIMMYT
31	P502	P502c2-185-3-4-2-3-B-2-B-B-B-B-B	CIMMYT
32	CML348	G26SEQC3-H83-1-1-2-1-B	CIMMYT
33	CL-RCW31	CL-RCW31 (CML-247*CL-G2415)-B-1-B-2-1-1-BB-B-B	CIMMYT
34	CL-RCW37	CL-RCW37	CIMMYT
35	La Posta Seq C7	La Posta Seq C7-F103-2-1-1-1xMIRTC5 Bco F80-4-2-1-1-1-3-1-B-B	CIMMYT
36	CL-RCW35	[CL-04317*v]-1-B-1-1-2-BBBB	CIMMYT
37	CL-02510	P25C5HC246-3-1-BB-2-#-BBBBBBB	CIMMYT
38	CML451	[NPH28-1*G25)*NPH28]-1-2-1-1-3-1-B*6	CIMMYT
39	CML202	ZSR923S4BULK-5-1-b-b	CIMMYT
40	CML444	P43C9-1-1-1-1-1-BBBB	CIMMYT
41	CML322	89[L/LMBR]17-B-5-3-1-4-B*4	CIMMYT

4.3.1.2. Extraction of DNA from maize kernels

Using a mortar grinder (Spex sample prep), each maize inbred line kernels were fine ground and following the manufactures instruction isolation of genomic DNA was done using IncloneTM prep kit. Briefly; 1.5mL microcentrifuge tube, 100mg of kernel powdered was added containing 600 µl of lysis buffer. Then incubated for 30 minutes at 65 ^{o}C after vortexing for 2 minutes later 1.5 µl of RNase A (4mg/ml) was added. Using 1.1% agarose gels, stained with Loading STARTM solution, extracted DNA was electrophoresed and photographed using gel image analysis system under ultraviolent light.

4.3.1.3. PCR conditions and electrophoresis for SSR analysis

From the MaizeGDB database, 25 SSR primers were selected based on bin location for polymerase chain reaction amplification (Table 4.2) (Legesse et al., 2006, Choukan et al., 2006). All the primers were stored at 20°C after diluting from the stock to a working solution of 10 µM. The reaction volume was 30 µl containing 0.3 units of Taq polymerase, 3 µl of 10x PCR buffer, 1.2 pmols each of primers, 0.6 µl of 10mM of dNP and 2 µl of 10ng/µl template DNA. The PCR conditions were 94 ^{o}C for 3 min, by 34 cycles of 98 ^{o}C for 10 seconds, Annealing for 30 sec and 72 ^{o}C for 1 min and 40 sec, followed by extension of 72 ^{o}C for 7 min. The annealing temperatures ranged from 55-65°C (Table 4.2) was used for different SSR primers. Products were run on 3% agarose gel stained loading STARTM dye and photographed using a gel image analysis system under ultraviolet light to confirm the amplification. To finely separate the nucleic acid fragments based on sizes, a capillary gel electrophoresis system QIAxcel was used.

Table 4. 2 Information on the 25 SSR loci used in this study, including name, repeat unit, bin location, and annealing temperature.

No	Marker	Repeat Type	Bin location	Ann Temp (^{o}C)
1	Bnlg1082	AG(11)	9.02	60
2	Bnlg1762	AG(15)	10.03	60
3	Bnlg2190	AG(31)	10.06	65
4	Bnlg238	-	6.00	65
5	Bnlg602	-	3.04	65
6	Nc003	AG	2.06	55
7	phi015	AAAC	8.08	62
8	phi032	AAAG	9.04	65
9	phi034	CCT	7.02	60
10	phi037	AG	1.08	60
11	phi079	AGATG	4.05	65
12	phi085	AACGC	5.06	65
13	phi109275	AGCT	1.03	55
14	phi115	AT/ATAC	8.03	65
15	phi427434	ACC	2.08	55
16	Umc1153	(TCA)4	5.09	65
17	Umc1296	(GGT)7	6.06	65
18	Umc1367	(CGA)6	10.03	65
19	Umc1568	(TAG)4	1.02	55
20	Umc1669	(AGA)4	4.01	65
21	Umc1677	(GGC)4	10.05	60
22	Umc2036	(GTC)4	5.01	65
23	Umc2038	(GAC)4	4.07	65
24	Umc2050	(CGC)4	3.07	65
25	Umc2214	(CTT)4	2.1	65

In comparison to 25-500 pb DNA markers, QIAexcel biocalculator software was used to score the fragments size basis on each peak. Patterns were double checked using agarose gel electrophoresis and Allelobin software (Prasanth et al., 1997) for quality control

4.3.2. Determination of aflatoxin accumulation in inbred lines

4.3.2.1. Field experimental design

A total of 36 inbred lines were used in the study. These were selected from the 41 lines included in diversity studies based on the genetic distances. The field trial was planted on station at the National Crops Resources Research Institute (NaCRRI), Namulonge. Namulonge falls in the mid-altitude agro-ecological zone, located at 0º 32'N and 32º 35´E, at 1150 meters above sea level.

The trial was conducted in main season of 2013 with 36 inbred lines. The experimental design was a 5 x 7 alpha lattice. The plot consisted of two rows of 5m long, 0.75m apart and 0.5m between plants. Two seeds per hill were planted and later thinned to one at four weeks after emergence leaving a population of 53,333 plants ha^{-1}. Cultural and other standard agronomic practices were followed and at harvest air dried maize was shelled and transferred to the laboratory for aflatoxin analysis.

4.3.2.2. Inoculation with *Aspergillus flavus*

Aspergillus flavus was grown on *Aspergillus* Differentiation Agar for 14 days at 28 ^{0}C with 12 hours of light. With 500 mL sterile distilled water containing 0.1% Tween 20 per litre, conidia were washed and filtered through sterile cheesecloth. The concentration was determined using hemacytometer and adjusted to 1×10^6 conidia per mL with sterile distilled water. Each plant, the primary ear was inoculated with 3.4mL of conidia suspension when 50% of the plants in a plot had silks. The suspension was delivered underneath the husk using an Indico tree-marking gun fitted with a 14-gauge hypodermic needle (Zummo and Scott, 1989; Abbas HK et al, 2011).

4.3.2.3. Determination of aflatoxin concentration in grain

The concentration of aflatoxin was analyzed as described by Simyung et al., (2013). Primary ears from 13 plants per plot were hand harvested, approximately 65 days after mid-silk, and air dried for seven days. The 13 ears were shelled using an Almaco maize ear shelled and bulked.

From the bulk sample, 4 g was weighed and mix thorough with 70% methanol and centrifuged, then 5 mL of the supernatant was drawn. This was diluted with distilled water (20mL) and passed in the immunoaffinity column. To remove the matric components, 10mL of distilled water was used to rinse the column. Later, 1.4 mL of methanol was added for the final quantitative elution. Added 2 mL of water to the eluate and passed through a 0.22 µm filter, then injected into the HPLC. The mobile phase consisted of methanol: water: acetonitrile at a ratio of 2:6:2. Excitation and emission wave-length were 435 and 365 nm.

Chromatographic analyses were performed on an Agilent HPLC system model HP-1100 (Agilant, USA) using zorbax SB-Aq (4.6x150mm; 5 um, Agilant, USA). Derivative formations were performed on Kobra cell (Rhone Diagnostics Technologies Ltd, Lyon, France) and 1046A Programmable Fluorescence Detector (Agilant, USA) as fluorescence Detector.

4.3.3 Statistical data analysis

The data were tested for normality using the Kolmogorov-Smirnov test before descriptive analysis via PROC UNIVARIATE of SAS (SAS Institute, 2011). Aflatoxin values were transformed into [ln(y + 1)], where y is the aflatoxin concentration in a sample before statistical analysis. Arithmetic means were calculated by converting logarithmic means back to the original units of measurement. For genetic diversity analysis, Gene diversity, polymorphic information content (PIC), allele frequency, and Genetic distance (GD) between lines was calculated based on Rogers distance

(Rogers, 1972) using PowerMarker version 3.25 (Liu and Muse, 2005). A dendrogram was constructed from the genetic distance matrix using the neighbour-joining technique and visualized using MEGA version 5.0 (Tamura et al., 2011).

4.4. Results

4.4.1. Genetic variation among maize inbred lines

There was variation in 27 loci across 41 inbred lines. 184 alleles were detected with an average of 7.36 alleles per locus and ranged from 2 (Umc1669 and Umc1367) to 19 (Bnlg2190) (Table 4.3). The average of polymorphic information content (PIC) value was 0.61 ranged from 0.16 (Umc1669) to 0.91 per SSR loci (Table 4.3). Fourteen SSR loci manifested values more than 0.6 (Bnlg2190, Bnlg1082, Bnlg238, Nc003, phi115, phi034, phi079, phi109275, Umc2214, Umc1153, Bnlg602, Umc1296, Umc2038 and phi427434). Among the SSR markers, the average heterozygosity was 4% ranging was from 0% to 20% (Table 4.3) with Umc2036 SSR marker having the maximum heterozygosity (20%). Average gene diversity was 0.65 and ranged from 0.92 (Bnlg2190) to 0.18 (Umc1669) (Table 4.3).

Out of 41 inbred lines, 10 pairs of lines had a genetic distance of more than 0.9, meaning they exhibited differences at 25 SSR loci studied. However, genetic distance, in general, ranged from 0.20 to 0.989. Cluster analyses using Neighbor-Joining proposed three among groups for inbred lines (Figure 4.2)

Table 4. 3 Number of alleles, major allele frequency, gene diversity, and polymorphic information content among 41 maize inbred lines.

No	Marker	Major allele frequency	Minor allele frequency	Number of alleles	Gene diversity	Observed heterozygosity	PIC
1	Bnlg1082	0.37	0.63	6	0.74	0.02	0.70
2	Bnlg1762	0.56	0.44	8	0.63	0.12	0.59
3	Bnlg2190	0.17	0.83	19	0.92	0.02	0.91
4	Bnlg238	0.29	0.71	13	0.85	0.05	0.84
5	Bnlg602	0.39	0.61	10	0.79	0.00	0.78
6	Nc003	0.37	0.63	9	0.77	0.00	0.74
7	phi015	0.75	0.25	4	0.41	0.00	0.38
8	phi032	0.71	0.29	3	0.45	0.00	0.40
9	phi034	0.46	0.54	9	0.72	0.02	0.69
10	phi037	0.22	0.78	13	0.86	0.02	0.85
11	phi079	0.51	0.49	9	0.70	0.00	0.68
12	phi085	0.61	0.39	4	0.57	0.00	0.52
13	phi109275	0.39	0.61	6	0.77	0.00	0.74
14	phi115	0.32	0.68	9	0.79	0.02	0.77
15	phi427434	0.27	0.73	5	0.79	0.00	0.75
16	Umc1153	0.29	0.71	7	0.79	0.00	0.76
17	Umc1296	0.49	0.51	6	0.65	0.00	0.60
18	Umc1367	0.90	0.1	2	0.18	0.00	0.17
19	Umc1568	0.51	0.49	5	0.62	0.00	0.56
20	Umc1669	0.90	0.1	2	0.18	0.00	0.16
21	Umc1677	0.66	0.34	4	0.51	0.03	0.46
22	Umc2036	0.77	0.23	4	0.38	0.20	0.35
23	Umc2038	0.48	0.52	7	0.66	0.17	0.61
24	Umc2050	0.65	0.35	5	0.54	0.15	0.50
25	Umc2214	0.22	0.78	15	0.87	0.10	0.86
	Mean	0.49	0.51	7.36	0.65	0.04	0.61

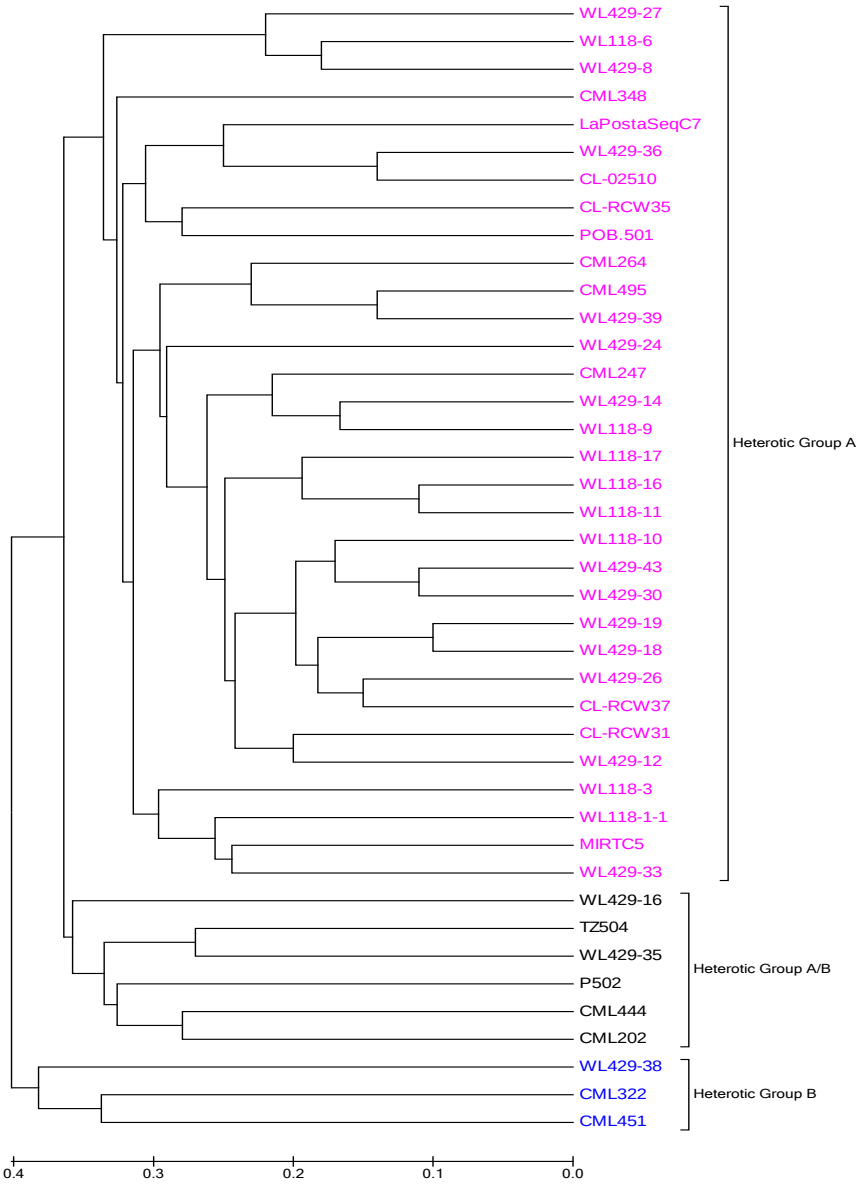

Figure 4. 1 Genetic relationship among maize inbred lines and their heterotic grouping.

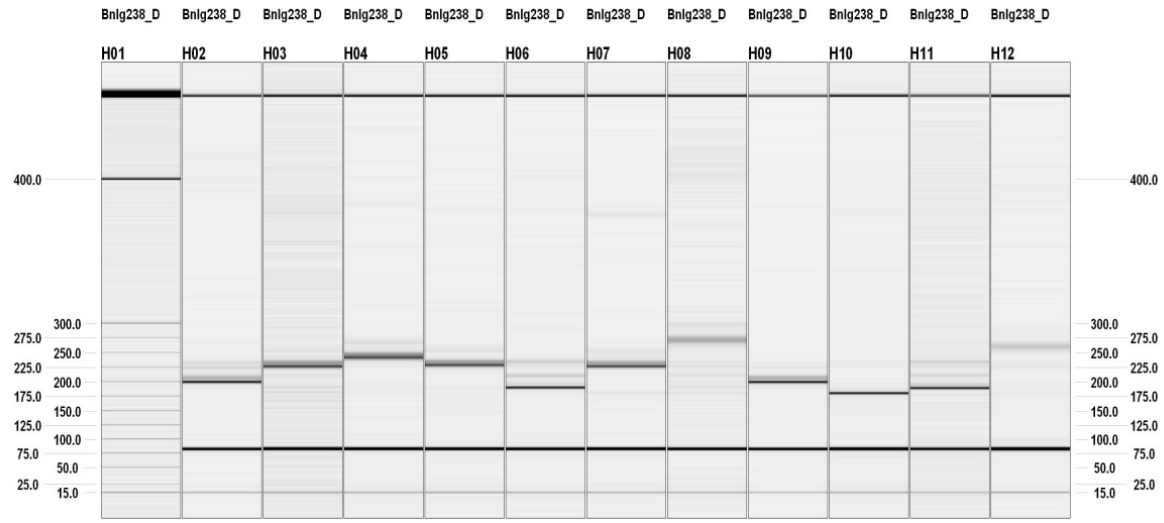

Figure 4. 2 The banding patterns from Capillary electrophoresis of different maize inbreeding lines.

4.4.2. Aflatoxin accumulation in maize inbred lines

The assessment of aflatoxin accumulation levels in different genotypes with the Codex Alimentarius international standards is summarized in Table 4.3. It was observed that 20% of the entire samples exceeded the Codex standard 10 ng/kg (EU Commission Regulation, 2010). Data show that out of the 35 inbred lines 80% would be recommended suitable for both human and animal consumption. The aflatoxin concentration among the 35 inbred lines varied widely from as low as 0 to as high as 142.36 ng/kg and average 8.75 (Table 4.4). The lines CL-RCW31, CML322, CML444, WL118-17, WL188-9, WL429-27 and WL429-43 showed consistently high concentrations of aflatoxin and CL-02510, CML202, CML451, WL188-1-1, WL118-16 WL118-6, WL429-12, WL429-16, and WL429-8 consistently moderate and below codex Alimentarius international standards, while lines WL118-3, Wl118-10, WL118-11, WL429-18, WL429-14, WL429-24, WL429-26, WL429-30, WL429-38, CML495, CML264, CML247, CML348, CL-RCW35, CL-RCW37, POB.501, MIRTC5, P502 AND

LaPostaSeqC7 has completely no detectable Aflatoxin levels (Table 4.3). Genotype "CML444" showed significantly high levels of accumulation of aflatoxin compared to all other genotypes.

Table 4. 4 Aflatoxin levels in inbred lines genotypes

Assay type	Samples analyzed	Negative samples	Positive samples	Exceeding Codex regulations (>10 ng/kg)	
				Number	Range(ng/kg)
High-Performance Liquid Chromatography	35	19	16	7(35)	10-142.36

Table 4. 5 Arithmetic mean analysis for aflatoxin accumulation in 35 maize inbred lines.

No	Lines	Aflatoxin Levels (ng/kg)
1	CL-02510	3.04
2	CL-RCW31	30.03
3	CL-RCW35	0.00
4	CL-RCW37	0.00
5	CML202	2.08
6	CML247	0.00
7	CML264	0.00
8	CML322	30.56
9	CML348	0.00
10	CML444	142.36
11	CML451	2.35
12	CML495	0.00
13	LaPostaSeqC7	0.00
14	MIRTC5	0.00
15	P502	0.00
16	POB.501	0.00
17	WL 118-10	0.00
18	WL 118-11	0.00
19	WL 118-1-1	1.74
20	WL 118-16	2.36
21	WL 118-17	12.00
22	WL 118-3	0.00
23	WL 118-6	1.60
24	WL 118-9	42.11
25	WL 429-12	1.46
26	WL 429-14	0.00
27	WL 429-16	5.74
28	WL 429-18	0.00
29	WL 429-24	0.00
30	WL 429-26	0.00
31	WL 429-27	11.55
32	WL 429-30	0.00
33	WL 429-38	0.00
34	WL 429-43	14.66
35	WL 429-8	2.58
	Mean	8.75
	Standard Error	4.28
	Minimum	0.00
	Maximum	142.36

4.6. Discussion

4.6.1. Genetic variation among maize inbred lines

Utilization of diverse sources of maize inbred lines is important for a new aflatoxin maize genetic enhancement program. Use of molecular markers to infer genetic diversity using simple sequence repeats in maize been reported in many studies (Armour et al., 1999, Warburton et al., 2002; Reif et al., 2003a; Van Inghelandt et al., 2010; Cömertpay et al., 2012; Frascaroli et al., 2013; Akinwale et al., 2014). The number of alleles recorded in this study was higher than that reported in studies with diverse maize germplasm (Cömertpay et al., 2012; Frascaroli et al., 2013; Akinwale et al., 2014; Pandit et al., 2016). However, they were lower than that reported in studies with diverse maize germplasm (Van Inghelandt et al., 2010). Discrepancies may be attributed to the use of different types of SSR with different repeats, the detection method for polymorphic markers, number of lines in the study and uniformity based on pedigrees. SSR markers with long motif tend to yield less number of alleles per markers than Dinucleotide SSR markers hence not commonly used due to difficulties in accurate allele sizing (Heckenberger et al., 2002, Choukan et al., 2006, Adetimirin et al., 2008). This would normally cause a good deal of common alleles, but each line in this study is potentially contributing unique alleles at many of the loci surveyed which should reflect a more diverse genetic pool for breeding maize for mid-altitude Africa.

The average gene diversity recorded in this study was lower than that reported by (Frascaroli et al., 2013) but higher than that reported in other studies (Akinwale et al., 2014). However, it was comparable to that reported by (Van Inghelandt et al., 2010) in

Flint and Lancaster inbred lines. This means that the expected heterozygosity in the materials used was diverse due to different genetic pools. The genetic distance between pairs of inbred lines in this study was smaller than that reported in most other studies (Van Inghelandt et al., 2010) but higher than that reported in other studies (Cömertpay et al., 2012; Akinwale et al., 2014). Large genetic distance estimates (0.65) between some of the pairs inbred lines in this set suggests that there is reasonable diversity to choose from and possibly high levels of heterosis between those pairs with high genetic distance. PIC values varied with 14 of the markers with a value of more than 0.6 and a high mean genetic distance signified their prospective informativeness to distinguish between maize lines. In overall, compared to tri, and tetra-nucleotide SSR markers, largest alleles and PIC mean was identified by dinucleotide SSR type, this is similar with other studies report by (Senior et al., 1998, Enoki et al., 2002, Adetimirin et al., 2008). However, these PIC values were higher than that reported (Shehata et al., 2009; Cömertpay et al., 2012; Akinwale et al., 2014).

The clustering of maize lines based on pedigree or origin is rarely straightforward unless a group of lines have been selected specifically to be diverse and represent different breeding programs, countries of origin, or growing environment (for example, Suwarno et al., 2015). The markers used in the study were able to separate the lines into groups. This means we can utilize this set of inbred lines using of both molecular-based grouping and pedigree of the lines where molecular information does not distinguish lines. In addition, a few representative lines from each of the clusters could be crossed

with testers from existing African heterotic groups to help determine which will form the best hybrids;

4.6.2. Aflatoxin accumulation levels in maize inbred lines

In any breeding program, it is a task worth taking to identify traits that contribute to resistance to aflatoxin in order to develop commercial hybrids with resistance. Data revealed that out of the 35 inbred lines 80% would be recommended suitable for breeding. The aflatoxin accumulation in inbreds reported in this study was lower compared to earlier experimental findings (Betrán et al., 2006; Dhakal et al., 2016; Brown et al., 2016; Guo et al., 2017) but higher than that reported by (Okoth et al., 2017). This means that the germplasm used in these studies different resistance to Aspergillus infection and aflatoxin accumulation and also the inoculation technique used.

Among the lines evaluated CML495, CML264, CML247 MIRTC5, and LaPostaSeqC7 had no aflatoxin content detected hence regard as resistant. This was in agreement with study reported by (Okoth et al., 2017). These inbred lines will enhance resistance breeding for aflatoxin through Identifying resistant maize germplasm. Hence this will pave way for further studies to determine specific mechanisms underlying *Aspergillus flavus* resistance.

Globally, Aflatoxin contamination in agricultural crops is has a direct impact to food security. To date, the best approach to lower accumulation of aflatoxin is through

resistance breeding. Results revealed that there was high genetic variation in the germplasm collections and 5 inbred lines were identified with no aflatoxin accumulation. This is a significant step development of hybrids with resistance to aflatoxin accumulation. This will also paved way for further study to understand the mechanisms of resistance to Aspergillus flavus.

CHAPTER FIVE

INHERITANCE OF RESISTANCE TO KERNEL INFECTION BY *ASPERGILLUS FLAVUS* IN TROPICAL MAIZE

5.1. Abstract

Knowledge concerning genetics of resistance is essential in developing a breeding strategy for *Aspergillus flavus* resistant germplasm with low aflatoxin accumulation. The study investigated the genetic action behind resistance infection by *Aspergillus flavus* and aflatoxin accumulation in 19 inbred lines. We used North Carolina II mating design to generate 90 hybrids. Single cross F1 hybrids along with five commercial checks were evaluated at five locations across year 2014 and 2015 while Aflatoxin levels were evaluated in year 2015. At harvest cobs from each entry were scored for kernel texture and thereafter a sample of 50 kernels was inoculated with a toxigenic strain of *Aspergillus flavus* in a laboratory to assess percentage kernel infection and measured aflatoxin accumulation using enzyme-linked immunosorbent assay procedure. Data on percent kernel infection were taken at seven, 14 and 21 days after inoculation. There were no significant differences in kernel infection by *Aspergillus flavus* at seven and 14 days after inoculation and also aflatoxin accumulation. Percentage kernel infection, however, varied significantly among hybrids at 21 days after inoculation. Analysis of variance for two years revealed that the year effect was significantly different ($P < 0.05$) for seven, 21 days after inoculation, and kernel texture. The environment plus, year x environment interaction were highly significant ($P < 0.001$) for percent kernel infection at seven, 14, 21 after inoculation days after inoculation, mean percent kernel infection and kernel texture. Inbred parents WL 118-3, WL 429-18, WL 429-14, CL-RCW35, CML495,

CML264, and WL 118-11 emerged as the best general combiners for *Aspergillus* ear rot while inbred CML247 and WL118-10 emerged as best combiners for aflatoxin accumulation. The non-additive effects were slightly more important than additive genetic effects for percent kernel infection and aflatoxin accumulation. Inbred lines with good general combining ability effects for Kernel infection resistance by *Aspergillus flavus* and aflatoxin accumulation are good candidates for breeding resistant varieties.

5.2. Introduction

In east Africa, maize produced is for commercial purposes hence it contribution to the economy. Most of the losses which result from grain quality in maize have been reported to result frequently from mycotoxins contamination and especially aflatoxins (Lewis et al., 2005, Kaaya et al., 2005, Munimbazi and Bullerman, 1996, Simyung et al., 2013). Use of maize kernels contaminated with aflatoxin as food poses a threat to human and animal health due to the mutagenic and carcinogenic effects of aflatoxin. Host plant resistance has proved to be the most cost effective and highly desirable approach to combat aflatoxin accumulation in maize (Lillehoj, 1987, Brown et al., 1999, Warburton et al., 2013).

Within tropical and temperate maize germplasm, several sources have been identified with resistance to *Aspergillus flavus* infection and aflatoxin accumulation (Campbell et al., 1993, Menkir et al., 2006; Brown et al., 2010). Efforts to understand mechanisms and genetics of resistance to *Aspergillus flavus* infection and aflatoxin accumulation in maize have been conducted (Zhang et al., 1997, Brown et al., 2001; Abbas et al.,

2017). Efforts have revealed that resistance is related to additive genetic effect with a strong genotype by environment component (Zuber et al 1978; Fountain et al., 2015), although other investigations indicate that resistance to aflatoxin contamination is polygenic (Cary et al., 2011). *Aspergillus flavus* infections and accumulation of Aflatoxin in grain occur simultaneously and hence they need to be handled separately. This means, one leads to another, in that if there is no kernel infection there will not be aflatoxin accumulation. Resistance to fungal infection and toxin accumulations can also be associated to the outer integument of developing kernels and ear components of maize (Darrah et al., 1987, Guo et al., 1995, Zhang et al., 1997; Cary et al., 2011).

International Maize and Wheat Improvement Center and partners have developed a number of inbred lines with tolerance to ears rots and other biotic stresses. Using these inbred lines to develop hybrids could potentially provide a solution to the aflatoxin contamination problems. However, there is limited or no information on resistance to kernel infection among these inbred lines. The objectives of the study were to assess the resistance of F1 to *Aspergillus flavus* kernel infection, aflatoxin accumulation under laboratory conditions and its relationship with grain texture, estimate general combining ability and specific combining ability for resistance to *Aspergillus flavus* kernel infection, aflatoxin accumulation and determine the mode of gene action and to investigate the role of incubation time on percent kernel colonization.

5.3. Materials and methods

5.3.1. Description of study sites

The five locations Namulonge, Serere, Bulindi, Ngetta, and Kasese were used as they are spread in different agro-ecologies in Uganda. The soil type at Namulonge (0^{o}36'N, 32^{o}36E; 1150 masl) is sandy clay loam and is classified as Orthic Ferrasol. The mean annual rainfall at Namulonge is 1270 mm with a bimodal distribution (March–July and September–November). The soil type at Serere (1^{o}31'N, 33^{o}27'E, 1080 masl) is sandy clay loams and black clays and is classified as Petric Plinthosol. The mean annual rainfall at Serere is 1419 mm, with a bimodal distribution (March–July and September–November). The soil type at Bulindi (0^{o}16'N, 32^{0}52'E'; 1144 masl) is Sandy loam and is classified as Acric Ferralsol. The mean annual rainfall at Bulindi is 1338 mm, with a bimodal distribution (March–July and September–November). The soil type at Ngetta (2^{o}16'N, 32^{o}52'E; 1300 masl) is sandy loam and is classified as Vertisol. The mean annual rainfall at Ngetta is 1483 mm, with a bimodal distribution (March–July and September–November). The soil type at Kasese (0^{o}10'S, 30^{o}04'E; 960 masl) is peaty sands and clay and is classified as Luvisol. The mean annual rainfall at Kasese is 1200 mm, with a bimodal distribution (March–July and September–November).

5.3.2. Plant materials and hybrid formation

In this study, nineteen white grain maize inbred lines were used (Table 5.1) were selected due to the low aflatoxin accumulation in the field trial and their genetically diversity. Nine lines obtained from NARO are adapted to mid-altitudes of East Africa had been previously selected for resistance to foliar disease and weevil infestation and

good agronomic traits. Ten inbred lines obtained from CIMMYT are adapted to the tropical lowland of Mexico and were selected based on resistance to *Aspergillus* and other ear rots (CIMMYT, unpublished report). Four of the lines from Mexico are released as CIMMYT maize lines (CMLs). North Carolina Design II mating scheme (Comstock and Robinson, 1948) was used to cross 19 inbred lines in which nine lines were used as females and 10 lines as males to produce 90 hybrids. The crosses were made at Kenya Agricultural & Livestock Research Organization, Kiboko Research Station in Kenya in 2012 and 2013.

Table 5. 1 Maize inbred lines used in the study, their pedigrees, and origin

Parent	Name	Pedigree	Origin
Females			
1	WL 118-3	[WEEVIL/387]-B-19-B-B-B-B-B-B	NARO[†]
2	WL 118-10	[WEEVIL/CML202]-B-7-B-B-B-B	NARO
3	WL 118-11	[WEEVIL/CML205]-B-24-B-B-B-B	NARO
4	WL 429-18	[WEEVIL/CML197]-B-12-B-B-B-B	NARO
5	WL 429-14	[WEEVIL/CML444]-B-22-B-B-B-B	NARO
6	WL 429-24	[WEEVIL/CML312]-B-1-B-B-B-B	NARO
7	WL 429-26	[WEEVIL/CML312]-B-23-B-B-B-B	NARO
8	WL 429-30	[WEEVIL/CML387]-B-8-B-B-B-B	NARO
9	WL 429-38	[WEEVIL/CML389]-B-17-B-B-B-B	NARO
Males			
1	CML495	[PNVABCO(S/D)/NPH-28]F32-B-1-B-1-2-BBBBBB	CIMMYT
2	CML264	Pob21C5F219-3-1-B-##-8-1-3-BBB-F	CIMMYT
3	CML247	(G24F119*G24F54)-6-4-1-1-BB-F	CIMMYT
4	CML348	G26SEQC3-H83-1-1-2-1-B	CIMMYT
5	CL-RCW35	[CL-04317*v]-1-B-1-1-2-BBBB	CIMMYT
6	CL-RCW37	(CL-04347*CL-04904)-B-109-2-1-B-B	CIMMYT
7	POB.501	POB.501c3 F2 20-3-1-2-B-B-B-B	CIMMYT
8	MIRTC5	MIRTC5 Bco F78-2-2-1-1-1xDERRc2 15-3-7-1-1-B-B-B	CIMMYT
9	P502	P502c2-185-3-4-2-3-B-2-B-B-B-B-B	CIMMYT
10	LaPostaSeqC7	LaPostaSeqC7-F103-2-1-1-1xMIRTC5BcoF80-4-2-1-1-1-3-1-B-B	CIMMYT

[†]NARO, National Agricultural Research Organization, Uganda. CIMMYT, International Maize and Wheat Improvement Center

5.3.3. Field evaluation of maize inbred lines

Ninety generated hybrids and five commercial hybrids (checks) were evaluated in a trial. The commercial hybrids used as checks were G91, G92, G93, G94, and G95. The hybrids were evaluated for grain yield and other agronomic traits under rain-fed conditions at five locations in Uganda in 2014 and 2015 (Table 5.2). The locations (Namulonge, Serere, Bulindi, Ngetta, and Kasese) used are spread in different agro-ecologies in Uganda. The experimental design was a 5 x 19 alpha lattice with two replications at each location. An experimental unit was a two-row plot, 5 m long, spaced 0.75 m apart and 0.25 m between plants. Two seeds were planted per hill and subsequently thinned to one plant per hill four weeks after emergence to give a final plant population density of 53,333 plants ha^{-1}. In all the experiments, standard agronomic and cultural practices including weeding and appropriate fertilizer applications were followed. At harvest, data on grain texture was recorded by scoring whole cobs from each plot on a scale of 1-5 where 1 = flint, round crown kernel with vitreous appearance and 5 = dent kernel with a floury endosperm. Grain moisture content was recorded at harvest for a representative sample of each plot using a moisture meter. The ears harvested from each plot were stored and for laboratory assessment. Kernel texture data was collected at harvest (1=flint, to 5=dent: (Betr'an et al., 2002).

5.3.4. Assay of maize kernels for *Aspergillus flavus* infection

The cobs harvested from each plot at all locations were transferred to National Crops Resources Research Institute (NaCRRI) for laboratory-based Infection Resistance

Screening (LIRS) experiment. The cobs from each plot were hand shelled and grain bulked into paper bags. The grain was then dried in paper bags to facilitate uniform drying and avoid direct heat on the kernels. The media-free, kernel-isolated incubation (MIKI) protocol was used to assay for the *Aspergillus flavus* infection (Ruming and Manjit, 2005). For each genotype 20 maize kernels were placed in a 500 ml beaker and sterilized in 70% ethanol and rinsed in three minutes and then rinsed thrice in distilled water. The kernels were then soaked in a 500 ml beaker with distilled water for 1 minute to enable them to gain approximately 30% moisture content, to simulate field moisture content of maize kernels at physiological maturity. The water was decanted off and using a pair of forceps, kernels were placed individually in a 90 mm x 15 mm diameter petri dish on disposable aluminum foil petri lined with wet cotton wool. Each petri dish contained 20 kernels. Each kernel in a petri was inoculated with a conidial suspension containing 1 x 10^6 conidia of *Aspergillus flavus* on the kernel surface. The conidial suspension *Aspergillus flavus* was delivered using a 20 µl micropipette. The petri dishes were incubated in a Panasonic electric oven, Japan at 31^oC and 95-100% relative humidity for 21 days. To ensure that humidity remained constant all the petri dishes were sealed off using parafilm, with moisture from the wet cotton wool maintaining humid incubation conditions.

The number of kernels infected with *Aspergillus flavus* was recorded at intervals of seven, 14 and 21 days after inoculation in every petri dish. The incidence of kernel infection was expressed as percent as follows:

$$\text{PKI} = \frac{(\text{Number of kernels infected with } A.\ flavus) \times 100}{\text{Total number of kernels incubated}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(iv)}$$

The scores of percent kernel infection were used to calculate the area under disease progress curve (AUDPC) (Shaner and Finney, 1977, Campbell and Madden, 1990) as follows:

$$AUDPC = \sum_{i=1}^{n-1} [(t_{i+1} - t_i)(y_i + y_{i+1})/2] \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(v)}$$

where t_i is a time in days of each reading, y_i is the percentage infection at each reading and n is the number of readings.

5.3.5. Sample extraction and quantification of total aflatoxin

Each genotype was ground into flour (≈0.5 mm particle diameter) using a blender (WARING Commercial® blender, 80104, Union, USA). Extraction of aflatoxin was done using 70% methanol (Reckitt Benkiser East Africa Limited, Nairobi, Kenya) from 5.0 grams of each genotype. Analysis was done using enzyme-linked immunosorbent assay (Helica Cat. N0 941 AFLOIM-96, Helica Biosystems Inc., Fullerton, CA) According to manufactures instructions sample extracts and standards were analyzed in duplicates using a plate reader (BioTek Instruments, Winooski, VT) with Gen5 software (Gen5TM version 1.11.5) at an absorbance filter of optical density 45 nm. To get the total aflatoxin concentration values were compared with corresponding concentration using a regression equation and adjusted the final concentration using dilution factor.

5.3.5.1. Validation method and quality control

To ensure quality of data validation was done prior to sample analysis by enzyme-linked immunosorbent assay. This involved calculation of coefficient of variation and spike recoveries of each aflatoxin standards and comparing it with the set level of coefficient of variation (5%). To assess the accuracy, reference certified corn materials from the State Chemist is used. The integrity of that data generated an in-house analytical method is used to assess the precision, accuracy and linearity each enzyme-linked immunosorbent assay plate were performed. With this method, regression coefficient of 0.98 is the least acceptable level.

Three difference ground certified corn with known concentration was used to test accuracy of the method. There sample used were divided into 5 mg/kg (±40%), 40 mg/kg (±34%) and 273 mg/kg (±20%). Enzyme-linked immunosorbent assay plates that had ranges way off compared to the reference material were repeated. Samples were diluted exceeding the upper limit of quantification limits of the kit of 20 ng/kg.

5.3.6. Analysis of variance

Analysis of variance for percent kernel infect, grain texture, and area under disease progress curve were done separately for each location and combined across environments using PROC MIXED procedure of SAS (SAS Institute, 2011). Aflatoxin values were transformed using [ln(y + 1)], where y is the aflatoxin concentration in the

sample before statistical analyses. The following model was used for analysis of the alpha lattice design:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + y_{k(j)} + \varepsilon_{ijk} \quad \text{.....(vi)}$$

where Y_{ijk} = is the response of the *i*th treatment in the *j*th replication and the *k*th incomplete block, μ is the population mean, α_i is the effect of the *i*th treatment, βj is the effect of the *j*th replication, $\gamma_{k(j)}$ is the effect of the *k*th incomplete block within the *j*th replication, and ε_{ijk} is the residual. Genotypes were considered fixed effects, while replications and incomplete blocks within replications were considered as random effects. The fixed effects model was used to obtain the adjusted means while the random effects model was used to estimate the variance components. Each location-year combination was considered an environment. Means were separated using the least significant difference (LSD).

5.3.7. Determination of combining ability

Across experiments and years analysis of variance for percent kernel, kernel texture, area under progress curve and Aflatoxin accumulation of the single cross hybrids was performed according to the North Caroline Design II (Comstock and Robinson, 1948) using PROC GLM of SAS (SAS Institute, 2011) following the linear model:

$$Y_{ijkq} = \mu + g_i + g_j + s_{ij} + y_q + r_k(y_q) + (gy)_{iq} + (gy)_{jq} + (sy)_{ijq} + e_{ijkq} \text{.....(vii)}$$

where i = 1, 2, …., 9; j = 1, 2, …., 10; k = 1, 2; q = 1, 2, 3, 4, 5; and Y_{ijkq} denotes the value of the hybrid of mating the ith female line and the jth male line in the kth replication, in the qth environment. The term μ denotes the grand mean, gi the general combining ability (GCA) effect common to all progeny of the ith female line, gj the general combining ability effect common to all progeny of the jth male line, sij the specific combining ability (SCA) effect specific to the hybrid obtained by mating the ith female line and the jth male line, y_q is the average effect of the qth environment, $r_k(y_q)$ is the effect of the kth replication nested within the qth environment, $(gy)_{iq}$ and $(gy)jq$ are the interactions between the general combining ability effects and environment, $(sy)_{ijq}$ is the interaction between the SCA effect and environment, and e_{ijkq} is the random experimental error.

The sums of squares for hybrids were partitioned into variation due to females, males, and female x male interaction. The hybrid x environment sums of squares were partitioned into sources of variation due to the female x environment, male x environment, and female x male x environment. The sources of variation due to females, males, and female x male were tested for significance against their interaction with the environment while the other terms were tested against the pooled error.

The general combining ability effects of each female and male line and SCA effects of the hybrids for the traits were calculated as

$$g_j = (y_{.j} - y_{..}) \quad \text{(viii)}$$

$$s_{ij} = (y_{ij} - y_{i.} - y_{.j} + y_{.}) \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(ix)}$$

where y_{ij} is the mean of the hybrid of mating the *i*th female and the *j*th male, $y_{i.}$ is the mean of all hybrids involving the *i*th female parent, $y_{.j}$ is the mean of all hybrids involving the *j*th male parent, and y is the mean of all the hybrids. Standard errors of the general combining ability (SE_{GCA}) and SCA (SE_{SCA}) effects for all traits were calculated according to Cox and Frey (1984). Two-tailed t-tests were used to test the significance of the general combining ability and SCA effects where t = GCA/SE_{GCA} or SCA/SE_{SCA}, respectively (Cox and Frey, 1984).

5.3.8. Estimate of heritability

Broad-sense heritability (H) for individual trials was estimated according to Hallauer et al. (2010):

$$H = \frac{\sigma_G^2}{\left[\sigma_G^2 + \left\{\frac{\sigma_E^2}{r}\right\}\right]} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(x)}$$

where; σ_G^2 is the genotypic variance, σ_E^2 is the error variance, and r the number of replications.

H for traits across environments was estimated using the variance components according to Hallauer et al. (2010) as:

$$H = \frac{\sigma_G^2}{\left[\sigma_G^2 + \frac{\sigma_{GxL}^2}{E} + \frac{\sigma_E^2}{ER}\right]} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(xi)}$$

where; σ_G^2, σ_{GxL}^2 and σ_E^2 are genotypic, genotype × location, and residual variance components, respectively: E is the number of environments, and R is the number of replications.

Narrow-sense heritability based on full-sib family means was estimated across environments using variance components according to Hallauer et al (2010) as:

$$h^2_{HSmales} = \frac{\sigma_m^2}{\frac{\sigma^2}{ref} + \frac{\sigma^2_{fme}}{ef} + \frac{\sigma^2_{me}}{e} + \frac{\sigma^2_{mf}}{f}\sigma_m^2} \quad \text{.....(xii)}$$

where is σ^2_m is variance component for males, σ^2_{me} variance component for male x environment, σ^2_{mf} is variance component for male x female, σ^2_{fme} is variance component for male x female x environment, r is a number of replications, f is a number of females, and e is a number of environments?

5.4. Results

5.4.1. Analysis of variance across the five environments

In year 2014, combined analysis of variance across the five environments shown that environment was highly significant ($P < 0.001$) for percent kernel infection at seven, 14, and 21 day after inoculation, Mean percent kernel infection, area under disease progress curve, and kernel texture. Genotype was highly significant ($P < 0.001$) for only

percent kernel infection at 21 day after inoculation, Mean percent kernel infection, area under disease progress curve, and kernel texture and significant ($P < 0.05$) for percent kernel infection at 14 day after inoculation, except percent kernel infection at 7 day after inoculation but environment x genotype was only significant for kernel texture (Table 5.3). There were no differences in the response of the test materials at the different environments. The F_1 hybrid was only highly significant ($P < 0.001$) for percent kernel infection at 21 day after inoculation, and kernel texture and significant ($P < 0.01$) for other traits except for percent kernel infection at seven day after inoculation. Also, F_1 hybrid x environment interaction was only highly significant ($P < 0.001$) for kernel texture (Table 5.3).

The F_1 hybrid mean square was partitioned into male general combining ability (GCA_m), female general combining ability (GCA_f), and specific combining abiliyt components. The mean square for GCA_m was highly significant ($P < 0.001$) for kernel texture, significant for percent kernel infection at 21 day after inoculation, Mean percent kernel infection, area under disease progress curve and significant ($P < 0.05$) for percent kernel infection at 14 day after inoculation but not significant for percent kernel infection at seven day after inoculation suggesting differences in general combining ability effects of the male parents for these traits (Table 3). The mean square for GCA_f was only highly significant ($P < 0.001$) for kernel texture and not significant for all other percent kernel infection at seven, 14, 21 day after inoculation, mean percent kernel infection, area under disease progress curve, and kernel texture.

Table 5. 2 Mean squares and degrees of freedom for the combined analysis of variance for percentage kernel infection and cob texture for 90 tropical maize hybrids in 5 environments of Uganda combined across year 2014.

Source	DF	Percent Kernel infection			Mean Percent kernel Infection	Area Under Disease progress Curve	Kernel Texture
		7 Days	14 Days	21 Days			
			(%)				(1-5)
Environment (Env)	4	167.35**	6187.91***	28276.97***	7266.71***	1367295.78***	64.60***
Rep(Env)	5	50.14^{ns}	670.51***	1152.69**	454.33***	102506.82***	0.71**
Genotypes	94	47.87^{ns}	163.77**	456.16***	146.73***	28873.41***	7.15***
Env*Genotype	376	42.41^{ns}	120.93^{ns}	246.82^{ns}	86.41^{ns}	17691.32^{ns}	0.49***
F1 Hybrids	89	30.64^{ns}	151.47**	410.91***	122.67**	24774.46**	6.69***
Env*Hybrids	356	37.61^{ns}	118.07^{ns}	229.31^{ns}	81.19^{ns}	16798.29^{ns}	0.45***
GCAm	9	46.98^{ns}	249.70*	865.30**	274.10**	51525.59**	30.74***
GCAf	8	23.16^{ns}	108.02^{ns}	240.60^{ns}	76.75^{ns}	17042.42^{ns}	27.53***
SCA	72	29.36^{ns}	147.78*	373.53**	109.75*	22622.49*	1.36***
GCAm *Env	36	43.20^{ns}	108.61^{ns}	253.56^{ns}	86.62^{ns}	18110.33^{ns}	1.25***
GCAf*Env	32	29.41^{ns}	172.73*	270.39^{ns}	113.71*	25239.10*	1.25***
SCA*Env	288	38.01^{ns}	116.32^{ns}	220.40^{ns}	77.11^{ns}	15960.71^{ns}	0.27**
Error	445	35.38	105.45	237.25	78.45	15771.82	0.2

* Significant at $P < 0.05$; ** Significant at $P < 0.01$; *** Significant at $P < 0.001$; ‡ns, not significant.

GCA_m, male general combining ability, $GCA_{f,}$ female general combining ability; SCA, specific combining ability

The specific combining ability mean square was highly significant ($P < 0.001$) for kernel texture, significant for percent kernel infection at 14 and 21 day after inoculation, and area under disease progress curve but not significant for percent kernel infection at seven days after inoculation (Table 5.3).

The mean square for GCA_m x environment interaction effect was not significant for all the traits percent kernel infection at seven, 14 and 21 day after inoculation, Mean percent kernel infection, area under disease progress curve and kernel texture except highly significant ($P < 0.001$) for kernel texture (Table 5.3). The mean square for GCA_f x environment interaction effect was not significant for percent kernel infection at seven, and 21 day after inoculation, though significant for ($P < 0.05$) for percent kernel infection at 14 day after inoculation, mean kernel infection, area under disease progress curve and highly significant for kernel texture (Table 3). The SCA x environment interaction was highly significant ($P < 0.01$ or $P < 0.001$) for only kernel texture and not significant for all other traits (Table 5.3).

In year 2014, the overall contribution of specific combining ability (SCA) sum of squares to the total variation was significantly higher for percent kernel infection at seven, 14 and 21 day after inoculation, Mean percent kernel infection, and area under disease progress curve than the contribution of general combining ability (GCA_m+ GCA_f) sum of squares but the reverse was true for kernel texture (Table 5.4).

Table 5. 3 Percentage of total genotypic sum of squares of percentage kernel infections, area under disease progress curve, kernel texture to general combining ability of (GCA_f) and (GCA_m) and specific combining ability (SCA) for year 2014, 2015 and across years (2014-2015).

Trait	Evaluation Year								
	2014			2015			Across years		
	GCA_m	GCA_f	SCA	GCA_m	GCA_f	SCA	GCA_m	GCA_f	SCA
Percentage kernel infection at 7 days	15.5	6.79	77.5	34.7	12.4	51.1	30.46	9.15	59.62
Percentage kernel infection at 14 days	16.67	6.41	78.93	44	16.8	39.5	33.75	11.18	54.72
Percentage kernel infection at 21 days	21.29	5.26	73.54	42.5	14.2	43.6	35.08	11.53	53.28
Mean percentage kernel infection	22.6	5.62	72.38	45.6	16.4	38.2	39.01	11.51	49.11
Area Under disease progress curve	21.03	6.18	73.87	45.8	16.1	38.5	38.26	11.42	49.92
Kernel texture	46.46	36.97	16.49	50.1	25.5	23.6	50.01	32.97	17.02
Aflatoxin	-	-	-	24.0	20.8	55.6	-	-	-

In year 2015, combined analysis of variance across the five environments shown that environment was only highly significant ($P < 0.001$) for percentage kernel infection at seven, 14 and 21 days after inoculation, mean percent kernel infection and kernel texture. Genotype was highly significant ($P < 0.001$) for kernel texture, significant ($P < 0.01$) for percentage kernel infection at 21 days after inoculation and significant ($P < 0.01$) for percentage kernel infection at 14 days after inoculation and mean percent kernel infection but not significant for percentage kernel infection at seven days after inoculation (Table 5.5). While environment x genotype was only highly significant for kernel texture. There were, therefore, no differences in the response of the test materials at the different environments based on percentage kernel infection (Table 5.5).

The F_1 hybrid was only highly significant ($P < 0.001$) for kernel texture, significant ($P < 0.01$) for percentage kernel infection at 21 days after inoculation and significant ($P <$

0.05) for mean percent kernel infection and area under disease progress curve but not significant for percentage kernel infection at seven and 14 days after inoculation. F_1 hybrid x environment interaction was only highly significant ($P < 0.001$) for kernel texture and not significant for any other trait (Table 5.). Again F_1 hybrid mean square was partitioned into male general combining ability (GCA_m), female general combining ability (GCA_f), and SCA components.

The mean square for GCA_m was highly significant ($P < 0.001$) for percentage kernel infection at 14 and 21 days after inoculation, mean percent kernel infection, area under disease progress curve and kernel texture, significant for percentage kernel infection at seven days after inoculation hence also suggesting differences in general combining ability effects of the male parents for these traits (Table 5.5). The mean square for GCA_f was only highly significant ($P < 0.001$) for kernel texture, significant for percentage kernel infection at 14 and 21 days after inoculation, mean percent kernel infection, area under disease progress curve and kernel texture. The specific combining ability mean square was only highly significant ($P < 0.001$) for kernel texture, and not significant for all other percentage kernel infection at seven, 14 and 21 days after inoculation, mean percent kernel infection and area under disease progress curve (Table 5.5).

Table 5. 4 Degrees of freedom and mean Squares for the combined analysis of variance for percentage kernel infection and cob texture for 90 tropical maize hybrids in 5 environments of Uganda combined across year 2015.

Source	DF	Percent Kernel infection at:			Mean Percent kernel Infection	Area Under Disease progress Curve	Kernel Texture
		7 Days	14 Days	21 Days			
			(%)				(1-5)
Environment (Env)	4	632.89***	3761.28***	5307.54***	2801.90***	588397.23***	2.73***
Rep(Env)	5	24.25^{ns}	87.67^{ns}	213.61^{ns}	64.92^{ns}	13641.53^{ns}	0.91*
Genotypes	94	24.92^{ns}	114.75^{ns}	265.15**	99.51*	19732.72*	3.77***
Env*Genotype	376	25.99^{ns}	80.95^{ns}	168.04^{ns}	66.08 ns	13348.51^{ns}	0.63***
F1 Hybrids	89	25.70^{ns}	118.25^{ns}	268.95**	101.87*	20255.97*	2.99***
Env*Hybrids	356	26.05^{ns}	79.28^{ns}	168.43^{ns}	65.42^{ns}	13182.14^{ns}	0.57***
GCA_m	9	88.09**	514.90***	1130.62***	458.93***	91832.13***	14.81***
GCA_f	8	35.41^{ns}	221.18*	423.75*	185.30*	36223.65*	8.48***
SCA	72	16.23^{ns}	57.75^{ns}	144.99^{ns}	48.04^{ns}	9632.72^{ns}	0.87***
GCA_m *Env	36	38.61*	98.66^{ns}	167.33^{ns}	79.17^{ns}	16606.82^{ns}	1.70***
GCA_f *Env	32	25.14^{ns}	69.57^{ns}	138.12^{ns}	57.86^{ns}	11885.25^{ns}	1.16***
SCA*Env	288	24.55^{ns}	77.29^{ns}	171.46^{ns}	64.21^{ns}	12800.14^{ns}	0.36^{ns}
Error	445	25.36	101.35	168.73	73.79	15452.24	0.33

* Significant at $P < 0.05$; ** Significant at $P < 0.01$; *** Significant at $P < 0.001$; ‡ns, not significant.

GCA_m, male general combining ability, $GCA_{f,}$ female general combining ability; SCA, specific combining ability

The mean square for GCA_m x environment interaction effect was not significant for percentage kernel infection at 14 and 21 days after inoculation, mean percent kernel infection, and area under disease progress curve but significant for percentage kernel infection at seven days after inoculation but highly significant ($P < 0.001$) for kernel texture (Table 5.5). The mean square for GCA_f x environment interaction effect was not significant for percentage kernel infection at seven, 14 and 21 days after inoculation, mean percentage kernel infection and area under disease progress curve but highly significant ($P < 0.001$) for kernel texture (Table 5.5). The SCA x environment interaction was not significant for all traits (Table 5.3).

In year 2015, the overall contribution of specific combining ability sum of squares to the total variation was higher for only percent kernel infection at seven days after inoculation than the contribution of general combining ability (GCA_m+ GCA_f) sum of squares. There was a change in the trend for the contribution of general combining ability (GCA_m+ GCA_f) sum of squares percentage kernel infection at 21 days after inoculation, mean percent kernel infection, and area under disease progress curve and kernel texture (Table 5.4).

5.4.2. Analysis of variance in five environments across years 2014 and 2015

Combined analysis of variance across two years of study shown that percent kernel infection at seven days after inoculation was significant ($P < 0.01$), 21 days after inoculation and kernel texture were highly significant ($P < 0.001$) (Table 5.6). The

environment was highly significant ($P < 0.001$) for percent kernel infection at seven, 14 and at 21 days after inoculation, mean percent kernel infection and kernel texture (Table 5.6).

Genotype was highly significant ($P < 0.001$) for percent kernel infection at 21 days after inoculation and kernel texture, significant ($P < 0.01$) for mean percent kernel infection, significant ($P < 0.05$) for area under disease progress curve, though not significant for percent kernel infection at seven and 14 days after inoculation (Table 5.6). Year x Environment interaction was highly significant ($P < 0.001$) for percent kernel infection at 7, 14 and 21 days after inoculation, mean percent kernel infection, area under disease progress curve and kernel texture (Table 5.6).

Hybrid x year interaction was highly significant ($P < 0.001$) for kernel texture and significant ($P < 0.01$) for percent kernel infection at 21 days after inoculation. Environment x hybrid was only significant ($P < 0.05$) for percent kernel infection at seven days after inoculation and highly significant ($P < 0.001$) for kernel texture (Table 5.6). The interaction of Year x Environment x hybrid was not significant for all the percentage kernel infection and area under disease progress curve except kernel texture for the grains (Table 5.6).

The F_1 hybrid was only highly significant ($P < 0.001$) for percent kernel infection at 21 days after inoculation and kernel texture but not significant ($P < 0.01$) for other traits (Table 5.6). The F_1 hybrid mean square was partitioned into male general combining

ability (GCA_m), female general combining ability (GCA_f), and specific combining ability components. The mean square for GCA_m was highly significant ($P < 0.001$) for percent kernel infection at 14 and 21 days after inoculation, mean percent infection, area under disease progress curve, kernel texture and significant for percent kernel infection at seven days after inoculation (Table 5.6).

The mean square for GCA_f was only highly significant ($P < 0.001$) for kernel texture and significant ($P < 0.05$) for percent kernel infection at 14 and 21 days after inoculation, mean percent kernel infection, area under disease progress curve but not significant for percent kernel infection at seven days after inoculation. The specific combining ability mean square was highly significant ($P < 0.001$) for kernel texture, significant for percent kernel infection at 21 days after inoculation and not significant for percent kernel infection at seven and 14 days after inoculation, mean percent kernel infection and area under disease progress curve (Table 5.6).

Table 5. 5 Degrees of freedom and mean Squares for the combined analysis of variance for percentage kernel infection and cob texture for 90 tropical maize hybrids in 5 environments of Uganda combined across years (2014 and 2015).

Source	DF	Percent Kernel infection at:			Mean Percent kernel Infection	Area Under Disease progress Curve	Kernel Texture
		7 Days	14 Days	21 Days			
			(%)				(1-5)
Year	1	268.11**	38.37	2536.50***	85.97	5715.66	84.72***
Environment (Env)	4	461.61***	7324.77***	27817.15***	8050.70***	1540724.35***	56.09***
Rep(Env)	5	43.80^{ns}	247.16*	531.89*	185.29*	37933.01*	0.91**
Genotypes	94	39.33^{ns}	132.86^{ns}	375.92***	115.86**	22694.08*	3.17***
Year*Env	4	651.57***	7087.84***	21895.68***	7151.79***	1397218.56***	38.22***
F1 Hybrids	89	25.56^{ns}	121.26^{ns}	332.51***	93.94^{ns}	18928.14^{ns}	3.15***
GCA_m	9	99.80**	544.94***	1394.54***	549.75***	107280.10***	41.15***
GCA_f	8	32.87^{ns}	211.32*	510.08*	183.45*	36548.87*	30.61***
SCA	72	24.51^{ns}	110.64^{ns}	264.48*	86.50^{ns}	17497.74^{ns}	1.71***
Year*Hybrids	89	23.07^{ns}	105.41^{ns}	277.77**	81.64^{ns}	16516.01^{ns}	1.34***
GCA_m *Year	9	35.41^{ns}	220.76*	598.32**	184.27*	37130.63*	4.30***
GCA_f *Year	8	24.39^{ns}	121.85^{ns}	146.01^{ns}	77.36^{ns}	16913.96^{ns}	5.45***
SCA*Year	72	21.43^{ns}	93.78^{ns}	253.67^{ns}	70.92^{ns}	14344.50^{ns}	0.53*
Env*Hybrid	356	36.94*	112.04^{ns}	220.00^{ns}	79.39^{ns}	16349.51^{ns}	0.51***
Env* GCA_m	36	35.15^{ns}	124.50^{ns}	192.29^{ns}	88.27^{ns}	18650.89^{ns}	1.08***
Env* GCA_f	32	28.57^{ns}	104.97^{ns}	221.06^{ns}	85.84^{ns}	17356.10^{ns}	1.21***
Env*SCA	288	29.22^{ns}	93.43^{ns}	192.20^{ns}	67.88^{ns}	13889.06^{ns}	0.32^{ns}
Year*Env*Hybrids	356	33.87^{ns}	100.79^{ns}	202.47^{ns}	75.00^{ns}	15341.33^{ns}	0.55***
Error	893	30.31	106.42	206.32	77.54	15958.33	0.27

* Significant at $P < 0.05$; ** Significant at $P < 0.01$; *** Significant at $P < 0.001$

GCA_m, male general combining ability, GCA_f, female general combining ability; SCA, specific combining ability,

^{‡ns}, not significant.

The year x hybrids interaction was only highly significant ($P < 0.001$) for kernel texture and significant ($P < 0.01$) but not significant for other traits (Table 5.6). The mean square for GCA_m x Year interactions was highly significant ($P < 0.001$) for kernel texture, significant ($P < 0.01$) for percent kernel infection at 21 and significant ($P < 0.05$) for 14 days after inoculation, mean percent kernel infection, and area under disease progress curve, and not significant for percent kernel infection at seven days after inoculation (Table 5.6). The mean square for both GCA_f x Year and specific combining ability x year interactions were highly significant ($P < 0.001$) and Significant ($P < 0.05$) for only kernel texture respectively but not significant for percent kernel infection at seven, 14 and 21 days after inoculation, mean percent kernel infection, and area under disease progress curve (Table 5.6).

The mean square for GCA_m x environment and GCA_f x environment effects were only highly significant ($P < 0.001$) for kernel texture and not significant for percent kernel infection at 7, 14 and 21 days after inoculation, mean percent kernel infection, and area under disease progress curve. Specific combining ability x environment interaction as not significant for all the traits of interest whereas Year x Environment x hybrids was only highly significant ($P < 0.001$) for only kernel texture and not significant for other traits (Table 5.6). Across the two years of testing, the overall contribution of specific combining ability sum of squares to the total variation was significantly higher for percent kernel infection at seven, 14 and 21 days after inoculation, mean percent kernel infection and area under disease progress curve than the contribution of general

combining ability (GCA_m+ GCA_f) sum of squares but the reverse was true for kernel texture (Table 5.4).

5.4.3. Analysis of variance for aflatoxin accumulation across environments

Combined analysis of variance across sites revealed that environment was highly significant ($P < 0.001$) but Genotype, environment x hybrid were not significant for aflatoxin accumulation (Table 5.7). Also, the F1 hybrid was not significant ($P < 0.01$) for aflatoxin accumulation (Table 5.7). The F1 hybrid mean square was partitioned into male general combining ability (GCA_m), female general combining ability (GCA_f), and specific combining ability components that were also not significant for aflatoxin accumulation (Table 5.7). The mean square for GCA_m x environment, GCA_f x environment effects, and specific combining ability x environment interaction as not significant (Table 5.7). Across testing sites, overall contribution of specific combining ability sum of squares to the total variation was significantly higher for aflatoxin accumulation than the contribution of general combining ability (GCA_m+ GCA_f) sum of squares but the reverse was true for kernel texture (Table 5.7).

Table 5. 6 Combined analysis of variance for Aflatoxin accumulation for 90 Tropical Maize hybrids, without checks across 5 environments of Uganda in 2015.

Source	DF	Mean Square	F Value	Pr > F
Environment(Env)	4	42.27	108.25	<.0001
Rep(Env)	5	0.38	27.44	<.0001
F1 Hybrids	89	0.35 ^{ns}	0.92	0.667225
Env*Hybrids	349	0.45 ^{ns}	1.18	0.110902
GCA_m	9	0.19 ^{ns}	0.48	0.8859
GCA_f	8	0.28 ^{ns}	0.71	0.6849
SCA	72	0.45 ^{ns}	1.15	0.2135
GCA_m *Env	36	0.38 ^{ns}	0.98	0.5039
GCA_f *Env	32	0.78 ^{ns}	2.01	0.0013
SCA*Env	281	0.42 ^{ns}	1.08	0.2397
GCA_m	346	0.39		

GCA_m, male general combining ability, GCA_f, female general combining ability; SCA, specific combining ability

5.4.4. Average genotypic response across environments in year 2014

Responses of genotypes were based on percent kernel infection at 21 days after inoculation since it is where we noted significant differences within and among genotypes; hence all genotypes were ranked based on percent kernel infection at 31 days after inoculation. Combined analyses of variance for average response across five environments for the top 15 hybrids were compared against 5 checks. The average response percentage score for all genotypes was 14.5% with a minimum response of 4% and a maximum of 40.5% (Table 5.7). The best performing hybrids (G16) across five environments had response advantage of 3.89 times higher than the average of checks. Also, area under disease progress curve of the best-performing hybrids (G16) across five environments was the lowest with 21 average disease accumulations. All the genotypes had comparable kernel texture ranging from 1.0 to 4.4 scores with a mean of 2.8 although the best hybrid kernel texture score was not significantly different from average of the checks (Table 5.7).

Table 5. 7 Mean performance of 15 best entries with low percentage kernel infection based on percent kernel infect at 21 days after inoculation among 95 tropical maize hybrids in year 2014

Rank	Genotype	Cross	Percent Kernel infection			Mean Percent kernel Infection	Area Under Disease progress Curve	Kernel Texture
		(F x M)	7 Days	14Days (%)	21Days			(1-5)
Top								
1	G16	7 x 1	0.00	1.00	4.00	1.67	21.00	3.60
2	G17	8 x 1	0.50	3.50	5.50	3.17	45.50	3.40
3	G74	2 x 10	1.50	2.00	5.50	3.00	38.50	1.20
4	G20	2 x 2	1.00	3.00	6.00	3.33	45.50	2.35
5	G29	2 x 7	1.00	3.50	6.00	3.50	49.00	2.80
6	G3	3 x 3	0.50	5.50	6.50	4.17	63.00	2.55
7	G41	5 x 8	1.00	3.00	6.50	3.50	47.25	2.95
8	G60	6 x 4	2.50	2.50	6.50	3.83	49.00	2.85
9	G54	9 x 9	1.50	4.50	7.00	4.33	61.25	1.00
10	G11	2 x 1	1.00	2.00	7.50	3.50	43.75	2.65
11	G33	6 x 7	0.50	4.50	8.00	4.33	61.25	3.15
12	G49	4 x 9	0.00	2.00	8.00	3.33	42.00	1.10
13	G38	2 x 8	0.96	6.79	8.19	5.31	56.00	2.50
14	G22	4 x 2	0.50	3.50	8.50	4.17	56.00	3.45
15	G73	1 x 10	2.50	6.50	8.50	5.83	84.00	1.50
Worst								
90	G87	6 x 5	2.00	7.50	25.00	11.50	147.00	3.05
91	G39	3 x 8	3.50	13.00	26.50	14.33	196.00	1.85
92	G88	7 x 5	1.50	16.00	30.00	15.83	222.25	3.95
93	G84	3 x 5	8.00	21.50	32.50	20.67	292.25	3.10
95	G81	9 x 10	2.50	21.00	40.50	21.33	297.50	1.25
Checks								
19	G91		0.00	5.00	9.00	4.67	66.50	4.40
37	G94		0.50	3.00	11.00	4.83	61.25	4.15
52	G92		1.00	5.00	13.50	6.50	85.75	3.65
88	G95		2.00	7.00	24.50	11.17	141.75	3.50
94	G93		15.00	20.00	36.50	23.83	320.25	4.05
	Mean		1.97	7.17	14.50	7.88	107.58	2.79
	Minimum		0.00	1.00	4.00	1.67	21.00	1.00
	Maximum		15.00	21.50	40.50	23.83	320.25	4.40
	LSD0.05		0.45	0.82	1.38	0.78	10.95	0.17

5.4.5. Average genotypes response across environments in year 2015

The average response percentage score for all genotypes was 11.9% with a minimum response of 3.5% and a maximum of 31% (Table 5.8). The best performing hybrids

(G22) across five environments had response advantage of 3.49 times higher than the average of checks. Also, area under disease progress curve of the best-performing hybrids (G22) across five environments had the lowest score with 35 average disease accumulations. All the genotypes had comparable kernel texture ranging from 1.3 to 3.9 scores with a mean of 2.4 and the best hybrid kernel texture score was significantly different from average of the checks (Table 5.8).

5.4.6. Average response of genotypes across environments in across years

Significant differences in all of these traits percent kernel infection at seven, 14 and 21 day after inoculation, and mean percent kernel infection were observed among hybrids. The ranges of average values for each trait over the two years were 0.0–15 % (at seven days after inoculation), 1.0–21.6 % (at 14 days after inoculation), 5-39.8 % (at 21 days after inoculation), and 1.9-24.2 % (mean percent kernel infection). The best performing hybrids (G20) across two years in five environments had response advantage of 2.19 times higher than the average of checks. Although area under disease progress curve of the best-performing hybrids (G20) across two years in five environments was not among the best hybrids with low disease accumulation. All the genotypes had comparable kernel texture ranging from 1.0 to 4.4 scores with a mean of 2.4 and the best hybrid kernel texture score was significantly different from average of the checks (Table 5.9). It's observed that although G16 responded the best in year 2014, it was not among the best 15 hybrids in year 2015 of which G22 did. G20 was consistent in both years being among the best 10 hybrids.

Table 5. 8 Mean performance of 15 best entries with lower Percentage Kernel Infection based on percent kernel infection at 21 days after inoculation among 95 Tropical maize hybrids in year 2015

Rank	Genotype	Cross	Percent Kernel infection			Mean Percent kernel Infection	Area Under Disease progress Curve	Kernel Texture
		(F x M)	7	14	21			(1-5)
				(%)				
Top								
1	G22	2 x 4	0.50	3.00	3.50	2.33	35.00	2.85
2	G13	1 x 4	1.00	4.00	4.00	3.00	45.50	1.70
3	G5	3 x 5	1.00	3.00	4.50	2.82	40.25	3.10
4	G8	3 x 8	1.50	3.50	4.50	3.17	45.50	3.55
5	G20	2 x 2	2.50	4.00	4.50	3.66	52.50	2.35
6	G37	8 x 1	1.00	4.00	4.50	3.16	47.25	2.25
7	G75	10 x 3	2.50	4.50	5.00	4.01	57.75	1.35
8	G9	3 x 9	0.50	2.00	5.50	2.67	35.00	2.45
9	G26	1 x 2	0.50	2.00	5.50	2.66	40.25	3.20
10	G11	1 x 9	1.00	2.50	5.50	2.99	54.25	2.00
11	G18	2 x 8	2.00	4.00	5.50	3.84	35.00	1.70
12	G44	3 x 4	2.50	4.00	6.00	4.16	61.25	2.85
13	G4	8 x 8	1.50	5.00	6.00	4.17	57.75	2.95
14	G10	1 x 1	0.00	3.50	6.50	3.34	47.25	2.00
15	G46	9 x 1	1.00	6.00	6.50	4.49	68.25	1.70
Worst								
91	G51	6 x 9	3.50	14.00	22.00	13.18	187.25	2.00
92	G48	3 x 9	3.00	8.50	23.50	11.65	152.25	1.65
93	G49	4 x 9	5.50	13.00	24.50	14.34	196.00	2.05
94	G69	6 x 6	8.50	18.50	24.50	17.18	245.00	2.45
95	G70	7 x 6	7.50	21.00	31.00	19.83	281.75	2.45
Checks								
25	G91		0.50	3.00	8.00	3.84	50.75	3.45
29	G93		2.50	6.00	9.00	5.85	82.25	3.85
46	G95		2.50	6.00	11.00	6.50	89.25	3.85
61	G94		3.00	8.00	12.50	7.84	110.25	3.55
89	G92		3.50	9.00	20.50	11.01	147.00	3.50
	Mean		2.64	7.37	11.89	7.30	102.37	2.40
	Minimum		0.00	2.00	3.50	2.33	35.00	1.30
	Maximum		8.50	21.00	31.00	19.83	281.75	3.90
	LSD0.05		0.32	0.69	1.05	0.64	9.09	0.10

Table 5. 9 Mean performance of 15 best entries with lower percentage kernel infection based on percent kernel infection at 21 days after inoculation among 95 tropical maize hybrids across years (2014 – 2015).

Rank	Genotype	Cross	Percent Kernel infection			Mean Percent kernel Infection	Area Under Disease progress Curve	Kernel Texture
		(F x M)	7	14	21			
				(%)				(1-5)
Top								
1	G20	2 x 2	1.75	3.50	5.25	3.50	49.00	2.35
2	G22	4 x 2	0.50	3.25	6.00	3.25	45.49	3.15
3	G16	7 x 1	0.75	2.25	6.00	3.01	39.39	3.03
4	G17	8 x 1	0.50	4.00	6.25	3.59	51.63	2.90
5	G11	2 x01	1.00	2.25	6.50	3.24	42.00	2.33
6	G5	5 x 3	1.25	5.00	6.75	4.33	62.98	3.48
7	G74	2 x 10	2.75	4.25	6.75	4.59	63.03	1.30
8	G37	1 x 8	1.00	5.25	7.25	4.50	65.61	2.93
9	G8	8 x 3	0.75	3.50	7.75	4.00	54.24	3.78
10	G18	9 x 1	2.00	3.75	7.75	4.50	60.37	1.78
11	G26	8 x 2	0.50	4.25	7.75	4.16	58.60	3.45
12	G3	3 x 3	1.75	5.50	7.75	5.01	71.76	2.13
13	G10	1 x 1	0.00	4.75	8.25	4.34	62.11	2.48
14	G12	3 x 1	2.00	5.50	8.25	5.25	74.37	1.65
15	G41	5 x 8	1.75	5.25	8.25	5.08	71.78	2.93
Worst								
90	G68	5 x 6	3.00	10.00	21.25	11.42	154.89	3.50
91	G70	7 x 6	4.50	13.01	21.51	13.01	182.11	2.63
92	G84	3 x 5	5.75	13.99	21.74	13.83	194.15	2.85
94	G69	6 x 6	6.75	16.75	24.25	15.93	225.77	2.90
95	G81	9 x 10	3.75	15.99	28.74	16.17	225.67	1.50
Checks								
16	G91		0.25	4.00	8.50	4.25	58.62	3.93
43	G94		1.75	5.50	11.75	6.34	85.78	3.85
79	G92		2.25	7.00	17.00	8.76	116.41	3.58
81	G95		2.25	6.50	17.74	8.84	115.47	3.68
93	G93		8.74	12.99	22.74	14.83	201.12	3.95
	Mean		2.31	7.27	13.20	7.59	105.16	2.57
	Minimum		0.00	2.25	5.25	3.01	39.39	1.28
	Maximum		8.74	16.75	28.74	16.17	225.77	3.95
	LSD0.05		0.29	0.59	0.93	0.56	7.82	0.14

5.4.7. Genotypes response to aflatoxin accumulation at individual location and across environments

Aflatoxin accumulation of the test hybrids varied significantly different among environments. The lowest aflatoxin accumulation was in hybrids that were planted at

Serere, and the highest aflatoxin accumulation was in hybrids that were planted at Kasese (Table 5.11). The hybrid with lowest aflatoxin accumulation recorded at Kasese, Namulonge, Serere, Bulindi, and Ngetta were 0.0 ng/kg, 0.29 ng/kg, 0.01 ng/kg, 0.43 ng/kg and 0.0 ng/kg, respectively. The best performing hybrids (G10) across five environments with lowest aflatoxin accumulation had 0.79 ng/kg.

5.4.8. Correlation between percentage kernel infection with grain texture

The simple correlation coefficients among traits are presented in Table 10. Correlations of different mean percent kernel infection with kernel texture were significant for year 2014 and across two years but not significant for year 2015. Overall percentage kernel infection was negatively correlated with grain texture. The highest significant correlation coefficient was between percent kernel infection at 21 days after inoculation and kernel texture in 2014. There was no significant correlation within and across years for percent kernel infection at seven days after inoculation and kernel texture (Table 5.10). Overall, the level of correlation between percentage kernel infection with grain texture increased from percent kernel infection at seven days after inoculation to 21 days after inoculation.

Table 5. 10 Mean aflatoxin content among 95 Tropical maize hybrids, and 5 checks within each and across five locations and in Uganda evaluated in year 2015.

Entry	Genotype	Bulindi	Kasese	Namulonge	Ngetta	Serere	Across sites
				ng/kg			
1	G1	11.41	4,328.20	1.16	4.08	1,496.93	83.84
2	G2	3.86	5,075.76	743,289.28	6.94	5.74	251.62
3	G3	0.47	752,183.90	15,730.75	1.97	0.07	154.91
4	G4	0.13	1,227.00	2.30	2.13	0.36	5.70
5	G5	0.15	20.51	2.01	0.95	0.06	1.69
6	G6	56,558.47	3.44	0.01	342.25	1.64	18.38
7	G7	169.43	429.58	0.76	5.47	7.16	22.97
8	G8	1.94	0.81	0.52	1.29	15.40	4.94
9	G9	0.39	497,218.91	1.97	7.14	0.28	90.93
10	G10	0.73	0.39	0.57	3.66	0.30	0.79
11	G11	3.83	38,877.70	0.38	6.94	0.11	30.88
12	G12	448.94	95,971.07	3.72	3.15	5.92	190.73
13	G13	0.36	37.16	2.83	4.50	0.06	1.62
14	G14	1.90	-	4.07	5.15	3.26	24.99
15	G15	0.24	26,298.72	181,874.64	4.41	0.02	111.61
16	G16	12.53	10,937.62	44,925.17	6.04	0.82	161.68
17	G17	202.67	5,765.54	176.56	1.47	0.19	64.16
18	G18	6.08	4,651.47	0.38	5.52	0.06	16.53
19	G19	3.76	1,515.04	2.70	5.97	0.16	10.98
20	G20	0.05	196,229.01	1,627.14	5.83	1.47	127.13
21	G21	0.74	7.35	0.12	8.13	1.09	1.08
22	G22	1.35	2,654.79	3,353.44	7.29	0.36	61.26
23	G23	21.28	23.51	0.07	5,997.61	0.02	19.64
24	G24	0.57	5.88	3.51	-	0.36	2.46
25	G25	0.00	3,301.69	2.06	5.75	0.30	7.23
26	G26	0.27	10,967.87	1.12	3.34	1.37	13.26
27	G27	2.69	63,210.38	1.11	7.52	0.01	32.96
28	G28	17.86	378,720.65	0.31	6.77	3.77	196.56
29	G29	10.61	4,723.94	4.42	2.95	0.08	14.57
30	G30	4.26	24,538.68	1.22	2.78	0.55	28.43
31	G31	1.63	29,272.43	0.34	6.11	0.89	18.21
32	G32	66.75	29.61	3.61	2.78	6.72	22.87
33	G33	0.05	653,733.52	0.38	2.96	0.48	19.29
34	G34	2.11	264,333.64	1.27	6.20	1.03	92.10
35	G35	75.76	7,111.32	109.95	2.44	0.32	70.11
36	G36	0.41	161,427.83	6.29	6.85	0.73	66.83
37	G37	13.87	8,462.47	0.77	12.79	2.55	35.04
38	G38	0.49	320.92	1.57	4.55	0.12	3.26
39	G39	4.82	12,250.78	-	5.23	0.02	19.93
40	G40	0.66	23.45	400.52	4.20	2.35	21.98
41	G41	14.00	0.86	2.82	5.78	2.76	13.11
42	G42	0.35	66,881.47	133.29	15.85	0.52	87.68
43	G43	174.15	16.33	4.67	9.79	713.75	49.98
44	G44	2.96	-	10.60	0.59	0.09	10.90
45	G45	6.24	125,645.11	0.07	3.00	0.46	38.79
46	G46	2.50	290.91	1,706.51	7.38	3.98	47.53
47	G47	0.20	2,871.10	4.95	2.71	0.10	6.93
48	G48	3.45	21,020.15	0.05	52.29	0.51	45.69
49	G49	0.40	1,530.41	1.05	0.81	48.28	8.08
50	G50	819.76	642.78	6.35	6.38	0.43	44.89
51	G51	1.06	0.30	28.35	7.53	0.21	2.33
52	G52	0.93	1,393,882.16	6.85	12.88	0.18	194.60
53	G53	2.82	3,873.48	5,028.38	1.47	0.24	77.90
54	G54	187.48	16,476.38	2.10	7.32	0.11	56.98
55	G55	0.28	188,873.11	1.01	11.98	0.16	49.38

56	G56	1.76	223,429.77	2.56	3.70	0.04	27.16
57	G57	0.45	11,341.20	2.57	5.45	0.15	15.51
58	G58	5.82	1.82	0.53	3.09	4,683.56	19.50
59	G59	1,787.65	343,685.02	1.60	462.03	5.77	617.31
60	G60	17.42	1,436.80	0.57	2.73	0.03	5.57
61	G61	2,822.27	14,247.56	18.58	0.45	0.33	164.92
62	G62	2.24	2,680.24	4.42	6.31	0.05	12.59
63	G63	138.19	19,979.71	0.16	2.28	0.00	24.46
64	G64	0.33	3,708.87	3.10	5.70	531.63	47.50
65	G65	212.90	339,196.77	4.63	5.22	1.76	243.91
66	G66	2.63	23,146.70	0.27	6.45	6.33	27.06
67	G67	0.89	0.54	-	3.40	0.39	1.68
68	G68	0.40	5,746.17	4.08	4.35	1.17	14.37
69	G69	0.72	442.16	3,194.62	-	2.35	52.57
70	G70	0.09	169,689.88	0.40	5.60	0.14	30.51
71	G71	1.48	229.13	1.56	2.99	0.22	4.05
72	G72	0.18	247,469.41	17,707.74	4.56	211.29	853.05
73	G73	1.20	8,083.24	0.83	1.28	0.06	10.21
74	G74	8.95	1,287,953.44	1.08	6.35	0.26	309.40
75	G75	3.31	1,631.03	0.94	0.43	1.24	7.93
76	G76	0.78	4,436.19	0.84	4.79	2,030.42	62.99
77	G77	3.19	24,351.79	0.63	121.60	1.21	54.01
78	G78	5.49	7,440.19	0.55	7.69	6.41	27.73
79	G79	0.28	73.19	0.53	2.27	0.68	3.08
80	G80	10.54	7,631.52	4.04	1.98	1,062.53	140.15
81	G81	1.26	0.29	0.15	9.26	0.50	2.57
82	G82	11.51	1.99	9.03	2.16	13.83	4.07
83	G83	0.26	33,184.29	0.11	319.84	0.02	49.21
84	G84	3.57	411,258.45	0.84	-	0.02	31.21
85	G85	1.09	7,090.84	730.20	0.39	0.77	54.08
86	G86	3.58	13,072.70	0.40	6.75	1.99	27.85
87	G87	1.75	57.82	3,204.61	3.59	0.25	43.50
88	G88	1.40	0.71	0.05	5.16	2.49	2.71
89	G89	3,931.52	35,243.33	0.36	8.62	0.06	150.30
90	G90	0.46	669,669.02	97.88	1.20	0.12	191.40
91	G91	1.03	22,759.88	0.24	5.72	1.53	19.35
92	G92	1.71	28,364.29	10.93	3.79	0.22	33.52
93	G93	0.24	453,882.80	4.54	3.58	0.48	38.90
94	G94	0.72	9,136.34	0.75	2.96	0.10	9.75
95	G95	62.16	14,781.95	0.06	3.11	0.43	34.65
	Mean	714.96	100362.46	10773.61	81.20	114.80	66.61
	Minimum	0.00	0.00	0.00	0.00	0.00	0.79
	Maximum	56,558.47	13,93,882.16	743,289.28	5,997.61	4,683.56	853.05
	Confidence Level (95.0%)	1184.48	49007.32	15962.55	125.71	113.18	24.08

Table 5. 11 Correlation coefficients between Percent-Kernel Infection with Kernel texture

	Kernel texture		
	2014	2015	Across years
Percentage kernel infection at 7 days after inoculation	-0.04	-0.02	-0.04
Percentage kernel infection at 14 days after inoculation	-0.18***	-0.05	-0.13***
Percentage kernel infection at 21 days after inoculation	-0.23***	-0.05	-0.15***
Mean percent kernel infection	-0.21***	-0.05	-0.14***
Area under disease progress curve	-0.21***	-0.04	-0.14***
Aflatoxin	-	0.02	-

5.4.9. Correlation between Percent-Kernel Infection and Kernel Texture with Aflatoxin accumulation traits

Overall aflatoxin accumulation was negatively correlated to percent-kernel infection but positively correlated with grain texture. Highest significant correlation coefficient was between Aflatoxin accumulation and percent kernel infection was observed during mean percent kernel infection however the correlation was not significant.

5.4.10. General combining ability and specific combining ability estimates for percentage kernel infection and grain texture

In year 2014 results from across location analysis, general combining ability effects for different percentage kernel infection intervals (percent kernel infection at seven, 14 and 21 days after inoculation), average percentage kernel infection, area under disease progress curve and kernel texture are presented in Table 5.11. Seven inbred lines parents with five male parents 1, 2, 3, 4 and 7 and two female parents 1 and 5 had negative general combining ability values for percent kernel infection at seven, 14 and 21 days after inoculation, mean percent kernel infection and area under disease

progress curve Six inbred lines parents with three male parents 8, 9, and 10 and three female parents 3, 8, and 9 had positive general combining ability values for percent kernel infection at seven, 14 and 21 days after inoculation, mean percent kernel infection and area under disease progress curve (Table 5.11). Inbred lines parent 10 (La Posta Seq C7) had the highest and positive general combining ability effects for percent kernel infection at seven, 14 and 21 days after inoculation, mean percent kernel infection and area under disease progress curve, and kernel texture, with significant ($P < 0.05$) percent kernel infection at seven and 14 days after inoculation, area under disease progress curve, significant ($P < 0.01$) at 21 days after inoculation, percent kernel infection at seven days after inoculation and highly significant ($P < 0.001$) kernel texture. The inbred line male parent 7 (POB.501) was the best in terms of traits studied with the lowest negative general combining ability effects [percent kernel infection at seven days (-0.07), at 14 days (-0.52), and at 21 days after inoculation (-0.24), mean percent kernel infection (-0.27), and area under disease progress curve (-1.69)]. For the best female parent, inbred line 1 (WL 118-3) was the best parent with the lowest negative general combining ability effects [percent kernel infection at seven days (-0.37), at 14 days (-0.71), and at 21 days after inoculation (-0.59), mean percent kernel infection (-0.56), and area under disease progress curve (-5.59)] (Table 5.11).

Table 5. 12 General combining ability effects for percent kernel infection and kernel texture of 19 inbred lines across locations in year 2014.

	Percent Kernel infection			Mean Percent kernel Infection	Area Under Disease progress Curve	Kernel Texture
	7	14	21			
	(%)					(1-5)
Males						
1	-0.38	-0.13	-0.76	-0.42	-4.83	0.33*
2	-0.99	-2.24	-4.03	-2.42*	-31.52	-0.11
3	-0.82	-1.69	-1.31	-1.27	-18.44	0.48***
4	-0.32	-1.19	-2.59	-1.37	-18.20	0.28
5	-0.09	1.28	-0.60	0.19	6.46	-0.03
6	0.59	-1.18	-2.64	-1.08	-16.47	-0.84***
7	-0.07	-0.52	-0.24	-0.27	-1.69	-0.07
8	0.51	1.70	4.35*	2.19	27.48	0.39***
9	0.12	1.04	2.58	1.25	16.40	-1.13***
10	1.45*	2.93*	5.24**	3.21**	43.60*	0.69***
SE	0.84	1.34	2.05	1.20		0.14
Females						
1	-0.37	-0.71	-0.59	-0.56	-5.59	0.22
2	0.28	-1.56	-3.12	-1.47	-19.70	-0.26
3	0.82	1.45	2.15	1.48	19.63	-0.47***
4	-0.78	0.57	-0.66	-0.29	-0.67	-0.71***
5	-0.33	-1.53	-0.86	-0.90	-15.51	0.69***
6	-0.18	-0.13	1.69	0.46	2.89	-0.14
7	-0.08	0.07	0.49	0.16	1.89	0.47***
8	0.42	0.92	0.24	0.53	9.73	0.66***
9	0.22	0.92	0.64	0.60	9.84	-0.45***
SE	0.66	1.60	2.00	1.30		0.14

In year 2015 results for across location, general combining ability effects for different percentage kernel infection intervals (percent kernel infection at seven, 14 and 21 days after inoculation), mean percentage kernel infection, area under disease progress curve and kernel texture are presented in Table 5.12. Ten inbred lines parents with five male parents 1, 2, 3, 4 and 5 and five female parents 1, 2, 5, 8 and 9 had negative general combining ability values for percent kernel infection at seven, 14 and 21 days after

inoculation, mean percent kernel infection and area under disease progress curve while eight inbred lines parents with four male parents 6, 7, 8, and 9 and four female parents 3, 4, 6 and 7 had positive general combining ability values for percent kernel infection at seven, 14 and 21 days after inoculation, mean percent kernel infection and area under disease progress curve (Table 5.12). Inbred lines parent 8 (WL 429-30) had the highest and positive significant general combining ability effects for percent kernel infection at seven and 14 days after inoculation, mean percent kernel infection, area under disease progress curve although their percent kernel infection at 21 days after inoculation and kernel texture were highest and positive but not significant. The inbred line male parent 4 (CML348) was the best in terms of traits studied with the lowest negative general combining ability effects [percent kernel infection at seven (-0.54), at 14 days (-0.22), and at 21 days after inoculation (-0.00), mean percent kernel infection (-0.23), and area under disease progress curve (-3.20)]. As for the best female parent, inbred line 8 (WL 429-30) was the best parent with the lowest negative general combining ability effects [percent kernel infection at seven (-0.10), at 14 days (-0.41), and 21 days after inoculation (-1.30), mean percent kernel infection (-0.63), and area under disease progress curve (-6.43)] (Table 5.12).

Table 5. 13 General combining ability effects for percent kernel infection and kernel texture of 19 inbred lines across locations in year 2015.

	Percent Kernel infection			Mean Percent kernel Infection	Area Under Disease progress Curve	Kernel Texture
	7	14	21			
	(%)					(1-5)
Males						
1	-0.59	-2.54	-3.72	-2.27	-32.89	0.33
2	-1.15	-3.42	-5.10	-3.22	-45.68	-0.34
3	-0.82	-1.99	-3.80	-2.21	-29.96	0.36
4	-0.54	-0.22	0.00	-0.23	-3.2	-0.23
5	-0.37	-0.73	-0.39	-0.49	-7.78	0.27
6	0.23	1.85	5.03	2.38	29.28	-0.37
7	0.75	1.82	1.76	1.42	21.52	-0.07
8	2.29*	4.68*	5.5	4.17*	61.78*	0.31
9	0.35	0.36	0.56	0.43	5.89	-0.74*
10	-0.21	0.06*	0.03	-0.05	-0.04	0.48
SE	0.94	2.23	3.40	2.14	30.20	0.38
Females						
1	-0.89	-1.98	-3.02	-1.99	-27.57	-0.04
2	-0.40	-1.05	-1.67	-1.02	-14.39	-0.21
3	0.35	0.6	1.27	0.72	9.94	-0.48
4	0.00	1.28	1.05	0.80	12.81	-0.06
5	-0.5	-0.93	-0.91	-0.77	-11.23	0.36
6	0.95	2.35	2.90	2.05	29.81	-0.18
7	0.75	1.52	2.71	1.67	20.80	0.27
8	-0.10	-0.41	-1.30	-0.63	-6.43	0.40
9	-0.20	-1.48	-1.14	-0.91	-14.77	-0.05
SE	0.55	1.38	1.91	1.27	17.65	0.27

Combined analysis across two years, general combining ability effects for different Percentage Kernel infection intervals (percent kernel infection at seven, 14, and 21 days after inoculation), average percentage kernel infection, and area under disease progress curve and kernel texture are presented in Table 5.13. Seven inbred lines parents with four male parents 1, 3, 4 and 5 and three female parents 1, 2, and 5 had negative general combining ability values for percent kernel infection at seven, 14 and 21 days after inoculation, mean percent kernel infection and area under disease progress curve.

Seven inbred lines parents with four male parents 7, 8, 9, and 10 and three female parents 3, 6, and 7 had positive general combining ability values for percent kernel infection at seven, 14 and 21 days after inoculation, mean kernel infection and area under disease progress curve (Table 5.13). Inbred line parent 9 (P502) had the overall highest and positive general combining ability effects for percent kernel infection at seven, 14 and 21 days after inoculation, and mean percent kernel infection, being significant for percent kernel infection at 14 and 21 days after inoculation, mean percent kernel infection, and kernel texture. The inbred line male parent 4 (CML348) was the best parent in terms of traits studied with the lowest negative general combining ability effects [percent kernel infection at seven (-0.43), 14 days (-1.87), and 21 days after inoculation (-2.52), mean percent kernel infection (-1.75), and area under disease progress curve (-10.29)]. As for the best female parent, inbred line 1 (WL 118-3) was the best with the lowest negative general combining ability effects [percent kernel infection at seven (-0.63), 14 days (-1.34), and 21 days after inoculation (-1.89), mean percent kernel infection (-1.28), and area under disease progress curve (-18.08)] (Table 5.13).

Table 5. 14 General combining ability effects of (GCA_f) and (GCA_m) for percent kernel infection and kernel texture of 19 inbred lines across years (2014 and 2015).

	Percent Kernel infection			Mean Percent kernel Infection	Area Under Disease progress Curve	Kernel Texture
	7	14	21			
	(%)					(1-5)
Males						
1	-0.49	-1.32	-2.26	-1.36	-18.9	0.33
2	-1.07	1.52	2.79	1.64	-39.01	-0.23
3	-0.82	-2.82	-4.39	-2.77	-24.91	0.42
4	-0.43	-1.87	-2.52	-1.75	-10.29	0.02
5	-0.24	-0.63	-1.28	-0.78	-1.52	0.12
6	0.43	0.24	-0.69	-0.22	8.75	-0.61
7	0.33	0.41	1.25	0.7	7.75	-0.06
8	1.40*	0.61	0.61	0.53	44.43*	0.36
9	0.23	3.23*	4.81*	3.15*	10.71	-0.93*
10	0.62	0.66	1.54	0.8	22.6	0.58
SE	0.71	1.64	2.56	1.63	22.85	0.45
Females						
1	-0.63	-1.34	-1.89	-1.28	-18.08	0.10
2	-0.07	-1.25	-2.39	-1.24	-17.34	-0.24
3	0.58	1.04	1.96	1.17	15.99	-0.48
4	-0.39	0.95	0.16	0.22	5.64	-0.39
5	-0.42	-1.21	-0.93	-0.84	-13.07	0.53
6	0.38	1.05	2.08	1.20	16.21	-0.16
7	0.33	0.78	1.52	0.89	12.04	0.36
8	0.16	0.28	-0.21	0.06	1.59	0.53
9	0.01	-0.27	-0.42	-0.23	-3.33	-0.25
SE	0.39	0.98	1.45	0.89	12.65	0.37

5.4.11. General Combining Ability and Specific Combining Ability estimates for aflatoxin accumulation

Combined analysis across locations, general combining ability effects for aflatoxin accumulation are presented in table 5.16. All female and male inbred lines parents had positive significant general combining ability values. Inbred lines male parent 9 (P502) had the overall highest and positive general combining ability effects for aflatoxin accumulation while Inbred lines female parent 4 (CML348) had the overall highest and

positive general combining ability effects for aflatoxin accumulation. The inbred line male parent 3 (CML247) was the best in terms of aflatoxin accumulation studied with the lowest GCA effects and the inbred line female parent 2 (WL 118-10) was the best in terms of aflatoxin accumulation studied with the lowest general combining ability effects (Table 5.16).

Table 5. 15 Estimates of general combining ability effects of (GCA_f) and (GCA_m) for Aflatoxin accumulation of 19 inbred lines across locations in 2015.

Parents	Male	Female	General combining Ability
CML495	1		1.13***
CML264	2		0.89***
CML247	3		0.87***
CML348	4		0.99***
CL-RCW35	5		1.04***
CL-RCW37	6		0.96***
POB.501	7		0.98***
MIRTC5	8		1.17***
P502	9		1.21***
La Posta Seq C7	10		0.91***
WL 118-3		1	1.19***
WL 118-10		2	0.88***
WL 118-11		3	0.93***
WL 429-18		4	1.25***
WL 429-14		5	1.01***
WL 429-24		6	0.92***
WL 429-26		7	1.06***
WL 429-30		8	1.01***
WL 429-38		9	0.89***

* Significant at $P < 0.05$; ** Significant at $P < 0.01$; *** Significant at $P < 0.001$

5.4.12. Specific combining ability effects for percentage kernel infection and grain texture

The results of the specific combining ability effects estimated for the different percentage kernel infection and kernel texture of the 90 hybrids under kernel inoculation are presented in Appendix 1, 2 and 3. In year 2014, the specific combining ability effect for a cross between parents L_9 x L_{10} was significant ($P < 0.01$) and negative for percent kernel infection at 21 days after inoculation, and area under disease progress curve but not significant negative for percent kernel infection at seven and 14 days after inoculation across locations. Also a cross between parents L_6 x L_7 was had negative significant specific combining ability ($P < 0.05$) for percent kernel infection at 21 days after inoculation.

In year 2015, the specific combining ability effect for a hybrid cross between parents L_2 x L_8 had negative significant specific combining ability ($P < 0.01$) for percent kernel infection at seven, 14 and 21 days after inoculation, mean percent kernel infection and area under disease progress curve across locations. Also a cross between parents L_3 x L_9 was L_9 x L_{10} had negative significant specific combining ability ($P < 0.01$) for percent kernel infection at 14 and 21 days after inoculation, mean percent kernel infection and area under disease progress curve but not significant negative for percent kernel infection at seven days after inoculation across locations.

Across two years, specific combining ability effect for a hybrid cross between parents L_2 x L_8 had negative significant specific combining ability ($P < 0.01$) for percent kernel infection at seven and 14 days after inoculation, across locations but negative non-significant percent kernel infection at 21 days after inoculation, mean kernel infection, and area under disease progress curve. Also a cross between parents L_3 x L_9 had negative significant specific combining ability ($P < 0.01$) for percent kernel infection at 14 days after inoculation, but not significant negative for percent kernel infection at 21 days after inoculation, mean percent kernel infection, and area under disease progress curve across locations.

5.4.13. Specific combining ability effects for aflatoxin accumulation

The results of the specific combining ability effects estimated for aflatoxin accumulation are presented in table 5.17. In year 2014, the specific combining ability effect for hybrid cross between parents 1x6, 5x7, 9 x 9 was highly significant ($P < 0.001$) for aflatoxin accumulation. Also a hybrid cross for 1x1, 2x3, 7x4, 9x8 and 10x7 was positively significant ($P < 0.05$) for aflatoxin accumulation. However, the hybrid cross 3x8 and 3x9 had the lowest negative specific combining ability though not significant but hybrid cross 7x1 had the highest negative specific combining ability.

5.4.14. Heritability of percent kernel infection

Medium to high broad sense coefficients heritability estimates was found in different traits except for percent kernel infection at seven days after inoculation (0.46) which was below 0.5. The highest heritability of 0.93 was recorded for kernel texture followed

by mean percent kernel infection (0.70), at 14 days (0.62) and at 21 days after inoculation (0.57) (Table 5.14). Narrow sense coefficients of genetic determination for percent-kernel-infection; percent kernel infection at seven, 14 and 21 days after inoculation, mean percent kernel infection and kernel texture were 0.46, 0.45, 0.51, 0.53 and 0.73, respectively, with kernel texture having the highest heritability of 0.73 (Table 5.14).

Table 5. 16 Estimates of Specific combining ability effects for Aflatoxin accumulation of 19 inbred lines across locations in year 2015.

Male	Female	Female*Male mean	Specific combining Ability	Rank
1	1	1.66	0.46*	6
1	2	1.13	-0.07	49
1	3	0.85	-0.35	88
1	4	0.86	-0.34	87
1	5	0.78	-0.42	89
1	6	2.01	0.81***	1
1	7	1.42	0.23	15
1	8	1.20	0.00	35
1	9	0.89	-0.31	86
2	1	0.95	-0.12	61
2	2	0.97	-0.10	55
2	3	1.52	0.45*	8
2	4	0.84	-0.22	75
2	5	1.09	0.02	33
2	6	0.85	-0.22	74
2	7	1.13	0.07	28
2	8	1.24	0.17	19
2	9	1.02	-0.05	43
3	1	1.01	0.08	25
3	2	0.90	-0.03	40
3	3	1.01	0.08	26
3	4	0.94	0.01	34
3	5	1.03	0.10	23
3	6	0.91	-0.02	39
3	7	0.72	-0.21	73
3	8	0.92	-0.01	36
3	9	0.92	-0.01	37
4	1	1.25	0.19	17
4	2	1.00	-0.07	51
4	3	1.06	-0.01	38
4	4	1.00	-0.07	48
4	5	1.36	0.29	12
4	6	0.79	-0.28	82
4	7	1.01	-0.06	45
4	8	1.24	0.17	21
4	9	0.91	-0.16	67

5	1	1.20	0.09	24
5	2	0.86	-0.24	79
5	3	0.91	-0.19	72
5	4	0.99	-0.11	57
5	5	1.25	0.14	22
5	6	0.92	-0.19	71
5	7	1.82	0.72***	2
5	8	0.95	-0.16	68
5	9	1.05	-0.06	46
6	1	1.14	0.06	29
6	2	0.81	-0.27	80
6	3	1.04	-0.04	42
6	4	1.16	0.08	27
6	5	1.44	0.36	11
6	6	0.97	-0.11	58
6	7	0.93	-0.16	66
6	8	0.99	-0.09	54
6	9	1.26	0.18	18
7	1	0.75	-0.42	90
7	2	0.89	-0.28	81
7	3	0.87	-0.29	83
7	4	1.77	0.60*	3
7	5	1.62	0.45	7
7	6	1.01	-0.15	65
7	7	1.54	0.38	10
7	8	0.94	-0.23	76
7	9	1.11	-0.06	44
8	1	1.39	0.29	13
8	2	1.37	0.28	14
8	3	1.15	0.05	30
8	4	0.96	-0.14	62
8	5	0.92	-0.17	69
8	6	1.03	-0.06	47
8	7	0.80	-0.30	85
8	8	0.97	-0.12	60
8	9	1.27	0.17	20
9	1	0.92	-0.23	77
9	2	1.05	-0.10	56
9	3	1.03	-0.12	59
9	4	1.55	0.39	9
9	5	1.07	-0.09	53
9	6	1.20	0.04	31
9	7	0.92	-0.24	78
9	8	1.68	0.52*	5
9	9	0.97	-0.18	70
10	1	1.27	0.21	16
10	2	0.76	-0.29	84
10	3	0.90	-0.15	64
10	4	0.98	-0.07	50
10	5	1.08	0.03	32
10	6	0.98	-0.08	52
10	7	1.02	-0.03	41
10	8	1.58	0.53*	4
10	9	0.90	-0.15	63

* Significant at $P < 0.05$; ** Significant at $P < 0.01$; *** Significant at $P < 0.001$

Table 5. 17 Heritability estimates for percent kernel infection and kernel texture of 19 inbred lines across years (2014 and 2015).

Obs	Percentage kernel infection at	Coefficient of genetic determination	
		Narrow_sense Heritability	Broad_sense Heritability
1	7 Days	0.46	0.46
2	14 Days	0.45	0.62
3	21 Days	0.51	0.57
4	Mean Percentage kernel infection	0.53	0.70
5	kernel texture	0.73	0.93

The low broad-sense heritability (0.16) for aflatoxin accumulation in this study suggested that the actual heritability estimates might be lower (Falconer and Mackay, 1996; Mutiga et al., 2017), which may lead to low genetic gain from selection for aflatoxin accumulation in the five test environments. For a trait measured from the same genotype in different environments, indirect selection can be applied given information on the heritability and the genetic correlation for the trait in the two environments (Makumbi et al., 2015).

5.5. Discussion

5.5.1. Genotypes response to kernel infection and aflatoxin accumulation at individual location and across environments

In this study there were significant variations among hybrids for percent kernel infection at 14 days and 21 days after inoculation, mean percent kernel infection and area under disease progress curve and kernel texture traits in 2014, percent kernel infection at 21 days after inoculation, mean percent kernel infection, area under disease progress curve and kernel texture traits in 2015 and percent kernel infection at 21 days after

inoculation, and kernel texture traits across years. These results are comparable to those reported by (Zhang et al., 1997, Ruming et al., 2002, Ruming and Manjit, 2006; Asea et al., 2012; Alunga et al., 2016). This suggests presence of large genetic variation among the hybrids for these traits which should allow progress in selection for percent kernel infection at 14 and 21 days after inoculation and mean percent kernel infection under artificial maize kernel inoculation with *Aspergillus flavus*.

There were no significant variation for genotype x interaction for percent kernel infection parameters. These results are not similar to that reported in other studies by (Zhang et al., 1997; Li and Manjit, 2006; Okoth et al., 2017). This suggests that the laboratory conditions under which kernel infection with *Aspergillus flavus* was evaluated in each year did not lead to differential response among genotypes over two years. However, Scott and Zummo (1988, 1990) also reported no significant genotype x year interaction for kernel infection in field inoculated temperate maize when the kernels were evaluated in the laboratory. Differences in germplasm and inoculation technique used may also explain differences in results between these studies (Dhakal et al., 2016).

For aflatoxin accumulation study, results show that there were differences in the environments this results are the similar to the study reported by (Okoth et al., 2017). However, there was no significant differences in genotypes this result are different from that reported in the study by (Chiuraise et al., 2016) this can be indicative of the difference in the germplasm evaluated and also error variation observed in some screening environments when attempting to quantify aflatoxin accumulations in field

trials. This is consistent with the earlier reports by Mayfield (2011). However, Payne (1992) reported that aflatoxin contamination of maize is associated with drought combined with high temperature as well as insect injury (Payne 1992; Farfan et al., 2015). This is evident with the description of the sites where the trials were conducted meaning these trials were exposed do different climatic conditions of the trials site. Kasese exhibited high leaves of accumulation of Aflatoxin due to high temperature and humidity. Aflatoxin accumulation depends on temperature, soil moisture and relative humidity, the genotype of the host, and the inoculation method used for screening (Zummo and Scott, 1989; Windham et al., 2009; Henry et al., 2010; Warburton et al., 2013).

5.5.2. General combining ability and specific combining ability estimates for percentage kernel infection, aflatoxin accumulation and grain texture

The mean squares for general combining ability were significant for percent kernel infection at 21 days after inoculation and mean percent kernel Infection. These results are similar to that reported in the study by (Asea et al., 2012). These findings suggest the importance of both additive and non-additive genes in the inheritance of resistance to artificial kernel infection by *Aspergillus flavus* (Williams et al., 2014). These findings are in line with findings by Zhang et al., (1997) and Li and Kang (2006) who also reported that general combining ability and Specific combining ability were important in determining resistance to kernel infection. Therefore breeding methods that target utilization of both general combining ability and specific combining ability should be considered in developing germplasm with kernel infection resistance.

The non-additive effects were slightly more important than additive genetic effects for percent kernel infection at 21 days after inoculation (53.3% to 46.6%). These findings are not similar to that reported in the study by (Williams et al., 2011). The high general combining ability/ specific combining ability ratio suggests that non-additive effects play a major role in the inheritance of resistance to kernel infection and that general combining ability will be more useful to identify elite parents to constitute hybrids with kernel infection resistance to *Aspergillus flavus* and aflatoxin accumulation.

The general combining ability and specific combining ability interaction with environment were not significant for all percent kernel Infection. These findings are not similar to that reported in the study by (Williams et al., 2011). This indicates that the general combining ability effects of the lines and specific combining ability hybrids were consistent under the varying environmental conditions used in this study. This suggests that it may not be necessary to test inbred lines in multiple environments under artificial inoculation to obtain reliable general combining ability effects and for identification of hybrids with *Aspergillus flavus* resistance. This result corroborates the findings report in the study on the Inheritance study of ear rot resistance by (Hamblin and White 1999; William et al., 2011).

General combining ability effects allow the identification of superior parents that could be used to make and select better crosses for direct use or for further breeding (Simmonds, 1979; Hallauer et al., 2010). In disease resistance studies negative general

combining ability effects are desirable as they indicate the presence of alleles that contribute towards resistance while positive general combining ability effects indicate the presence of alleles that increase susceptibility. Seven inbred lines (four males namely CML495, CML247, CML348, and CL-RCW35; and three females namely WL 118-3, WL 118-10, and WL 429-14) had negative general combining ability effects for percent kernel infection at seven, 14 and 21 days after inoculation, mean percent kernel infection and area under disease progress curve.

This suggests that these inbred lines likely possess favorable alleles for resistance to kernel infection by *Aspergillus* ear rot. These lines could be good candidates for use in further studies on the inheritance of *Aspergillus* ear rot resistance through classical genetic studies like generation mean analysis or *Aspergillus*-resistance quantitative trait loci (QTL) validation and/or identification through the development of mapping populations. Such mapping populations could be developed using this inbred line and other resistant inbred lines identified as resistant to *Aspergillus* ear rot (Hamblin and White, 2000; Busboom and White, 2004; Menkir et al., 2006; Zhang et al., 2007, Williams et al., 2011; Dhakal et al., 2016) in crosses with known susceptible inbred lines.

These inbred lines can also be used to start bi-parental breeding populations to develop new lines with resistance or tolerance to *Aspergillus* ear rot. Inbred line [WEEVIL/CML202]-B-7-B-B-B-B (female 2) which showed negative general combining ability effects for percent kernel infection was reported as source of resistance to both

Stenocarpella maydis and *Fusarium graminearum* (Tembo et al., 2013), suggesting that this inbred line might be a source of multiple ear rot resistance. Inbred line CML495 (male 1) which also showed negative general combining ability effects for percent kernel infection is resistant to *fusarium* ear rot (Chen et al., 2016) which makes it another potential source of multiple ear rot resistance. Further studies will be needed to confirm these lines as sources of multiple disease resistance. Also, large doubled haploid (DH) population using these lines showing potential as one of the parents (crossed to known susceptible lines). The DH populations could be testcrossed and genome wide association studies used to identify agro-ecological zones important for kernel infection resistance to *Aspergillus flavus*.

The specific combining ability effects for percentage kernel infection across test years varied in magnitude with 30% of the crosses having negative specific combining ability effects for percent kernel infection at seven, 14 and 21 days after inoculation. This suggests that there is higher possibility of developing hybrids form specific inbred line crosses than basing on average performance of inbred lines. Only one hybrid specific combining ability effect (L_2 x L_8) was significant and negative for percent kernel infection at 7, 14 days and 21 days after inoculation while another specific combining ability effects (L_3 x L_9) had percent kernel infection at 14 and 21 days after inoculation significant and negative for *Aspergillus* ear rot infection.

Five hybrids with negative specific combining ability effects for *Aspergillus* ear rot infection had inbred lines WL 429-14, CL-RCW35, WL 118-10, and WL 118-11 as one

of the parents. These inbred lines had good general combining ability effects for kernel infection resistance to *A. flavus.* The hybrids showing negative specific combining ability effects for *Aspergillus* ear rot infection need to be tested further in multiple locations under artificial inoculation to confirm resistance or tolerance to *Aspergillus* ear rot infection and aflatoxin accumulation. In this study disease pressure was sufficient to differentiate hybrids and all the test hybrids (G20, G22, G16, G17, G 11, G5, G74, G37, G8, G18, G26, G3, G10, G12, and G41) had lower percentage kernel infection than the commercial hybrids. These results suggest that there are single cross hybrids with good potential resistance to kernel infection that can be used as parents in new hybrid combinations that can be tested for adaptability and possible release.

5.5.3. Phenotypic correlation among percent kernel infect and kernel texture

Association between traits may either be due to pleiotropy, linkage, or as the result of environmental effects. In this study, the three measures of percent kernel infection; (percent kernel infection at seven, 14 and 21 days after inoculation) were negatively correlated with kernel texture in 2014 and across years, also aflatoxin accumulation was negatively correlated to percent-kernel infection. These findings are not similar to that reported in the study by (Asea et al., 2012). This suggests that entries showing lower percent kernel infection by *Aspergillus flavus* and aflatoxin accumulation were associated with flint or semi-flint kernel texture. The magnitude of the correlations between kernel infection and grain texture was weak so was that for aflatoxin accumulation, implying that application of indirect selection in this germplasm is not possible/easy.

Also quantities of aflatoxin in these samples were inconsistent with studies that reported a positive association between endosperm texture and aflatoxin content in some temperate germplasm (Betrán et al., 2006; Guo et al., 2017). This might attributed to a hard endosperm maintains integrity of the kernel of perhaps has a direct effect on the pathogen (Betrán et al., 2006; Balconi et al., 2010; Mideros et al., 2012). Various physical factors in the kernel including wax content and wax composition in the outer pericarp layer and cutin layers (Guo et al., 1995; Guo et al., 1995; Russin et al., 1997; Brown et al., 2001; Betrán et al., 2006; Balconi et al., 2010; Mideros et al., 2012; Dhakal et al., 2016) have been implicated in maize resistance to *Aspergillus flavus*. Also, the genetic correlations between percentage kernel infection and kernel texture among locations across the two years were negative and highly significant. Although there were low genetic correlations, kernel texture has some levels of influence on kernel infection. The negative correlation indicated that Kernel with good texture has less kernel infection and the reverse is true.

5.5.4. Heritability of percent kernel infection

Heritability is the proportion of total variability accounted for by genetic variance. Medium to high Broad sense coefficients heritability estimates were found for different traits. This is consistent with the earlier studies on the inheritance of resistance to *Aspergillus* ear rots (Hamblin and White, 2000; Clements and White, 2004; Warburton and Williams, 2014; Dolezal et al., 2014). Low percent kernel infection in maize should have good character transmissibility between generations. Previous studies reported

low to moderate heritabilities for aflatoxin (Walker and White, 2001; Campbell and White, 1995; Warburton et al., 2009; Dolezal et al., 2014), and our results reported herein are consistent with those studies. The low heritabilities in our study were a function of low separation of entries in all environments when compared with aflatoxin measurement error and G × E. Perhaps the greatest barrier to breeding for *Aspergillus* ear rot resistance is the considerable amount of variation that may exist between years. In some years, environmental conditions favor fungal infection. In those years, selection of resistance based on fungal infection under natural infection will allow for progress. This calls for artificial kernel inoculation which subjects the test materials to uniform amount of inoculum. From this study, it's evident that percent kernel infection trait can be used as an indirect selection for Aflatoxin accumulation resistance.

This study was undertaken to estimate general combining ability effects of maize inbred lines and specific combining ability effects of hybrids and elucidate the mode of gene action to *Aspergillus flavus* resistance and aflatoxin accumulation in mid-altitude and tropical maize inbred lines under artificial kernel inoculation over two seasons. The presence of genetic differences, reliable pathogen isolation, and screening techniques are essential for a successful disease resistance breeding program.

CHAPTER SIX:

GENERAL DISCUSSION, CONCLUSIONS, AND RECOMMENDATIONS

6.1. General discussion

The study on the prevalence of *Aspergillus flavus* across Uganda elucidated for the first time the distribution and diversity of the plant pathogen among different agroecologies across Uganda. Studying disease prevalence is critical since it gives an insight on agricultural risk factors which guides research. These enables to development and implement sustainable disease control mechanism but also shapes policy decisions and evidence-based practice hence targets for preventive epidemics as well as increase knowledge about which factors contribute to such circumstances. In this study, it's evident that across Uganda, the fungus which produces aflatoxin in most of the food crops is prevalent. Distribution of this fungus is influenced by the elements of the disease triangle which include; a susceptible host, virulent pathogen, and conducive environment (Agrios, 2005; Scholthof, 2006; Balint-Kurti and Holland, 2015). For a disease to occur all three of these must be present. The fourth element for an epidemic to occur is time. As long as all three of these elements are present disease can initiate, an epidemic will only ensue if all three continue to be present.

From diversity study, among the lines used in the experiment, the results showed that inbred lines from different breeding programs i.e. NARO and CIMMYT were clearly divergent. This meant that the genetic makeup of these inbred lines were different. This genetic diversity serves as a way for populations to adapt and perform better to changing environments. The findings suggest also that there are three genetic pools of

maize lines which can be utilized during breeding processes. Selection of desirable parents is an important task to initiate a hybrid breeding program. Because heterosis is associated with the interaction of different alleles at a locus (Jones, 1945; Birchler et al., 2003; Birchler et al., 2010; Schnable and Springer, 2013), it has been suggested that molecular marker diversity may be used to select parents for hybridization.

In Understanding the inheritance mechanism of *Aspergillus flavus* Kernel infection, results shown variations in different factors attributed to *Aspergillus flavus* infection on maize, environment, genotypes plus their interactions. Breeding for this trait is difficult due to its quantitative nature and high genotype-by-environment (G x E) interaction (Campbell and White, 1995, Hamblin et al., 2007). Environmental stress can significantly increase pre-harvest infection of *A. flavus* and aflatoxin accumulation in grain (Widstrom, 1996; Cotty and Jaime-Garcia, 2007; Windham et al., 2009; Hell et al., 2010; Warburton and Williams, 2014). Maize physiological stresses caused by atmospheric temperatures, type of soil, amount of rainfall, make it prone to fungal colonization and hence results to accumulation of aflatoxin at harvest (Cotty and Jaime-Garcia, 2007; Daves et al., 2010).

Since the mid-1970s, studies on inheritance of *Aspergillus flavus* resistance and aflatoxin accumulation have been conducted (Darrah et al., 1987; Guo et al., 2017) and still continue. However the methods of screening germplasm using appropriate techniques have affected the results (Campbell and White, 1994; Dhakal et al., 2016). *Aspergillus flavus* resistance and subsequent aflatoxin contamination in some

germplasm is associated to kernel proteins that impede colonization or aflatoxin production. A 14-kDa trypsin-inhibiting protein has demonstrated to be useful in conferring resistance therefore more useful in maker assisted breeding programs (Brown et al., 1999; Cary et al., 2011; Chen et al., 2015; Fountain et al., 2015).

Aspergillus ear rot and Aflatoxin accumulation resistance are quantitatively inherited (Walker and White, 2001; Williams et al., 2015). This current study, non-additive effects were slightly more important than additive genetic effects. However, earlier studies determined that resistance was highly quantitative hence inherited in an additive manner and led to high general combining ability in hybrids (Williams et al., 2008a, Warburton and Williams, 2014) but occasionally, dominant, reciprocal and epistatic effects have also been seen in diallel experiments, possibly limiting the utility of resistance in hybrids (Williams et al., 2008b; Warburton and Williams, 2014).

6.2. Conclusions

Although the first study examined samples from few fields in the district, the results clearly indicated that *Aspergillus flavus* occurs at varying levels of its incidence and severity among Agroecologies, districts, and fields in Uganda. The second study, demonstrated that the simplicity of laboratory assays for SSR is an attractive method for the analysis of genetic diversity among maize landraces. The polymorphism detected among the accessions can be used in breeding programs to maximize the use of genetic resources. Also, the materials used in the study had varying levels of aflatoxin accumulations due to the difference in their genetic constitution.

From the combining ability studies, 15 Hybrids (G20, G22, G16, G17, G11, G5, G74, G37, G8, G18, G26, G3, G10, G12, and G41; were identified as the best hybrids in *Aspergillus* ear rot resistance in artificial inoculation. These hybrids and their parents need to be re-evaluated in a larger number of locations under artificial inoculation with *Aspergillus* ear rot to verify their performance. Such a study should also include quantification of aflatoxin content in these hybrids. The study also showed important correlations between texture and *Aspergillus* ear rot. Selection for *Aspergillus* ear rot resistance will be effective in both hybrid and parent line as well as shown by the significant additive and non-additive gene effects. In this case, favorable alleles can be accumulated in hybrid parents through recurrent selection before crossing to make a final product. Moreover, a line with dominant alleles for *Aspergillus* ear rot resistance can be crossed to other complementary lines in the opposite heterotic group to develop potentially resistant hybrids.

Inbred parents WL 118-3, WL 429-18, WL 429-14, CL-RCW35, CML495, CML264, and WL 118-11 emerged as the best general combiners for *Aspergillus* ear rot. These lines can also be used to develop superior synthetics and open-pollinated varieties. The study also demonstrated that grain texture plays a vital role in resistance or susceptibility to *Aspergillus* infection. It’s important to use a representative number of kernels from the sample to minimize bias. Also from this study, it’s clear that percentage kernel infection within 7 days after inoculation was not significant, hence we recommend that more than one data record points should be considered and also area under

disease progress curve is important for the assessing the disease development. Aflatoxin accumulation in maize is a genetically heritable trait. Within the traits measured, grain texture appeared to be most related to aflatoxin accumulations.

6.3 Recommendations and future perspectives

Based on the results from the research studies, we recommend the following;

i. Routine surveillance to monitor aflatoxin levels in different agricultural commodities in order to enforce management guidelines.
ii. From the diversity studies, hybrids should be developed and tested from distantly related inbred line with lower levels of aflatoxin to maximize heterosis.
iii. The panel of SSR markers used should be utilized in future germplasm characterization studies and also in inbred line development in maize.
iv. A local breeding program should focus on screening a wide range of inbred lines and the development of a resistant local check.
v. Further testing for other agronomic traits for the outstanding hybrids and inbred lines, identified in the present study should be conducted with a focus to enhancing maize productivity and yield stability for smallholder farmers.

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Appendix 1 Specific combining ability effects of percent kernel infection and kernel texture for 90 hybrids across locations in 2014.

Genotype	Female	Male	Percent Kernel infection at			Mean Percent kernel Infection	Area Under Disease progress Curve	Kernel Texture
			7 Days	14 Days	21 Days			
				(%)				(1-5)
G1	1	3	0.32	-0.55	3.82	1.18	10.98	-0.07
G2	2	3	-0.31	-0.99	-2.58	-1.41	-19.27	-0.60*
G3	3	3	-1.88	1.11	-1.05	-0.67	-3.11	-0.25
G4	4	3	0.23	-2.53	-3.61	-1.92	-28.98	0.99**
G5	5	3	0.28	-1.43	1.19	0.043	-4.76	-0.23
G6	6	3	-0.37	-0.83	2.56	0.41	1.69	0.01
G7	7	3	1.03	1.97	2.97	2.2	30.61	-0.09
G8	8	3	-0.97	0.12	-3.24	-1.32	-13.57	-0.18
G9	9	3	1.73	2.62	0.42	1.45	23.92	0.43
G10	1	1	5.37***	3.89	0.54	3.26	47.94	-0.47
G11	2	1	-1.26	-2.05	0.93	-0.719	-13.76	0.06
G12	3	1	-1.82	-2.95	-9.01	-4.5	-57.41	-0.02
G13	4	1	-0.22	5.42	11.30*	5.41*	75.19	-0.03
G14	5	1	0.33	1.52	-1.38	-0.04	3.93	0.13
G15	6	1	-0.82	1.62	2.77	1.25	18.79	-0.85**
G16	7	1	1.58	0.42	-0.92	0.14	2.83	0.59*
G17	8	1	-1.92	-4.43	-3.87	-3.18	-48.73	0.21
G18	9	1	-1.22	-3.93	0.14	-1.67	-31.29	0.37
G19	1	2	-0.52	1.51	-0.85	0.1	7.35	0.12
G20	2	2	-0.14	-1.44	1.26	-0.24	-8.27	0.34
G21	3	2	-0.71	-0.33	-2.67	-1.24	-14.73	-0.19
G22	4	2	-0.11	2.53	10.27*	4.34	55	-0.14
G23	5	2	1.44	3.13	3.96	2.851	40.075	0.02
G24	6	2	0.79	2.73	-0.34	1.21	23.34	-0.53
G25	7	2	-0.81	-3.97	-6.71	-3.92	-55	0.54
G26	8	2	-0.81	-2.32	-4.34	-2.49	-34.38	0.11
G27	9	2	0.89	-2.32	-0.09	-0.67	-15.89	-0.27
G28	1	7	-0.3	5.88	6.08	3.9	60.85	0.48
G29	2	7	-0.06	1.62	-0.28	0.48	11.17	0.07
G30	3	7	-2.05	-3.13	-5.74	-3.7	-48.91	-0.13
G31	4	7	0.98	-0.41	-4.05	-1.28	-14.47	-0.32
G32	5	7	-0.47	-2.31	6.6	1.4	5.875	-0.14
G33	6	7	0.88	-4.21	-9.69*	-4.39	-60.62	0.31
G34	7	7	0.28	0.09	0.63	0.35	3.31	0.12
G35	8	7	-1.22	0.74	5.11	1.64	20.6	-0.3
G36	9	7	1.98	1.24	1.83	1.56	19.69	-0.11
G37	1	8	-2.02	-0.94	1.39	-0.49	-7.51	0.13
G38	2	8	-1.64	-1.89	3.57	-0.05	-7.62	-0.05
G39	3	8	0.79	-2.78	-5.13	-2.37	-34.88	0.41
G40	4	8	-0.11	4.58	5.27	3.27	49.39	-0.15
G41	5	8	-1.06	0.18	6.25	2.02	22.64	-0.01
G42	6	8	2.79	6.28	3.37	4.234	66.27	0.33
G43	7	8	-0.81	-3.92	-6.61	-3.767	-53.53	-0.82**
G44	8	8	3.19*	0.23	-1.2	0.551	6.42	0.02
G45	9	8	-1.11	-2.27	-6.41	-3.435	-43.67	0.13
G46	1	9	0.87	-1.27	-4.5	-1.538	-22.76	-0.29
G47	2	9	-0.76	-4.72	-7.19	-4.319	-62.47	-0.19
G48	3	9	-0.32	-4.12	2.22	-0.616	-20.46	0.08
G49	4	9	-0.22	-0.75	-5.84	-2.181	-25.22	0.41
G50	5	9	-0.67	-2.15	-5.75	-2.658	-34.05	-0.1
G51	6	9	-0.82	-5.05	-1.84	-2.777	-47.58	0.19

G52	7	9	-0.42	-2.25	-1.07	-1.273	-21.75	-0.1
G53	8	9	2.08	7.9	2.68	4.02	70.83	-0.07
G54	9	9	0.28	11.90***	21.76***	11.30***	160.94***	0.08
G55	1	4	0.32	0.45	-0.11	0.35	6.38	0.08
G56	2	4	-0.81	-0.99	-0.41	-0.87	-13.98	0.07
G57	3	4	-1.88	-4.39	-0.53	-2.35	-41.71	0.3
G58	4	4	2.23	-0.53	-1.78	-0.24	-4.78	-0.11
G59	5	4	0.78	2.07	-2.11	0.23	10.51	0.1
G60	6	4	-0.87	-1.33	-6.94	-2.99	-34.97	0.25
G61	7	4	2.53	1.97	0.22	1.43	22.03	-0.72*
G62	8	4	-1.47	1.12	10.76*	3.69	42.89	0.1
G63	9	4	-0.77	1.12	1.39	0.7	11.12	-0.09
G64	1	6	-1.66	-4.75	4.31	-0.78	-25.01	-0.47
G65	2	6	2.75	7.40*	6.5	5.54*	83.86*	0.11
G66	3	6	3.18*	3.01	2.48	2.5	36.21	-0.03
G67	4	6	-1.71	-4.63	-4.4	-3.51	-52.31	-0.06
G68	5	6	-0.66	4.47	0.17	1.15	27.48	0.60*
G69	6	6	-1.81	-2.93	0.48	-1.22	-22.66	0.27
G70	7	6	-0.41	0.37	-2.45	-0.64	-5.87	0.06
G71	8	6	1.59	-0.98	-0.85	-0.15	-5.31	-0.06
G72	9	6	-1.21	-2.48	-5.76	-2.93	-38.92	-0.41
G73	1	10	-1.96	-3.16	-8.11	-4.559	-59.149	-0.2
G74	2	10	3.41*	2.89	3.56	3.36	46.3	0.18
G75	3	10	3.84*	1.00**	11.10*	8.48**	124.25**	0.1
G76	4	10	-2.05	-3.64	-4.96	-3.49	-49.21	-0.48
G77	5	10	0.5	-2.04	-3.42	-1.83	-25.92	0.02
G78	6	10	-1.15	-2.44	5.78	0.64	-2.86	-0.18
G79	7	10	-1.75	5.86	10.05*	4.71	70.02	0.07
G80	8	10	1.25	-2.99	-3.82	-1.83	-30.78	-0.09
G81	9	10	-2.05	-4.99	-9.68*	-5.53*	-75.13	0.59*
G82	1	5	-0.39	-1.63	-2.04	-1.48	-21.86	0.69*
G83	2	5	-1.14	-0.39	-4.82	-1.82	-18.76	0.02
G84	3	5	0.91	3.03	8.88	4.42	57.96	-0.28
G85	4	5	1.02	-0.61	-1.64	-0.44	-7.38	-0.1
G86	5	5	-0.43	-4	-4.97	-3.21	-48.55	-0.41
G87	6	5	1.42	5.6	4.38	3.58	55.81	0.21
G88	7	5	-1.18	-1.1	4.46	0.72	4.56	0.34
G89	8	5	-1.68	0.05	-0.69	-0.99	-10.75	0.26
G90	9	5	1.52	-1.45	-3.06	-0.82	-13.55	-0.72*
SE‡			**1.54**	**3.42**	**4.88**	**2.79**	**40.71**	**0.31**

* Significant at $P < 0.05$

** Significant at $P < 0.01$

*** Significant at $P < 0.001$

Appendix 2 Specific combining ability effects for percent kernel infection and kernel texture for 90 hybrids across locations in 2015.

Genotype	Female	Male	Percent Kernel infection at:			Mean Percent kernel Infection	Area Under Disease progress Curve	Kernel Texture
			7 Days	14 Days	21 Days			
				(%)				(1-5)
G1	1	3	0.56	1.28	1.9	1.34	17.95	-0.06
G2	2	3	1.07	-0.38	-1.84	-0.4	-5.39	-0.08
G3	3	3	0.32	3.35	5.81	3.1	44.77	-0.36
G4	4	3	-1.33	-3.65	-5.55	-3.53	-49.57	0.26
G5	5	3	-0.83	-0.67	0.73	-0.23	-5.3	-0.15
G6	6	3	0.72	-1.21	-3.28	-1.38	-18.64	-0.01
G7	7	3	-0.08	1.21	-0.32	0.25	8.7	0.14
G8	8	3	-1.23	-3.25	-1.42	-1.87	-33.01	0.15
G9	9	3	0.87	3.43	4.09	2.8	41.51	0.11
G10	1	1	0.34	3.57	9.22*	4.40*	58.36*	-0.34
G11	2	1	0.84	2.18	0.95	1.29	21.22	0.25
G12	3	1	0.59	-0.05	-0.56	0.02	-0.57	-0.45
G13	4	1	-0.56	-1.32	-3.16	-1.69	-22.02	0.39
G14	5	1	-0.56	-0.92	-2.72	-1.45	-18.15	0.09
G15	6	1	1.49	1.34	0.94	1.23	17.59	-0.69*
G16	7	1	-0.31	-2.11	-0.43	-0.92	-14.9	0.31
G17	8	1	-0.46	-0.94	-2.4	-1.27	-18.42	0.53*
G18	9	1	-1.36	-1.62	-1.71	-1.53	-21.99	-0.1
G19	1	2	-0.61	1.44	2.73	1.21	17.36	0.09
G20	2	2	-0.1	-0.73	0.26	-0.142	-4.29	0.25
G21	3	2	1.15	0.51	-0.52	0.36	5.38	-0.15
G22	4	2	-0.5	-1.61	-4.04	-1.99	-27.14	-0.17
G23	5	2	-0.5	-0.49	1.18	0.02	-1.33	0.07
G24	6	2	1.55	0.46	0.49	0.77	10.01	-0.17
G25	7	2	-0.75	-2.14	-1.49	-1.42	-20.55	0.24
G26	8	2	-0.9	1.19	1.64	0.61	9.54	0.03
G27	9	2	0.7	1.47	-0.12	0.66	12.04	-0.19
G28	1	7	0.11	-2.06	-4.37	-2.21	-29.89	0.38
G29	2	7	-0.01	2.73	3.05	1.95	29.77	0.18
G30	3	7	1.75	2.21	1.7	1.91	27.77	-0.14
G31	4	7	-0.9	1.55	4.54	1.71	23.68	-0.22
G32	5	7	-0.4	-1.17	-0.65	-0.75	-11.87	-0.33
G33	6	7	-1.35	-2.69	-2.13	-1.99	-31.23	0.16
G34	7	7	0.35	-1.63	-4.24	-1.87	-23.29	0.06
G35	8	7	-0.3	1.19	3.35	1.35	17.1	-0.47
G36	9	7	0.8	-0.02	-1.11	-0.04	-1	0.38
G37	1	8	0.95	-3.58	-1.33	-1.32	-28.09	0.07
G38	2	8	-4.04***	-6.04*	-6.66*	-5.63*	-81.72*	-0.39
G39	3	8	-2.79*	-2.39	-2.98	-2.79	-38.89	0.33
G40	4	8	-0.44	0.1	-1.44	-0.64	-7.9	0.25
G41	5	8	0.56	1.3	1	0.93	12.6	0.223
G42	6	8	2.61*	4.16*	4.35	3.69*	51.99*	0.04
G43	7	8	1.81	6.94**	10.79**	6.59**	93.49**	-0.41
G44	8	8	1.66	2.11	0.78	1.58	34.7	0.08
G45	9	8	-0.24	-2.49	-4.39	-2.33	-35.15	-0.21
G46	1	9	-1.11	1.14	0.12	0.07	4.7	0.13
G47	2	9	1.4	-0.2	-2.75	-0.53	-6.06	0.06
G48	3	9	-0.85	-4.28*	-8.88*	-4.55*	-63.74*	0.28

G49	4	9	0.5	-3.2	-4.087	-2.24	-35.11	-0.19
G50	5	9	-4.4	1.95	3.4	1.82	25.66	-0.46
G51	6	9	-1.95	-1.79	2.25	-0.56	-11.95	0.27
G52	7	9	0.25	0.34	2.94	1.13	15.3	-0.33
G53	8	9	-0.4	1.37	1.49	0.77	11.72	-0.01
G54	9	9	2.20*	4.77*	5.65	4.18*	60.51*	0.24
G55	1	4	0.78	-0.18	0.64	0.39	3.3	0.27
G56	2	4	0.29	2.44	4.03	2.33	32.27	-0.13
G57	3	4	-0.46	2.06	0.76	0.82	15.57	0.22
G58	4	4	1.89	0.31	-1.34	0.18	3.55	0.04
G59	5	4	-0.61	0.49	0.94	0.29	4.43	-0.28
G60	6	4	-1.06	-1.41	-2.39	-1.55	-21.34	0.02
G61	7	4	-0.36	-0.76	-4.67	-1.95	-20.96	-0.36
G62	8	4	1.49	-0.81	2.85	1.21	8.078	0.28
G63	9	4	-1.91	-2.02	-0.68	-1.64	-23.85	-0.05
G64	1	6	-0.03	0.42	-5.63	-1.77	-14.88	-0.06
G65	2	6	-0.98	-0.19	2.36	0.35	5.42	0.01
G66	3	6	-0.23	-1.32	5.43	1.28	11.42	0.19
G67	4	6	2.62*	2.3	6.47*	3.81*	49.88	0.2
G68	5	6	-0.38	-1.46	-5.59	-2.46	-28.4	0.28
G69	6	6	-0.33	2.2	2.19	1.41	24.45	0.25
G70	7	6	0.37	0.88	1.41	0.86	-4.05	-0.18
G71	8	6	-0.28	-0.19	-3.07	-1.2	-12.62	-0.47
G72	9	6	-0.68	-2.53	-3.45	-2.2	-30.17	-0.22
G73	1	10	-0.55	-1.33	0.68	-0.4	-8.86	-0.22
G74	2	10	-0.54	0.6	-0.28	-0.1	1.1	-0.35
G75	3	10	0.71	-1.33	-2.07	-0.93	-14.55	0.32
G76	4	10	-0.94	1.98	2.49	1.19	19.06	-0.29
G77	5	10	2.06	-0.53	2.47	1.33	11.72	0.57*
G78	6	10	0.61	1.76	-0.75	0.55	12.01	-0.2
G79	7	10	-0.69	-1.97	-3.13	-1.92	-25.05	0.01
G80	8	10	0.16	1.49	0.99	0.9	13.2	-0.03
G81	9	10	-0.74	-0.54	-0.28	-0.54	-7.59	0.2
G82	1	5	-0.39	-0.58	-3.81	-1.63	-18.79	-0.26
G83	2	5	2.12	-0.28	1.03	0.98	8.82	0.19
G84	3	5	-0.13	1.37	1.44	0.86	13.98	-0.22
G85	4	5	-0.28	3.65	6.26	3.28	46.71	-0.26
G86	5	5	0.72	1.63	-0.62	0.58	11.79	-0.03
G87	6	5	-2.23*	-2.69	-1.53	-2.09	-31.74	0.32
G88	7	5	-0.53	-0.62	-0.73	-0.64	-7.44	0.51*
G89	8	5	0.32	-2.04	-4.07	-1.99	-29.14	-0.08
G90	9	5	0.42	-0.32	2.15	0.73	6.84	-0.17
SE‡			**1.14**	**2.07**	**3.36**	**1.94**	**27.23**	**0.26**

* Significant at $P < 0.05$

** Significant at $P < 0.01$

*** Significant at $P < 0.001$

Appendix 3 Specific combining ability effects for percent kernel infection and kernel texture for 90 hybrids across years (2014 and 2015).

Genotype	Female	Male	Percent Kernel infection at:			Mean Percent kernel Infection	Area Under Disease progress Curve	Kernel Texture
			7 Days	14 Days (%)	21 Days			(1-5)
G1	1	3	0.43	0.63	2.77	1.32	15.94	-0.07
G2	2	3	0.37	-0.49	-2.3	-0.8	-10.07	-0.34
G3	3	3	-0.78	2.04	2.14	1.14	19.11	-0.3
G4	4	3	-0.55	-3.15	-4.56	-2.79	-40.36	0.61*
G5	5	3	-0.28	-0.97	0.95	-0.1	-4.47	-0.2
G6	6	3	0.17	-0.98	0.12	-0.25	-5.76	-0.02
G7	7	3	0.47	1.48	1.73	1.27	18.55	0.04
G8	8	3	-1.1	-1.49	-2.41	-1.65	-22.69	-0.01
G9	9	3	1.3	2.9	1.7	1.91	30.09	0.28
G10	1	1	2.85**	3.79	4.99	3.92*	54.38*	-0.44
G11	2	1	-0.21	0.02	1.31	0.37	4.11	0.15
G12	3	1	-0.61	-1.56	-5.56	-2.53	-32.04	-0.24
G13	4	1	-0.39	2.14	4.38	2.04	28.85	0.17
G14	5	1	-0.11	0.38	-2.54	-0.8	-7.02	0.11
G15	6	1	0.34	1.35	1.39	1.01	15.28	-0.78**
G16	7	1	0.64	-0.81	-0.43	-0.26	-5.64	0.50*
G17	8	1	-1.19	-2.67	-2.93	-2.25	-32.87	0.4
G18	9	1	-1.29	-2.68	-0.48	-1.46	-24.71	0.11
G19	1	2	-0.57	1.54	0.89	0.64	12.07	0.1
G20	2	2	-0.13	-1.12	-0.13	-0.45	-8.7	0.28
G21	3	2	0.22	0.24	-1.35	-0.32	-2.36	-0.15
G22	4	2	-0.3	0.55	2.89	1.07	13.21	-0.14
G23	5	2	0.47	1.43	2.62	1.52	20.98	0.04
G24	6	2	1.17	1.43	0.88	1.17	17.33	-0.35
G25	7	2	-0.78	-2.94	-3.75	-2.51	-36.69	0.38
G26	8	2	-0.85	-0.65	-1.63	-1.04	-13.18	0.09
G27	9	2	0.8	-0.52	-0.3	-0.04	-2.31	-0.25
G28	1	7	-0.08	0.72	-0.31	0.09	3.57	0.49
G29	2	7	-0.03	2.34	1.08	1.2	20.72	0.11
G30	3	7	-0.18	-0.2	-0.95	-0.48	-5.8	-0.12
G31	4	7	0.04	0.64	0.64	0.41	6.48	-0.29
G32	5	7	-0.43	-1.68	2.1	0.06	-5.17	-0.23
G33	6	7	-0.23	-3.17	-5.68	-3.02	-42.74	0.23
G34	7	7	0.32	-0.62	-1.97	-0.8	-10.53	0.06
G35	8	7	-0.76	1.05	5	1.78	22.4	-0.41
G36	9	7	1.39	0.88	0.23	0.8	11.41	0.13
G37	1	8	-0.54	-2.32	0.52	-0.77	-16.28	0.07
G38	2	8	-2.85*	-3.91*	-1.8	-2.88	-43.97	-0.25
G39	3	8	-1	-2.65	-3.85	-2.5	-35.48	0.36
G40	4	8	-0.28	2.38	1.16	1.1	19.96	0.07
G41	5	8	-0.25	0.79	4.07	1.59	19.47	0.11
G42	6	8	2.70*	5.28*	4.42	4.11*	61.62*	0.2
G43	7	8	0.5	1.55	1.41	1.15	17.42	-0.60*
G44	8	8	2.42*	1.22	-0.41	1.07	15.55	0.05
G45	9	8	-0.68	-2.36	-5.38	-2.82	-37.94	-0.04
G46	1	9	-0.12	0.34	-2.9	-0.94	-8.58	-0.1
G47	2	9	0.32	-2.44	-5.39	-2.5	-34.92	-0.04
G48	3	9	-0.58	-4.06*	-3.18	-2.59	-41.47	0.18

G49	4	9	0.14	-1.9	-4.88	-2.15	-29.19	0.09
G50	5	9	-0.33	-0.27	-0.23	-0.31	-4.32	-0.25
G51	6	9	-1.38	-3.52	-0.41	-1.79	-31.13	0.23
G52	7	9	-0.08	-0.95	0.9	-0.02	-3.55	-0.24
G53	8	9	0.84	4.62*	2.58	2.64	44.01	-0.06
G54	9	9	1.24	8.16	13.64	7.71	109.48***	0.18
G55	1	4	0.54	0.2	1.15	0.64	7.5	0.2
G56	2	4	-0.26	0.92	1.11	0.58	9.15	-0.04
G57	3	4	-1.17	-1.09	-1.22	-1.16	-15.85	0.25
G58	4	4	2.06*	-0.29	-1.15	0.13	0.34	-0.02
G59	5	4	0.08	1.34	-0.15	0.41	8.9	-0.08
G60	6	4	-0.97	-1.32	-4.49	-2.24	-28.13	0.15
G61	7	4	1.08	0.56	-2.35	-0.27	-0.87	-0.57*
G62	8	4	0.01	0.11	6.88*	2.4	25.44	0.16
G63	9	4	-1.34	-0.46	0.36	-0.45	-6.13	-0.05
G64	1	6	-0.81	-1.79	0.57	-0.74	-13.99	-0.26
G65	2	6	0.87	3.77	4.55	3.05	45.44	0.07
G66	3	6	1.47	0.73	3.53	1.89	22.34	0.08
G67	4	6	0.45	-1.41	1.04	0.05	-4.61	0.06
G68	5	6	-0.53	1.4	-3.35	-0.83	-3.82	0.4
G69	6	6	-1.08	-0.34	1.12	-0.09	-2.24	0.26
G70	7	6	-0.03	0.55	-1.01	-0.08	1.11	-0.04
G71	8	6	0.65	-0.54	-2.04	-0.69	-9.14	-0.26
G72	9	6	-0.95	-2.4	-4.27	-2.52	-34.74	-0.32
G73	1	10	-1.26	-2.12	-4.11	-2.47	-33.25	-0.21
G74	2	10	1.43	1.71	2.69	1.92	26.08	-0.1
G75	3	10	2.28*	4.24*	4.08	3.57*	52.31*	0.24
G76	4	10	-1.5	-0.65	-0.81	-1.02	-12.79	-0.4
G77	5	10	1.28	-1.39	-0.42	-0.15	-6.37	0.31
G78	6	10	-0.27	-0.26	2.2	0.51	4.41	-0.21
G79	7	10	-1.22	1.97	3.4	1.41	21.65	0.02
G80	8	10	0.7	-0.68	-1.79	-0.64	-9.19	-0.05
G81	9	10	-1.4	-2.84	-5.11	-3.1	-42.5	0.39
G82	1	5	-0.4	-1.01	-3.41	-1.65	-20.98	0.2
G83	2	5	0.52	-0.84	-0.98	-0.44	-7.46	0.14
G84	3	5	0.39	2.27	6.52*	3.02	39.62	-0.3
G85	4	5	0.37	1.65	1.46	1.21	18.49	-0.16
G86	5	5	0.14	-1.07	-2.89	-1.34	-17.79	-0.23
G87	6	5	-0.41	1.49	0.6	0.64	11.74	0.26
G88	7	5	-0.86	-0.81	2.21	0.16	-1.07	0.44
G89	8	5	-0.68	-0.99	-3.11	-1.57	-19.95	0.07
G90	9	5	0.97	-0.72	-0.24	0.02	-2.28	-0.42
	SE‡		**0.99**	**2.08**	**3.13**	**1.82**	**25.95**	**0.26**

* Significant at $P < 0.05$

** Significant at $P < 0.01$

*** Significant at $P < 0.001$

Appendix 4 Mean performance of 95 best entries across five locations in Uganda in 2014

GENOTYPE	Percent Kernel infection at:			Mean Percent kernel Infection	Area Under Disease progress Curve	Kernel Texture
	7 Days	14 Days	21 Days			
	(%)					(1-5)
G16	0.00	1.00	4.00	1.67	21.00	3.60
G17	0.50	3.50	5.50	3.17	45.50	3.40
G74	1.50	2.00	5.50	3.00	38.50	1.20
G20	1.00	3.00	6.00	3.33	45.50	2.35
G29	1.00	3.50	6.00	3.50	49.00	2.80
G3	0.50	5.50	6.50	4.17	63.00	2.55
G41	1.00	3.00	6.50	3.50	47.25	2.95
G60	2.50	2.50	6.50	3.83	49.00	2.85
G54	1.50	4.50	7.00	4.33	61.25	1.00
G11	1.00	2.00	7.50	3.50	43.75	2.65
G33	0.50	4.50	8.00	4.33	61.25	3.15
G49	0.00	2.00	8.00	3.33	42.00	1.10
G38	0.96	6.79	8.19	5.31	56.00	2.50
G22	0.50	3.50	8.50	4.17	56.00	3.45
G73	2.50	6.50	8.50	5.83	84.00	1.50
G5	1.50	7.00	9.00	5.83	85.75	3.85
G12	1.00	6.00	9.00	5.33	77.00	2.00
G31	3.00	6.00	9.00	6.00	84.00	2.20
G91	0.00	5.00	9.00	4.67	66.50	4.40
G32	2.00	6.50	9.50	6.00	85.75	3.80
G52	2.00	6.50	9.50	6.00	85.75	2.45
G10	0.00	6.00	10.00	5.33	77.00	2.95
G18	2.00	3.50	10.00	5.17	66.50	1.85
G26	0.50	6.50	10.00	5.67	82.25	3.70
G37	1.00	6.50	10.00	5.83	84.00	3.60
G50	1.50	9.00	10.00	6.83	103.25	3.10
G53	4.50	6.00	10.00	6.83	92.75	2.50
G58	2.00	7.00	10.00	6.33	91.00	1.60
G82	1.00	6.50	10.00	5.83	84.00	3.45
G30	0.50	3.00	10.50	4.67	59.50	2.80
G40	2.00	8.50	10.50	7.00	103.25	1.90
G76	1.00	8.00	10.50	6.50	96.25	1.25
G8	0.00	3.50	11.00	4.83	63.00	4.00
G45	3.50	8.00	11.00	7.50	106.75	1.55
G56	2.00	7.00	11.00	6.67	94.50	2.45
G90	1.50	6.00	11.00	6.17	85.75	3.55
G94	0.50	3.00	11.00	4.83	61.25	4.15
G57	0.59	5.20	11.94	5.91	77.00	2.10
G34	4.00	8.00	12.00	8.00	112.00	2.70
G44	0.50	9.50	12.00	7.33	110.25	3.60
G70	1.50	5.00	12.00	6.17	82.25	2.80
G2	0.50	3.50	12.50	5.50	70.00	2.85
G7	3.00	7.50	12.50	7.67	106.75	4.20
G14	2.00	6.50	12.50	7.00	96.25	3.35
G23	1.00	2.50	13.00	5.50	66.50	3.65
G28	1.50	6.00	13.00	6.83	92.75	3.35
G72	1.50	7.50	13.00	7.33	103.25	2.80
G77	1.00	4.50	13.00	6.17	80.50	2.25
G15	1.50	7.50	13.50	7.50	105.00	1.95

G27	3.00	9.00	13.50	8.50	120.75	3.20
G36	1.00	8.00	13.50	7.50	106.75	2.50
G92	1.00	5.00	13.50	6.50	85.75	3.65
G1	6.50	10.50	14.00	10.33	145.25	2.75
G21	0.00	8.00	14.00	7.33	105.00	2.50
G51	0.50	3.00	14.00	5.83	71.75	2.00
G85	0.50	7.00	14.00	7.17	99.75	2.20
G9	0.50	4.00	14.50	6.33	80.50	2.95
G47	5.50	12.00	14.50	10.67	154.00	1.75
G61	2.00	7.00	14.50	7.83	106.75	3.20
G89	5.00	8.00	14.50	9.17	124.25	4.00
G63	4.00	9.00	15.00	9.33	129.50	2.10
G78	1.00	3.00	15.00	6.33	77.00	1.65
G46	0.50	1.50	15.50	5.83	66.50	1.60
G48	6.50	10.50	15.50	10.83	150.50	1.35
G66	4.00	7.50	15.50	9.00	120.75	3.05
G79	1.50	6.00	16.00	7.83	103.25	1.90
G19	1.00	4.50	16.50	7.33	92.75	3.35
G86	3.50	6.50	16.50	8.83	115.50	4.15
G24	0.50	4.50	17.00	7.33	92.75	3.05
G6	0.50	8.50	17.50	8.83	122.50	2.05
G71	6.00	10.00	17.50	11.17	152.25	3.80
G25	2.00	7.50	18.00	9.17	122.50	3.60
G55	1.00	7.50	18.00	8.83	119.00	3.35
G65	1.00	5.50	18.00	8.17	105.00	2.75
G42	3.00	14.00	19.00	12.00	175.00	2.75
G43	0.50	7.50	19.00	9.00	120.75	3.50
G59	1.00	3.00	19.00	7.67	91.00	3.25
G13	0.00	8.00	20.00	9.33	126.00	1.80
G62	1.00	8.50	20.50	10.00	134.75	3.00
G80	4.50	17.00	20.50	14.00	206.50	2.15
G64	0.00	7.50	21.00	9.50	126.00	3.45
G67	1.50	14.00	21.50	12.33	178.50	2.30
G75	2.50	5.50	22.00	10.00	124.25	1.20
G83	7.00	11.50	22.00	13.50	182.00	3.30
G35	0.50	8.00	23.50	10.67	140.00	3.70
G4	0.50	13.00	24.00	12.50	176.75	2.30
G69	5.00	15.00	24.00	14.67	206.50	3.35
G95	2.00	7.00	24.50	11.17	141.75	3.50
G68	1.00	7.50	25.00	11.17	143.50	3.80
G87	2.00	7.50	25.00	11.50	147.00	3.05
G39	3.50	13.00	26.50	14.33	196.00	1.85
G88	1.50	16.00	30.00	15.83	222.25	3.95
G84	8.00	21.50	32.50	20.67	292.25	3.10
G93	15.00	20.00	36.50	23.83	320.25	4.05
G81	2.50	21.00	40.50	21.33	297.50	1.25
Mean	1.97	7.17	14.50	7.88	107.58	2.79
Minimum	0.00	1.00	4.00	1.67	21.00	1.00
Maximum	15.00	21.50	40.50	23.83	320.25	4.40
Confidence Level (95.0%)	0.45	0.82	1.38	0.78	10.95	0.17

Appendix 5 Mean performance of 95 best entries across five locations in Uganda in 2015

No.	GENOTYPE	Percent Kernel infection			Mean Percent kernel Infection	Area Under Disease progress Curve	Kernel Texture
		7 Days	14 Days	21 Days			
		(%)					(1-5)
1	G22	0.50	3.00	3.50	2.33	35.00	2.85
2	G13	1.00	4.00	4.00	3.00	45.50	1.70
3	G5	1.00	3.00	4.50	2.82	40.25	3.10
4	G8	1.50	3.50	4.50	3.17	45.50	3.55
5	G20	2.50	4.00	4.50	3.66	52.50	2.35
6	G37	1.00	4.00	4.50	3.16	47.25	2.25
7	G75	2.50	4.50	5.00	4.01	57.75	1.35
8	G9	0.50	2.00	5.50	2.67	35.00	2.45
9	G11	1.00	2.50	5.50	2.99	40.25	2.00
10	G18	2.00	4.00	5.50	3.84	54.25	1.70
11	G26	0.50	2.00	5.50	2.66	35.00	3.20
12	G4	1.50	5.00	6.00	4.17	61.25	2.95
13	G44	2.50	4.00	6.00	4.16	57.75	2.85
14	G10	0.00	3.50	6.50	3.34	47.25	2.00
15	G46	1.00	6.00	6.50	4.49	68.25	1.70
16	G14	0.50	2.50	7.00	3.33	43.75	2.35
17	G17	0.50	4.50	7.00	4.00	57.75	2.40
18	G19	1.50	5.00	7.00	4.51	64.75	2.55
19	G2	2.50	6.00	7.50	5.33	77.00	2.65
20	G12	3.00	5.00	7.50	5.16	71.75	1.30
21	G24	3.50	6.00	7.50	5.66	80.50	2.45
22	G16	1.50	3.50	8.00	4.35	57.75	2.45
23	G23	0.50	4.00	8.00	4.16	57.75	2.85
24	G74	4.00	6.50	8.00	6.17	87.50	1.40
25	G91	0.50	3.00	8.00	3.84	50.75	3.45
26	G55	4.00	7.00	8.50	6.50	92.75	2.65
27	G3	3.00	5.50	9.00	5.84	80.50	1.70
28	G65	0.50	5.00	9.00	4.83	68.25	2.00
29	G93	2.50	6.00	9.00	5.85	82.25	3.85
30	G28	2.00	5.00	9.50	5.49	75.25	2.30
31	G73	1.00	7.00	9.50	5.83	85.75	1.65
32	G76	3.50	6.00	9.50	6.34	87.50	1.30
33	G82	1.00	4.00	9.50	4.83	64.75	2.50
34	G15	4.00	6.50	10.00	6.84	94.50	1.60
35	G34	2.50	8.00	10.00	6.82	99.75	1.95
36	G36	0.00	3.50	10.00	4.49	59.50	1.95
37	G41	2.50	7.50	10.00	6.66	96.25	2.90
38	G83	1.50	7.00	10.00	6.16	89.25	2.20
39	G7	2.50	4.50	10.50	5.83	77.00	3.20
40	G25	2.50	8.00	10.50	7.00	101.50	3.05
41	G50	2.00	7.00	10.50	6.49	92.75	2.55
42	G90	1.50	5.50	10.50	5.83	80.50	2.90
43	G27	2.50	7.50	11.00	7.00	99.75	2.70
44	G38	4.00	5.50	11.00	6.83	91.00	2.55
45	G84	3.50	6.50	11.00	7.00	96.25	2.60
46	G95	2.50	6.00	11.00	6.50	89.25	3.85
47	G31	4.00	8.50	11.50	8.00	113.75	2.05
48	G63	4.00	8.00	11.50	7.84	110.25	2.55
49	G88	2.50	7.00	11.50	7.00	98.00	3.00

50	G89	2.50	8.50	11.50	7.50	108.50	3.15
51	G6	4.50	8.50	12.00	8.34	117.25	1.75
52	G32	1.00	7.00	12.00	6.67	94.50	2.15
53	G59	2.50	7.00	12.00	7.17	99.75	2.25
54	G61	4.50	9.00	12.00	8.50	120.75	2.55
55	G72	4.50	8.50	12.00	8.33	117.25	2.35
56	G33	2.00	8.50	12.50	7.66	110.25	1.90
57	G45	2.50	5.00	12.50	6.66	87.50	2.35
58	G53	2.50	8.50	12.50	7.84	112.00	1.85
59	G54	2.00	5.50	12.50	6.66	89.25	1.65
60	G80	2.50	8.50	12.50	7.82	112.00	1.95
61	G94	3.00	8.00	12.50	7.84	110.25	3.55
62	G42	1.00	6.50	13.00	6.84	94.50	2.70
63	G64	5.00	6.50	13.00	8.17	108.50	2.60
64	G35	3.50	6.00	13.50	7.68	101.50	2.75
65	G43	2.50	7.50	13.50	7.84	108.50	3.35
66	G86	4.00	6.00	13.50	7.84	103.25	3.70
67	G30	2.00	10.00	14.00	8.66	126.00	1.80
68	G39	2.50	8.50	14.00	8.34	117.25	1.85
69	G87	4.00	11.50	14.00	9.84	143.50	2.40
70	G1	1.50	6.50	14.50	7.50	101.50	2.25
71	G29	2.00	9.00	14.50	8.50	120.75	1.70
72	G60	3.00	9.00	14.50	8.84	124.25	2.20
73	G21	2.50	9.00	15.00	8.83	124.25	1.80
74	G56	3.00	11.00	15.00	9.67	140.00	2.20
75	G77	2.50	9.00	15.00	8.83	124.25	1.45
76	G62	3.00	9.50	15.50	9.34	131.25	2.15
77	G66	2.50	10.00	15.50	9.33	133.00	2.45
78	G85	1.50	11.00	15.50	9.32	136.50	2.40
79	G57	5.50	12.00	16.50	11.33	161.00	1.60
80	G67	4.50	13.50	17.00	11.67	169.75	2.80
81	G71	6.50	14.00	17.00	12.49	193.41	3.10
82	G81	5.00	11.00	17.00	11.02	154.00	1.75
83	G47	1.50	8.00	17.50	9.00	122.50	1.70
84	G68	5.00	12.50	17.50	11.67	166.25	3.20
85	G78	2.00	8.00	17.50	9.17	124.25	1.65
86	G79	4.00	9.50	18.00	10.50	143.50	1.50
87	G40	2.00	12.00	19.00	11.00	157.50	2.25
88	G58	2.50	12.00	19.00	11.18	159.25	1.95
89	G92	3.50	9.00	20.50	11.01	147.00	3.50
90	G52	4.00	11.50	21.00	12.18	148.38	2.00
91	G51	3.50	14.00	22.00	13.18	187.25	2.00
92	G48	3.00	8.50	23.50	11.65	152.25	1.65
93	G49	5.50	13.00	24.50	14.34	196.00	2.05
94	G69	8.50	18.50	24.50	17.18	245.00	2.45
95	G70	7.50	21.00	31.00	19.83	281.75	2.45
	Mean	2.64	7.37	11.89	7.30	102.37	2.36
	Minimum	0.00	2.00	3.50	2.33	35.00	1.30
	Maximum	8.50	21.00	31.00	19.83	281.75	3.85
	Confidence Level (95.0%)	0.32	0.69	1.05	0.64	9.09	0.13

Appendix 6 Mean performance of 95 best entries across five locations in Uganda combined across 2014 and 2015

No.	GENOTYPE	Percent Kernel infection at:			Mean Percent kernel Infection	Area Under Disease progress Curve	Kernel Texture
		7 Days	14 Days	21 Days			
			(%)				(1-5)
1	G20	1.75	3.50	5.25	3.50	49.00	2.35
2	G22	0.50	3.25	6.00	3.25	45.49	3.15
3	G16	0.75	2.25	6.00	3.01	39.39	3.03
4	G17	0.50	4.00	6.25	3.59	51.63	2.90
5	G11	1.00	2.25	6.50	3.24	42.00	2.33
6	G5	1.25	5.00	6.75	4.33	62.98	3.48
7	G74	2.75	4.25	6.75	4.59	63.03	1.30
8	G37	1.00	5.25	7.25	4.50	65.61	2.93
9	G8	0.75	3.50	7.75	4.00	54.24	3.78
10	G18	2.00	3.75	7.75	4.50	60.37	1.78
11	G26	0.50	4.25	7.75	4.16	58.60	3.45
12	G3	1.75	5.50	7.75	5.01	71.76	2.13
13	G10	0.00	4.75	8.25	4.34	62.11	2.48
14	G12	2.00	5.50	8.25	5.25	74.37	1.65
15	G41	1.75	5.25	8.25	5.08	71.78	2.93
16	G91	0.25	4.00	8.50	4.25	58.62	3.93
17	G44	1.50	6.75	9.00	5.75	83.97	3.23
18	G73	1.75	6.75	9.00	5.83	84.88	1.58
19	G14	1.25	4.50	9.75	5.17	69.97	2.85
20	G82	1.00	5.25	9.75	5.33	74.36	2.98
21	G54	1.75	5.00	9.75	5.50	75.26	1.33
22	G38	2.49	6.34	9.83	6.22	87.47	2.53
23	G9	0.50	3.00	10.00	4.50	57.73	2.70
24	G2	1.50	4.75	10.00	5.40	73.50	2.75
25	G76	2.25	7.00	10.00	6.41	91.87	1.28
26	G50	1.75	8.00	10.25	6.66	97.99	2.83
27	G31	3.50	7.25	10.25	7.00	98.89	2.13
28	G33	1.25	6.50	10.25	6.00	85.78	2.53
29	G29	1.50	6.25	10.25	6.01	84.91	2.25
30	G23	0.75	3.25	10.50	4.83	62.12	3.25
31	G60	2.75	5.75	10.50	6.34	86.66	2.53
32	G90	1.50	5.75	10.75	5.99	83.12	3.23
33	G32	1.50	6.75	10.75	6.34	90.13	2.98
34	G46	0.75	3.75	11.00	5.16	67.38	1.65
35	G34	3.25	8.00	11.00	7.41	105.87	2.33
36	G28	1.75	5.50	11.25	6.16	83.99	2.83
37	G53	3.50	7.25	11.25	7.34	102.39	2.18
38	G7	2.75	6.00	11.50	6.74	91.86	3.70
39	G19	1.25	4.75	11.74	5.92	78.74	2.95
40	G15	2.75	7.00	11.75	7.17	99.74	1.78
41	G36	0.50	5.75	11.75	5.99	83.10	2.23
42	G45	3.00	6.50	11.75	7.08	97.11	1.95
43	G94	1.75	5.50	11.75	6.34	85.78	3.85
44	G13	0.50	6.00	11.99	6.17	85.71	1.75
45	G24	2.00	5.25	12.24	6.49	86.62	2.75
46	G27	2.75	8.25	12.25	7.75	110.24	2.95
47	G30	1.25	6.50	12.25	6.66	92.79	2.30
48	G72	3.00	8.00	12.50	7.84	110.26	2.58
49	G89	3.75	8.25	13.00	8.33	116.37	3.58

50	G56	2.50	9.00	13.00	8.17	117.27	2.33
51	G55	2.50	7.25	13.24	7.67	105.86	3.00
52	G63	4.00	8.50	13.25	8.58	119.86	2.33
53	G61	3.25	8.00	13.25	8.17	113.76	2.88
54	G75	2.50	5.00	13.49	7.00	90.96	1.28
55	G65	0.75	5.25	13.50	6.50	86.61	2.38
56	G77	1.75	6.75	14.00	7.50	102.40	1.85
57	G57	3.01	8.53	14.16	8.57	119.81	1.85
58	G25	2.25	7.75	14.25	8.08	111.99	3.33
59	G1	4.00	8.50	14.25	8.91	123.35	2.50
60	G21	1.25	8.50	14.50	8.08	114.64	2.15
61	G58	2.25	9.50	14.50	8.76	125.16	1.78
62	G6	2.50	8.50	14.75	8.58	119.87	1.90
63	G85	1.00	9.00	14.75	8.24	118.14	2.30
64	G40	2.00	10.25	14.75	9.00	130.40	2.08
65	G4	1.00	9.00	14.99	8.33	118.94	2.63
66	G86	3.75	6.25	15.00	8.33	109.37	3.93
67	G52	3.00	9.00	15.26	9.09	126.92	2.23
68	G59	1.75	5.00	15.50	7.41	95.38	2.75
69	G66	3.25	8.75	15.50	9.16	126.88	2.75
70	G83	4.25	9.25	15.99	9.83	135.58	2.75
71	G42	2.00	10.25	16.00	9.42	134.71	2.73
72	G47	3.50	10.00	16.00	9.83	138.23	1.73
73	G43	1.50	7.50	16.25	8.41	114.62	3.43
74	G78	1.50	5.50	16.25	7.76	100.65	1.65
75	G49	2.75	7.51	16.26	8.85	119.08	1.58
76	G80	3.50	12.75	16.50	10.90	159.20	2.05
77	G64	2.50	7.00	17.00	8.83	117.24	3.03
78	G79	2.75	7.75	17.00	9.17	123.40	1.70
79	G92	2.25	7.00	17.00	8.76	116.41	3.58
80	G71	6.25	12.00	17.25	11.82	166.26	3.45
81	G95	2.25	6.50	17.74	8.84	115.47	3.68
82	G62	2.00	9.00	18.00	9.67	133.00	2.58
83	G51	2.00	8.51	18.00	9.51	129.56	2.00
84	G35	2.00	7.00	18.49	9.17	120.73	3.23
85	G67	3.00	13.75	19.25	12.00	174.12	2.55
86	G87	3.00	9.50	19.49	10.67	145.25	2.73
87	G48	4.75	9.50	19.50	11.25	151.38	1.50
88	G39	3.00	10.75	20.24	11.33	156.58	1.85
89	G88	2.00	11.50	20.74	11.42	160.06	3.48
90	G68	3.00	10.00	21.25	11.42	154.89	3.50
91	G70	4.50	13.01	21.51	13.01	182.11	2.63
92	G84	5.75	13.99	21.74	13.83	194.15	2.85
93	G93	8.74	12.99	22.74	14.83	201.12	3.95
94	G69	6.75	16.75	24.25	15.93	225.77	2.90
95	G81	3.75	15.99	28.74	16.17	225.67	1.50
	Mean	2.31	7.27	13.20	7.59	105.16	2.57
	Minimum	0.00	2.25	5.25	3.01	39.39	1.28
	Maximum	8.74	16.75	28.74	16.17	225.77	3.95
	Confidence Level (95.0%)	0.29	0.59	0.93	0.56	7.82	0.14