GENETIC ANALYSIS OF RESISTANCE TO *ASPERGILLUS* EAR ROT AND AFLATOXIN ACCUMULATION IN MAIZE (*Zea mays* L.) INBRED LINES

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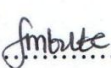**COLLEGE OF AGRICULTURE AND VETERINARY SCIENCES**
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DECLARATION

This thesis is my original work and has not been submitted for the award of any degree in any university.

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This thesis is dedicated to my loving mum Jecinter and my grandparents Eliakim and Monica for their unconditional support and their belief in my academic growth, also my siblings for their love and encouragement.

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LIST OF ABBREVIATIONS

AFB1-Aflatoxin B1

AGRA-Alliance for Green Revolution in Africa

AFB2- Aflatoxin B2

CIMMYT-International Maize and Wheat Improvement Centre

DAP-Di Ammonium Phosphate Fertilizer

FAO-Food and Agricultural Organization of the United Nations

FAOSTAT-FAO Statistical Databases

GCA- General combining ability

IFPRI-International Food Policy Research Institute

GC-gas chromatography

LC-liquid chromatography

TLC-thin layer chromatography

HPLC-high-performance liquid chromatography

KALRO- Kenya Agricultural and Livestock Research Organization

NCDII- North Carolina II Design

PDA -Potato Dextrose Agar

SCA- Specific combining ability

USDA-ARD- US Department of Agriculture, Agricultural Research Service

ANOVA-analysis of variance

HCC- Hepatocellular carcinoma

QPM-Quality Protein Maize

RAPs- Resistance-Associated Proteins

RFLP- restriction fragment length polymorphism

ELISA-enzyme-linked immunosorbent assay

ABSTRACT

Recurrent aflatoxin contamination in maize has been a major problem in Kenya as it consistently causes loss of produce and lives resulting to massive economic losses. Kenyan maize germplasm are susceptible to aflatoxin accumulation hence there is need to incorporate resistance genes into these germplasm. Therefore, the objective of this study was to improve quality and safety of maize through development of hybrids that are resistant to *Aspergillus flavus* and aflatoxin contamination.

Seventy F_1 hybrids were generated from North Carolina II (NCDII) cross among seventeen inbred lines. The trial was planted in a 5x15 Alpha lattice design in two replicates at KALRO Kiboko and Katumani in 2015. Ears were artificially inoculated with *Aspergillus flavus* spores at mid-silk stage. Data was collected on days to flowering, plant height, ear height, lodging, husk cover, stem borer infestation, ear rots and grain yield. After harvesting, grain was tested for *Aspergillus flavus* infection by plating. Quantification of aflatoxin content in the grains was done by ELISA technique using Accuscan Pro-reader kits.

The level of *Aspergillus flavus* among the hybrids ranged between 100 cfu/g and 2500 cfu/g while that of aflatoxin accumulation was between 2 ng/g and 15000 ng/g. The grain yield ranged from 1.39 t/ha to 5.8 t/ha. Hybrids 18, 31, 37, 56, 59, 60, 58, 65 and 68 were identified as the most resistant with high grain yields. *Aspergillus* ear rot in these hybrids was at 1.9%, while ear damage by stem borer was at 7.2%. The hybrids had an average starch content of 70% and 5% for oil content across the sites. *Aspergillus* ear rot, aflatoxin accumulation and poor husk cover were observed to be directly correlated.

However, these traits were indirectly correlated to grain yield. Parents P329, Mp 313E, CKL05003 and Mp719 were identified as the best combiners for resistance with high negative general combining ability (GCA) effects for aflatoxin accumulation and *Aspergillus flavus*. Inbred lines NC298 and (CKL05003/La Posta Seq C7-F180-3-1-1-1-B-B -B)DH152-B-B were the best combiners for grain yield with the highest positive GCA effects of 0.68 and 0.72 respectively. Parents (CKL05003/La Posta Seq C7-F180-3-1-1-1-B-B -B) DH56-B-B/Mp717, CKL05019/Mp 715   and

        ([CML444/CML395//DTPWC8F31-1-1-2-2-BB]-4-2-2-1-1-B*4/(9071xBabamgoyo)-3-1-BBB)-B-1-2-3-1-3-B-B/Hi27 had the highest specific combining abilities for resistance to aflatoxin accumulation and parents   CKL05003/Hi27,   (ZM621A-10-1-1-1-2-B*8/PHG35)-B-16-2-2-B-B/Mp719 and (CKL05003/La Posta Seq C7-F180-3-1-1-1-B-B -B) DH152-B-B/Mp 313E for *Aspergillus flavus* resistance.

This study showed that genetic variations existed among the genotypes hence they were genetically diverse. Husk cover was noted as an important secondary trait in phenotypic selection for resistance to *Aspergillus flavus* and aflatoxin accumulation in maize. Hybrids resistant to *Aspergillus flavus* and aflatoxin accumulation were identified from the study. These germplasm could be incorporated into local breeding programs for improved safe maize productivity. Marker-assisted selection should be considered as an avenue to propel this research further as it is more effective and convenient.

Key words: Maize, Aflatoxin, *Aspergillus flavus*, Hybrid, General combining ability, Specific combining ability.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Maize is the primary staple food for most African countries accounting for up to 50% of total calories consumed in Eastern Africa (Hassan *et al*., 2001). Nutritionally maize is rich in starch, protein, fiber, vitamins (A, B_1, B_2, B_3, and C), minerals (calcium, phosphorus, iron, potassium, zinc, sodium) and lipids. The crop is normally grown for food, livestock feed, bio-fuel and raw material for manufacturing industries.

Maize is widely adapted and it grows over vast environments ranging from 58^oN to 40^oS (Dowsell *et al*., 1996). The growth cycle ranges from 3-13 months depending on the cultivar and the agro-ecological zone of production (Pingali, 2001). Ideally the crop requires well drained loamy or alluvial soils with a pH of 5.5-7. It performs well on altitudes of between 0-2200 meters above sea level and at an optimum temperature of 30° C. Temperatures much lower than this extend the maturity period of the crop while higher temperatures fasten the rate of maturity although this lowers yields. Maize requires an annual rainfall of 600mm-900mm evenly distributed throughout the growing period. Limited rainfall during the critical stages of growth (flowering) interferes with pollination resulting in low yields. However towards harvesting the crop requires dry conditions to facilitate drying of the kernels (Betrán *et al*., 2003).

In Kenya, maize production stands at 1.6 tons per hectare (FAOSTAT, 2013) most (70%) of which is produced by small scale farmers. Maize production is greatly limited by low soil fertility, poor infrastructure, low quality seed, high cost of inputs, unreliable rainfall, pest and disease infestation (Abera *et al*., 2013). Diseases in maize are mainly caused by fungal pathogens which cause ear rots resulting to significant

yield losses (Farrell and O'Keeffe, 2007). The major fungal pathogens responsible for causing ear rots in maize include: *Fusarium, Aspergillus, Penicillium* and *Stenocarpella* species (Koehler, 1959; De Leon and Jeffers, 2004; Dragich and Nelson, 2014). Once they infect the crop, they produce poisonous mycotoxins such as ochratoxin, fumonisin, tricothecenes, zearalenone and aflatoxin (Farrell and O'Keeffe, 2007) as they metabolize rendering the produce unpalatable (Paterson and Lima, 2010). Aflatoxin is the most dangerous mycotoxin due to its carcinogenic potential. It is produced by *Aspergillus* species the major producers being *Aspergillus flavus* (Peterson *et al.*, 2001). Produce confirmed to be contaminated by aflatoxin have to be destroyed to prevent entry into the market stream and this results to famine due to inadequate food supply and massive economic losses.

1.2 Problem statement

Aflatoxin contamination occurs in most crops including cassava, cotton seeds, oil crops such as sunflower and peanuts and cereal crops such as maize, sorghum, millet and rice (Gourami and Bullerman, 1995; Wild and Gong, 2010). The toxin also finds its way into milk, meat and eggs obtained from infected animals through biotransformation (Frobish *et al.*, 1986).

Currently more than 25% of the world's produce is contaminated by aflatoxin, the mostly affected crop being maize (Munkvold, 2003). In relation to this, more than 5 billion people are exposed to aflatoxin contamination in the world (Shephard, 2003; Williams *et al.*, 2004; CDC, 2004), hence placing them at a risk of impaired growth, immune suppression and hepatocellular carcinoma-HCC (Strosnider *et al.*, 2006). In 2010, more than 10% of maize harvested in Kenya was destroyed due to contamination and the loss accrued was valued at KES 89 billion (IFPRI, 2010).

Consequently the less informed farmers opt to feed their livestock and poultry with the contaminated grains rather than destroying it, leading to death and health deterioration (ICRISAT, 2015).

The first major case of aflatoxin contamination in Kenya was reported in 1981 (Ngindu *et al*., 1982) and since then there has been recurrent outbreaks of aflatoxicosis as a result of aflatoxin contamination. A case study conducted in Eastern Kenya between 2005 and 2007 showed that approximately 35% of the samples tested were contaminated by 48,000 ppb of aflatoxin (Daniel *et al*., 2011). The region also recorded the highest level of aflatoxin B1 contamination in the world (Unnevehr and Grace, 2013). In 2010 a similar study was conducted by IFPRI testing samples from different agro-ecological zones in Kenya namely; Kisii, Makueni, Embu and Mbeere and it was reported that the occurrence of aflatoxin contamination in maize is widely varied in different parts of the country, warm and humid environments reporting higher contamination levels (IFPRI, 2010).

Unexpected rainfall during the harvesting season and poor post harvest handling of produce have been reported to predispose maize to aflatoxin contamination as the fungus thrives in warm and humid conditions (Strosnider *et al*., 2006; IFPRI, 2010). Aflatoxin contamination in maize is a major problem in Eastern Kenya because the region experiences warm and humid climate making it favorable for the fungus to colonize the produce (Strosnider *et al*., 2006). In 2004, an outbreak occurred in Eastern Kenya where up to 125 people succumbed (Lewis *et al*., 2005).

1.3 Justification
Several efforts have been made in the search for a solution to aflatoxin accumulation and contamination for instance proper drying, packaging and storing of produce, good agronomic practices and development of atoxigenic strains of *Aspergillus* (Turner *et*

al., 2005; Fandohan *et al*., 2005; Strosnider *et al*., 2006; IFPRI, 2010). Despite these efforts, a sustainable remedy is yet to be found. The common farmer cannot afford resources and technology for optimal drying and storage of produce after harvesting. Frequent droughts as a result of climate change predispose the crops to infection by *Aspergillus flavus* and aflatoxin accumulation. This is because plant stress has been reported to exacerbate aflatoxin production by the fungus. This makes it difficult for the local farmers to manage this crisis since they lack access to irrigation water and cannot afford the available irrigation technologies.

According to IFPRI (2010), host plant resistance is considered the most viable option for control of aflatoxin accumulation since it will be affordable and readily accessible by most farmers. This has driven the search for resistant germplasm in which inbred lines that confer natural resistance to aflatoxin accumulation were identified. However, these lines are late maturing, prone to lodging and produce low grain yield (Brown *et al*., 1999; Warburton *et al*., 2013). To benefit from the lines, it is necessary to cross them with the locally adapted, high yielding genotypes. The International Maize and Wheat Improvement Centre (CIMMYT) have acquired some of these lines to start a breeding program for incorporating the resistance into locally bred inbred lines.

To ensure success of the program, it is necessary to determine the combining abilities of these lines and the genetics of resistance to *Aspergillus flavus* and aflatoxin accumulation in maize.

1.4 Objectives

The overall objective was to improve quality and safety of maize through development of hybrid lines that are resistant to *Aspergillus flavus* and aflatoxin accumulation.

1.4.1 Specific objectives

1) Evaluate maize hybrids for response to *Aspergillus flavus* infection and aflatoxin accumulation**.**

2) Evaluate the combining ability for resistance to *Aspergillus flavus* and aflatoxin accumulation in maize.

1.4.2 Hypothesis

1) Productivity of maize hybrids is not influenced by *Aspergillus flavus* infection and aflatoxin accumulation**.**

2) Resistance to *Aspergillus flavus* and aflatoxin accumulation in maize inbred lines is conditioned by both additive and non-additive gene action.

CHAPTER TWO

LITERATURE REVIEW

2.1 Botany and origin of maize

Maize *(Zea mays* L*.)* is a diploid crop with 10 chromosomes (2n=20). It belongs to the family *Poaceae* and genus *Zea*. Maize has two major wild relatives, *Teosinte* and *Tripsacum*. The genus *Zea* has four species, three of which are wild grasses of Mexican and Central American origin and the fourth is *Zea mays* which is the only member of this genus having economic value (Doebley, 1990). Maize is self-compatible and it is 95% cross pollinated translating to high genetic variability (Sleper and Poehlman, 2006). The centre of origin of maize is believed to be the highlands of Central America, particularly Mexico (Watson and Dallwitz, 1992). The crop was domesticated more than 6000 years ago in Mexico and Central America from where it spread to North America, Europe, Africa, and Asia and to other parts of the world (Matsouka *et al*., 2002).

2.2 Economic importance of maize

Maize ranks third among the world's food crops after wheat and rice. The major maize producing regions are United States, China, Brazil and Mexico. It is the primary staple food for most African countries accounting for up to 50% of total calories consumed in Eastern Africa (Hassan *et al*., 2001). Nutritionally maize is rich in starch, protein, fiber, vitamins (A, B_1, B_2, B_3, and C), minerals (calcium, phosphorus, iron, potassium, zinc, sodium) and lipids. It is however deficient in lysine and tryptophan amino acids although these are supplemented through breeding for quality protein maize (QPM). Maize is normally grown for food, livestock feed, bio-fuel and raw material for manufacturing industries (Dowsell *et al*., 1996; Birch *et al*., 2003).

2.3 Constraints to maize production

Production of maize is greatly constrained by abiotic and biotic factors which reduce yields. Effects and magnitude of biotic and abiotic factors vary considerably with environmental conditions. Some of the abiotic constraints include low nutrition, water deficiency and high salinity. Maize requires nitrogen, phosphorus and potassium in adequate quantities for optimum production (Birch *et al*., 2003). Nitrogen is essential to enable capture and utilization of radiation while potassium is necessary for opening and closing of stomata, enzyme activation and photosynthesis (Birch *et al*., 2003). Inadequate availability of these nutrients hinders growth and development of the crop. Moisture is also essential especially during the critical stages of growth and inadequate moisture supply has been reported to cause massive losses in yield (Birch *et al*., 2003). Saline soils restrict the ecological zones in which maize can be produced as it causes reduced germination, reduced vegetative growth and consequently low yields (Birch *et al*., 2003).

Biotic constraints to maize production include weeds, pests and diseases. Weeds reduce crop performance by competing for growth resources such as nutrients, water, space and sunlight. Common weeds that interfere with maize production include pigweed (*Porttulaca spp*), witch-weed (*Striga spp*) and nutgrass (*Cyperus rotundus*) (Dowsell *et al*., 1996). Insect pests attack maize crops during establishment, development and storage. Soil insects such as cutworms, wireworms (*Elateridae* family) and beetles can be very destructive during establishment causing losses of up to 30% and in some cases may necessitate replanting (O'Gara, 2007). During development the crop faces challenges of attack by armyworms, leafhoppers, aphids, spider mites, thrips and weevils. These insects suck nutrients from the plant in form of sap causing the leaves to curl, transmit diseases and provide entry points for other

pathogens into the plant. During storage maize grains are often attacked by several insets of Lepidoptera and Coleoptera orders which grind the grains resulting to greatly reduced weight and market value of the grains.

Diseases in maize are mainly caused by pathogens such as bacteria, fungi, viruses and mycoplasmas infect maize resulting to massive reduction in yield. They cause diseases such as maize streak, maize lethal necrosis, grey leaf spot, and northern leaf blight. They are also responsible for ear rot diseases leading to accumulation of mycotoxins translating to massive yield losses (Farrell and O'Keeffe, 2007).

2.3.1 Ear rot diseases in maize

Ear rot refers to the occurrence of molds in grains both in the field and in storage leading to deterioration in quality and weight loss. Ear rot diseases in maize occur mainly during pollination and grain filling affecting the ears, kernels and cobs (Zummo and Scott, 1992). Development and spread of ear rots is majorly aggravated by poor sanitation, high temperature and relative humidity. Some of the most common ear rot diseases in maize include: *Diplodia, Fusarium, Gibberella, Cladosporium, Nigrospora, Penicillium and Aspergillus ear rots* (Koehler, 1959).

Fusarium ear rot is the most prevalent and is characterized by the presence of whitish to pinkish mycelia at the tip of the ear. *Gibberella* ear rot affects the kernels causing a reddish discoloration beginning at the ear tip (Dragich and Nelson, 2014). *Cladosporium* ear rot is characterized by dark grey mycelia on the kernels causing a blotched appearance from the point of kernel attachment to the cob progressing upwards. *Penicillium* and *Trichoderma* are characterized by powdery green mold and dark green mold respectively. In *Diplodia* the husks appear bleached as a result of a

white mold which begins to form at the base of the cob two to four weeks after silking (Malvick, 2001).

Aspergillus ear rots on the other hand are majorly characterized by the presence of olive green, dark green or golden brown fungal mycelia which occur between kernels especially near the ear tip depending on the species. For instance; *Aspergillus niger* (Van Tiegham) appear as a black mold, *Aspergillus glaucus* appear as a green mold while *Aspergillus flavus* (Link) appear as greenish-yellow mold (Koehler, 1959; Frisvad *et al.*, 2005). Ear rots can be controlled by proper field sanitation, destruction of crop residues that harbor insect pests which act as vectors, proper drying of produce before storage and maintaining standard conditions during storage (Malvick, 2001).

2.3.2 *Aspergillus* species involved in ear rot of maize

Aspergillus ear rots are associated with production of poisonous mycotoxins such as ochratoxin, fumonisin, tricothecenes, zearalenone and aflatoxin (Farrell and O'Keeffe, 2007). Over 14 *Aspergillus* species have been reported to cause ear rots in maize as a result of production of aflatoxins. These species are: *Aspergillus flavus, Aspergillus togoensis, Aspergillus pseudotamarii, Aspergillus arachidicola, Aspergillus parasiticus, Aspergillus nomius, Aspergillus minisclerottigenes, Aspergillus bombycis, Aspergillus parvisclerotigenus, Aspergillus rambellii, Aspergillus ochraceoroseus, Emericella astellata, Emericella olivicola* and *Emericella venezuelensis* (Varga *et al.*, 2009; Rank *et al.*, 2011). However the largest production of aflatoxin has been reported for *Aspergillus flavus* which is classified into two: *Aspergillus flavus* var *columnaris* and *Aspergillus flavus* var *parvisclerotigenus*, (Goto *et al.*, 1996; Yoko *et al.*, 2001; Peterson *et al.*, 2001). Members of this species require an optimum temperature of 37°C for growth. They inhabit soils, decaying

vegetation, hay and grains undergoing microbial deterioration and are phylogenetically related (Frisvad *et al.*, 2005).

2.3.3 Aflatoxin production by *Aspergillus* species

Aflatoxin is a mycotoxin produced by *Aspergillus* species as a secondary substrate during their metabolism (Paterson and Lima, 2010). Production requires temperatures of $20^oC - 40^oC$, the optimum being 35^oC and relative humidity of 70% - 90%, the optimum being 85%. A moisture content of 16.5% - 18% in grains also exacerbates its production. Therefore aflatoxin contamination is mostly prevalent in geographical locations between latitude 40° N and 40° S of the equator which are associated with high temperature and drought conditions (Rustom, 1997; Payne, 1998; Strosnider *et al.*, 2006).

Aflatoxin is produced in large quantities by *A. flavus* and *A. parasiticus* with *A. flavus* producing only aflatoxin B_1 ($AFB_{1)}$ and aflatoxin B_2 (AFB_2) types while *A. parasiticus* produces aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1) and aflatoxin G_2 (AFG_2). These classes are based on the color produced when aflatoxins are viewed under ultraviolet light (UV light). AFB_1 and AFB_2 produce blue fluorescence while AFG_1 and AFG_2 produce green fluorescence (Dalvi, 1986; Strosnider *et al.*, 2006). AFB_1 is the most common and the most potent (Creppy, 2002) therefore its levels are strictly monitored in produce before delivery into the market stream. In most countries, the maximum allowable content of aflatoxin in food and feed is 20 ppb which is equivalent to 10ng/g, (US Food and Drug Administration-FDA Guidelines for Aflatoxin Levels Policy guide 683.100).

2.3.4 Effects of aflatoxin contamination

Developing countries are the most adversely affected by aflatoxin contamination since they wholesomely rely on susceptible crops as their staple food (Soubra *et al*., 2009; Shundo *et al*., 2009; Bankole *et al*., 2010). Sub-Saharan Africa, Southeast Asia and China rank top in order of losses incurred in the world (Liu and Wu, 2010). Aflatoxin contamination causes a reduction in fertility, increase in abortion and lower birth weight in cattle. It also results in reduced appetite and increased susceptibility to diseases both in poultry and cattle translating to lower productivity in animals (Mwacharo *et al*., 2004). Subsequently it results in deterioration of food and feed quality and hence hinders trade between countries and regions since the contaminated produce cannot be allowed into the market stream (ICRISAT, 2015). Consuming high levels of aflatoxin contaminated produce results to acute aflatoxicosis in humans which is characterized by vomiting, abdominal pains and liver damage. It is estimated that approximately 4.6% - 28.2% of annual liver cancer (HCC) cases are as a result of aflatoxin contamination (Liu and Wu, 2010). Consumption of low to moderate levels of contaminated produce results in chronic aflatoxicosis which causes stunting in children and results in impaired immune system (Strosnider *et al*., 2006; Jiang *et al*., 2008). It has been reported that long exposure to aflatoxins exacerbate epidemics such as malaria, HIV/AIDS and tuberculosis (Jiang *et al*., 2008). This not only lowers human productivity but also results to death eventually.

Case studies conducted in Eastern Kenya following aflatoxicosis outbreaks have always recorded death tolls. For instance in 1981, there were 20 cases of aflatoxicosis and 12 of these individuals succumbed to death (Mehan *et al*., 1991). Out of the 317 cases reported in 2004, over 125 individuals in the region succumbed to death (Center for Disease Control and Prevention-CDC, 2004). Subsequent outbreaks have occurred

since the first case was reported claiming more lives (Center for Disease Control and Prevention-CDC, 2004; Probst *et al*., 2007).

2.4 Management strategies for *Aspegillus flavus* and aflatoxin contamination

Aflatoxin is the most widely studied mycotoxin (Brown *et al*., 1998, Dorner *et al*., 1999) and as a result several approaches have been adopted to reduce crop susceptibility to aflatoxin accumulation. These strategies involve creating awareness to the public on cultural and biological control strategies applied both at the pre-harvest and post-harvest stages. Cultural control measures include: irrigation to minimize crop susceptibility, field sanitation by proper disposal of crop residues, proper drying of produce before storage, improvement of storage conditions by modifying environmental conditions within the storage area, proper packaging of produce before storage and disposal of moldy grains (Turner *et al*., 2005; Fandohan *et al*., 2005; Strosnider *et al*., 2006; IFPRI, 2010).

Measures such as the use of cultivars that possess traits which hinder entry of the pathogen, controlling insect pests that vector fungal spores and planting hybrids that are resistant to drought stress have been adopted (Atehnkeng *et al*., 2008). Several approaches have been adopted to reduce crop susceptibility to aflatoxin accumulation for instance application of proper cultural control measures recommended by IFPRI (2010). Bio-control strategy involving the use of atoxigenic strains of *Aspergillus* to outcompete the toxigenic strains is also being recommended whereby the spores of atoxigenic strains are mixed depending on the target agro ecosystem and coated on the grain surface before planting (Atehnkeng *et al*., 2008; Probst *et al*. 2011). This approach has been implemented in Nigeria and has been reported to be 80% - 90% effective in Nigeria (Marechera and Ndwiga, 2015). In an attempt to introduce the

bio-control program in Kenya, a study was conducted to estimate the potential of its adoption among smallholder maize farmers and a positive response of 82% was reported (Marechera and Ndwiga, 2015).

Secondary traits in certain maize cultivars such as drooping ears, good husk cover and resistance to insects have been associated with resistance to aflatoxin (Betran *et al.*, 2002). This is because the traits prevent entry of *Aspergillus* into the maize kernels (IFPRI, 2010). Identification of these secondary traits facilitates easier selection of resistant or tolerant cultivars by ordinary farmers. Betran *et al.* (2002) further recommended that pyramiding the genes responsible for drooping ears, good husk cover and resistance to insects and introgressing them into adapted cultivars would be a viable strategy to mitigating aflatoxin contamination in maize.

Inbred lines resistant to aflatoxin contamination have also been identified and efforts are being made to introduce this trait into well adapted cultivars through breeding (Brown *et al.*, 1999). This is aimed at preventing infection by *Aspergillus*, preventing multiplication of *Aspergillus* in case it infects the crop, inhibiting production of afltoxins and/or enabling the plant or the fungus to degrade the toxins once they are produced (Probst *et al.*, 2011).

2.4.1 Breeding for resistance to *Aspergillus flavus* and aflatoxin contamination

This approach has gained momentum with most researchers especially after the discovery of genotypes with natural resistance to aflatoxin contamination (Brown *et al.*, 1999). Some of these lines include CML 176, CML 269, CML 322, Tx 114, GT-MAS: gk, MP 717 and MP 715 (Betran *et al.*, 2002; Williams and Windham, 2006). However these genotypes are late maturing and do not have the desirable agronomic quality hence they are of little value to farmers (Brown *et al.*, 1999). The process of

screening for resistance is limited by the fact that there are still no standard resistant materials that can be used as control across locations, natural infestation is unreliable due to varying environmental conditions across locations and in addition, artificial inoculation techniques sometimes fail to produce adequate differences that can be used to distinguish among genotypes (Brown *et al*., 1999; Asea *et al*., 2012). Betran *et al*. (2002) recommended that pyramiding the genes conditioning resistance and introgressing them into adapted cultivars would be a viable strategy to mitigating aflatoxin contamination in maize.

Betran *et al*. (2002) studied the inheritance patterns of aflatoxin resistance using diallel mating design and concluded that the additive effects played an important role in this inheritance. However, Campbell and White (1995) reported that both additive and non-additive gene effects were important in inheritance of aflatoxin resistance. Warbuton (2011) reported that the trait which confers aflatoxin resistance in maize is highly polygenic. It is therefore necessary to conduct studies on the inheritance patterns of this trait and test it over a wide range of agro-ecological zones. Consequently it is essential to obtain information on the combining abilities of these resistant germplasm and the commercial cultivars as this will determine the success of introgression of this trait into the commercial varieties.

Kelley *et al*. (2010) developed a database to integrate data obtained from several studies in aflatoxin resistance in maize (Corn Fungal Resistance Associated Sequences Database-CFRAS-DB). This has enabled researchers to identify the most promising lines and prioritize their activities. It has also allowed them to identify gene sequences that have a role in resistance to aflatoxin accumulation.

2.4.1.1 North Carolina mating design

Mating designs are schematic crosses between plants that are used to generate genetic pedigrees, genetic information and materials that can be used in a breeding program to ultimately improve plants (Acquaah, 2012). Correct choice of a mating design is paramount in determination of success of a breeding program (Gardner, 1963; Jinks and Mather, 1982; Hill *et al*., 1998; Acquaah, 2012). North Carolina designs were devised by Comstock *et al*. (1952) as it seemed less labor intensive. There are three North Carolina designs: North Carolina design I (NCDI), North Carolina design II (NCDII) and North Carolina design III (NCDIII).

NCDI involves mating each male parent to a series of female parents and the progenies are either halfsibs or fullsibs. It is suitable for both self and cross pollinated species that are highly prolific (Nduwumuremyi *et al*., 2013). It is commonly applied in animal breeding and it estimates additive and dominance variation (Acquaah, 2012). NCDIII involves backcrossing F_2 plants to their original descendant parents although it has been modified to include a third tester hence the name changes to triple test cross (Kearsey and Jinks, 1968; Acquaah, 2012). It estimates non-allelic interactions (epistasis), additive and dominance variance and can also be used to assess an array of populations regardless of their mating systems and gene frequencies (Hill *et al*., 1998).

The NC II involves a group of individuals or families that are used as male parents and a group of individuals or families used as female parents. Each male parent is crossed to each female parent in a factorial scheme creating female half-sib (HS) groups and male half-sib groups. The crosses generated in NC II mating scheme are evaluated in field trials at single or multiple environments. Studies of inheritance of

resistance to *Aspergillus* ear rot and grey leaf spot in maize conducted by Campbell and White (1995); Asea *et al*. (2012) and Gethi *et al*. (2013) respectively suggest that NC II gives two independent estimates of general combining ability for males and females and the specific combining ability and estimates of additive variance, dominance and heritability. A general analysis of variance (ANOVA) for a NC II at a single location is illustrated in Table 2.1.

Table 2. 1: ANOVA table for a NCD II mating design in a single location

Source of variation	Df	Ms	expected mean squares
Replications (r)	r-1		
Males (m)	m-1	m1	$\sigma^2 w + r \sigma^2 mf + rf\sigma^2 m$
Females (f)	f-1	m2	$\sigma^2 w + r \sigma^2 mf + rf \sigma^2 f$
Females × Males	(m-1) (f-1)	m3	$\sigma^2 w + r \sigma^2 mf$
Within progenies	mf (r-1)	m4	$\sigma^2 w$
Error	(r-1) (mf-1)	m5	
Total	rmf-1		

Source: Kearsey *et al*. (1997)

2.4.1.2 Analysis of combining ability for maize inbred lines

Combining ability is a very crucial aspect in development of hybrids as it shows the ability of inbred lines to nick and produce viable offspring. The concept of combining ability in plants was first described by Sprague and Tatum (1942). There are two types of combining abilities: General combining ability (GCA) and Specific combining ability (SCA). General combining ability is the average performance of an individual when crossed to a number of different lines while Specific combining ability is the deviation from average performance of the lines involved in a cross (Falconer Mackay, 1996). General combining ability implies additive gene action while specific combining ability shows non-additive gene action or dominance (Sprague and Tatum, 1942). A linear model devised by Hallauer *et al*. (2010) is applied when performing the combining ability test:

$$X_{ijk} = \mu + r_k + g_i + s_{ij} + e_{ijk} \quad \text{..} \quad \text{Equation 2.1}$$

Where:

X_{ijk} is the observed performance of the cross between i^{th} and j^{th} parents in the kth replication, μ is the population mean, rk is the replication effect, gi is the GCA effect for the ith parent, g_j is the GCA effect for the jth parent, s_{ij} is the SCA effect for the cross between i^{th} and j^{th} parents and e_{ijk} is the experimental error for the X_{ijk} observation.

2.4.2 Mechanisms of resistance to *Aspergillus flavus* and aflatoxin contamination

Several studies have been undertaken to understand the mechanism of resistance to aflatoxin accumulation in maize. Drooping ears and good husk cover prevent entry of *Aspergillus* spores into the maize kernels (Betran *et al*., 2002; IFPRI, 2010). Maize silks have also been studied and three chitinases have been identified to confer resistance in them. At the same time, restriction fragment length polymorphism (RFLP) analysis has been done on three resistant lines: R001, LB31 and Tex 6 and chromosome arms 2L, 3L, 4S and 8S have been identified to be associated with aflatoxin resistance in maize (White *et al*., 1998; Brown *et al*., 2010).

Brown *et al*. (2010) characterized proteins associated with resistance to aflatoxin in maize and reported that kernel proteins play a critical role in resistance to aflatoxin. Brown *et al*. (2010) analyzed resistant and susceptible lines through comparative proteomics and reported that resistant lines have high levels of constitutively produced proteins than the susceptible lines. The study by Brown *et al*. (2010) led to the identification and characterization of resistance-associated proteins (RAPs). On the basis of their peptide sequence homology, RAPs are grouped into three classes: storage proteins such as globulins and late embryogenesis abundant proteins, stress responsive proteins such as aldose reductase, glyoxalase and heat shock proteins and antifungal proteins (Brown *et al*., 2010). The study by Brown *et al*. (2010)

corroborated the results of Guo *et al.* (1996) that both inducible and constitutive proteins are required for maize kernels to resist aflatoxin accumulation.

Betran *et al.* (2002) evaluated accumulation of aflatoxin in white and yellow inbred lines in different locations in Texas and reported that yellow hybrids were more susceptible to aflatoxin compared to the white hybrids. However they pointed out that the susceptibility to aflatoxin is not associated to grain color but is as a result of other genes. White inbreds of sub-tropical origin had less contamination compared to those of temperate origin. On the other hand, yellow inbreds of temperate origin had lower aflatoxin compared to those of subtropical origin (Betran *et al.*, 2002).

2.5 Inoculation techniques applied in breeding for resistance to *Aspergillus flavus* and aflatoxin accumulation

To effectively screen the hybrids for aflatoxin resistance, it is necessary to use artificial inoculation techniques that can effectively distinguish between the resistant and susceptible lines. There are six major techniques applied in inoculation of fungal conidia in maize plants namely toothpick in the ear, toothpick in the silk channel, string around the silks, side needle through the husk and needle into the silk channel techniques (Zummo and Scott, 1989).

While screening for resistance to *Aspergillus flavus* in maize, King and Scott, (1982) compared the effectiveness of toothpick technique against that of the pinbar technique and concluded that the pinbar technique caused damage to the maize kernels. Toothpick technique involves boiling the toothpicks in water for 30 minutes, placing them in Erlenmeyer flasks with cotton plugs and autoclaving for 20 minutes. The toothpicks are then inoculated with fungal conidia and incubated at 28° C for 14 days (King and Scott, 1982). At mid-silk stage, the toothpicks are inserted into the middle

part of the ear either through the husk or through the silk channel (King and Scott, 1982). The pin bar on the other hand entails mounting sewing pins on a plastic bar and dipping their ends in conidial suspension. After incubation, the dipped ends are inserted into the kernels through the husks (King and Scott, 1982).

Similar studies were conducted by Zummo and Scott, (1989) using the string and the needle method. The string method involves boiling the strings in distilled water and autoclaving them for 20 minutes. They are then inoculated with fungal suspension and placed in a fungal growth medium for incubation at 28° C for 14 days. At mid-silk stage, the strings are tied around the maize silks (Zummo and Scott, 1989). In side needle conidial suspension is drawn into a 14-gauge hypodermic needle to a desired level. This is then injected into the maize plant kernels through the husk. Needle into the silk channel technique involves drawing conidia into the needle and injecting it through the silk channel of the maize two weeks after silking (Zummo and Scott 1989).

Zummo and Scott, (1989) further evaluated the effectiveness of these inoculation techniques and reported that toothpick and string techniques resulted in relatively low infection hence cannot easily distinguish resistant genotypes from resistant ones. The silk channel and side needle technique produced high levels of infection hence could be used to clearly distinguish susceptible genotypes from the resistant ones. These findings were later validated by Betran *et al*. (2002) who reported that pinbar technique produced the highest level of infection although the method used damaged the maize kernels. He recommended the use of silk channel inoculation due to its ability to produce significant differences between the inoculated and the non-inoculated samples in the field.

2.6 Quantification of aflatoxin content in maize

Techniques used in quantification of aflatoxins are based on electrochemical and optical principles such as chromatography, fluorescence, immunochemical assay tests, UV absorption and spectrometry (Espinosa *et al*., 2011). The most common techniques used in aflatoxin quantification are chromatography and serology (Espinosa *et al*., 2011; Berardo *et al*., 2011).

Serological technique employs the antigen-antibody reaction whereby the proteins in the sample compete with conjugate aflatoxins for antibodies through an enzymatic process. The reaction creates a change in electrical conductivity, whereby a great change implies low aflatoxin concentration while a small change implies high aflatoxin concentration (Espinosa *et al*., 2011; Berardo *et al*., 2011). The most commonly used serological technique is the enzyme linked immunosorbent assay (ELISA) due to its simplicity and speed.

While quantifying aflatoxin levels in food products, Leszczyńska *et al*, (2001) applied Elisa technique to test cereals, milk and other dairy products and reported that it is highly sensitive and selective hence recommended. This technique has also been reported to be effective in quantifying aflatoxin concentration in grains, animal feed and dairy products with a detection range of 0.1 ppb up to 1000 ppb (AflaTest WB instruction manual).

Chromatography entails an interaction between a stationery phase and a mobile phase. There are several types of chromatography, gas chromatography (GC), liquid chromatography (LC), thin layer chromatography (TLC) and high-performance liquid chromatography (HPLC) (Balcony *et al*., 2008). Thin-layer chromatography consists of a stationery phase immobilized on an inert material. In gas chromatography, the

mobile phase is a gas which carries along the vaporized sample through the stationery phase (Wacoo *et al*., 2014).

Herzallah, (2009) in a study to determine the level of aflatoxin in meat and dairy products reported that HPLC can detect a wide range of mycotoxins over a short period of time and is highly sensitive. However, the technology is expensive and requires skilled personnel. Frisvad *et al*. (2005); Balcony *et al*. (2008) and Wacoo *et al*. (2014) reported high sensitivity of HPLC in a study to quantify aflatoxin levels in agricultural food crops. The technique involves injecting the sample to be analyzed into the stationary phase and carried along by the mobile phase utilizing pressure being delivered into the system by a pump.

CHAPTER THREE

RESPONSE OF MAIZE HYBRIDS TO *ASPERGILLUS* EAR ROT AND AFLATOXIN ACCUMULATION

Abstract

Maize (*Zea mays* L.) is the primary staple food in sub-Saharan Africa (SSA) accounting for up to 50% of the total calories consumed in the Eastern Africa region. Aflatoxin contamination in maize has continued to be a problem in Kenya since 1981 when the first outbreak was recorded. Post-harvest technologies have been devised to help solve this situation, although most small scale farmers in the region lack resources to acquire the necessary technology. Host plant resistance is considered the most viable option hence the objective of this study was to determine the response of temperate by mid-altitude maize hybrids to *Aspergillus* ear rot and aflatoxin accumulation. Seventeen maize inbred lines were crossed in North Carolina II (NCII) mating design. The resultant F_1 progenies were laid out in Alpha lattice design, in two replications at KALRO Kiboko and KALRO Katumani. The top ear was artificially inoculated with *Aspergillus flavus* at mid-silk and data was collected on plant height, ear height, stem lodging, yield parameters and stem borer infestation. After harvesting, the levels of *Aspergillus flavus* and aflatoxin accumulation were determined by plating and ELISA technique respectively. The level of aflatoxin contamination varied from 2 ng/g to1500 ng/g across the two sites. The level of *Aspergillus flavus* varied from 100 cfu/g and 2400 cfu/g. Hybrids 18, 31, 37, 56, 59, 60, 58, 65 and 68 were identified to be resistant to *Aspergillus* ear rot with high grain yield of 3 t/ha. *Aspergillus* ear rot in these hybrids was at 1.9%, while ear damage by stem borer was at 7.2%. The hybrids had an average starch content of 70% and 5% for oil content across the sites. The significant variations (at *p<0.05*) noted among genotypes in this study imply that the germplasm was genetically diverse for

Aspergillus ear rot, aflatoxin accumulation and grain yield. Theses genotypes should be screened further in multiple environments to validate their resistance and harness the genes responsible for resistance and high grain yield in them.

3.1 Introduction

Maize ranks as the third most important crop in the world and it is grown both for food, animal feed and raw material for manufacturing industries (Hassan *et al*., 2001). However, aflatoxin contamination has proved to be a major challenge in maize production especially in the sub-Saharan Africa where yield losses are most prevalent (Liu and Wu, 2010). Over the years, Kenya has recorded the highest level of aflatoxin contamination in maize in the world (Unnevehr and Grace, 2013). However, this contamination is widely varied across the country, with the Eastern region ranking top (IFPRI, 2010). Aflatoxin contamination in maize leads to reduction in grain quality rendering the maize unpalatable. Consumption of contaminated maize leads to low productivity in poultry and cattle, aflatoxicosis in humans associated with immune suppression, stunting in children, liver cancer and eventually death (Strosnider *et al*., 2006; Jiang *et al*., 2008).

Aflatoxin is a toxin produced by *Aspergillus* species of fungi during their secondary metabolism (Peterson *et al*., 2001; Agag, 2004). It is mostly prevalent in cereal crops containing starch and oil as these are fungal substrates (Wild and Gong, 2010). Optimum production of aflatoxin by *Aspergillus* species is achieved under conditions of high temperature and relative humidity (Strosnider *et al*., 2006). This makes it more prevalent in the tropics and subtropics as the temperature and relative humidity is generally high (Kaaya and Warren, 2005; Asea *et al*., 2012). More than 5 billion

people are exposed to aflatoxin contamination globally (Williams *et al*., 2004; CDC, 2004).

The maximum allowable aflatoxin contamination in produce is 20ppb (US Food and Drug Administration-FDA Guidelines for Aflatoxin Levels Policy guide 683.100). This creates a barrier to trade between and within countries resulting to huge economic losses (Wild and Hall, 2000; ICRISAT, 2015). Previously, aflatoxin contamination in maize had been managed through improved storage, packaging, disposal of moldy grains, field sanitation and controlling insect vectors that spread fungal spores (Turner *et al*., 2005; Fandohan *et al*., 2005; Strosnider *et al*., 2006; Atehnkeng *et al*., 2008; IFPRI, 2010). The use of atoxigenic strains of *Aspergillus* to outcompete the toxigenic strains is a new technique being piloted in the country for extensive use (Atehnkeng *et al*., 2008; Probst *et al*., 2011).

Despite these efforts, host-plant resistance has been recommended as the most viable option and this has driven the search to identify germplasm that are resistant to *Aspergillus flavus* and aflatoxin contamination (Brown *et al*., 1999; Asea *et al*., 2012; Warburton *et al*., 2013). The US Department of Agriculture, Agricultural Research Service (USDA-ARD) has identified temperate lines Mp717, Mp313E and Mp719 to be potentially resistant to *Aspergillus flavus* and aflatoxin accumulation (Scott and Zummo, 1990; Williams and Windham, 2006; Williams and Windham, 2012; Williams *et al*., 2014; Williams and Windham, 2015). The objective of this study was therefore to determine the response of maize hybrids to *Aspergillus flavus* and aflatoxin accumulation in Kenya.

3.2 Materials and methods

3.2.1 Experimental materials

The germplasm used comprised of 17 maize inbred lines of diverse origin ranging from Mexico, USA-Mississippi, Hawaii and Kenya (Table 3.1). The exotic lines have putative resistance to aflatoxin accumulation and they were used as the male parents. The local lines are adapted and exhibit superior agronomic traits although they are potentially susceptible to aflatoxin accumulation and they were used as the female parents.

Table 3. 1: Entry and origin of inbred lines used in the study

	Entry	Inbred line	Origin
Susceptible Female lines	1	CKL05003	Kenya
	2	P329	Kenya
	3	CKL05019 ([CML444/CML395//DTPWC8F31-1-1-2-2-BB]-4-2-2-1-1-B*4/(9071xBabamgoyo)-3-1-BBB)-B-1-2-3-1-3-B-B	Kenya
	4	(CKL05003/La Posta Seq C7-F180-3-1-1-1-B-B -B)DH56-B-B	Kenya
	5	(CKL05003/La Posta Seq C7-F180-3-1-1-1-B-B -B)DH152-B-B	Kenya
	6	(ZM621A-10-1-1-1-2-B*8/PHG35)-B-16-2-2-B-B	Kenya
	7		Kenya
Resistant Male lines	1	CML11	Mexico
	2	CML343	Mexico
	3	CML247	Mexico
	4	Mp715	USA
	5	Mp717	USA
	6	Mp719	USA
	7	NC298	USA
	8	NC334	USA
	9	Hi27	USA
	10	Mp 313E	USA

3.2.2 Description of experimental site

The experiment was conducted at KALRO Kiboko and KALRO Katumani. Kiboko is located in Makueni County at latitude 2° 15'S and longitude 37° 45'E. The centre lies

at an altitude of 993 meters above sea level and receives a total annual rainfall of 560 mm for the two rainy seasons. The minimum and maximum temperatures recorded in the region are 17.4^o C and 30.6^o C respectively. Katumani is in Machakos County located at an altitude of 1600 m above sea level and experiences semi-arid tropical climate with a total annual rainfall of 655 mm and the mean minimum and maximum temperatures are 13.7^{o}C and 24.7^{o}C respectively (Mwacharo *et al*., 2004). These areas were selected for evaluation to obtain optimum conditions for *Aspergillus* infection and aflatoxin production since they repeatedly record the highest levels of aflatoxin accumulation (CDC, 2004; IFPRI, 2010; ICRISAT, 2015).

3.2.3 Generation of crosses

The 17 maize inbred lines were planted in the nursery at a spacing of 20 cm within the rows and 75 cm between the rows. This was done by including one line of the male parent after every ten lines of female parents. Di-ammonium Phosphate fertilizer was applied at the rate of 10g/hill and supplemental irrigation done to facilitate germination of the seeds and plant growth. As soon as ear shooting began, bagging was done both to the emerging shoots and tassels. This was aimed at controlling pollination by restricting entry of foreign pollen and also tapping pollen to be used during pollination. Crosses were generated following North Carolina II mating design (NCDII) whereby each female was mated by all male parental lines to generate F_1 seeds. Grain filling was at 80% -100% indicating success of the crosses.

3.2.4. Evaluation of F_1 maize hybrids for response to *Aspergillus* ear rot and aflatoxin

The 70 F_1 hybrids generated were planted at KALRO-Kiboko and KALRO-Katumani for evaluation. Five checks were included in the trial, four of which are commercial varieties and one an elite inbred line from CIMMYT. Two seeds were

planted per hill in 4 m row plots at a spacing of 20 cm by 75 cm following 5*15 Alpha lattice designs in two replications. DAP fertilizer was applied at the rate of 10g/hill to facilitate rooting and later urea to enhance vegetative growth. At mid-silking stage, the plants were inoculated with *Aspergillus flavus* and data collected on agronomic parameters, yield parameters, ear rot and aflatoxin content.

Table 3. 2: Pedigree of F_1 hybrid genotypes

Entry	Female	Male	Pedigree
1	1	1	CKL05003/CML 11
2	1	2	CKL05003/CML 343
3	1	3	CKL05003/CML247
4	1	4	CKL05003/Mp 715
5	1	5	CKL05003/Mp717
6	1	6	CKL05003/Mp719
7	1	7	CKL05003/NC298
8	1	8	CKL05003/NC334
9	1	9	CKL05003/Hi27
10	1	10	CKL05003/Mp 313E
11	2	1	P329/CML 11
12	2	2	P329/CML 343
13	2	3	P329/CML247
14	2	4	P329/Mp 715
15	2	5	P329/Mp717
16	2	6	P329/Mp719
17	2	7	P329/NC298
18	2	8	P329/NC334
19	2	9	P329/Hi27
20	2	10	P329/Mp 313E
21	3	1	CKL05019/CML 11
22	3	2	CKL05019/CML 343
23	3	3	CKL05019/CML247
24	3	4	CKL05019/Mp 715
25	3	5	CKL05019/Mp717
26	3	6	CKL05019/Mp719
27	3	7	CKL05019/NC298
28	3	8	CKL05019/NC334
29	3	9	CKL05019/Hi27
30	3	10	CKL05019/Mp 313E
31	4	1	([CML444/CML395//DTPWC8F31-1-1-2-2-BB]-4-2-2-1-1-B*4/(9071xBabamgoyo)-3-1-BBB)-B-1-2-3-1-3-B-B/CML 11
32	4	2	([CML444/CML395//DTPWC8F31-1-1-2-2-BB]-4-2-2-1-1-B*4/(9071xBabamgoyo)-3-1-BBB)-B-1-2-3-1-3-B-B/CML 343
33	4	3	([CML444/CML395//DTPWC8F31-1-1-2-2-BB]-4-2-2-1-1-B*4/(9071xBabamgoyo)-3-1-BBB)-B-1-2-3-1-3-B-B/CML247
34	4	4	([CML444/CML395//DTPWC8F31-1-1-2-2-BB]-4-2-2-1-1-B*4/(9071xBabamgoyo)-3-1-BBB)-B-1-2-3-1-3-B-B/Mp 715
35	4	5	([CML444/CML395//DTPWC8F31-1-1-2-2-BB]-4-2-2-1-1-B*4/(9071xBabamgoyo)-3-1-BBB)-B-1-2-3-1-3-B-B/Mp717
36	4	6	([CML444/CML395//DTPWC8F31-1-1-2-2-BB]-4-2-2-1-1-B*4/(9071xBabamgoyo)-3-1-BBB)-B-1-2-3-1-3-B-B/Mp719
37	4	7	([CML444/CML395//DTPWC8F31-1-1-2-2-BB]-4-2-2-1-1-B*4/(9071xBabamgoyo)-3-1-BBB)-B-1-2-3-1-3-B-B/NC298
38	4	8	([CML444/CML395//DTPWC8F31-1-1-2-2-BB]-4-2-2-1-1-B*4/(9071xBabamgoyo)-3-1-

Table 3. 2: Pedigree of F$_1$ hybrid genotypes

Entry	Female	Male	Pedigree
			BBB)-B-1-2-3-1-3-B-B/NC334
39	4	9	([CML444/CML395//DTPWC8F31-1-1-2-2-BB]-4-2-2-1-1-B*4/(9071xBabamgoyo)-3-1-BBB)-B-1-2-3-1-3-B-B/Hi27
40	4	10	([CML444/CML395//DTPWC8F31-1-1-2-2-BB]-4-2-2-1-1-B*4/(9071xBabamgoyo)-3-1-BBB)-B-1-2-3-1-3-B-B/Mp 313E
41	5	1	(CKL05003/La Posta Seq C7-F180-3-1-1-1-B-B -B)DH56-B-B/CML 11
42	5	2	(CKL05003/La Posta Seq C7-F180-3-1-1-1-B-B -B)DH56-B-B/CML 343
43	5	3	(CKL05003/La Posta Seq C7-F180-3-1-1-1-B-B -B)DH56-B-B/CML247
44	5	4	(CKL05003/La Posta Seq C7-F180-3-1-1-1-B-B -B)DH56-B-B/Mp 715
45	5	5	(CKL05003/La Posta Seq C7-F180-3-1-1-1-B-B -B)DH56-B-B/Mp717
46	5	6	(CKL05003/La Posta Seq C7-F180-3-1-1-1-B-B -B)DH56-B-B/Mp719
47	5	7	(CKL05003/La Posta Seq C7-F180-3-1-1-1-B-B -B)DH56-B-B/NC298
48	5	8	(CKL05003/La Posta Seq C7-F180-3-1-1-1-B-B -B)DH56-B-B/NC334
49	5	9	(CKL05003/La Posta Seq C7-F180-3-1-1-1-B-B -B)DH56-B-B/Hi27
50	5	10	(CKL05003/La Posta Seq C7-F180-3-1-1-1-B-B -B)DH56-B-B/Mp 313E
51	6	1	(CKL05003/La Posta Seq C7-F180-3-1-1-1-B-B -B)DH152-B-B/CML 11
52	6	2	(CKL05003/La Posta Seq C7-F180-3-1-1-1-B-B -B)DH152-B-B/CML 343
53	6	3	(CKL05003/La Posta Seq C7-F180-3-1-1-1-B-B -B)DH152-B-B/CML247
54	6	4	(CKL05003/La Posta Seq C7-F180-3-1-1-1-B-B -B)DH152-B-B/Mp 715
55	6	5	(CKL05003/La Posta Seq C7-F180-3-1-1-1-B-B -B)DH152-B-B/Mp717
56	6	6	(CKL05003/La Posta Seq C7-F180-3-1-1-1-B-B -B)DH152-B-B/Mp719
57	6	7	(CKL05003/La Posta Seq C7-F180-3-1-1-1-B-B -B)DH152-B-B/NC298
58	6	8	(CKL05003/La Posta Seq C7-F180-3-1-1-1-B-B -B)DH152-B-B/NC334
59	6	9	(CKL05003/La Posta Seq C7-F180-3-1-1-1-B-B -B)DH152-B-B/Hi27
60	6	10	(CKL05003/La Posta Seq C7-F180-3-1-1-1-B-B -B)DH152-B-B/Mp 313E
61	7	1	(ZM621A-10-1-1-1-2-B*8/PHG35)-B-16-2-2-B-B/CML 11
62	7	2	(ZM621A-10-1-1-1-2-B*8/PHG35)-B-16-2-2-B-B/CML 343
63	7	3	(ZM621A-10-1-1-1-2-B*8/PHG35)-B-16-2-2-B-B/CML247
64	7	4	(ZM621A-10-1-1-1-2-B*8/PHG35)-B-16-2-2-B-B/Mp 715
65	7	5	(ZM621A-10-1-1-1-2-B*8/PHG35)-B-16-2-2-B-B/Mp717
66	7	6	(ZM621A-10-1-1-1-2-B*8/PHG35)-B-16-2-2-B-B/Mp719
67	7	7	(ZM621A-10-1-1-1-2-B*8/PHG35)-B-16-2-2-B-B/NC298
68	7	8	(ZM621A-10-1-1-1-2-B*8/PHG35)-B-16-2-2-B-B/NC334
69	7	9	(ZM621A-10-1-1-1-2-B*8/PHG35)-B-16-2-2-B-B/Hi27
70	7	10	(ZM621A-10-1-1-1-2-B*8/PHG35)-B-16-2-2-B-B/Mp 313E
71			CKL05003/CKL05007//CML536
72			H517
73			WH401
74			WH505
75			WH507

Entries 1-70: Hybrids, Entries 71-75: Checks

3.2.5 Isolation and culturing of *Aspergillus flavus*

Maize kernels were surface sterilized in 2-3% Sodium hypochlorite (NaOCl) and rinsed in three changes of sterile water. A growth medium was prepared by dissolving 39 g of Potato Dextrose Agar (PDA) in 1 liter of distilled water. This was then heated for one minute to ensure that the media dissolves properly and autoclaved at 121°C for 15 minutes (Acumedia, 2011). The media was allowed to cool in a water

bath after which it was poured in Petri dishes. The sterilized maize kernels were then plated on the media under sterile conditions and then incubated at 25°C-30°C for 7 days. *Aspergillus* colonies were identified based on color of colonies. Sub-cultures were then made by scooping a portion of the *Aspergillus* culture using a wire loop and plating it on fresh media in the Petri dishes. Morphological characteristics were identified based on microscopy after which pure cultures were made following the same procedure (Nissen, 2012; Mutegi *et al*., 2013). During the entire period, the cultures were exposed to light to maximize characteristic color formation of *Aspergillus* (Mutegi *et al*., 2013).

3.2.6 Inoculum preparation and inoculation

Spores of *Aspergillus flavus* were harvested by flooding the cultures with distilled water and gently scrapping with wire loop. The resultant solution was then filtered through cheese cloth. This was then serially diluted by a factor of 6 to allow easy counting of cells. The haemocytometer chamber and cover slips were then carefully cleaned using a lens paper and ethanol. The counting surface was then charged and the cover slip placed on top. A micropipette was used to draw cell suspension from the universal bottle which was then added onto the V shaped well of the haemocytometer and allowed to fill by capillary action. This was then observed under a microscope counting the number of cells in the four corner squares and the middle square after which the average cell count was determined. Cell count per ml= average number of spores per ml x dilution factor x 10^{4}. The suspension was then adjusted to contain 10^{7} spores per milliliter using the haemocytometer (Krishnan and Damle, 1954; Hoffman, 2006). Inoculation was done 7-10 days after silk emergence through the silk channel inoculation technique. The process entailed drawing 3 ml of conidial suspension from a container using a syringe and injecting it into the top most ear of

the maize plant through the silk channel (Zummo and Scott, 1989). After inoculation, each plant was marked by a red ribbon to distinguish it from the ones that had not yet been inoculated. After two weeks, the cobs were checked for spore formation as a confirmation of inoculation.

3.2.7 Assessment of agronomic parameters

To assess the agronomic parameters data was taken on stand count flowering, plant height, ear height, stalk lodging and husk cover. Stand count was taken after thinning by counting the total number of plants per entry. Flowering was taken as soon as 50% of the plants in an entry began producing silks and shedding pollen. It was done by counting the number of days from planting to 50% silk emergence and pollen shedding. Plant height and ear height were taken at physiological maturity stage when no more increase in plant height was evident. It was taken using a ruler calibrated in centimeters. Plant height was recorded by measuring the plant from the base to the main tassel branch. Ear height on the other hand was taken by measuring height from the base of the plant to the insertion of the top ear of the same plant. Root and stalk lodging was taken at maturity by counting the number of plants leaning at an angle more than 30° from the vertical and by counting plants with broken stalk below the main ear respectively. These were then expressed as a percentage of the total number of plants in an entry. Husk cover was taken by counting the number of plants with ears that are not completely covered by the husks for each entry.

3.2.8 Assessment of stem borer and ear rots

During vegetative growth, the degree of stem borer damage was recorded by counting the number of pinhole damages on the leaves and cobs of sampled maize plants per entry (Makueti *et al*., 2012). At maturity the cobs, kernels and ears were examined for

symptoms of fungal infection such as growth of mycelia or rotting. Ear rots were measured by counting the number of cobs with rotten ears and expressed as a percentage of total number of ears at maturity. In addition, the degree of resistance was ascertained through a scale based on Reid *et al.*, (1996) where; 1- no symptoms, 2- 1%- 3% infection, 3- 4%- 10% symptoms observed, 4- 11%-25% infection, 4- 26%- 50%, 5- 51%-75% infection and 6- 76%-100%.

3.2.9 Assessment of yield attributes

Before harvesting, the number of plants per entry was ascertained by counting all plants in that entry excluding the end plants in each row. After harvesting, data was recorded on number of ears, ear aspect, ears per plant, field weight and moisture content. Number of ears was taken by counting the total number of ears harvested per entry. Ear aspect was measured on a scale of 1-5, where 1 represents nice and uniform cobs with the preferred texture while 5 represents ugly cobs with undesirable texture. Field weight was taken using a weighing balance by measuring the total weight of the harvested ears for each entry. Ears per plant were ascertained by taking the average number of ears with at least one fully developed grain. Near Infrared (NIR) analysis was also carried out to ascertain the oil, protein and starch content of the harvested grains for each entry according to Salami *et al.* (2003). Grain yield was calculated based on field weight, grain moisture and shelling percentage (80%) using the formula by Salami *et al.* (2003).

$$\text{Grain yield} = \{\text{grain weight} \times (100 - \text{grain moisture}) \times (\text{shelling percentage} \times$$
$$10000)/(100 - 12.5) \times (\text{plot area})$$

………..……… Equation 3.1

3.2.10 Determination of level of contamination by *Aspergillus flavus* in grain

Maize grains were ground, 1g of the sample obtained and added to 10 ml distilled water. This was then thoroughly mixed using a mechanical shaker for 15 minutes and the suspension serially diluted at 10^0 to 10^{-2} (Nazir, 2007). One ml of the suspension was transferred into Petri dishes containing PDA media using a pipette and spread using a glass spreader. This was incubated at 28° C to facilitate fungal growth (Nazir, 2007). Colony forming units per gram (cfu/g) was computed based on Sutton, 2011 formula:

$$cfu/g = \frac{\text{Number of colonies observed}}{\text{Dilution factor}}$$

…..Equation 3.2

3.2.11 Analysis of aflatoxin content in grains

A sample of 1kg of the shelled grains was obtained and ground into fine powder for analysis on the content of aflatoxin using Accuscan Pro Reader. Fifty grams of flour was obtained from each sample and mixed with 250 ml of 65% ethanol to obtain a ratio of 1:5 parts and shaken in the mechanical shaker at 200RPM for 3 minutes. The mixture was allowed to settle and then 0.1ml of the filtrate was pipetted and mixed with 0.5 ml of sample diluent. Exactly 0.1 ml of the mixture was transferred into a new sample cap and a test strip inserted in the sample. After 6 minutes, the strip was removed and inserted into the Accuscan Pro Reader to obtain results (Neogen Reveal Q+ for aflatoxin using Accuscan III and Accuscan Pro Readers manual).

3.2.12 Statistical data analysis

i.) General analysis of variance was carried for all traits following the general linear model using PROC GLM procedure of SAS program (SAS, 2003). The means

obtained were separated using Fisher's protected least significant difference (LSD) method (Frederick, 1999).

ii.) The PROC CORR procedure of SAS was used to compute phenotypic correlations between traits based on Pearson correlation coefficients.

3.3 Results

3.3.1: Performance of hybrids with respect to agronomic traits and ear rot

Significant differences were noted among the genotypes at p<0.05 for all traits except ear and leaf damage, anthesis-silking interval, root and stalk lodging, ears per plant and plant height (Table 3.3). Across the sites, significant differences were observed at p<0.05 for all traits except leaf damage. The genotype by environment interaction (SxE) was significantly different for all traits except leaf and ear damage, ears per plant, anthesis-silking interval, husk cover, plant height and stem lodging.

3.3.2: Response of hybrids to *Aspergillus flavus* inoculation at individual sites

The resistant hybrids had relatively high yield as compared to the susceptible ones at both sites. The checks used in this study had higher levels of aflatoxin accumulation as compared to the hybrids. The mean grain yield of the top 20 resistant hybrids was higher than that of the checks in this trial. The highest yielding hybrids achieved flowering faster compared to the others and they also had a relatively short interval of 2days between anthesis and silking. The percentage of hybrids with poor husk cover and ear rot was relatively low as compared to the checks.

At Kiboko, maize had higher levels of *Aspergillus flavus* and aflatoxin accumulation as compared to those at Katumani (Table 3.5). The mean aflatoxin concentration was 65.05 ng/g while that of *Aspergillus flavus* was 628.9 cfu/g. Nine hybrids namely 11, 13, 18, 30, 31, 37, 56, 59 and 60 had low levels of *Aspergillus flavus* (<400 cfu/g) and

aflatoxin accumulation (<10 ng/g) with the high grain yield of >3.5 t/ha. Hybrids 19, 45 and 50 had low levels of aflatoxin concentration (<10 ng/g) although their grain yield was low (<2.5 t/ha). Most of the hybrids at Kiboko were very tall, growing up to 220 cm and hence their ear placement was also high. For this reason, a high percentage of stalk lodging was recorded in most entries. The top 20 resistant hybrids had relatively low incidences of ear rots, recording a mean of only 1.8%.

Table 3. 3: Mean squares for *Aspergillus* ear rot, aflatoxin content, grain yield and agronomic traits

Source	Df	GY	ED	LD	ASI	HC	ER	EPP	PH	SL	OIL	PROT	STAR	ASP	AFL
		t/ha	%	%	days	%	%	No.	cm	%	%	%	%	cfu/g	ng/g
Rep	1	0.83	8.73	6.05	2.09	45.80	9.23	0.00	630.00	467.52	0.08	0.21	0.33	0.03	0.54
Site(S)	1	79.13*	0.94*	0.03	95.20*	4867*	401.05*	2.43*	146574*	18466*	49.94*	199.59*	174.80*	0.41*	2.85*
Entry(E)	74	1.49*	4.84	5.81	5.13	96.62*	14.15*	0.01	982.01	434.47	0.74*	2.82*	2.95*	0.16*	0.75*
SXE	74	1.44*	4.02	7.58	4.03	62.47	14.51*	0.01	875.40	258.55	0.15*	0.44*	0.63*	0.10*	0.47
Residual	92	0.23	3.24	4.24	2.30	32.29	2.51	0.01	489.93	206.75	0.05	0.12	0.24	0.01	0.39
Total	299														

*-significant at $p<0.05$, GY-grain yield, ED-ear damage, LD-leaf damage, ASI-anthesis-silking interval, HC-husk cover, ER-ear rot, EPP-ears per plant, ,PH-plant height, SL-stalk lodging, PROT-protein content, ASP-*Aspergillus flavus*, AFL-aflatoxin content, STAR-starch content.

Table 3. 4: Weather averages for Kiboko and Katumani during the experimental period

	Kiboko			Katumani		
	Mean	Max	Min	Mean	Max	Min
Temperature (°C)	26	31	20	21	24	18
Relative Humidity (%)	70	100	40	62	83	50
Rainfall (mm)	320			520		

At Katumani, *Aspergillus* ear rot and aflatoxin levels were relatively low with a mean of 541 cfu/g and 40.29 ng/g respectively (Table 3.6). Hybrids 16, 55, 58, 59, 60, 65 and 68 had low levels of aflatoxin concentration with high grain yield of >3 t/ha. Although hybrids 11, 12, 18 and 19 had low levels of *A.flavus* and aflatoxin concentrations, their grain yields were low (<2.3 t/ha). The top 20 resistant hybrids took averagely 70 days to achieve 50% flowering, having an interval of 2 days in-between anthesis and silking. The plants at Katumani were very tall, others growing up to 350 cm high resulting to a high occurrence of stalk lodging among the hybrids.

3.3.2: Correlations between *Aspergillus* ear rot, grain yield and agronomic traits

In this study, significant correlations were observed between *Aspergillus flavus* and all other traits except days to flowering, ear damage, ear rot, stem lodging and plant height at $p < 0.05$. Aflatoxin accumulation was significantly correlated to all traits except days to flowering, plant height, lodging, oil content, ear damage and leaf damage. Significant correlations were noted between grain yield and all other traits except ear damage and leaf damage at $p < 0.05$ (Table 3.7). Hybrids with poor husk cover had high levels of *Aspergillus* ear rot and aflatoxin accumulation with coefficients of 0.07 and 0.05 respectively. Hybrids high in oil and starch contents had corresponding high levels of aflatoxin content with a coefficient of 0.23 and 0.05 respectively. Hybrids susceptible to ear damage by stem borer had subsequently high levels of aflatoxin content with a coefficient of 0.53.

Table 3. 5: Grain yield, agronomic traits, *Aspergillus* and aflatoxin content of hybrids and checks at KALRO Kiboko

	Entry	Cross	GY	AD	ASI	PH	SL	HC	ER	OIL	STAR	ASP	AFL	AFL
		(fxm)	t/ha	Days	days	cm	%	%	%	%	%	cfu/g	ng/g	original
Top	10	1x10	3	57	2	209	70.9	2.7	2.3	5.1	68.8	167	2.4	2.4
resistant	68	7x8	2.8	57	3	212	7.1	13.3	4.2	5.6	66.5	160	2.5	2.6
hybrids	11	2x1	3.7	58	4	217	10.3	3.3	2.2	6.2	65.4	267	2.6	2.7
	20	2x10	3.1	57	2	185	35.5	1.8	0	5.2	66.6	233	2.7	2.7
	31	4x1	4.1	59	6	201	1.2	21.2	0	5.5	67.9	367	2.9	2.9
	30	3x10	3.6	56	1	206	38.5	4	0	5.2	67.2	267	3	3.0
	18	2x8	3.9	56	4	209	0	7.4	0	6	65.7	200	3	3.1
	70	7x10	2.5	57	2	200	22.3	3.9	6.3	4.7	67.3	267	3.1	3.1
	13	2x3	3.5	55	3	216	28.3	3.8	3.4	5.3	66.3	400	3.2	3.2
	59	6x9	4.4	54	1	217	8.6	20.5	1.5	5	67.6	767	3.2	3.2
	56	6x6	5.1	57	1	205	33.3	9	1.6	5.9	66.4	567	3.4	3.4
	39	4x9	3.1	55	3	199	26.2	14	6.2	4.7	68.3	700	3.6	3.6
Bottom hybrids	43	5x3	2.8	58	4	210	18.1	25.4	0.0	5.7	67.9	633	130.6	170.5
	42	5x2	3.6	60	6	204	1.2	21.9	0.0	5.5	67.4	233	239.2	240.0
	25	3x5	2.0	57	4	218	7.6	24.0	0.0	5.9	66.0	300	260.8	270.0
Checks	71		3.2	59	3	213	7.2	3.6	6.8	5.4	68.9	900	80.6	156.2
	72		2.5	62	6	199	16.1	14.5	8.5	5.5	68.2	1133	24.8	28.4
	73		3.5	57	5	210	13.2	6.1	5.9	5.6	68.1	1100	59.0	298.0
	74		3.0	61	3	207	9.8	6.0	0.0	5.5	68.5	187	2.4	2.5
	75		2.1	61	7	207	9.8	3.0	1.9	5.4	68.0	1133	86.3	204.6
	Mean		3.3	57.6	3.5	210.7	15.7	11.7	2.5	5.4	67.4	628.9	65.0	75.0
	Lsd (0.05)		1.4	2.5	3.9	27.6	25.4	14.7	6	0.5	1.1	20.6	1.4	381.2
	CV(%)		20.7	2.2	55.4	6.6	11.2	22.6	12.4	4.2	0.8	13.9	61.7	255.1

*-significant at p<0.05, GY-grain yield, ED-ear damage, LD-leaf damage, ASI-anthesis-silking interval, HC-husk cover, ER-ear rot, PH-plant height, SL-stalk lodging, OIL-oil content, ASP-*Aspergillus flavus*, AFL-aflatoxin content, STAR-starch content, AD- days to anthesis.

Table 3. 6: Grain yield, agronomic traits, *Aspergillus* and aflatoxin content of hybrids and checks at KALRO Katumani

	Entry	Cross Fxm	GY t/ha	AD Days	ASI days	PH cm	SL %	HC %	ER %	OIL %	STAR %	ASP cfu/g	AFL ng/g	AFL Original
Top	65	7x5	3.2	69	2	242	34.4	0	0	4.9	68.4	133.3	2.1	2.1
resistant	11	2x1	1.4	75	3	254	23.8	0	0	5.6	67.5	166.7	2.5	2.6
hybrids	61	7x1	2.8	70	2	247	21.7	2.7	0	4.7	69.2	436	2.5	2.6
	16	2x6	3.2	71	2	260	38.3	2.7	0	5	69.2	467	2.6	2.6
	55	6x5	3.0	66	2	234	48.7	0	0	5	68.6	200	2.8	2.8
	68	7x8	3.1	69	2	243	31.7	0	0	5.3	67.4	185	2.9	3.0
	12	2x2	1.6	73	2	246	38.7	0	0	4.9	67.7	567	3	3.1
	18	2x8	2.1	71	3	227	41.3	1.3	0	5.6	66.8	200	3	3.1
	60	6x10	3.2	68	2	224	20.8	0	0	4.3	69.4	167	3.2	3.3
	59	6x9	3.3	65	3	227	28.6	0	0	4.5	67.5	367	3.3	3.3
	58	6x8	3.8	67	2	227	47.2	0	0	5.3	68.9	167	3.4	3.5
	67	7x7	4.3	67	2	249	23.6	0	0	4.7	68.9	800	3.4	4.0
Bottom hybrids	4	1x4	1.8	79	3	267	51.9	1.3	0.0	4.3	69.7	500	374.7	375.0
	46	5x6	2.5	74	2	253	12.1	1.3	0.0	5.0	69.0	600	724.6	925.0
	34	4x4	2.1	77	2	271	25.1	6.0	0.0	3.6	70.4	567	875.6	895.0
Checks	71		2.9	72	2	268	26.9	0.0	0.0	4.4	70.9	367	7.7	8.1
	72		2.6	70	3	280	48.1	0.0	1.7	5.3	68.9	367	37.3	172.1
	73		2.5	71	2	259	25.3	0.0	0.0	4.6	70.4	667	5.6	5.9
	74		2.4	71	2	257	18.0	0.0	0.0	4.2	70.2	700	10.1	13.3
	75		1.3	72	2	255	74.7	1.6	0.0	4.4	69.9	633	8.2	9.9
	Mean		2.7	71	2	250.5	31.4	1	0.2	4.6	69.1	541	40.3	77.8
	LSD (0.05)		1	3	2	58.4	37.8	5.7	1.3	0.6	0.9	6.4	1.1	487.4
	CV (%)		17.9	2	32	11.6	60.3	275.6	379.3	6.2	0.7	19.2	63.7	314.4

*-significant at p<0.05, GY-grain yield, ED-ear damage, LD-leaf damage, ASI-anthesis-silking interval, HC-husk cover, ER-ear rot, PH-plant height, SL-stalk lodging, OIL-oil content, ASP-*Aspergillus flavus*, AFL-aflatoxin content, STAR-starch content, AD- days to anthesis.

Table 3. 7: Correlations between *Aspergillus* ear rot, grain yield and other selected traits

	GY	AD	ASI	PH	SL	HC	ER	LD	ED	OIL	STAR	ASP	AFL
GY	---												
AD	-0.54*												
ASI	-0.24*	-0.20*											
PH	-0.22*	0.60*	-0.19*										
SL	-0.23*	0.31*	-0.19*	0.17*									
HC	0.18*	-0.44*	0.26*	-0.31*	-0.26*								
ER	0.09	-0.35*	0.02	-0.21*	-0.11*	0.27*							
LD	-0.02	0.01	0.02	0.06	0.04	-0.11	-0.02						
ED	-0.11	0.09	0.03	0.05	-0.04	-0.08	-0.03	0.25*					
OIL	0.34*	-0.56*	0.29*	-0.36*	-0.22*	0.26*	0.05	0.10	-0.03				
STAR	-0.23*	0.65*	-0.23*	0.44*	0.24*	-0.34*	-0.10	-0.08	0.03	-0.74*			
ASP	-0.02*	0.15	-0.02	0.09	-0.13	0.07*	0.07	0.07	-0.53*	-0.6*	-0.46*		
AFL	-0.24*	0.43	-0.06	-0.32	-0.14	0.05*	0.37*	0.1	-0.11	-0.05	-0.23*	0.26*	---

*-significant at p<0.05, GY-grain yield, ED-ear damage, LD-leaf damage, ASI-anthesis-silking interval, HC-husk cover, ER-ear rot, STAR-starch content, SL-stalk lodging, EH-ear height, PH-plant height, AFL-aflatoxin, ASP-*A.flavus*

3.6 Discussion

The study showed large genetic variation among the hybrids for various traits. The mean *Aspergillus* ear rot in the hybrids was low compared to that of the commercial checks. Incidences of ear rot were low in Katumani as compared to Kiboko.

Eller *et al*. (2008) in a study to determine host resistance for *Aspergillus flavus* in maize lines reported that the inbred lines responded variably. While studying combining ability for resistance to *Gibberrella* ear rot, Henry *et al*. (2009) reported that the maize hybrids responded variably under conditions of artificial inoculation. Hefny *et al*. (2012), Hung and Holland (2012) and Balconi *et al*. (2014) also reported phenotypic variability among maize genotypes while studying heritability for resistance to *Fusarium*. They also reported low mean ear rot in the hybrids studied. Eller *et al*. (2008) conducted their study in three different environments and reported low ear rot incidence in one of the environments relating it to low plant stress experienced during the season.

Variations among genotypes for *Aspergillus* ear rot observed in this study suggest that the germplasm used in the study was genetically diverse. Genetic diversity is a prerequisite in breeding as it allows for genetic improvement through selection (Eller *et al*., 2008 and Henry *et al*., 2009; Hefny *et al*., 2012; Hung and Holland, 2012; Gethi *et al*., 2013 and Balconi *et al*., 2014). The hybrids showed low levels of ear rot because the resistance in their respective parents was passed onto them. This implies that high heritability existed among the germplasm (Hefny *et al*., 2012; Hung and Holland, 2012 and Balconi *et al*., 2014). Mechanisms of resistance to *Aspergillus* ear rot observed in the hybrids could be due to traits such as good husk cover, drooping ears and resistance to insects as earlier reported (Betran *et al*., 2002; IFPRI, 2010). Low incidences of ear rot observed in hybrids at Katumani could be attributed to low

plant stress experienced during the season. Low plant stress denies the pathogen optimum conditions for growth hence plant stress is a prerequisite for effective pathogenicity (Payne *et al*., 1986; Oren *et al*., 2003 and Eller *et al*., 2008).

Significant variations were noted for aflatoxin concentration among the genotypes in this trial. Genotype by environment interaction was significant for aflatoxin concentration among the genotypes. The checks had a high mean of aflatoxin concentration as compared to the hybrids.

Eller *et al*. (2008), Henry *et al*. (2009) Hefny *et al*. (2012) and Williams and Windham (2015) reported variations in aflatoxin concentration among the maize genotypes used in their studies. In studies of aflatoxin accumulation in maize and inheritance of resistance to aflatoxin, Betran *et al*. (2002), Warburton *et al*. (2011), Asea *et al*. (2012) and Warburton and Williams (2014) reported that the genotype by environment interaction played an important role in the resistance to aflatoxin accumulation in maize.

From these studies, the hybrids were reported to be more resistant as compared to the inbred lines used in their development. The interaction between the environment and the genotypes played an important role in expression of resistance to aflatoxin accumulation. This could be attributed to the fact that resistance is quantitatively inherited and is highly influenced by the environment (Betran *et al*., 2002; Warburton *et al*., 2011; Asea *et al*., 2012; Warburton and Williams, 2014). The significance in GxE interaction could be reduced by testing the genotypes extensively in multiple environments (Warburton and Williams, 2014).

The high levels of aflatoxin content observed in Kiboko could be explained by high temperature and drought experienced in the region. High temperature, relative

humidity and drought have been reported to increase aflatoxin production (Payne, 1998; Strosnider *et al*., 2006; Eller *et al*., 2008). The low aflatoxin concentration observed among the top resistant hybrids lies within the Food and Drug Administration (FDA) of the US provisions (US-FDA guidelines for aflatoxin levels). However, most inbred lines identified to be sources of resistance to aflatoxin accumulation are late maturing, very tall hence prone to lodging and are low yielding (Warburton and Williams 2014). It is therefore necessary to test these hybrids further to ensure that they posses traits preferred by the farmers.

The mean number of ears per plant and grain yield of the F_1 hybrids was higher than that of commercial checks. Gethi *et al*. (2013) in a study to determine combining ability for resistance to grey leaf spot and grain yield in maize lines reported that the inbred lines had significant yield differences. Hefny *et al*. (2012), Hung and Holland, (2012) and Balconi *et al*. (2014) also reported significant differences in grain yield among the germplasm used in their study. Combining ability studies by Reif *et al*. (2005) and Khorzoght *et al*. (2010) reported high grain yield and starch contents in the hybrids as compared to their parental inbred lines.

The high grain yield and nutritional contents in the hybrids reported in this study is due to heterosis which results to better performance in the progenies obtained from a single cross (Reif *et al*., 2005; Khorzoght *et al*., 2010 and Gethi *et al*., 2013).

These findings are consistent with Gethi *et al*. (2013) who reported a large anthesis-silking interval in grey leaf resistant maize hybrids. Betran *et al*. (2003) also reported large intervals between silking and tasselling in managed drought trials with the emphasis that supplemental irrigation hastens silk production in maize. Trials conducted by Bolaños and Edmeades, (1996) and Aslam *et al*. (2013) registered

minimal or no flowering in maize lines under drought stress in a study to identify water efficient maize. In a study to identify maize lines resistant to aflatoxin accumulation in Uganda, Asea *et al*. (2012) reported that the F_1 hybrids were very tall and hence not recommended for direct use by the farmers since it was associated with proneness to stalk.

Generally the hybrids took long to produce silks in Kiboko because there was no supplemental irrigation during plant growth, implying that water availability is imperative during flowering (Bolaños and Edmeades, 1996; Betrán *et al*., 2003 and Aslam *et al*., 2013). The high plant and ear heights observed in this study is as a result of hybrid vigor which results to improved performance in the progenies obtained from a cross (Reif *et al*., 2005; Khorzoght *et al*., 2010). Tall plants are prone to stalk lodging as a result of weak stems and increased centre of gravity (Asea *et al*., 2012; Gethi *et al*., 2013 and Amaefula *et al*., 2014).

Aspergillus ear rot, husk cover aflatoxin content and grain yield were observed to be significantly correlated. *Aspergillus* ear rot was negatively correlated to grain yield while it had a positive correlation with aflatoxin accumulation. Husk cover was positively correlated to *Aspergillus* ear rot and aflatoxin accumulation. Horne *et al*. (2016) reported a negative correlation between grain yield and *Fusarium* ear rot in a study of recurrent selection for reduced ear rot in maize. While studying aflatoxin accumulation in maize hybrids of different maturities, Betran and isakeit (2004) reported low grain yield in hybrids susceptible to ear rot. Warfield and Davis (1999), Betran *et al*. (2002), Atehnkeng *et al*. (2008) and IFPRI (2010) reported that maize lines with good husk cover have low incidences of ear rot and accumulate low levels of aflatoxin in resistance studies for *Aspergillus flavus* and aflatoxin accumulation.

The negative and significant correlation between *Aspergillus* ear rot and grain yield observed in this study suggests that high susceptibility to ear rot results to low grain yield. This could be attributed to the fact that susceptible plants fail to achieve optimum productivity (Moreno and Kang, 1999; Betr'an and Isakeit, 2004; Eller *et al*., 2008). *Aspergillus* ear rot was positively correlated with aflatoxin accumulation implying that hybrids which are susceptible to *Aspergillus* ear rot accumulate high levels of aflatoxin. This suggests that as the fungi metabolize, they release large quantities of aflatoxin as secondary metabolites (Strosnider *et al*., 2006; Paterson and Lima, 2010).

The positive correlation between husk cover, *Aspergillus* ear rot and aflatoxin accumulation could suggest that genotypes with tight husks minimize entry of fungal pathogens and hence low levels of aflatoxin (Betran *et al*., 2002; IFPRI, 2010). Presence of a good husk cover hinders entry of fungal spores into maize kernels preventing aflatoxin accumulation in grains. Grain yield and flowering were negatively correlated suggesting that early maturing hybrids were more productive than the late maturing ones. This could have been as a result of late maturing hybrids failing to tassel before the onset of drought hindering cob formation and kernel production (Bolaños and Edmeades, 1996 and Aslam *et al*., 2013).

Correlation enables the identification of candidate traits that can be used in indirect selection for resistance to *Aspergillus* ear rot and aflatoxin accumulation. In breeding programs, simultaneous selection for a number of traits can hasten the progress in selection and ultimately hybrid development (Edwards, 2006). This can be achieved when the traits of interest are highly and significantly correlated.

The hybrids evaluated responded variably under artificial inoculation by *Aspergillus flavus* in the different environments. Hybrids 11, 13, 18, 30, 31, 37, 56, 59, 60, 16, 55, 58, 59, 60, 65 and 68 have been identified to be resistant to *Aspergillus* ear rot and aflatoxin accumulation. These hybrids also maintained a high yield across the environments and could therefore be introduced into the local breeding programs for development of varieties resistance to *Aspergillus flavus* and aflatoxin accumulation. Hybrids 19, 45, 50, 11, 12 and 18 were also identified to be resistant to *Aspergillus* ear rot and aflatoxin accumulation although they are not highly productive.

The parents of these hybrids could be traced and used as donors to improve the performance of the adapted but rather susceptible genotypes. Good husk cover could be used as an indicator during selection for germplasm resistant to *Aspergillus* ear rot and aflatoxin accumulation. The study has revealed that maturity is not an important trait in resistance to *Aspergillus* ear rot and therefore it cannot serve as a secondary trait in selection for resistance.

CHAPTER FOUR

COMBINING ABILITY OF MAIZE INBRED LINES FOR RESISTANCE TO *ASPERGILLUS* EAR ROT AND AFLATOXIN ACCUMULATION

Abstract

Aflatoxin contamination in maize has continued to be a challenge in Kenya, consistently causing loss of produce and death as a result of aflatoxicosis. This contamination is as a result of *Aspergillus flavus* fungi infecting the crop both at the field and in storage. The search for resistant germplasm has led to the identification of some genotypes native to the temperate region. Therefore the aim of this study was to determine how these genotypes combine with the local elite inbred lines for resistance. The genetics of resistance was studied among seventy F_1 progenies generated from seventeen maize inbred lines following North Carolina II (NCDII) mating design. The progenies were evaluated at two locations; KALRO Kiboko and KALRO Katumani. Experiments were laid out in Alpha lattice design with two replications. Combining ability analysis was conducted using Line by Tester (LxT) method in the SAS program. Large genetic variations were noted among the genotypes implying genetic variability. The effect of additive gene action was more profound in the inheritance of resistance. The best combiners for resistance were inbred lines P329, Mp 313E, CKL05003 and Mp719 giving the highest negative GCA effects. Parents (CKL05003/La Posta Seq C7-F180-3-1-1-1-B-B-B)DH56-B-B/Mp717, ([CML444/CML395//DTPWC8F31-1-1-2-2-BB]-4-2-2-1-1-B*4/(9071xBabamgoyo)-3-1-BBB)-B-1-2-3-1-3-B-B/Hi27 and CKL05019/Mp715 had the best specific combining abilities (SCA) for resistance to aflatoxin accumulation while parents CKL05003/Hi27, (ZM621A-10-1-1-1-2-B*8/PHG35)-B-16-2-2-B-B/Mp719 and (CKL05003/La Posta Seq C7-F180-3-1-1-1-B-B -B) DH152-B-B/Mp 313E were the best for *A.flavus* resistance.The genes in these genotypes

could be incorporated into elite lines through breeding to improve maize productivity in Kenya.

4.1 Introduction

Maize is an important food crop and a staple for most African countries accounting for up to 50% of calories consumed (Hassan *et al*., 2001). It is projected that the demand for maize as a food crop will have doubled by the year 2050 (Rosegrant *et al*., 2009). Maize production in Kenya is constrained by post-harvest losses majorly as a result of fungal ear rots (Asea *et al*., 2012). This is associated with deterioration in grain quality due to discoloration and accumulation of mycotoxins (Chandrashekar *et al*., 2000) and health hazard to humans and animals (Agag, 2004).

Management strategies such as field sanitation, proper drying of grains before storage and packaging have been previously used (Turner *et al*., 2005; Fandohan *et al*., 2005; Strosnider *et al*., 2006). Recently, there has been development of bio-control through atoxigenic strains of *Aspergillus* that outcompete the toxigenic ones (IFPRI, 2010). However these options are largely dependent on climatic conditions and are uneconomical to most small scale farmers hence host plant resistance is still considered as the most viable option in combating *A.flavus* and aflatoxin accumulation in maize grains (Williams and Windham, 2015).

Several resistant germplasm have been identified and registered (Scott and Zummo, 1990; Williams and Windham, 2006; Williams and Windham, 2012; Williams *et al*., 2014; Williams and Windham, 2015). However they have poor agronomic traits therefore it is necessary to hybridize them with the genotypes possessing desirable traits (Kakani *et al*., 2007; Warburton and Williams 2014). Combining abilities of these germplasm should be established in order to understand the nature of gene

action controlling resistance to *A.flavus* and aflatoxin accumulation in maize for successful breeding (Asea *et al*., 2012; Williams and Windham, 2015; Patial *et al*., 2016). Combining abilities comprise of general combining ability-GCA) and specific combining ability-SCA (Sprague and Tatum, 1942). General combining ability enables breeders to identify superior genotypes that can be used to make better crosses whereas specific combining ability enables the identification of useful single cross combinations that result into heterosis (Simmonds, 1979; Patial and Kumar, 2016). Previous studies have reported the significance of GCA effects in resistance to *A.flavus* and aflatoxin accumulation implying that inheritance of resistance is mainly conditioned by additive gene action (Khorzoght *et al*., 2010; Asea *et al*., 2012; Williams and Windham, 2015). The objective of this study was therefore to determine the combining ability of maize inbred lines for resistance to *Aspergillus* ear rot and aflatoxin accumulation.

4.2 Materials and Methods

4.2.1 Experimental material

The germplasm used comprised of 17 maize inbred lines of diverse origin ranging from Mexico, USA-Mississippi, Hawaii and Kenya (Table 3.1). The exotic lines have putative resistance to aflatoxin accumulation and they were used as the male parents. The local lines are adapted and exhibit superior agronomic traits although they are potentially susceptible to aflatoxin accumulation and they were used as the female parents (Table 3.1).

4.2.2 Description of experimental sites

The experiment was conducted at the agricultural research stations in Kiboko and Katumani as described in section 3.2.2 of chapter three.

4.2.3 Experimental design and layout

The 17 inbred lines were planted in alpha lattice design at a spacing of spacing of 20 cm within rows and 75 cm between rows as described in section 3.2.3 of chapter 3.

4.2.4 Generation of crosses

Crosses were generated from the 17 maize inbred lines following North Carolina II mating design (Table 3.2).

4.2.5 Evaluation of crosses

The 70 F_1 hybrids generated were planted at KALRO-Kiboko and KALRO-Katumani for evaluation. Five checks were included in the trial, four of which are commercial varieties and one an elite inbred line from CIMMYT. Planting was done in 4 m row plots following 5*15 Alpha lattice designs in two replications. Each entry was planted in two row plots at a spacing of 20 cm within rows and 75 cm between rows. Evaluation of the crosses was done as described in section 3.2.4 of chapter 3.

4.2.6 Data analysis

Analysis of variance for NC II was carried out according to the model below using SAS program (Frederick, 1999).

$$Y_{ijk} = \mu + M_i + F_j + MF_{ij} + R_k + \varepsilon_{ijk} \dots\dots\dots\dots\dots\dots\dots\dots\text{ Equation 4.1}$$

Where,

Y_{ijk} = observed trait value, μ = mean effect, M_i = effect of the ith male, F_j = effect of the jth female, MF_{ij} = effect of interaction between i^{th} male and j^{th} female, R_k = effect of kth replication, and ε_{ijk} = experimental error.

Combining ability analysis was performed following a linear model using SAS program (Hallauer *et al.*, 2010).

$$X_{ijk} = \mu + r_k + g_i + s_{ij} + e_{ijk}$$ .... Equation 4.2

Where,

X_{ijk} is the observed performance of the cross between i[th] and j[th] parents in the kth replication, μ is the population mean, rk is the replication effect, g_i is the GCA effect for the ith parent, g_j is the general combining ability effect for the jth parent, s_{ij} is the specific combining ability effect for the cross between i[th] and j[th] parents and e_{ijk} is the experimental error for the X_{ijk} observation.

Mean sum of squares of the hybrids were partitioned into male and female GCA and their interaction was ascertained as SCA. The proportion of GCA: SCA importance was determined using Baker's ratio (Baker, 1978).

$$Baker's\ ratio = \frac{2MS_{GCA}}{2MS_{GCA} + MS_{SCA}}$$ .... Equation 4.3

4.3 Results

4.3.1 Variations for the combining ability of maize inbred lines

Significant differences were noted among the genotypes for all traits except leaf damage $p < 0.05$ (Table 4.1). Across the sites, significant differences were observed for all traits except stalk lodging and leaf damage. Significant GCA effects were observed among the female inbred lines for all traits except stalk lodging and leaf damage. Significant GCA effects were noted among the male inbred lines for all traits except aflatoxin accumulation. SCA effects were significantly different for all traits except flowering, plant height, stalk lodging. The female GCA by environment interaction (GCA_f x E) was significantly different for all traits except root and stalk lodging, leaf damage, *A. flavus* and aflatoxin accumulation. The male GCA by environment interaction (GCA_m x E) was significantly different for all traits except anthesis-

silking interval, stalk lodging, ear rot, leaf damage, *Aspergillus flavus* and aflatoxin accumulation. The SCA by environment interaction (SCAxE) was only significantly different for grain yield, ear rot, leaf damage, oil and starch contents. The variance component ratio (Baker's ratio >0.5) revealed that GCA was more predominant than SCA for all traits.

4.3.2 *Aspergillus* ear rot and aflatoxin content of hybids and checks

The level of aflatoxin contamination was lower in Katumani as compared to Kiboko both for the hybrids and the checks. Female parent 2 and male parents 9 and 10 produced crosses with the least levels of aflatoxin content. Female parent 5 produced crosses with the highest levels of aflatoxin content both at individual stes and across sites. The most resistant hybrids had very low levels of aflatoxin content ranging from 2- 4 ng/g while the most susceptible ones had very high levels of aflatoxin of upto 900 ng/g. Check 74 had relatively low levels of aflatoxin content both at individual sites and across sites. Hybrids 11, 20, 18 and 60 had low levels of aflatoxin content both at individual sites and across sites while hybrid 46 was among the most susceptible at individual sites and across sites.

Table 4. 1: Mean squares for combining ability for *Aspergillus* ear rot, aflatoxin accumulation grain yield and agronomic traits across sites

Source	Df	GY	AD	ASI	PH	SL	HC	ER	LD	OIL	STAR	ASP	AFL
		t/ha	Days	Days	Cm	%	%	%		%	%	cfu/g	ng/g
Rep	1	0.7	145.7	1.0	62.7	5916.3	323.1	19.6	33.1	0.0	0.7	0.0	0.5
Envt (E)	1	75.0*	11494.4*	77.2*	128390.4*	15455.0	4920.7*	330.3*	0.1	46.0*	164.2*	0.4*	2.9*
Entry	69	1.5*	23.8*	5.2*	1034.4*	432.5*	99.9*	13.8*	5.8	0.8*	2.8*	0.2*	0.8*
GCA$_f$	6	6.2*	68.8*	25.0*	3823.4*	246.7	306.3*	52.0*	2.2	1.9*	14.9*	0.3*	2.4*
GCA$_m$	9	2.9*	121.3*	7.5*	2062.1*	822.8*	174.1*	9.4*	13.9*	4.1*	7.7*	0.1	0.7
SCA	54	0.7*	2.6	2.7	553.4	388.1	64.6*	10.3*	4.9	0.1*	0.7*	0.2*	0.5*
GCA$_f$ xE	6	5.1*	43.9*	21.2*	2766.2*	334.7	176.6*	53.3*	6.7	0.5*	1.0*	0.2	0.8
GCA$_m$ xE	9	4.1*	9.1*	3.3	1368.3*	437.4	128.9*	7.2	7.3	0.2*	1.4*	0.1	0.6
SCAxE	54	0.7*	2.5	2.4	613.4	192.0	41.0	11.7*	8.1*	0.1*	0.5*	0.1	0.5
Error	139	0.5	2.3	2.1	570.1	263.5	38.0	4.8	4.8	0.1	0.3	0.0	0.4
Baker's ratio		0.9	1.0	0.9	0.9	0.7	0.9	0.9	0.8	1.0	1.0	0.8	0.9

*-significant at *p<0.05*, GY-grain yield, LD-leaf damage, ASI-anthesis-silking interval, HC-husk cover, ER-ear rot, ASP-*Aspergillus flavus*, PH-plant height, SL-stalk lodging, AFL-aflatoxin, STAR-starch.

Table 4. 2: Mean squares for combining ability of *Aspergillus* ear rot, aflatoxin accumulation, grain yield and agronomic traits at Kiboko

Source	Df	GY t/ha	AD Days	ASI Days	PH Cm	SL %	HC %	ER %	ASP cfu/g	AFL ng/g
Rep	1	0.0	17.9	0.0	93.0	5916.5	859.7	42.5	0.0	1.4
Entry	69	2.2*	8.5*	8.8*	215.8	309.3*	150.2*	27.6*	158134.2	0.6
GCA$_f$	6	7.9*	19.0*	45.1*	444.6*	352.1	462.1*	103.5*	318970.6	1.3*
GCA$_m$	9	4.4*	39.6*	10.0*	305.8	899.0*	290.5*	16.0	153946.5	0.8
SCA	54	1.2*	2.2	4.6	175.3	206.3	92.1*	21.1*	140961.5	0.5
Error	69	0.7	1.7	3.5	185.4	170.9	59.8	9.0	0.0	0.5
Baker's ratio		0.9	0.9	0.9	0.8	0.9	0.9	0.8	0.8	0.8

GY-grain yield, LD-leaf damage, ASI-anthesis-silking interval, HC-husk cover, ER-ear rot, *significant at *p<0.05*, ASP-*Aspergillus flavus* colony forming units, PH-plant height, SL-stalk lodging, AFL-aflatoxin content, STAR-starch.

Table 4. 3: Mean squares for combining ability of *Aspergillus* ear rot, aflatoxin accumulation, grain yield and agronomic traits at Katumani

Source	Df	GY t/ha	AD Days	ASI Days	PH Cm	SL %	HC %	ER %	ASP cfu/g	AFL ng/g
Rep	1	1.6	165.0	2.3	434.4	1015.0	15.2	0.1	0.0	0.0
Entry	69	0.8*	22.3	0.6	1717.8*	359.6	14.0*	0.9*	181405.5	0.7*
GCA$_f$	6	3.4*	93.8*	1.1	6145.1*	229.3	20.8*	1.8*	291093.6	1.9*
GCA$_m$	9	2.6*	90.8*	0.8	3124.5*	361.2	12.4	0.6*	262366.0	0.6
SCA	54	0.2	2.9	0.5	991.4	373.8	13.5*	0.8*	155724.5	0.5*
Error	69	0.2	2.3	0.6	956.3	345.1	8.7	0.4	0.0	0.3
Baker's ratio		0.9	0.9	0.8	0.9	0.6	0.7	0.8	0.8	0.8

GY-grain yield, LD-leaf damage, ASI-anthesis-silking interval, HC-husk cover, ER-ear rot, *significant at *p<0.05*, ASP-*Aspergillus flavus* colony forming units, PH-plant height, SL-stalk lodging, AFL-aflatoxin content, STAR-starch.

Table 4. 4: *Aspergillus* **ear rot and aflatoxin content of hybrids and checks**

	Entry	Cross (fxm)	Across ASP cfu/g	AFL ng/g	AFL Original	Entry	Cross (fxm)	Kiboko ASP cfu/g	AFL ng/g	AFL original	Entry	Cross (fxm)	Katumani ASP cfu/g	AFL ng/g	AFL Original
Top	11	2x1	130	2.6	2.8	10	1x10	167	2.4	2.4	65	7x5	133.3	2.1	2.1
resistant	68	7x8	165	2.8	2.9	68	7x8	160	2.5	2.6	11	2x1	166.7	2.5	2.6
hybrids	20	2x10	183	2.9	3.0	11	2x1	267	2.6	2.7	61	7x1	436	2.5	2.6
	18	2x8	195	3.1	3.2	20	2x10	233	2.7	2.7	16	2x6	467	2.6	2.6
	59	6x9	200	3.3	3.4	31	4x1	367	2.9	2.9	55	6x5	200	2.8	2.8
	15	2x5	267	3.4	3.5	30	3x10	267	3	3	68	7x8	185	2.9	3
	13	2x3	300	3.4	3.5	18	2x8	200	3	3.1	12	2x2	567	3	3.1
	19	2x9	300	3.6	3.7	70	7x10	267	3.1	3.1	18	2x8	200	3	3.1
	39	4x9	325	3.6	3.7	13	2x3	400	3.2	3.2	60	6x10	167	3.2	3.3
	45	5x5	333	3.7	3.8	59	6x9	767	3.2	3.2	59	6x9	367	3.3	3.3
	60	6x10	350	3.8	3.9	56	6x6	567	3.4	3.4	58	6x8	167	3.4	3.5
	21	3x1	350	4.0	4.1	39	4x9	700	3.6	3.6	67	7x7	800	3.4	4
Bottom hybrids	34	4x4	900	459.1	459.2	43	5x3	633	130.6	170.5	4	1x4	500	374.7	375
	28	3x8	1000	469.1	469.2	42	5x2	233	239.2	240	46	5x6	600	724.6	925
	46	5x6	1033	475.8	475.9	25	3x5	300	260.8	270	34	4x4	567	875.6	895
Checks	71		633	82.1	82.2	71		900	80.6	156.2	71		367	7.7	8.1
	72		750	100.2	100.3	72		1133	24.8	28.4	72		367	37.3	172.1
	73		883	151.9	152.0	73		1100	59	298	73		667	5.6	5.9
	74		633	7.9	8.0	74		187	2.4	2.5	74		700	10.1	13.3
	75		883	107.2	107.3	75		1133	86.3	204.6	75		633	8.2	9.9
	Mean		584.9	76.4	78			628.9	65	75			541	40.3	77.8
	Lsd (0.05)		20.6	1.4	381.2			20.6	1.4	381.2			6.4	1.1	487.4
	CV(%)		13.9	61.7	255.1			13.9	61.7	255.1			19.2	63.7	314.4

ASP-*A.flavus* colony forming units, AFL-aflatoxin content

4.3.3: General combining ability of inbred lines for *Aspergillus* ear rot, aflatoxin accumulation, grain yield and agronomic traits

Across the two sites, female parent 2 and male parent 10 had the highest negative GCA effects of -0.36 and -0.33 respectively for aflatoxin accumulation (Table 4.2). Male parents 3 and 6 and female parent 1 had the highest negative GCA effects for *Aspergillus flavus*. Female parents 1 and 5 and male parent 4 had high GCA effects (0.36, 0.20 and 0.26 respectively) for susceptibility. Male parent 7 and female parent 6 had the highest positive GCA effects of 0.72 and 0.68 for grain yield. Male parents 5, 7, 9 and female parent 6 had the highest negative GCA values for flowering. Female parents 1, 2 and male parent 10 had the highest negative GCA values for poor husk cover.

At KALRO Kiboko, male parent 10 and female parent 2 had the highest negative GCA values of -0.52 and -0.35 for aflatoxin accumulation while female parent 1 had the highest negative GCA effects for *Aspergillus flavus*. Female parent 5 had high positive GCA values for both aflatoxin accumulation and *A.flavus*. Female parent 6 and male parents 7, 8 and 2 had high GCA effects of 0.92, 0.79, 0.67 and 0.62 respectively for grain yield. Male parents 7 and 9 were the best combiners for early maturity. Female parent 1 and male parent 10 had the highest negative GCA effects of -5.33 and -7.67 respectively for poor husk cover (Table 4.3).

At KALRO Katumani, female inbred lines 2 and 6 had the highest negative GCA values for aflatoxin accumulation and *Aspergillus flavus* respectively. Female parents 1 and 4 and male parents 4 and 6 had the highest GCA effects for susceptibility to aflatoxin accumulation. Both male and female lines 6 and 7 were the best combiners for grain yield while female lines 3 and 6 and males 5 and 9 were the best combiners for early maturity. Male inbred line 10 had the best general combining ability for good husk cover (Table 4.4).

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Table 4. 5: General combining ability for *Aspergillus* ear rot, aflatoxin, grain yield and agronomic traits across all sites

	Parents	GY t/ha	AD Days	ASI Days	PH Cm	SL %	HC %	ER %	ASP cfu/g	AFL ng/g
Female	1	0.14	2.09*	0.4	9.36	4.76*	-2.95	-0.47	-156.85	0.36*
	2	-0.34	-0.29	-0.15	-6.47	1.72	-2.32	-0.62	25.65	-0.36*
	3	-0.21	-0.59	0.07	17.42*	-2.18	-2.23	-1.03	103.99	0.02
	4	0.17	0.69	0.4	-0.05	-1.53	0.42	0.31	28.99	0.09
	5	-0.51	0.84	1.12	-8.34	0.16	1.56	-0.4	41.98	0.2
	6	0.68*	-1.91*	-1.4*	-5.76	-1.38	0.61	-0.21	-72.68	-0.17
	7	0.07	-0.84	-0.43	-6.16	-1.55	4.89*	2.42*	28.91	-0.15
SE		0.33	0.97	0.67	7.70	2.68	1.95	1.07	81.69	0.13
Male	1	-0.15	0.73	0.37	-0.75	0.7	-0.01	-0.48	-3.42	-0.08
	2	-0.01	2.76	0.08	-0.74	-4.38	1.14	-0.75	51.13	-0.01
	3	-0.13	-0.66	-0.13	-1.53	3.89	4.5	0.29	-113.87	0.02
	4	-0.27	3.84	-0.31	14.24	-0.67	-1.45	-0.65	-3.63	0.26
	5	-0.35	-1.38	0.69	-6.9	3.97	0.85	-0.21	143.99	0.06
	6	-0.1	0.94	0.23	13.36	-1.21	-0.65	0.87	-149.10	0.12
	7	0.72*	-1.37	-0.45	3.98	-5.59	-1.96	0.88	82.08	0.14
	8	0.4	-0.95	0.05	-1.28	-2.4	-0.78	-0.19	-57.69	-0.05
	9	-0.12	-3.06	0.55	-11.84	-6.07	2.74	-0.07	37.48	-0.12
	10	0.01	-0.84	-1.06	-8.53	11.75	-4.38	0.32	13.03	-0.33*
SE		0.36	0.54	0.33	6.63	3.75	2.04	0.48	79.75	0.14

* -significant at p<0.05 probability, GY-grain yield, ASI-anthesis-silking interval, HC-husk cover, PH-plant height, SL-stalk lodging, SE-standard error, ER-ear rot, ASP-*A.flavus*, AFL- aflatoxin, AD-days to anthesis

Table 4. 6: General combining ability for *Aspergillus* ear rot, aflatoxin, grain yield and agronomic traits at Kiboko

	Parents	GY t/ha	AD days	ASI Days	PH Cm	SL %	HC %	ER %	ASP cfu/g	AFL ng/g
Female	1	0.44**	0.32	0.42	8.45*	8.8**	-5.33*	-0.78	-236.81	0.30*
	2	-0.1	-1.08***	-0.33	-1.47	-1.33	-4.07*	-1.09*	-11.48	-0.35*
	3	-0.66***	0.87**	0.02	1.05	0.03	-4.15*	-1.9**	-4.79	0.16
	4	0.47**	1.02***	0.62	-3.2	-0.66	0.25	-0.01	-1.47	-0.19
	5	-0.69***	0.82**	2.42***	-3.58	-2.44	3.56*	-0.64	105.18	0.30*
	6	0.92***	-1.13***	-2.58***	3.55	-4.47*	2.04	-0.56	-18.15	-0.16
	7	-0.39**	-0.83**	-0.58	-4.8	0.06	7.71**	4.99***	167.52	-0.06
SE		0.11	0.18	0.37	2.59	2.14	1.54	0.44	0.00	0.11
Male	1	0.05	0.39	0.52	1.21	-0.47	0.52	-0.8	-33.38	-0.18
	2	0.62**	2.46***	0.09	2.99	-7.88*	2.81	-1.35*	9.48	0.16
	3	0.05	-0.33	-0.12	2.15	2.91	7.28**	0.74	-107.67	0.21
	4	0.02	3.1***	-0.69	-1.65	0.6	-3.22	-1.59*	133.28	0.18
	5	-0.86***	-0.61*	1.31*	-6.96*	2.68	2.78	-0.27	76.15	0.15
	6	-0.6**	0.53*	0.52	6.3*	3.89	-2.53	1.41*	-162.42	-0.13
	7	0.79***	-1.69***	-0.69	4.24	-5.39*	-3.98*	1.51*	-52.42	0.22
	8	0.67***	-1.26***	-0.05	1.83	-9.6**	-1.04	-0.22	-48.64	0.03
	9	-0.45**	-2.04***	0.74	-2.03	-5.09*	5.04*	0.01	176.13	-0.12
	10	-0.27*	-0.54*	-1.62**	-8.08*	18.36***	-7.67**	0.57	9.49	-0.52*
SE		0.14	0.23	0.45	3.17	2.63	1.89	0.54	0.00	0.14

* , ** and *** - significant at p<0.05, p<0.01 and p<0.001 probability respectively, GY-grain yield, ASI-anthesis-silking interval, HC-husk cover, AD-days to anthesis, ASI-anthesis-silking interval, ER-ear rot, ASP-*A.flavus*, PH-plant height, SL-stalk lodging, AFL- aflatoxin.

Table 4. 7: General combining ability for *Aspergillus* ear rot, aflatoxin, grain yield and agronomic traits at Katumani

	Parents	GY t/ha	AD days	ASI Days	PH Cm	SL %	HC %	ER %	ASP cfu/g	AFL ng/g
Female	1	-0.17*	3.86***	0.37*	10.26	0.71	-0.56	-0.16	-76.89	0.43*
	2	-0.58***	0.51	0.02	-11.47	4.77	-0.56	-0.16	62.78	-0.37*
	3	0.25*	-2.04***	0.12	33.79**	-4.4	-0.31	-0.16	212.77	-0.12
	4	-0.14	0.36	0.17	3.11	-2.4	0.6	0.63**	59.45	0.38*
	5	-0.32**	0.86*	-0.18	-13.1*	2.76	-0.43	-0.16	-21.22	0.1
	6	0.43**	-2.69***	-0.23	-15.06*	1.71	-0.82	0.15	-127.22	-0.18
	7	0.53***	-0.84*	-0.28	-7.53	-3.16	2.07**	-0.16	-109.70	-0.24
SE		0.09	0.29	0.16	6.38	3.65	0.58	0.12	0.00	0.12
Male	1	-0.35**	1.07*	0.21	3.53	1.87	-0.54	-0.16	26.54	0.02
	2	-0.63***	3.07***	0.07	-2.31	-0.87	-0.53	-0.16	92.77	-0.18
	3	-0.3*	-1*	-0.14	-12.72**	4.88	1.73*	-0.16	-120.07	-0.17
	4	-0.56***	4.57***	0.07	27.5***	-1.93	0.32	0.29*	-140.54	0.34*
	5	0.15	-2.14***	0.07	-12.2**	5.27	-1.09	-0.16	211.83	-0.04
	6	0.41**	1.36**	-0.07	15.65***	-6.32	1.22	0.33*	-135.78	0.36*
	7	0.66***	-1.07*	-0.21	12.43**	-5.8	0.05	0.25	216.59	0.06
	8	0.13	-0.64	0.14	-6.58*	4.8	-0.52	-0.16	-66.74	-0.14
	9	0.21*	-4.07***	0.36*	-13.83**	-7.05	0.44	-0.16	-101.17	-0.12
	10	0.28*	-1.14**	-0.5*	-11.48**	5.14	-1.09	0.08	16.57	-0.14
SE		0.11	0.36	0.20	7.81	4.47	0.71	0.15	0.00	0.14

* , ** and *** - significant at p<0.05, p<0.01 and p<0.001 probability respectively, SE= Standard error, GY-grain yield, ASI-anthesis-silking interval, HC-husk cover, AD-days to anthesis, ASI-anthesis-silking interval, ER-ear rot, ASP-*A.flavus*, PH-plant height, SL-stalk lodging, AFL- aflatoxin.

4.3.4: Specific combining ability of inbred lines for *Aspergillus* ear rot, aflatoxin accumulation, grain yield and agronomic traits across all sites

Single cross hybrids with the highest resistance to aflatoxin accumulation were 5/5, 2/4 and 4/9 with the lowest SCA values of -0.68, -0.51 and – 0.42 respectively. Those with the highest resistance to *Aspergillus* ear rot were 1/9, 7/6 and 6/10. The most susceptible single cross hybrid combinations were 2/8, 5/6 and 4/4. Single cross hybrids with the highest grain yield were 2/6, 4/8, and 3/6 with SCA values of 0.63, 0.65 and 0.61 respectively (Table 4.5).

Table 4. 8: Specific Combining ability for *A.flavus*, aflatoxin, grain yield and other agronomic traits across sites

Entry	Female	Male	GY (t/ha)	AD (days)	ASI (Days)	PH (Cm)	SL (%)	HC (%)	ER (%)	ASP (cfu/g)	AFL(ng/g)
1	1	1	0.17	-0.05	0.78	-0.27	1.29	-1.07	0.51	0.29*	0.43
2	1	2	0.3	1.66*	0.07	2.86	-7.85	0.18	-0.02	-0.07	-0.36
3	1	3	-0.13	-0.41	-0.47	-8.12	11.82*	-4.08	-0.1	-0.16	-0.7*
4	1	4	0.28	0.09	-0.04	-16.1	11.25*	0.49	-0.12	0.2	0.6*
5	1	5	0.1	-0.45	-0.29	9.22	-4.36	-0.55	-0.56	0.2	0.54
6	1	6	-0.94*	0.73	1.43*	3.5	-3.73	-0.36	0.63	0.16	-0.03
7	1	7	0.25	-0.95	-0.15	0.87	-6.03	2.77	0.08	0.14	0.04
8	1	8	0.18	0.13	-0.4	0.76	-12.21*	4.4	0.25	-0.17	0.03
9	1	9	0.12	0.48	-0.9	3.54	-9.22	-3.82	-0.7	-0.4*	-0.38
10	1	10	-0.33	-1.23*	-0.04	3.73	19.03*	2.04	0.04	-0.13	-0.19
11	2	1	-0.15	1.82*	0.33	10.24	-8.84	-2.29	0.95	-0.16	-0.14
12	2	2	-0.55	0.04	0.87	10.81	-0.88	2.45	1.86	0.03	0.2
13	2	3	-0.11	-1.04	-0.17	-6.1	4.71	-6.71*	0.76	-0.06	-0.12
14	2	4	0.01	-0.54	0.76	-6.82	-11.91*	6.78*	0.03	0.2	-0.09
15	2	5	-0.06	-0.32	-1.49*	1.02	19.97*	1.28	-0.41	0.1	-0.16
16	2	6	0.63*	-1.14	-0.03	-3.88	4.57	1.68	-1.49	0.01	-0.16
17	2	7	0.53	0.68	0.4	5	-1.24	-0.37	0.22	-0.01	0.37
18	2	8	-0.24	0.75	0.15	-4.72	-2.2	0.82	-0.43	-0.12	-0.09
19	2	9	-0.04	-0.64	-1.1	2.8	-6.43	-5.04*	-0.55	-0.01	0.04
20	2	10	-0.03	0.39	0.26	-8.36	2.23	1.42	-0.94	0.02	0.15
21	3	1	-0.05	-0.13	-1.39*	-12.93	14.2*	-0.46	0.27	-0.3*	-0.34
22	3	2	0.4	-0.16	-0.11	-11.38	-0.36	-2.4	0.54	0.03	-0.21
23	3	3	-0.48	0.01	0.86	41.41*	3.45	-2.48	0.81	0.05	0.05
24	3	4	-0.19	0.51	-0.71	48.16*	-5.1	-0.99	1.27	-0.05	-0.51
25	3	5	0.35	-0.52	-0.46	-9.2	-12.46*	6.14*	0	-0.2*	0.45
26	3	6	0.61*	0.41	-0.25	-7.5	-8.08	0.16	-1.08	-0.04	-0.06

Table 4. 8: Specific Combining ability for *A.flavus*, aflatoxin, grain yield and other agronomic traits across sites

Entry	Female	Male	GY (t/ha)	AD (days)	ASI (Days)	PH (Cm)	SL (%)	HC (%)	ER (%)	ASP (cfu/g)	AFL(ng/g)
27	3	7	-0.31	0.23	0.93	-9.58	1.91	0.27	-1.1	0.09	-0.11
28	3	8	-0.77*	0.05	0.93	-7.81	-0.46	-0.99	-0.03	0.18	0.89*
29	3	9	0.15	-0.09	0.43	-22.42*	-2.26	-2	-0.14	0.15	-0.16
30	3	10	0.29	-0.31	-0.21	-8.75	9.16	2.76	-0.54	0.17	0
31	4	1	0.06	0.85	0.03	-3.06	-10.13*	4.09	-1.07	-0.01	0.16
32	4	2	0.32	0.31	0.07	-4.26	3.37	-4.97*	0.09	0.08	-0.37
33	4	3	-0.21	-0.01	1.03	-2.49	-12.96*	0.62	0.85	-0.26	0.08
34	4	4	-0.13	1.24*	0.46	-1.18	-1.02	1.98	-0.9	0.05	0.67*
35	4	5	0.08	-0.05	0.21	1.3	3.65	-0.44	1.83	-0.31*	-0.01
36	4	6	-0.6	-0.87	-0.33	1.03	3.53	2.95	-0.72	0.11	-0.03
37	4	7	-0.05	0.45	-0.65	4.47	12.58*	-1.73	-1.01	-0.01	0.18
38	4	8	0.65*	-0.98	0.6	5.81	4.47	-2.51	-1.37	0.08	-0.39
39	4	9	-0.1	-1.37*	-0.9	-7.11	8.95	-2.39	1.62	0.05	-0.42
40	4	10	-0.03	0.42	-0.54	5.49	-12.43*	2.39	0.68	0.22	0.13
41	5	1	-0.07	-1.05	0.56	4.33	1.21	-0.75	-0.36	-0.04	-0.3
42	5	2	-0.02	-1.09	-0.16	2.71	4.1	2.02	-0.09	-0.29*	0.38
43	5	3	0.05	0.59	-0.94	-5.29	-1.45	0.21	-1.14	0.22	0.2
44	5	4	0.51	-0.16	-1.01	-12.62	3.2	-2.08	0.77	-0.12	0
45	5	5	-0.4	-0.2	1.49*	-3.26	-17.85*	-4.31	-0.63	0.17	-0.68*
46	5	6	0.17	0.73	-0.05	6.89	-7.32	3.05	-1.71	0.18	0.73*
47	5	7	-0.09	1.05	-1.12*	-4.84	2.3	-2.97	-1.73	0.06	-0.23
48	5	8	0.47	-0.13	-0.87	2.35	4.92	-1.09	2.16	-0.24	-0.36
49	5	9	-0.67*	0.73	2.38*	8.09	-4.52	7.81*	1.4	0.02	0.51
50	5	10	0.06	-0.48	-0.26	1.64	15.42*	-1.89	1.33	0.04	-0.24
51	6	1	0.18	-0.8	-0.42	6.28	3.19	-1.57	-0.56	0.17	-0.03
52	6	2	-0.85*	-0.34	0.12	3.18	-9.47	1.24	0.52	0.21	0.04
53	6	3	0.32	1.09	-0.17	-4.88	2.18	-2.47	0.19	0.03	0.39

Table 4. 8: Specific Combining ability for *A.flavus*, aflatoxin, grain yield and other agronomic traits across sites

Entry	Female	Male	GY (t/ha)	AD (days)	ASI (Days)	PH (Cm)	SL (%)	HC (%)	ER (%)	ASP (cfu/g)	AFL(ng/g)
54	6	4	-0.66*	-0.16	0.01	-8.84	0.87	-1.23	1.96	-0.02	-0.28
55	6	5	0.2	0.55	-0.74	1.61	6.99	-2.01	0.1	0.03	-0.14
56	6	6	0.49	-0.02	-0.53	-8.53	17.92*	-1.34	-1.12	-0.06	-0.21
57	6	7	-0.47	-0.2	0.9	-2.81	-9.45	5.12*	-1.92	-0.03	0.06
58	6	8	0.31	-0.38	-0.1	-2.49	5.61	3.22	0.82	-0.09	0.27
59	6	9	0.35	0.23	-0.1	10.44	2.5	0.55	-0.23	0.13	-0.19
60	6	10	0.14	0.02	1.01	6.05	-20.34*	-1.51	0.25	-0.35*	0.08
61	7	1	-0.13	-0.63	0.11	-4.59	-0.92	2.05	0.27	0.07	0.23
62	7	2	0.4	-0.41	-0.86	-3.92	11.1*	1.49	-2.91*	0.01	0.32
63	7	3	0.55	-0.24	-0.14	-14.54	-7.74	14.91*	-1.37	0.18	0.13
64	7	4	0.19	-0.99	0.54	-2.61	2.71	-4.95*	-3.01*	-0.27	-0.44
65	7	5	-0.27	0.98	1.29*	-0.7	4.07	-0.12	-0.32	0.08	0
66	7	6	-0.38	0.16	-0.25	8.5	-6.91	-6.13*	5.5*	-0.36*	-0.24
67	7	7	0.16	-1.27*	-0.32	6.89	-0.08	-3.08	5.45*	-0.23	-0.31
68	7	8	-0.6	0.55	-0.32	6.1	-0.13	-3.85	-1.39	0.36*	-0.35
69	7	9	0.19	0.66	0.18	4.67	10.97*	4.88*	-1.41	0.13	0.59*
70	7	10	-0.11	1.19*	-0.21	0.2	-13.08*	-5.21*	-0.81	0.05	0.07

GY-grain yield, ASI-anthesis-silking interval, HC-husk cover, AD-days to anthesis, ASI-anthesis-silking interval, ER-ear rot, ASP-*A.flavus*, PH-plant height, RL-root lodging, SL-stalk lodging, AFL- aflatoxin, * - significant at p<0.05 probability

4.4 Discussion

Analysis of variance showed highly significant differences among parents implying that the inbred lines were genetically diverse. Both GCA and SCA and their interaction with the environment were significant in the expression of traits. The Baker's ratio between the GCA and SCA was high for all traits in this study.

Genetic diversity in germplam is a prerequisite for a successful selection procedure in breeding (Khorzhoght *et al*., 2010; Asea *et al*., 2012; Hefny *et al*., 2012; Williams and Windham, 2015). The significant differences noted across the sites could be attributed to Kiboko and Katumani lying in different agro ecological zones (Mwacharo *et al*., 2004). Significant GCA and SCA mean squares suggest that both additive and non-additive gene action was important in the expression of traits in the F_1 hybrids hence contributions by parental inbred lines to their progenies were varied (Asea *et al*., 2012; Gethi *et al*., 2013).

The significance of SCA suggests that multiple testers may be needed in order to screen for disease resistance (Asea *et al*., 2012). The significant differences observed between the GCA and SCA interaction by the environment suggested that combining ability effects were influenced by variations in the environment. This implies that the parental lines could be selected at specific environments therefore the use of multiple environments to obtain reliable combining ability effects is recommended (Bhatnagar *et al*., 2004; Gethi *et al*, 2013 and Dar *et al*., 2014).

The high GCA: SCA ratio noted in this study suggests that additive gene action was more predominant than non-additive gene action in the expression of traits. This implies that parents greatly influenced the expression of traits in their progenies and therefore productivity could be enhanced by pyramiding genes in the parents before

making crosses to achieve genetic improvement (Hamblin and white, 1999; Khorzoght *et al*., 2010; Asea *et al*., 2012 and Williams and Windham, 2015).

These findings are consistent with Betran *et al*. (2002); Asea *et al*. (2012); Williams and Windham, (2009) and Warburton and Williams, (2014) who reported significant differences in maize inbred lines in combining ability studies for resistance to ear rot in maize. In a study to determine the combining ability of maize inbred lines for resistance to grey leaf spot and grain yield, Gethi *et al*. (2013) reported that both GCA and SCA were significant. Asea *et al*. (2012) also reported that both GCA and SCA played an important role in inheritance of resistance to *A.flavus* and aflatoxin accumulation.

However, Khorzoght *et al*. (2010) reported non-significant SCA for *Gibberella* ear rot in combining ability studies in maize. Mukanga *et al*. (2010) and Asea *et al*. (2012) also reported the significance of interaction between GCA by environment and SCA by environment in tropical maize using diallel analysis. Combining ability studies for resistance to *Aspergillus flavus* and aflatoxin accumulation in maize by Hamblin and white (1999), Khorzoght *et al*. (2010), Asea *et al*. (2012) and Williams and Windham (2015) reported a high GCA: SCA ratio in the inheritance of resistance.

Male parents 10 and 6 had negative GCA for *Aspergillus* ear rot and aflatoxin accumulation across the two sites. Male inbred line 4 had a high GCA for susceptibility to *Aspergillus* ear rot and aflatoxin accumulation. Male inbred line 7 and female inbred line 6 had high GCA for grain yield and low GCA for flowering. Single crosses between 5/5, 3/4 and 4/9 produced the most resistant hybrids.

These lines possessing resistance to *Aspergillus* ear rot and flatoxin accumulation had been previously registered and released as resistant germplasm (Williams and

Windham, 2001; Williams and Windham, 2006; Williams and Windham, 2012). Williams and Windham (2012) reported male inbred line 4 to be resistant to *Aspergillus* ear rot and flatoxin accumulation although in this study it had a high GCA for susceptibility. General combining ability (GCA) effects enable the identification of superior parents that could be used to make better crosses in a breeding program (Simmonds, 1979). Negative GCA effects are desirable in disease resistance studies as they indicate contribution of a genotype towards resistance while positive GCA effects indicate a tendency towards susceptibility (Simmonds, 1979).

The negative GCA observed in male parents 10 and 6 across all sites suggested that these lines could be used as sources of resistance to *Aspergillus* ear rot and aflatoxin accumulation in breeding programs. In this study, male inbred line 4 had a high GCA for susceptibility to *Aspergillus* ear rot and aflatoxin accumulation, although it had earlier been reported to be resistant (Williams and Windham, 2012). This could also be as a result of the resistance being polygenic hence it is controlled by many minor genes that are highly influenced by changes in the environment (Betran *et al*., 2002; Asea *et al*., 2012; Warburton and Williams, 2014). Male inbred line 7 and female inbred line 6 had high GCA for grain yield and low GCA for flowering implying that early maturity could have translated to the high grain yield. These lines could be incorporated into resistance breeding programs for improved productivity.

Most of the promising single cross hybrid combinations were from parents with good combining abilities for resistance and grain yield implying that the resistant hybrids had more grain yield compared to the susceptible hybrids. These findings suggest that additive and non-additive gene action in the inbred lines complement to produce resistant hybrids with high grain yield (Solanki and Gupta, 2001). The crosses producing the most resistant hybrids involved inbred lines MP 717, MP 715 and Hi27

which had earlier been reported to be resistant (Williams and Windham, 2006; Williams and Windham, 2012). These single cross hybrids could be further tested across multiple locations and released to farmers.

The genetic diversity of germplasm revealed in this study is very important to breeders as it can be exploited in breeding programs to develop superior genotypes. Female inbred line 2 and male inbred line 10 have been identified as the best combiners for resistance. These lines should be extensively tested under different agro ecological zones to validate their performance across locations. The most resistant single crosses have been identified as those between parents 5/5, 3/4 and 4/9. These crosses should be further tested before being released to farmers.

CHAPTER FIVE

CONCLUSSION AND RECOMMENDATIONS

5.1 Conclusion

Seventy single cross hybrids were developed from seventeen maize inbred lines following North Carolina II (NCII) mating design. These hybrids were evaluated under two different environments together with five hybrid checks. There was great genetic variability among the single cross hybrids across all environments. Hybrids D1192-18, D1192-20, D1192-31, D1192-59, D1192-60, D1192-66, D1192-69 and D1192-68 were identified as the top performers for grain yield, resistance to *Aspergillus* ear rot and aflatoxin accumulation across the sites. Consequently, these hybrids are from parental lines that also emerged as the best combiners for grain yield, *Aspergillus* ear rot and aflatoxin accumulation. Hybrids D1192-37, D1192-56 and D1192-67 also had high grain yield with low aflatoxin concentrations.

Additive gene action was identified to be predominant over non-additive gene action in the inheritance of resistance to *Aspergillus* ear rot and aflatoxin accumulation in the hybrids. Parents Mp 313E and Mp719 and P329 were identified as the best combiners for resistance to *Aspergillus* ear rot and aflatoxin accumulation while parents NC298 and (CKL05003/La Posta Seq C7-F180-3-1-1-1-B-B -B)DH152-B-B were identified as the best combiners for grain yield. Important phenotypic correlations were observed between agronomic traits, *Aspergillus* ear rot and aflatoxin accumulation reaffirming the need to develop resistant lines for reduced yield losses and aflatoxin contamination. Husk cover was identified as an important guide when carrying out phenotypic selection for resistance to *Aspergillus* ear rot and aflatoxin accumulation. It was noted that days to maturity *per se* does not influence resistance to *Aspergillus* ear rot and aflatoxin accumulation. However, timely planting should be encouraged to

ensure that plants form cobs early enough before the onset of drought since aflatoxin accumulation is exacerbated by plant stress.

The effectiveness of selection for resistance to *Aspergillus* ear rot and aflatoxin accumulation can be enhanced by pyramiding genes conferring resistance in parental inbred lines such that crossing the parents will result in highly superior hybrids. This will be made possible by the presence of significant additive and non additive gene effects observed in this study. It is also necessary to carry out heterotic grouping of the inbred lines used in this study in order to devise schemes for effective crossing and thereby develop potentially high yielding and resistant hybrids.

5.2 Recommendations

i.   The germplasm identified to be resistant to *Aspergillus* ear rot and aflatoxin accumulation and possessing desirable agronomic traits with high grain yield should be evaluated further in multiple locations to validate their performance and released to farmers.

ii. The germplasm identified to be resistant to *Aspergillus* ear rot and aflatoxin accumulation but with poor agronomic traits should be used as donor materials for introgression of the resistance into the locally adapted genotypes.

iii. The susceptible inbred lines and single crosses identified in this study could be used as checks in studies involving resistance to *Aspergillus flavus* and aflatoxin accumulation.

iv. The resistance observed in the genotypes in this study could be enhanced by pyramiding genes conferring resistance to *Aspergillus* ear rot and aflatoxin accumulation in parental inbred lines before using them to develop hybrids.

v.   The duration of breeding for resistance to *Aspergillus* ear rot and aflatoxin accumulation could be shortened by adoption of marker assisted selection.

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APPENDICES

Appendix 1: Grain yield, agronomic traits, *Aspergillus* and aflatoxin content of all entries at Kiboko

Entry	GY	AD	ASI	PH	SL	HC	ER	LD	ED	OIL	STAR	ASP	AFL
	t/ha	Days	days	Cm	%	%	%	%	%	%	%	cfu/g	ng/g
1	3.7	58	5	218	23.9	7	1.6	4.1	6.5	6.2	67.5	566.7	91.9
2	5.4	62	4	223	4.9	7.5	0	6.9	6.7	5.9	67.6	500	10.6
3	3.6	56	4	215	40.9	8.9	1.9	7.8	6.8	5.5	68.8	180	5.12
4	4.9	60	3	208	25.9	2.5	0	4.8	8.5	5.5	68.4	633.3	86.5
5	3.3	56	5	222	27.5	7.6	0	5.9	7.3	6.1	67.2	400	82.7
6	1.8	58	8	226	25.5	2.4	4.6	7.1	6.1	5.9	66.8	400	9.55
7	5.2	55	4	215	11.3	7.3	3.5	5.9	7.2	5.7	67.7	500	110
8	4.6	58	3	217	7.5	14.8	1.7	4.8	8.4	6.4	66.7	233.3	69.3
9	3	57	4	240	10.1	5.6	0	6	6.3	5.2	66.6	100	10.8
10	3	57	2	209	70.9	2.7	2.3	4.9	7.2	5.1	68.8	166.7	2.37
11	3.7	57	4	217	10.3	3.3	2.2	6.1	6.1	6.2	65.4	266.7	2.57
12	2.7	60	6	218	1.2	15.7	3.5	4.6	7.1	5.3	67.2	800	14.2
13	3.5	55	3	216	28.3	3.8	3.4	6.7	7.2	5.3	66.3	400	3.19
14	3.1	58	4	215	3.6	20.9	0	3.9	8.5	5.2	67.2	900	9.24
15	2.6	55	2	211	34	11.8	0	6.5	5	6	66	2410	3.09
16	2.9	56	4	210	18.7	9.3	0	5.6	6.6	5.7	67.5	433.3	5.7
17	4.5	54	3	213	9	2.4	3.5	6.4	4.7	5.7	66.3	666.7	47.1
18	3.9	56	4	209	0	7.4	0	4.9	8	6	65.7	200	3.02
19	2.4	54	2	201	6.4	4.8	0	4.1	7.6	4.9	67.1	533.3	3.59
20	3.1	57	2	185	35.5	1.8	0	4.8	7.8	5.2	66.6	233.3	2.7
21	2.7	58	2	212	19.8	5.9	0	6.4	7.1	6.3	66.6	500	4.45
22	3.5	60	3	217	27.5	7.7	0	7.7	7	5.6	67.6	566.7	6.16

Appendix 1: Grain yield, agronomic traits, *Aspergillus* and aflatoxin content of all entries at Kiboko

Entry	GY	AD	ASI	PH	SL	HC	ER	LD	ED	OIL	STAR	ASP	AFL
23	2.2	60	5	217	16.2	10.3	2.7	4.9	9.3	5.6	66.6	466.7	45.2
24	2	63	2	209	19.5	4.4	1.7	7.2	7.5	5.1	68.5	833.3	10.3
25	2	57	4	218	7.6	24	0	7.5	6.7	5.9	66	300	261
26	2.4	59	4	213	12.6	7.4	0	4.9	5.2	4.7	68.7	366.7	17.2
27	2.7	57	5	223	8.7	4	0	3.8	7.2	5.6	66.4	466.7	31.4
28	2.7	57	5	215	1.2	3.6	0	4.5	9.5	6	66	1000	67.9
29	3.2	56	6	191	8.8	8.4	0	3.5	8	4.7	67.6	933.3	8.2
30	3.6	56	1	206	38.5	4	0	4	7.2	5.2	67.2	266.7	2.96
31	4.1	59	6	201	1.2	21.2	0	4.9	6.1	5.5	67.9	366.7	2.87
32	4.4	62	4	202	7.4	7.1	1.8	5.8	5.9	5.2	69	900	6.36
33	3.7	58	6	211	3.8	23.4	5.4	5.3	9.2	5.5	67.7	266.7	52.1
34	2.8	63	5	211	15.5	10.3	0	6.5	6.7	4.6	68.5	566.7	11.6
35	2.6	57	6	194	9.9	16.4	6.4	8.3	6.2	5.9	67	666.7	6.71
36	2.9	59	4	217	19.1	9.4	0	5.2	8	5.5	67.9	533.3	14.2
37	5.1	57	2	207	26.7	2.8	0	6.4	7.9	5.6	67.5	700	4.1
38	5.8	55	5	220	18.4	8.1	0	4.9	5.2	6	67.8	433.3	6
39	3.1	55	3	199	26.2	14	6.2	5.5	7.5	4.7	68.3	700	3.43
40	3.7	59	2	216	25.5	11	3.5	5.6	7	4.9	68.6	900	10.8
41	2.7	59	8	224	14.6	14.5	0	6.2	6.9	6.2	66.8	1033.3	4.25
42	3.6	60	6	204	1.2	21.9	0	3.6	6	5.5	67.4	233.3	239
43	2.8	58	4	210	18.1	25.4	0	4.6	6	5.7	67.9	633.3	131
44	3.8	61	3	204	10.6	8.2	1.9	6.9	8.9	5.4	68.5	1033.3	93.6
45	0.9	58	10	187	9	9.7	0	8	7	5.9	66.8	833.3	3.33
46	2.9	59	7	225	18.2	18.2	0	6.1	6.3	6	66.9	566.7	16.3
47	4.3	57	3	213	6.2	2.8	0	6.9	6.2	5.8	67.2	766.7	37.5

Appendix 1: Grain yield, agronomic traits, *Aspergillus* and aflatoxin content of all entries at Kiboko

Entry	GY	AD	ASI	PH	SL	HC	ER	LD	ED	OIL	STAR	ASP	AFL
48	3.8	57	4	210	7.1	11.1	5.6	7.8	7.1	6.4	66.1	533.3	9.06
49	1.2	56	11	208	9.3	36.1	4.4	4	7.2	5.3	66.5	1000	101
50	2.3	56	4	189	41.7	1.7	5	5.6	9.4	4.9	68.6	466.7	3.59
51	4.7	56	1	217	17.1	11.1	0	4.2	5.9	5.8	67.7	433.3	4.69
52	4	57	1	223	5.1	16.7	1.6	4.9	7.5	5	68.4	633.3	7.44
53	4.6	56	1	228	11.2	20.7	3	4.8	7.4	5	68	833.3	22.4
54	3.5	60	1	207	8.9	7.1	1.6	4.6	8	4.9	68.2	766.7	14
55	3.6	57	1	206	17.4	13.3	1.9	5.7	6.8	5.8	66.8	500	10.9
56	5.1	57	1	205	33.3	9	1.6	5.3	6.5	5.9	66.4	566.7	3.39
57	3.9	56	2	219	3.9	20.9	0	6.1	7.2	5.6	66.8	633.3	8.67
58	5.5	54	1	206	3.5	17.1	3.4	4.9	7	5.9	67.3	533.3	35
59	4.4	54	1	217	8.6	20.5	1.5	2.7	5.6	5	67.6	766.7	3.19
60	4.8	55	1	216	6.2	1.3	3.3	8	7.8	4.8	67.6	200	4.26
61	2.6	57	4	195	21.7	25.2	6.9	5.9	6.8	5.4	67.5	833.3	36.5
62	4.8	58	2	212	9.7	26.5	0	3.6	5.5	5.3	67.8	666.7	53.3
63	3.1	56	2	195	13.9	45.2	5.2	4.7	6.1	5	66.8	700	9.95
64	3.2	58	4	210	32.4	9.2	0	4.7	8.2	5	67.3	433.3	5.8
65	1.4	57	7	191	25.6	25.1	6.3	4.6	7.4	5.5	67.2	1066.7	29.3
66	1.6	57	3	225	11.8	11	20.1	4.5	6.8	5	68.4	230	6.66
67	3.3	54	2	215	8.9	14.3	20	5.2	7	4.9	68	133.3	6.15
68	2.8	57	3	212	7.1	13.3	4.2	6	9.5	5.6	66.5	160	2.49
69	2.6	56	4	206	7.3	30.6	4.4	5.7	8.6	4.8	66.1	1433.3	17.4
70	2.5	57	2	200	22.3	3.9	6.3	5.4	7.3	4.7	67.3	266.7	3.07
71	3.2	59	3	213	7.2	3.6	6.8	6.1	7.8	5.4	68.9	900	80.6
72	2.5	62	6	199	16.1	14.5	8.5	4.1	9	5.5	68.2	1133.3	24.8

Appendix 1: Grain yield, agronomic traits, *Aspergillus* and aflatoxin content of all entries at Kiboko

Entry	GY	AD	ASI	PH	SL	HC	ER	LD	ED	OIL	STAR	ASP	AFL
73	3.5	57	5	210	13.2	6.1	5.9	3.6	6.6	5.6	68.1	1100	59
74	3	61	3	207	9.8	6	0	4.2	6.9	5.5	68.5	186.7	2.45
75	2.1	61	7	207	9.8	3	1.9	3.9	8.4	5.4	68	1133.3	86.3
Mean	3.3	57.6	3.5	210.7	15.7	11.7	2.5	5.4	7.2	5.4	67.4	628.9	65
LSD (0.05)	1.4	2.5	3.9	27.6	25.4	14.7	6	3.3	2.7	0.5	1.1	20.6	1.4
CV (%)	20.7	2.2	55.4	6.6	81.2	62.6	122.4	30.6	19	4.2	0.8	13.9	61.7

*-significant at p<0.05, GY-grain yield, ED-ear damage, LD-leaf damage, ASI-anthesis-silking interval, HC-husk cover, ER-ear rot, STAR-starch content, SL-stalk lodging, EH-ear height, PH-plant height, AFL-aflatoxin, ASP-*A.flavus*

Appendix 2: Grain yield, agronomic traits, *Aspergillus* and aflatoxin content of all entries at Katumani

Entry	GY	AD	ASI	PH	RL	SL	HC	ER	LD	ED	OIL	STAR	ASP	AFL
	t/ha	days	days	cm	%	%	%	%	%	%	%	%	cfu/g	ng/g
1	2.1	76	5	253	3.3	36.5	0	0	8.4	11.4	5	69.42	733	26.2
2	1.95	78	3	260	0.3	27.2	1.3	0	3.8	8.7	5	69.21	267	8.7
3	2.17	74	2	251	-0.1	47	0	0	4.4	9.6	3.9	71.46	210	3.7
4	1.79	79	3	267	2.9	51.9	1.3	0	4.5	10	4.3	69.71	500	374.7
5	2.49	72	3	253	14.9	28.2	0	0	9	10.9	5.4	68.92	1200	93.2
6	3.11	76	2	291	8	21.2	1.4	0	4.9	10.9	4.6	69.45	400	76.5
7	3.15	72	2	280	-0.1	22	1.3	0	3.5	8.9	4.6	69.61	567	10.3
8	3.1	72	3	265	0	19.8	0	0	9.5	10.2	5.4	68.96	167	6.4
9	2.73	71	2	219	1.8	15.9	0	0	3.4	9.2	4	69.12	160	4.7
10	2.75	72	2	260	6.8	47.1	0	0	4.6	7.5	4.2	70.27	433	18.8
11	1.36	75	3	254	4	23.8	0	0	3.9	7.7	5.6	67.51	167	2.5

Appendix 2: Grain yield, agronomic traits, *Aspergillus* and aflatoxin content of all entries at Katumani

Entry	GY	AD	ASI	PH	RL	SL	HC	ER	LD	ED	OIL	STAR	ASP	AFL
12	1.58	73	2	246	4.3	38.7	0	0	11.3	12.4	4.9	67.67	567	3
13	1.59	69	2	219	1.4	39.3	0	0	7.8	7.8	4.5	68.31	367	3.6
14	1.3	76	3	250	0.1	21.6	0	0	3.8	9.6	4.6	68.86	767	4.3
15	2.26	68	2	222	33.1	64.3	0	0	9.7	10.1	5.5	66.81	800	3.6
16	3.23	71	2	260	2.5	38.3	2.7	0	2.3	10.7	5	69.16	467	2.6
17	3	72	2	249	1.8	27.8	1.3	0	7.3	13.5	4.7	68.42	633	4
18	2.12	71	3	227	0.2	41.3	1.3	0	3.8	8.4	5.6	66.75	200	3
19	2.24	66	2	227	0.4	19.1	0	0	3.9	8.6	4.6	66.07	633	3.5
20	2.55	69	3	230	1.5	42.9	0	0	2.8	7.8	5.2	68.01	233	2.9
21	2.75	70	2	260	0	52.7	1.2	0	3.5	8.2	4.8	69.06	200	3.3
22	2.71	72	3	263	2.6	5.6	0	0	2.9	7.6	4.5	69.46	933	6.1
23	2.43	66	3	355	0.5	41.1	1.4	0	9.8	10	4.4	69.37	300	3.1
24	2.37	73	2	409	1.7	11.5	0	0	7.6	10.3	4.7	69.27	433	3.1
25	3.14	66	3	252	5.3	18.1	0	0	2.2	9.7	5.5	68.21	667	4.1
26	3.84	71	2	290	0.5	11.3	1.3	0	5.3	11	4.8	69.47	467	7.8
27	3.16	68	2	261	4.4	26.5	2.7	0	6	8.8	4.5	69.21	1600	3.9
28	2.88	68	3	269	3.8	35.7	0	0	7.9	9.9	5.5	68.2	633	73.5
29	2.68	64	3	233	0.5	17.2	1.2	0	6.1	10.2	4.6	67.36	767	3.5
30	3.24	67	2	260	1.3	45.9	0	0	2.9	10.6	4.3	69.25	1133	7.7
31	2.54	73	2	255	0.1	23.8	0	0	3	9.5	4.5	70.31	767	71.4
32	2.58	73	3	252	1.4	34.6	0	0	4.8	10.3	4.4	70.05	700	4
33	2.15	70	3	236	1.9	22	0	0	7.3	13.9	4	71.12	267	4.4
34	2.11	77	2	271	1.8	25.1	6	0	6.5	11.4	3.6	70.37	567	875.6
35	2.66	69	3	251	0.5	49.4	0	0	4.1	9.7	4.5	70.17	200	27.4
36	2.27	70	3	273	2.8	29.5	8.3	3.4	5.6	10.8	4	71.31	667	15.2

Appendix 2: Grain yield, agronomic traits, *Aspergillus* and aflatoxin content of all entries at Katumani

Entry	GY	AD	ASI	PH	RL	SL	HC	ER	LD	ED	OIL	STAR	ASP	AFL
37	3.2	71	2	275	0	31.2	1.4	2.9	3.5	9.4	4.2	70.21	667	151
38	2.59	70	3	249	0.3	29.6	0	0	7.8	8.4	5.4	69.06	733	3.1
39	2.67	65	3	219	0.6	23.4	1.3	0	4	7.7	3.7	69.37	600	3.4
40	2.62	69	1	245	-0.1	17	0	1.7	3.2	9	3.8	70.66	833	5.5
41	2	71	2	232	0	36.4	0	0	3.1	8.9	5.4	68.61	267	9.5
42	1.4	73	2	236	0.8	45.6	0	0	3.5	9.1	4.6	69.07	433	5.6
43	2.2	71	2	222	0.1	34.2	0	0	1.9	8.4	4.7	69.56	733	5
44	1.56	76	2	242	0.3	41.8	1.3	0	4.8	7.7	4.2	69.71	167	8.4
45	2.73	68	2	240	0.1	10.6	0	0	6.1	10.2	5.2	68.75	1167	4
46	2.53	74	2	253	0.7	12.1	1.3	0	3.1	9	5	68.97	600	724.6
47	2.65	71	2	226	0.4	34.6	1.4	0	5.1	7.8	5	68.96	700	4.3
48	2.83	70	2	243	-0.1	45.2	2.7	0	3.9	8.1	5.4	68.15	117	4
49	2.65	69	3	231	3.9	16.8	0	0	3.6	6.2	4.7	68.01	310	14.6
50	3.16	71	1	238	6.9	60	0	0	4.8	9.3	4.4	69.27	700	4.8
51	3.1	68	2	250	-0.1	34.8	0	0	2.4	6.4	4.8	69.26	900	5.5
52	1.96	72	2	234	0.1	11.5	1.3	0	5.8	8.5	5.2	69.16	1000	6.6
53	2.67	69	2	206	0.4	45.1	0	0	7.5	6.9	4.5	69.31	167	12.7
54	2.54	71	2	251	1.8	35.7	0	3.2	7.1	8.6	4.2	70.11	200	2.7
55	3.03	66	2	234	41.1	48.7	0	0	4.8	9.1	5	68.57	200	2.8
56	3.33	68	2	249	1.9	44.3	1.4	0	4.6	8	4.5	69.41	133	8
57	3.79	65	3	228	0.5	10.3	0	0	5.6	11.4	4.4	69.56	333	12.3
58	3.8	67	2	226	0.8	47.2	0	0	6.1	6.7	5.3	68.87	167	3.4
59	3.27	65	3	227	1.6	28.6	0	0	4.9	8.4	4.5	67.52	367	3.3
60	3.15	68	2	224	3	20.8	0	0	2.6	8.2	4.3	69.42	167	3.2
61	2.76	70	2	247	0.1	21.7	2.7	0	2.6	8.2	4.7	69.16	436	2.5

Appendix 2: Grain yield, agronomic traits, *Aspergillus* and aflatoxin content of all entries at Katumani

Entry	GY	AD	ASI	PH	RL	SL	HC	ER	LD	ED	OIL	STAR	ASP	AFL
62	2.76	72	2	231	1.4	47.5	1.3	0	5.6	8.6	4.3	69.3	533	3.7
63	3.24	69	2	219	0.4	22.3	18.3	0	5.5	9.6	4.2	68.87	500	9.1
64	2.68	74	2	264	9.1	15.5	1.3	0	7.9	13.9	4.1	69.56	167	3.5
65	3.22	69	2	242	0.3	34.4	0	0	8.4	9.4	4.9	68.42	133	2.1
66	3.28	71	2	264	0.4	15.8	0	0	6.1	9.1	4.2	69.71	100	3.9
67	4.28	67	2	249	1.9	23.6	0	0	4.2	8.7	4.7	68.86	800	3.4
68	3.08	69	2	242	0.5	31.7	0	0	5.3	11.2	5.3	67.41	185	2.9
69	3.3	66	3	220	7.5	46.5	8.1	0	3.4	8.5	4.3	67.82	239	23.2
70	3.43	70	1	235	1.4	19.1	0	0	4.4	7.1	4	69.66	200	4.5
71	2.95	72	2	268	3.2	26.9	0	0	3.1	10	4.4	70.87	367	7.7
72	2.62	70	3	280	1.5	48.1	0	1.7	2.1	10.9	5.3	68.91	367	37.3
73	2.48	71	2	259	0.9	25.3	0	0	3.8	8.8	4.6	70.37	667	5.6
74	2.44	71	2	257	0.8	18	0	0	4.3	7	4.2	70.22	700	10.1
75	1.29	72	2	255	1.3	74.7	1.6	0	6.9	10.1	4.4	69.86	633	8.2
Mean	2.7	70	2	250	2.8	31.4	1	0.2	5.1	9.3	4.6	69.1	541	40.3
LSD (0.05)	1	3	2	58	15	37.8	5.7	1.3	5.1	5	0.6	0.9	6.4	1.1
CV (%)	17.9	2	32	12	264.8	60.3	275.6	379.3	49.4	26.7	6.2	0.7	19.2	63.7

*-significant at p<0.05, GY-grain yield, ED-ear damage, LD-leaf damage, ASI-anthesis-silking interval, HC-husk cover, ER-ear rot, STAR-starch content, SL-stalk lodging, EH-ear height, PH-plant height, AFL-aflatoxin, ASP-*A.flavus*

Appendix 3: Grain yield, agronomic traits, *Aspergillus* and aflatoxin content of all entries across sites

Entry	GY	AD	ASI	PH	RL	SL	HC	ER	LD	ED	OIL	STAR	ASP	AFL
	t/ha	days	days	cm	%	%	%	%	%	%	%	%	cfu/g	ng/g
1	3.4	66	4.6	240.6	1.6	30.5	1.4	0.3	6.6	7.3	5.6	68.6	566.7	91.9
2	4	68.9	3.6	243.8	2	19.1	3.3	0.7	5.4	6.9	5.4	68.6	500	10.6
3	3.5	64	2.7	232	-0.1	46.2	2.9	1.7	6.4	6.8	4.7	70.2	180	5.1
4	3.7	68.9	3.1	239.8	1.4	41.6	2	-0.5	4.9	8.9	4.9	69.2	633.3	86.5
5	3.1	64.4	3.7	244	7	25.6	2.7	0.6	7.4	7.8	5.7	68.2	400	82.7
6	2.9	66.2	5	258.5	5.3	24.1	1.5	2	6	7.4	5.2	68.2	400	9.5
7	4.4	63	2.8	246.5	0.1	13.4	3.6	1.6	4.8	7.1	5.2	68.7	500	110.2
8	4.3	64.1	3.2	241.1	-0.3	15.7	6.1	0.2	7.2	8.7	5.9	67.9	233.3	69.3
9	3.4	63.2	3.2	233.4	0.5	16.2	1.7	0.2	4.7	6.1	4.6	68	100	10.8
10	3.5	63.7	2.3	236.9	4	54.4	-0.5	0.7	4.9	6.2	4.6	69.6	166.7	2.4
11	2.7	65.2	3.6	235.3	2.5	18.2	1.2	2.2	4.9	6.3	5.9	66.5	266.7	2.6
12	2.5	65.6	3.7	235.9	2.3	20.2	7	2	8.1	9.5	5.1	67.6	800	14.2
13	2.9	61.3	2.6	218.2	0.4	36.1	-0.1	0.3	7.5	6	4.9	67.4	400	3.2
14	2.9	66.2	3.4	233.3	0.6	9	8.5	-0.4	4.1	7.6	4.9	68.1	900	9.2
15	2.7	62	2.1	220	18.8	47.8	5	0.8	7.7	6.3	5.7	66.4	2410	3.1
16	3.8	62.8	2.9	235.3	1.2	27.6	5	0.5	4.1	7.3	5.4	68.4	433.3	5.7
17	4.1	62.4	2.8	234.8	-0.8	20.6	0.6	1.4	7.3	7.5	5.2	67.6	666.7	47.1
18	3.5	62.3	3.1	219.8	-0.1	19.5	3.8	0.9	4.7	7	5.8	66.3	200	3
19	2.8	59.7	2.2	216.8	0.7	11.1	0.1	-0.4	4	7.2	4.7	66.7	533.3	3.6
20	3.3	62	2	208.9	0.5	40.6	-0.2	0.3	4.1	6.3	5.2	67.4	233.3	2.7

Appendix 3: Grain yield, agronomic traits, *Aspergillus* and aflatoxin content of all entries across sites

Entry	GY	AD	ASI	PH	RL	SL	HC	ER	LD	ED	OIL	STAR	ASP	AFL
21	3.4	62.7	2	236	5.8	36.8	1.9	0.4	5.2	6.5	5.5	67.9	500	4.4
22	3.7	65.5	3.1	237.6	-1	14.3	1.7	-1.2	5.2	5.5	5.1	68.6	566.7	6.2
23	2.7	62.1	3.7	289.6	0.2	28.6	4.2	1.6	7.4	8.5	5	68	466.7	45.2
24	2.5	66.8	2.1	312.1	1.3	18.3	1.4	1.6	7.9	7.4	4.8	69	833.3	10.3
25	3.1	61.2	3.4	233.6	2.9	15.5	10.6	0.8	4.6	6.8	5.7	67.3	300	260.8
26	3.7	64.5	3	255.6	0.4	15.2	4.5	0.2	5.3	6.4	4.8	69.1	366.7	17.2
27	3.5	62	3.4	244.1	8.4	17.9	1.2	-0.3	4.7	7.4	5	68.1	466.7	31.4
28	3.3	61.1	3.8	240.6	1.6	19.1	0.2	0.9	6.2	9.2	5.7	67.2	1000	67.9
29	3.3	58.9	4	215.5	5.5	15.8	3.4	0	4.9	8.1	4.6	67.6	933.3	8.2
30	3.8	61.1	1.7	232.4	2.1	42.1	0.9	0.7	3.7	7.6	4.7	68.3	266.7	3
31	3.7	65.3	3.9	228.4	3.2	11.1	9.8	0.2	4.2	6.5	5	69.2	366.7	2.9
32	3.7	67.1	3.5	227.3	0.7	20	1.6	0.6	5.5	7.5	4.8	69.6	900	6.4
33	3.3	63.4	4.2	228.2	0.9	13.7	10.8	3	6.3	10	4.7	69.4	266.7	52.1
34	3.2	69.5	3.3	245.3	0.6	22.5	5.7	-0.4	6.6	6.9	4.1	69.6	566.7	11.6
35	3	63.3	4.4	226.7	1.2	25.2	6.4	4.1	6.5	7.3	5.2	68.8	666.7	6.7
36	3.1	63.8	3.2	246.6	1	21.6	7.4	1.9	5.3	8.6	4.7	69.7	533.3	14.2
37	4.5	63.4	2.4	240.7	0.4	30.4	2.2	0.9	5	7.6	4.8	68.9	700	4.1
38	4.8	62.8	4	236.8	-1.6	22.5	2.7	0.8	6.4	5.8	5.7	68.5	433.3	6
39	3.6	59.8	3.2	213.3	-0.2	26	5.7	2.8	4.8	6.6	4.2	68.9	700	3.4
40	3.8	63.1	1.5	229.2	1.5	19.2	4	3.4	4.5	6.6	4.3	69.7	900	10.8
41	2.7	63.7	4.9	227.5	2.5	29.4	6.1	-0.4	4.7	6.3	5.7	67.8	1033.3	4.2
42	3	65.2	4.1	225.9	0.4	26.3	10	0.4	3.9	6.7	5	68.3	233.3	239.2

Appendix 3: Grain yield, agronomic traits, *Aspergillus* and aflatoxin content of all entries across sites

Entry	GY	AD	ASI	PH	RL	SL	HC	ER	LD	ED	OIL	STAR	ASP	AFL
43	3.2	63.4	2.9	217.2	0.9	26.7	11.3	0.7	3.6	6.1	5.2	68.8	633.3	130.6
44	3.4	68.2	2.7	225.6	-0.2	24.1	3.1	0.4	5.7	7.6	4.8	69.2	1033.3	93.6
45	2.4	62.2	5.9	213.8	-1.2	12.5	2.8	0.2	6.9	7.4	5.6	67.9	833.3	3.3
46	3.4	65.9	4.3	244.2	1.1	15.2	8.9	0.5	4.8	6.5	5.5	68	566.7	16.3
47	4	63.7	2.4	223.1	0.4	17	2.1	0.5	6.2	6.9	5.3	68.2	766.7	37.5
48	3.6	63.2	3.3	225	-0.5	24.2	4.8	3.2	6.3	7.3	5.9	67.3	533.3	9.1
49	2.4	61.5	7	220.2	2.1	12.2	17	2.5	4.2	6.1	5	67.3	1000	100.7
50	3.5	62.4	2.5	217.1	3.2	51.7	0.2	2.4	5.7	7.7	4.6	69	466.7	3.6
51	4.4	61.5	1.5	232.1	0.3	23.7	4.4	-0.1	3.4	4.7	5.3	68.4	433.3	4.7
52	3.4	64.4	1.7	229	0.5	11.6	8.4	0.7	5.4	6.3	5.1	68.8	633.3	7.4
53	4.4	61.8	1.2	220.1	0.1	24	8.5	1.6	6.3	6.6	4.8	68.8	833.3	22.4
54	3.4	64.8	1.2	231.9	2.5	19.4	3.2	3.2	5.9	7.5	4.5	69.2	766.7	14
55	3.8	60.7	1.6	221.3	21	33.1	4	-1.2	5.3	6.2	5.4	67.9	500	10.9
56	4.7	62	1.3	231.4	2	37.8	3.8	0.5	5.3	6.6	5.2	68	566.7	3.4
57	4.4	59.2	2	227.7	0.8	8.6	9.5	0.2	6	8	5	68.3	633.3	8.7
58	4.8	60.1	1.6	222.8	0.1	23.7	7.7	1.6	5.8	5.6	5.6	68.2	533.3	35
59	4.5	58.7	1.9	225.1	0.4	17.1	9.8	-0.6	3.9	5.9	4.8	67.6	766.7	3.2
60	4.3	60.9	1.6	224.1	1.8	14.3	-0.7	2.1	5.2	6.6	4.5	68.6	200	4.3
61	3.2	63.2	3.1	220.8	0.5	23.2	12.5	2.4	4.4	6.2	5	68.4	833.3	36.5
62	4.2	64.6	1.9	221.5	0.3	26.6	12.7	0.4	4.8	6.2	4.8	68.5	666.7	53.3
63	3.5	62.6	2.5	210.1	0.1	18.7	29.6	1.4	5	6.3	4.6	68	700	10

Appendix 3: Grain yield, agronomic traits, *Aspergillus* and aflatoxin content of all entries across sites

Entry	GY	AD	ASI	PH	RL	SL	HC	ER	LD	ED	OIL	STAR	ASP	AFL
64	3.6	65.6	2.6	237.8	2.6	28.2	2.9	0.2	6.6	9.2	4.5	68.6	433.3	5.8
65	2.9	62.2	4.4	218.5	0.3	31.4	10.6	1.9	6.6	6.7	5.2	68	1066.7	29.3
66	3.2	63.3	2.6	248	2.2	9.8	3.5	9.5	5.2	6.7	4.6	69.2	230	6.7
67	4.5	59.3	1.9	237	1.6	15.8	5.7	9.5	4.8	6.6	4.8	68.5	133.3	6.1
68	3.2	62.5	2.4	231	-1.2	19.2	5.8	2.6	5.2	9.1	5.4	67.1	160	2.5
69	3.4	60.3	3.1	219	3.4	28.5	17.4	2.1	5	7	4.5	67.2	1433.3	17.4
70	3.6	62	1.1	217.8	0.8	22.4	0.4	2.4	5.4	6.5	4.3	68.5	266.7	3.1
71	3.5	64.5	2.4	244.3	1.5	16	0.7	3.3	4.9	8.4	4.9	70	900	80.6
72	3.4	64.8	4.4	241.2	0.6	30.2	5.4	5	3.2	8.7	5.3	68.7	1133.3	24.8
73	3.5	63.8	3.2	241.4	0.1	18.8	2.9	3.2	4.3	7.2	5	69.3	1100	59
74	3.5	64.9	2.5	237.6	0.7	13.5	1.7	-0.1	4.9	6	4.8	69.5	186.7	2.4
75	2.7	65.3	4	230.6	0.2	42.7	1.2	0.3	5.6	7.7	4.9	69	1133.3	86.3
Mean	3.51E+00	63.35	2.98	232.75	1.79	23.6	5.06	1.33	5.4	7.09	5.04	68.35	628.9	65
CV (%)	20.68	2.2	55.4	6.6	259.8	81.2	62.6	122.4	30.6	19	4.2	0.84	13.9	61.7
LSD	1.56	3.39	2.52	40.87	8.78	21.39	10.81	4.95	3.21	2.38	0.53	1.05	20.6	1.4

*-significant at p<0.05, GY-grain yield, ED-ear damage, LD-leaf damage, ASI-anthesis-silking interval, HC-husk cover, ER-ear rot, STAR-starch content, SL-stalk lodging, EH-ear height, PH-plant height, AFL-aflatoxin, ASP-*A.flavus*