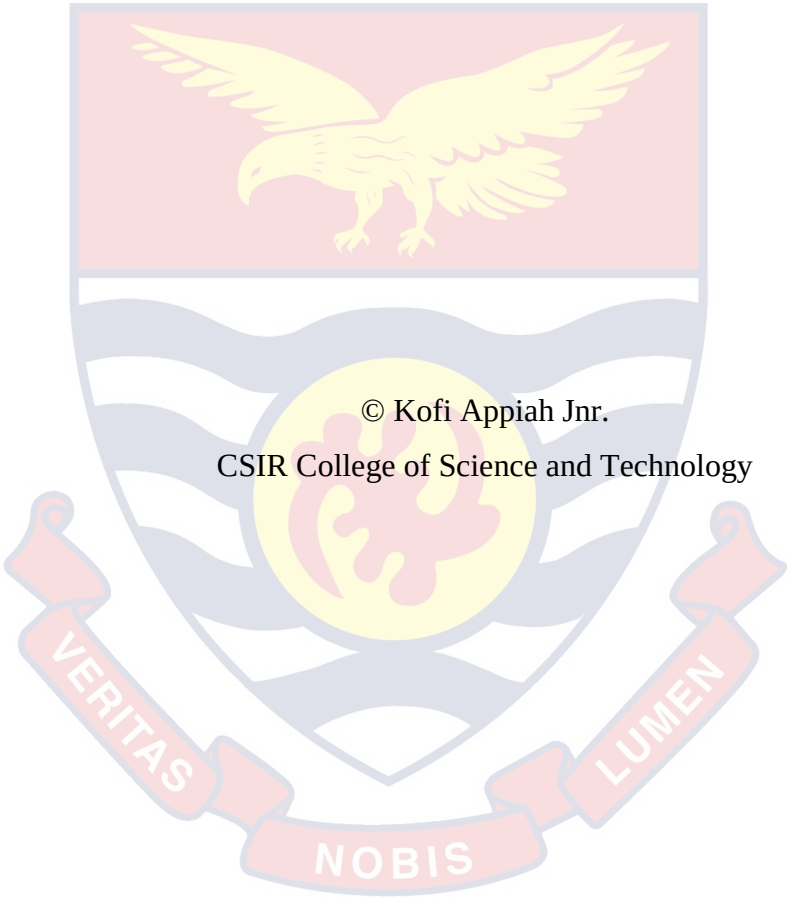
CSIR COLLEGE OF SCIENCE AND TECHNOLOGY

# ASSESSING THE PERFORMANCE OF PROMISING MAIZE HYBRIDS RESISTANT TO AFLATOXIN ACCUMULATION IN THE FOREST-TRANSITION AGRO-ECOLOGIES OF GHANA

KOFI APPIAH JNR.

2020



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# ASSESSING THE PERFORMANCE OF PROMISING MAIZE HYBRIDS RESISTANT TO AFLATOXIN ACCUMULATION IN THE FOREST-TRANSITION AGRO-ECOLOGIES OF GHANA

By

KOFI APPIAH JNR.

Thesis submitted to the Department of Plant Resources Development of the CSIR College of Science and Technology, in partial fulfilment of the requirement for the award of Master of Philosophy degree in Plant Breeding and Biotechnology

DECEMBER 2020

# DECLARATION

## Candidate's Declaration

I hereby declare that this thesis is the result of my own original research and that no part of it has been presented for another degree in this College or elsewhere.

Candidate's signature ……...Date…….

Name: Kofi Appiah Jnr

## Supervisors' Declaration

We hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision of the thesis laid down by the CSIR College of Science and Technology.

Principal Supervisor's Signature……Date……

Name: Dr. Allen Oppong

Co-Supervisor's Signature……. Date……...

Name: Rev. Prof. Hans Adu-Dapaah

## ABSTRACT

The production of maize in Ghana is affected by Aflatoxin contaminations which reduce grain quality and possess high health risk. Unsafe Aflatoxin levels above 20ppb have been reported from farmers' fields. The study was conducted to assess the performance of 18 maize hybrids resistant to aflatoxin accumulation in the forest transition ecologies in Ghana. The study comprised fourteen hybrids and four local checks evaluated using Randomized Complete Block Design across six ecologies in Ghana. Stability of the genotypes was estimated using the GGE Biplot model. Inoculation was done using the side needle method at a concentration of $9\times10^7$conidia/ml. Levels of aflatoxin were determined using high performance liquid chromatography. Results from the study revealed that, genotypic and environmental effects on some traits were consistently significant across environments. However, a non-significant genotype by environment interaction was observed for grain yield. Based on the GGE biplot analyses, MO826-12FxCML-343, MO826-7FxCML-343, ENT-85xCML-247, ENT-5xCML-287, ENT-5xTZ1-8, ENT-70xCML-247, were the highest yielding and stable genotypes whereas MO826-12FxCML-343, ENT-70xCML-247 were most stable hybrids. Genotypes ENT-5xCML-11, ENT-5xK1-3 and MO826-7FxTZ1-8 were lowest yielding whereas ENT-5xK1-3 was low yielding yet most stable. The study revealed MO826-12FxCML-343 and ENT-70xCML-247 as most stable among the top yielding hybrids with 14.30ppb and 25ppb aflatoxin levels after inoculation respectively. It is recommended that genotype MO826-12FxCML-343 and ENT-70xCML-247 should be further evaluated and released to farmers.

**KEY WORDS**

Aflatoxin contamination

Aflatoxin detection

Aflatoxin synthesis

Aflatoxin toxicity

Safe aflatoxin levels

Yield stability

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The contribution of my family to this accomplishment was enormous. I wish to thank them for their encouragement and prayers throughout the period. I do not have the right words to thank my mother and my siblings for their support, encouragement and prayers. I really appreciate you all. God richly bless you.

## DEDICATION

To my priceless dad and mum,

Kofi Appiah, Ama Dankwaa.

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| | |
|---|---|
| ANOVA | Analysis of Variance |
| ASI | Anthesis Silking Interval |
| CIMMYT | International Maize and Wheat Improvement Center |
| EVN | Environment |
| FAOSTAT | Statistical Database of the Food and Agriculture of the United Nations |
| GEI | Genotypes by environments interactions |
| HPLC | High performance liquid chromatography |
| LR | Root lodging |
| LS | Stalk lodging |
| METs | Multi-environment trials |
| MSV | Maize streak virus |
| MOFA | Ministry of Food and Agriculture |
| QPM | Quality Protein Maize |
| REP | Replication |

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# CHAPTER ONE

# INTRODUCTION

Maize (*Zea mays*) is a major cereal crop for both human and animal nutrition worldwide (Atmaca, Guvenc, & Aksoy, 2015). In Ghana, maize is the most important cereal crop accounting for 58% of local cereal that is produced and consumed (Scheiterle & Birner, 2016). Maize is cultivated in all the agroecological zones (Agyare, Asare, Sogbedji, & Clottey, 2014), on an area of about 865,000 ha with a national average yield of 1.7mt in farmers' fields (Ministry of Food and Agriculture[MoFA], 2017).

Maize production is constrained by both biotic and abiotic stresses. Biotic stresses such as diseases and pests' attacks as well as aflatoxin contamination caused by *Aspergillus flavus* affect productivity, quality of grains and income of farmers. Abiotic factors such as poor soil conditions and climatic factors also expose maize crop to yield losses which can result in food insecurity.

## Background of the Study

The production and consumption of maize in Ghana is particularly affected by mycotoxins (aflatoxin) contamination which results in reduced grain quality and its wholesomeness to be used as food or feed (Espinosa-calderón, et al., 2009). Aflatoxins are secondary fungal toxic metabolites that are produced by *Aspergillus* species such as *Aspergillus parasiticus*, *Aspergillus nomius* and *Aspergillus flavus* mainly on grains and nuts. Example of crops that can be infested with aflatoxin contamination includes: maize, sorghum, cottonseed,

peanuts, pistachio nuts, copra, cereals, fruits, oilseeds, dried fruits, cocoa, spices and beer in the field and during storage (Espinosa-calderón *et al*., 2009). There are more than ten (10) compounds classified as aflatoxin with the most important ones being B1, B2, B2a, G1, G2, M1, M2, Q1, R0 and P1. Aflatoxins B1, B2, G1, G2 are all considered harmful in this order of toxicity B1>G1>B2>G2 $LD_{50}$ 0.36, 0.78, 1.70,3.44 mg/kg respectively to humans (Kang, 2017).

Aflatoxin production on the field is influenced by high temperature and humidity which is favorable to the *Aspergillus* growth and contamination of grains on the field (Namjoo, Salamat, Rajabli, Hajihoseeini, Niknejad, Kohsar & Joshaghani, 2016). Its presence is also enhanced by factors such as stress or injury to the crop due to drought before harvest, insect activity, soil type and poor storage conditions (Alcaide-Molina, Ruiz-Jiménez, Mata-Granados, & de Castro, 2009).

The presence of Aflatoxins in food and feed makes them unsafe for consumption as there are reports of serious health hazards (Kang, 2017). When ingested, inhaled or adsorbed into the body it can lead to primary hepatocellular carcinoma (Namjoo *et al*., 2016) , hepatotoxic (capable of causing liver cancer), teratogenic (capable of effecting deformities in embryo) and mutagenic effects in human and animals at very low concentrations (Espinosa-calderón *et al*., 2009). Kenya recorded an incidence of deaths as a result of consumption of Aflatoxin contaminated commodity in 2004 (Probst, Njapau, & Cotty, 2007). According to (EU-European Commision, 2018) food commodities from Ghana were rejected at the European border due to high level of detectable Aflatoxins. Aflatoxin

contamination has negative impact on economic growth and trade (Bandyopadhyay et al., 2017) because food and feed commodities with high levels of Aflatoxin cannot enter the premium markets of developed countries with strict testing procedures.

Due to the health threat of Aflatoxin to humans and animals and its potential effect on the trade of commodities, there are prescribed acceptable safe levels of Aflatoxin contamination in food and feed. The European Union (EU) which has a rigorous regulation has set the limit for aflatoxins at 5 and 10µg/kg for Aflatoxin B1 and Total Aflatoxin respectively (Espinosa-calderón *et al*., 2009). According to Food and Agriculture Organisation [F A O] (2011), maize for human consumption should not exceed 5µg/kg for Aflatoxin contamination. In Ghana, Aflatoxin limit is set at 8µg/kg for maize produced in the country by Ghana Standard Authority[GSA] (2018).

There are many interventional technologies for the control of aflatoxin contamination. These include good agricultural practices, monitoring and crop destruction, postharvest interventions (Bandyopadhyay *et al*., 2017) and use of genotypes resistant to aflatoxin contamination (Warburton & Williams, 2014).

## Statement of the Problem

In Ghana, the commodities market is less developed and hence there is very little enforcement of standards and grades. This situation means health and safety of consumers cannot be assured (Adu-Appiah et al., 2017). Therefore, the safe measure to aflatoxin contamination in this part of the world and for most

developing countries where food quality standard checks and monitoring is less developed will be the use of resistant maize varieties.

Currently, there are no released maize varieties with resistance to aflatoxin contamination in the country; hence any approach that contributes to the release of aflatoxin resistant varieties will be a great step in the area of aflatoxin research for food safety for both consumers and maize farmers.

## Purpose of the Study

The purpose of the study is to develop maize hybrid resistant to aflatoxin accumulation in Ghana.

## Research Objectives

This study was therefore conducted with the main objective to identify high yielding stable hybrids with low levels of aflatoxin contamination.

The specific objective of the study was:

- To evaluate the performance of 18 CSIR-CRI maize genotypes for resistance to aflatoxin contamination across 12 environments in Ghana.

## Significance of the Study

The results from this study could be used to select promising maize hybrids for breeding programs and subsequently release them for farmers. This will help reduce aflatoxin health risk on consumers and protected farmers from economic loss arising from market rejection of maize exports.

## Delimitation

The research was conducted in only forest-transition agro-ecologies of Ghana specifically; Ejura, Akumadan, Wenchi, Fumesua, Kpeve and Ohawu. Other agro-ecologies were excluded because; these selected areas are the major maize growing hubs in Ghana.

## Limitations

Geographically there are no established aflatoxin hotspots for aflatoxin research in Ghana which could affect the results of the un-inoculated maize sample levels of aflatoxin accumulation.

## Organisation of the Study

In summary the study is organized in chapters, the next chapter focuses on literatures on aflatoxin. Chapter three also presents the research methods employed to achieve the set objective. Chapter four present results obtained from the experiment as well as discussions. Chapter five which is the final chapter presents conclusion and recommendations made from the study.

# CHAPTER TWO

# LITERATURE REVIEW

The purpose of this study was to evaluate promising maize hybrid for resistance to aflatoxin accumulation. Therefore, this chapter gives an overview of literature concerning this study and theoretical framework for the study.

## Origin, Botany and Distribution of Maize

Maize (*Zea mays* L.) belongs to the grass family (*Poaceae*) and is globally cultivated as one of the most important cereal crops. The centre of origin for maize has been established as Mexico and Central America (Watson & Dallwitz, 1992). The maize flower is characterized with staminate spikelets embedded in spike-like racemes which spreads out its terminal panicles (tassels). The female pistillate inflorescence is located in the leaf axils in which the spikelets occur on a thickened, woody axis (cob). The ear is covered with large foliaceous bracts (husk) and a mass of long styles (silks) that obtrude from the tip as a mass of silky threads (Badu-Apraku & Fakorede, 2017).

The male inflorescence known as the tassel is responsible for pollen production in the entire staminate inflorescence, while the eggs are located in the pistillate (female) inflorescence. The plant is usually pollinated by wind in either self or cross pollination mechanism. It releases pollen which usually remains viable for 10 to 30 minutes but may remain viable for longer periods under favorable conditions (Coe, Nueffer & Hoisington, 1988).

Maize is an adaptable crop grown over a range of agro climatic zones, usually from 58$^{o}$N to 40$^{o}$S, below sea level to altitudes higher than 3000 m, and in areas with 250 mm to more than 5000 mm of rainfall per year (Dowswell, Paliwal & Cantrell, 1996) It has a life cycle ranging from three to thirteen months (CIMMYT, 2000). Presently, maize is grown widely across the world with major producers located in temperate regions of the globe. The United States, China, Brazil and Argentina account for 70% of global production, whilst South Africa and Nigeria account for 6.9% of the world production. (Food and Agriculture Statistics [FAOSTAT], 2018). Hussan, Haqqani and Shafeeq (2003) classified maize as an important cereal fodder and grain crop which is able to perform well with good yield under both irrigated and rain-fed agricultural systems in the semi-arid and arid tropics.

**Importance of Maize**

Maize is adaptable and, grows on a vast range of continents with exception of Antarctica (Tagne, Feujio, & Sonna, 2008). Presently in Ghana, maize is the most cultivated cereal accounting for 58% of total grain output which serves as food for humans as well as animal feed (Scheiterle & Birner, 2016). It also provides raw material for the production of many industrial products such as corn starch, maltodextrins, corn oil, corn syrup and products of fermentation and distillation industries (World Agricultural Production [WAP], 2013). Recently the use of maize as raw material for biofuel production is emerging in developed industrialized countries such as USA (WAP). In developing countries, maize

cultivation employs a great number of farmers and hence it's a major source of income (Tagne et al.). The crop has good nutritional qualities consisting of 72% starch, 10% protein, 4.8% oil, 8.5 % fibre, 3.0 % sugar and 1.7 % minerals, and generally yield better and easy to cultivate (Chaudhary, 1983). In Ghana the per capital consumption of maize in the year 2018/19 was estimated at 62.56kg per head (MoFA, 2018) and an estimated national consumption of 943000 MT in 2017 (Statistic Research and Information Directorate [SRID], 2018).

**Constraints in Maize Production**

The wide range of challenges that are associated with maize cultivation can be grouped into abiotic such as poor soil condition, drought and heat, which currently threaten about 25% of maize production (Jones & Thornton, 2013) and biotic factors such as downy mildew, rust, leaf blight, maize streak virus (MSV) and most recently, maize lethal necrosis (MLN) which has led to total loss of maize crops in some parts of Kenya contributing significantly to the decline in yield and production (Wangai et al., 2012). Insect pests such as the stem borer also cause 20 - 40% yield loss whilst the fall armyworm in most cases can cause complete yield loss if not controlled early (Magan, Medina, & Aldred, 2011). Climate change reports predict global temperatures rise, which is likely to alter previously suitable lands to become more drier, becoming deserts through desertification, polluted by human activities and decreased access to fresh water and thus more suitable for aflatoxin production in maize as drought stressed maize are susceptible (Medina, Rodriguez, Sultan, & Magan, 2015).

In order to meet the growing demand for food due to increasing human population it is projected that there should be increase in total crop production by 60% by the year 2050 which will also mean there will be high demand for maize over other crops (Alexandratos & Bruinsma, 2012). To meet this increasing demand will require around a 2.4% per year increase in maize yield (Alexandratos & Bruinsma).

On the other hand, yield assessments suggest that annual global yield increases for maize will be as low as 1.6% due to climate change, exhausted sources of fertilizer and irrigation inputs will make increases yet more unattainable (Varga et al., 2012).

Besides the USA and Asia which records annual yield increments, Africa is characterized by intense disparities in maize yields. Some countries continue to record a drop in annual yields of over 7%. Others report extremely low average yields of 1.5 tons per hectare, which is a fifth of what is recorded by the leading maize producers (FAOSTAT, 2018). Besides the decline in yield, mould and other fungi contaminate maize with their mycotoxogenic products such as aflatoxin, fumonisin, cyclopiazonic acid, and ochratoxin which renders the grain unwholesome for consumption as food or feed (Varga et al., 2012).

**Maize Breeding in Ghana**

After the introduction of maize into Ghana from Mesoamerica, breeding of maize began in the late 30's. Before then farmers resorted to cultivation of landraces which they obtained from Americans which they eventually shifted to

open pollinated varieties but yield was generally low (Sallah, Twumasi-Afriyie, & Frimpong-Manso, 1997). It therefore triggered formal research into developing varieties which would give good and stable yield in different agro-ecological zones. Efforts into these researches resulted in the development of the C50 variety between 1939 and 1942 (Ghana Grains Development Project [GGDP], 1984; Sallah, 1986). The formation of the Ghana Grains Development Project (GGDP) in the 1979 funded by the Government of Ghana and the Canadian Government to champion research for the development of maize and legume with multiple objectives of improving yields, resistance to pest, disease and lodging became the game changer in maize breeding in Ghana (Azuni, 2014).The approach of the Ghana Grains Development Project (GGDP) resulted in the development of 15 maize varieties including (Okomasa, Abeleehi and Dorke SR), fertilizer recommendations and plant configuration recommendations ( Azuni).

As yield improvement was achieved, quality protein maize (QPM) development was initiated in 1989 at the Crops Research Institute with the sole goal of improving protein content in open pollinated variety (OPV) maize. The programme resulted in the development and release of Obatanpa which saw nationwide endorsement and adoption in some African countries. The international collaboration led to the release of several maize varieties and hybrids in 1984. Dobidi, Aburotia, Kawanzie, Golden Crystal and Safita-2 were improved open pollinated varieties released in 1984(Azuni, 2014).

Along the improvement of Obatanpa, a QPM hybrid maize development programme was also launched in 1991. The goal of the project was for the

development of a three (3) way QPM hybrids, which included, GH110 - 5 (Mamaba), GH132-28 (Dadaba), and GH2328-88 (CIDA-ba). Yields of the 3-way hybrids were superior and ranged between 6.3 and 7.3 t/ha on experimental plots, representing an increase of 19 to 38 percent over Obatanpa. Quality protein maize hybrids were afterwards released for commercial production in 1997 (Morris, Tripp & Dankyi, 1999).

Maize breeding in the 90s focused on the improvement of OPV's, QPM and drought resistant hybrids instead of breeding for resistance to biotic stresses such as aflatoxin which has become a world food concern due to scientific predictions of increasing global temperature by the year 2100 (Wu, 2015). With such rise in temperature, aflatoxin could become a major problem in all growing seasons of the crop.

Atongbiik, Achaglinkame, Opoku and Amagloh (2017) reported an average aflatoxin contamination level of 3276µg/kg in market groundnut samples in Ghana. However, in the Ejura-Sekyedumase district of Ghana, a range of 7.9 to 500ppb aflatoxin was detected in 36 food samples locally prepared as infant foods from a proportional blend of groundnut, beans and maize (Darwish, Ikenaka, Nakayama, & Ishizuka, 2014). Out of this, 30 of the samples exceeded the 20 ppb limit for aflatoxin. Such findings have shifted the focus of CRI and other research institutions from the development of open pollinated maize varieties towards the attainment of high yielding hybrid varieties that are aflatoxin resistant such that future release of aflatoxin resistant hybrid varieties for commercial production

will be appropriate to ensure food security, promote commodity export and improve health of maize consumers in Ghana and around the world.

**Brief History of Aflatoxin**

There are wide range of fungi that are associated with food and feed which have attracted major food security and food safety concerns. Aflatoxins are toxic substance produced by *Aspergillus spp.*

*Aspergillus spp* in literature was reported before the 20$^{th}$ century as associated with toxicosis disease in horse. The result of the outspread resulted in significant number of horses deaths. The characteristic symptom of the syndrome was later known as mouldy maize poisons (Morgavi & Riley, 2007). Fungal infections of maize kernel are enhanced by field conditions hence contamination becomes a serious challenge (Cotty & Jaime-Garcia, 2007). The presence of insects in the field, moisture and temperature is known to be important factors that enhance ear infection, but little emphasis was placed on the *Aspergillus spp* as contributing to the disease infestation (Moreno & Kang, 1999).

In the 1950's reports by Sippel, Burnside and Atwood (1953) suggesting Aflatoxicosis as the major cause to the feeding challenge sparked research to be focused on *Aspergillus spp* as the possible primary source of the disease in farm animals. However, contamination was only considered to be limited to stored maize (Christensen, 1957). Following the outbreak of Turkey X disease in the 1962 in England following a situation that resulted in the demise of about 100,000 young turkeys due to intake of contaminated food containing secondary

metabolites produced by *Aspergillus flavus* and *Aspergillus parasiticus*, much effort was directed toward the identification of the causal agent of the toxicity and the successful identification of the disease opened the gateway to an entirely new scientific aspect known as mycotoxicology (Morgavi & Riley, 2007). The causal agent was discovered as *A. flavus* in 1961 by Blout and the toxin produced was named aflatoxin due to origin (letter "A" for the genus *Aspergillus*, the next set of three letters, "FLA", for the species *flavus*, and the noun Toxin, means poison (Dadzie, 2019). The *Aspergillus* fungi, belongs to the Deuteromycetes (Fungi Imperfecti; Hyphomycetes); their teleomorphs can be found in the Ascomycetes (Akrobotu, 2008). Several other animal species such as cattle were later reported to be infected by aflatoxins (Morgavi & Riley). The fungi adapt easily on food commodities as substrate for growth because of the vast number of enzymes it secretes for substrate digestion during its development (Hell, 1997).

**Types of Aflatoxin**

Mycotoxins are compounds that are produced directly or indirectly by fungi that may be poisonous to living organisms particularly, humans and animals when ingested through food or feed. There are about 40 known species of *Aspergillus* that are of importance to food, beverage and feed industries (Kang, 2017) of which at least 10 of the species can produce one or more of the 30 known mycotoxins. Aflatoxin presence in food is a 44 years old problem in Ghana (Blepony & Akoto, 2018) that is produced by some strains of *Aspergillus flavus*, most strains of *Aspergillus parasiticus* and *aspergillus nomius* (Kang). But

among them, the most common aflatoxin-producing species worldwide s *Aspergillus flavus* Link (Klich &Pitt, 2007) which produces aflatoxin B1 which has an extremely high carcinogenic potency to some species of animals and a widespread occurrence in some food items. This species can be further subdivided into two distinct morphotypes, L and S, which vary in morphology, epidemiology and physiology, including their potential to produce aflatoxins (Mehl et al., 2012). The L morphotype produces fewer, larger sclerotia (avg. diameter > four hundred µm), many conidia, and ranging levels of aflatoxins whereas the S morphotype produces numerous small sclerotia (avg. diameter < four hundred µm), few conidia, and consistently high levels of aflatoxin (Cotty, 1989). The S morphotype often forms a minor proportion of the aflatoxigenic communities linked with a crop but is considered a key causal agent of contamination due to its ability to produce high aflatoxin levels (Probst, Schulthess, & Cotty, 2010).

Fungal analysis of toxigenic communities across the world has shown that there are several lineages of fungi with S morphotype with some of them producing large concentrations of both B and G aflatoxins. According to Probst et al. (2014) there is a group of fungi with S morphotype in West Africa that uniformly produces both B and G aflatoxins and identified as unnamed taxon $S_{BG}$. According to Thakare, Zhang, Wing, Cotty and Schmidt (2017) there are at least 20 compounds named aflatoxin. However, Kang, (2017) confirmed these B1, B2, B2a, M1, M2, G2, G2a, P1, Q1, M1 and R0 as the most important. The letters B and G refers to the blue and green florescence produced by the compounds when exposed under UV light (Espinosa-calderón et al., 2011), whereas the numbers 1

and 2 represent major and minor compounds respectively. Aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), and aflatoxin G2 (AFG2) are of importance to food security and food safety because these four are usually found in food and feed.

Pure AFB1 is faded-white to yellow crystalline, odorless and it's in stable form. Aflatoxins can be dissolved in methanol, chloroform, acetone and acetonitrile. It is highly toxic substance which can kill up to 50% Chang liver cells at a concentration as low as 0.1µg/ml and duck embryo cultured primary cells (Kang, 2017). AFM1 is the monohydroxylated metabolite of AFB1 produced in the liver by the use of microsomal cytochrome P450-related enzymes and excreted by way of body fluids similar to milk, urine, faeces and blood (Umesha, et al., 2017).

Aflatoxin B1 (AFB1) is metabolized to aflatoxin M1 (AFM1) in the liver and excreted in the milk of dairy cattle however, in birds, AFB1 is metabolized to AFMI and carried over in the egg products. Generally, the amount of AFM1 excreted in milk and poultry products is only 1-2 % of the total AFB1 ingested as feed (Sirma et al., 2018).

**Structure and Chemical Properties of Aflatoxins**

Van der Zijden, (1962) characterized the chemical and physical nature of the aflatoxins B1, B2, G1 and G2. Chemically, aflatoxins are of difurocoumarin derivatives known as difurocoumarolactones. Their structure consists of a bifuran ring fused to a coumarin nucleus with a pentanone ring (as found in B and M

aflatoxins) or a six membered lactone ring in G aflatoxins (Dhanasekaran, Shanmugapriya, Thajuddin, & Panneerselvam, 1993). The four compounds are best distinguished by the color of their fluorescence under long wave (Devero, 1999) ultraviolet illumination (B=blue, G=green) while M is the mammalian metabolites of aflatoxin B. The figure 1 shows the structure of aflatoxin B1, B2, G1, G2 and M1

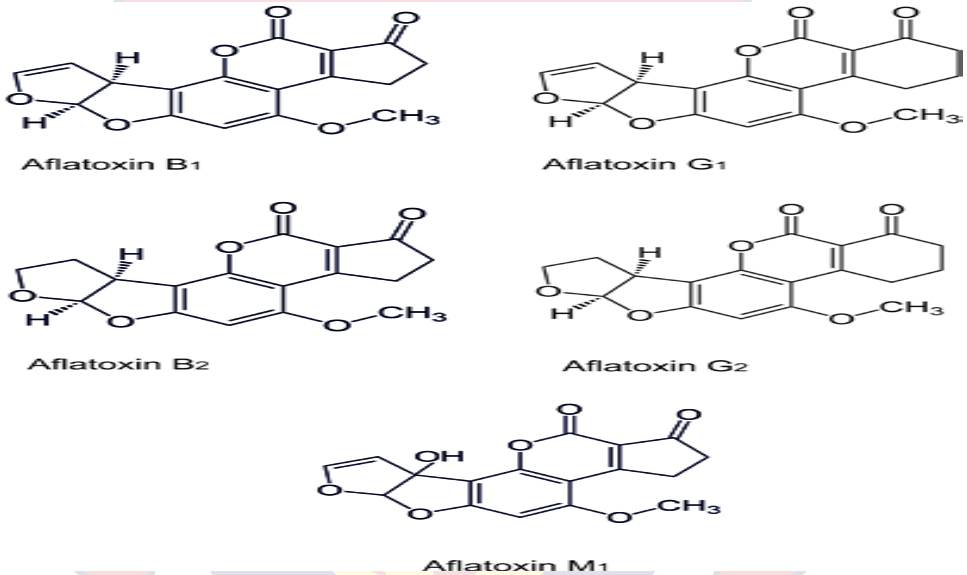


*Figure 1* - Structure of aflatoxin B1, B2, G1, G2 and M1.

Source**:** Cole and Cox (1981)

Table 1 shows the chemical formula, molecular weight and melting point of aflatoxin B1, B2, G1, G2

Table 1 -*Properties of Aflatoxin*

| Aflatoxin | Chemical Formula | Molecular Weight | Melting Point |
|---|---|---|---|
| Aflatoxin B1 | C17H12O6 | 312 | 268-269* |
| Aflatoxin B2 | C17H14O6 | 314 | 287-289* |
| Aflatoxin G1 | C17H12O7 | 328 | 244-249* |
| Aflatoxin G2 | C17H14O7 | 330 | 237-240* |

*Decomposes

Source: Dhanasekaran,Shanmugapriya,Thajudin and Panneerselvam (1993)

**Environmental Influence on Aflatoxin Accumulation**

A number of factors are important for the presence of the fungi and its ability to produce toxins. These factors could influence the presence and growth (population) of fungi and subsequently affects the production of aflatoxin: extrinsic factors are; temperature, moisture, insect attack, relative humidity, soil properties and mechanical injury to food.

**Temperature**

Atongbiik et al. (2017) reported that whether there is high or low temperature, fungal growth and its resultant mycotoxin production are inescapable. Atanda, Akpan and Enikuomehin. (2006) observed that temperatures below 20 $^{o}$C were detrimental whereas above 20$^{o}$C enhanced growth of Aspergillus species. They also reported that food products such as cereals and legumes were more prone to Aspergillus species than any other toxin-producing fungi, more so during storage due to the temperatures involved. However, fungal

activity and toxin production have been reported elsewhere to be optimum at 25-37°C in the presence of other favoring conditions. This range of temperature is the ambient temperature in Ghana (Atongbiik et al.)

Abdel-Hadi, Schmidt-Heydt, Para, Geisen and Magan (2012) also reported maximum Aspergillus growth rate of 6.9 mm/day at 35°C and maximum aflatoxin production rate of 2278-3082µg/g at 37°C in maize. However, the effect of temperature and that of moisture are inseparable.

**Moisture**

The amount of water present in grains is an important factor that affects both the grade and storability of grains and legumes as it significantly impacts microbial growth and toxin production. It is however, a key determinant of aflatoxin development in food and feed. Atongbiik *et al*. (2017) observed that 13% moisture or relative humidity of 65% (water activity, $a_w$ of 0.65) is optimum for storage pest like aspergillus for growth and toxin production. However, moisture above 77% is optimum for growth and proliferation (Shuaib et al, 2012).

When examining the impact of water activity on both A. flavus and aflatoxin (AFB) production in peanuts at 25°C, Abdel-Hadi et al. (2012) observed a maximum growth of *Aspergillus flavus* at 0.95 $a_w$ and 0.95 $a_w$ with maximum aflatoxin production at 0.90 $a_w$ after three weeks of storage. The researchers reported a significant positive correlation between A. flavus population and aflatoxin production, A. flavus population and water activity, and aflatoxin

production and water activity with respective correlation coefficients of 0.849, 0.75 and 0.68. Water activity is however known to increase with storage period; this, together with inappropriate drying predisposes stored cereals and legumes to fungal infestation, growth and aflatoxin development.

With maturity and harvest of food crops at the end of the raining season, the threat of A. flavus and metabolites buildup might be high in Ghana. Traditional drying techniques involve field- and bare ground-drying; and this immensely contributes to fungal contamination (Okello & Kaaya, 2010). These methods are labour-intensive and time-consuming, involving lots of crop handling that may not adequately accomplish efficient drying. This is sometimes compounded by heavy rainfall during harvesting and drying, which makes it difficult to attain the recommended moisture level for safe storage (Okello & Kaaya).

**Impact of soil on aflatoxin accumulation**

Makun, Dutton, Njobeh, Ayinla and Ogbadu (2002) identified the soil as a great natural determinant factor that has a key influence on fungal incidence in many agricultural produces. Thus, crops grown on different soils may have significantly different levels of aflatoxin contamination. Research conducted by Alimentarius Commission (2004), indicates that light sandy soils aid growth of the fungi, mostly under dry conditions, whereas heavier soils result in less contamination due to their high water retention capacity that helps in the reduction of drought stress. Though Ghana is predominantly made of various

types of soil such as sandy, loamy and clayey, the specific form of soil of a specific area could depend on the part of the country it is found. Predominantly the northern part of the country has largely sandy and sandy loamy soils, while the southern part is made of soil types ranging from clayey loamy to dark loamy (Fearon, 2000). However, most of the soils of Ghana where cereals and legumes such as maize, millet, groundnuts, bambara beans, and beans are grown range from light sandy to sandy loam (Fearon,). This might partly be the rationale for the high aflatoxin contamination in a number of some of these crops. Fussei (2015), also reported that light sandy soils promote the rapid proliferation of Aspergillus flavus especially, in adverse dry conditions.

Soil moisture stress has also been observed to have a great influence on pre-harvest aflatoxin contamination of produce. Fearon (2000) observed that excessive drought causes strains on seed coats that serve as entry points for fungi while excessive moisture weakens the seed coats causing a similar effect. Droughts are common in Ghana, especially in the northern part of the country, where rainfall is between May and September while the remaining part of the year forms the dry season (Atongbiik et al., 2017). This extreme period of no rain poses drought stress on legumes such as groundnuts and Bambara beans, which most often are harvested within the periods of little or no rainfall, causing strains on their pods and seed coats which may serve as access points to fungi. However, crops that are harvested within the period of severe rains may also face pod and seed coat weakening as a result of excessive moisture hence making the seeds highly susceptible to fungal infestation (Okello & Kaaya, 2010).

**Impact of nutrient composition of crop on accumulation of aflatoxin**

Without overlooking the fact that fungi have the genetic ability to produce a particular mycotoxin, the level of toxin and rate of production would partly be influenced by available nutrients (Makun et al., 2002). As a result, different food substrates may have varying effects on aflatoxin production due to differences in nutrient compositions.

However, the effect of nutrients in substrates (corn, wheat, peanut, soybean, corn germ and corn endosperm) on aflatoxin B production showed a slight A. flavus contamination and relatively low levels of AFB1 in defatted substrates (Liu et al., 2016). However, AFB1 levels sharply increased with the addition of corn oil. The levels of AFB1 in full-fat substrates were also higher than in the defatted substrates. Hence, processing complementary foods from full-fat cereals has the tendency to increase the potential of aflatoxin contamination

A study conducted in Ghana revealed that cereal-legume combinations containing maize and groundnuts had high levels of total aflatoxin contamination with some samples having values exceeding 500 ppb. It has also been reported that low concentration of soluble sugars in substrate limited the production of AFB1. However, concentration reached 3% and 6% there was significant amount of aflatoxin. Moreover, at a concentration of 3.0% for each of the sugars, sucrose significantly enhanced AFB1 production (39782.61 ng/30 mL), followed by maltose and fructose (23687.29 ng/30 mL), with the least effect (Liu et al., 2016).

On the other hand, increasing amino acids concentration generally decreased the production of aflatoxin. Nevertheless, 0.5% concentration of

glutamic acid, aspartic acid and glycine and all concentrations of arginine significantly promoted AFB1 production (Liu et al., 2016). Trace elements such as copper, iron, and manganese were found not to have any significant positive impact on AFB1 production with the exception of zinc which is directly involved in the synthesis of Aflatoxin.

Therefore as the concentration of zinc increased there is a corresponding increase in aflatoxin production (Atongbiik et al., 2017). The results from Liu et al. (2016) shows that there is a close relationship between oil content and aflatoxin production and that sucrose, maltose, glucose, arginine, aspartic acid, glutamic acid as well as zinc contents of food may predispose it to aflatoxin contamination. Thus, the levels of aflatoxin prevalence in cereals and legumes could partly be attributed to the varying contents of the nutrients mentioned above since these grains have relatively high concentrations of these nutrients (Liu et al., 2016).

**Aflatoxin Levels in Food/Feed and Other Products**

Cereals, though widely cultivated and consumed in Africa, are highly exposed to aflatoxin contamination. Maize is one of the most important staple foods in Africa and is now widely grown for animal feed. It is the third most cultivated food commodity after cassava and sugar cane however in terms of supply of food energy it is ranked first in Africa. Aflatoxins are regularly detected in maize throughout the world and the recent serious contamination which was

associated with drought led to fatal human aflatoxicosis in Kenya (Makun et al., 2002).

A study conducted by James et al. (2007) showed aflatoxin contamination in maize grains from a total of 38 samples collected from major store markets in Ghana, Togo and Benin. Their analysis showed that aflatoxin levels in contaminated maize samples ranged from 24 to 117.5 ng/g in Benin, from 0.4 to 490.6 ng/g in Ghana, and from 0.7 to 108.8 ng/g in Togo. Furthermore, a study taken in three districts in Ghana, namely Ejura-Sekyedumase, North-Kwahu and Nkoranza showed aflatoxin contamination levels in maize ranging from 12 to 30 ppb. Sugri et al. (2015) observed aflatoxin occurrence range of 0.011 to 308 ppb in maize samples taken in six (6) districts in the Upper East and Upper West regions of Ghana.

Additionally, Darwish et al. (2014) also observed that all maize samples taken from silos and warehouses in Ghana contained 20µg/kg to 355 µg/kg levels of aflatoxin while 31 out of 32 fermented maize dough samples taken from key processing areas across the nation also were contaminated with up to 310µg/kg of the toxin. Furthermore, 15 maize samples taken from major processing sites in, Accra, were analyzed and reported to contain aflatoxin levels ranging from 2 to 662µg/kg (Awuah & Kpodo 1996). Atongbiik et al., (2017) also reported an average aflatoxin contamination level of 3276µg/kg in market groundnut samples in Ghana. In the Ejura-Sekyedumase district of Ghana, a range of 7.9 to 500 ppb aflatoxin was detected in 36 food samples locally prepared as infant foods from a

proportional blend of groundnut, beans and maize (Darwish et al.,). Out of this, 30 of the samples exceeded the 20 ppb limit for aflatoxin.

Dadzie (2019) also reported of *A. flavus* contamination in grain samples *across* Fumesua, Wenchi, Ejura and Akomadan communities. Average percentage contamination of 56.7%, 30.6%, 53.5% and 45.6% were recorded from the communities respectively. The total aflatoxins observed in the samples were in the range below the limit of detection (LOD) 692 ng/g, 23 ng/g, 945 ng/g and 112 ng/g for Fumesua, Wenchi, Ejura and Akomadan, respectively.

**Impact of Aflatoxin Contamination on Health and the Economy**

The effect of exposure to aflatoxins contamination could lead to several health-related altered conditions. Humans are primarily exposed to aflatoxin through the consumption of contaminated agricultural or animal products and rarely through the inhalation of toxins (Makun et al., 2002) . Human exposure to aflatoxin has a negative impact on health throughout the globe. Exposure can lead to acute or chronic aflatoxicosis; based on the duration and amount of exposure it can lead to health issues or the risk of disease transmission ( EU-European Commision , 2018).

Since the liver is the main detoxification organ, it is the first organ to be exposed to the effect of Aflatoxicosis. Severe exposure of the liver could trigger and escalate conditions such as Kwashiokor, cirrhosis, hepatitis and other complicating illness (Lewis et al., 2005). In Ghana and Africa in general where aflatoxin contamination levels is very high, it has been reported that aflatoxin

plays a part in the prevalence of kwashiorkor during high humidity conditions (Makun et al., 2002). This assumption is supported by reports of general occurrence of Aflatoxin in children's excreta (de Vries, Maxwell, & Hendrickse, 1989); liver, serum (Hatem, Hassab, Al-Rahman, El-Deeb, & Ahmed, 2005); and its simple product, aflatoxicol, in the livers of children in Ghana (Apeagyei, Lamplugh, Hendricks, Affram, & Lucas., 1986). However, Makun et al. reports of a direct link with aflatoxicosis as a contributing factor to liver cirrhosis, hepatitis, hepatocellular carcinoma in humans. Other studies done in Ghana with HIV positive and negative persons revealed lower levels of CD4+,T regulatory cells with positive patients with higher aflatoxin accumulation(Wu, 2015).

Due to the toxic and carcinogenic effects of aflatoxin in humans and animals, more than 100 countries have set up regulatory standards on maximum tolerated levels of aflatoxin in food and feed (Adu-Appiah et al., 2017). Therefore, beside its impact on health, aflatoxin contamination can also reduce the price paid for food crops or in extreme cases, can cause market rejection of entire food or feed exports. Estimated global average price loss per annum due to aflatoxin contamination is reported to be around $1.5M from 2000 – 2014 (Udomkun et al., 2017). Contamination can directly reduce availability of food for low-income people. Farmers who produce contaminated crops may also experience income reduction due to product rejection, lower market value, or exclusion from high-value markets. Several direct results stem from lower income including limited ability to purchase food, which translates into reduced access to food (Udomkun et al.).

It is estimated that 16 million tons of maize is lost globally each year to aflatoxin contamination (Wu, 2015). Contamination of aflatoxin in food and feed accounts for an annual estimated agricultural loss of $270 million in the United States. The occurrence of aflatoxins in the food chain affects people's livelihoods, agricultural development, food security, and human health. Ghana received 23 separate red alert notifications on unfit agricultural commodities exported to Europe in 2016 (Udomkun et al., 2017). Aflatoxin contamination of commodities in Africa causes annual losses of more than USD 750 million. However, EU regulation of Aflatoxins is reportedly costing African food exporters USD 40 million annually (Wu,).

**Control Strategies of Aflatoxin**

To curb the menace associated with aflatoxin contamination, several preventive strategies have been proposed. These include good agronomic practices to decrease the ability of the fungus to grow, biocontrol with atoxigenic Aspergillus strains (Blepony & Akoto, 2018), improved postharvest storage methods, and the use of trapping agents to block toxin uptake (Bandyopadhyay et al., 2017), but each of these strategies have their set-backs rendering them inadequate (Thakare et al., 2017).

Biocontrol methods have effectively been applied to reduce aflatoxin levels in maize under field conditions in some parts of the United States and Africa. A study by Cotty and Bhatnagar (1994) found multiple strains of atoxigenic *A. flavus* that could inhibit aflatoxin production of toxigenic strains *in*

*vitro.* During the study, *A. flavus* strain AF36 was found to cause lowered aflatoxin concentration in cotton seed in the field, by competing with the toxigenic strains. There could be the possibility of vegetative recombination of atoxigenic strains with toxigenic strains besides the additional cost in purchasing the product.

Biotechnological approaches, such as host-induced gene silencing (HIGS), which comprises of the expression of double-stranded RNA molecules in plants to silence genes expressed by pests and pathogens, offer a possible alternative for controlling aflatoxin contamination (Thakare et al., 2017) but it's very expensive to carry out especially in developing countries where less resources are committed to research aside the misconceptions that is associated with transgenic food items. Plant breeding offers a promising approach which could curb the contamination in Africa where environmental conditions are favorable for fungi growth and local regulatory checks in various markets is almost non-existent. This implies that, consumers may be directly consuming contaminated commodities at unsafe levels. The success of such breeding programme is dependent on the availability of genetic variability for resistance and accessibility to secure plants infection. Breeding approaches had been developed for deciding on oblique instruments for determination of resistance to pre-harvest infection. These are to facilitate breeding for the development of resistant germplasm immune to fungal and eventually reducing the cost in screening of contaminated commodities (Umesha et al., 2017).

It is reported that most aflatoxin resistant varieties originated from a common ancestry of Tuxpeno germplasm, which exhibits characteristics of tropical germplasm such as late maturity and excessive height when grown in temperate environments (Warburton & Williams, 2014). Earlier aflatoxin resistant studies focused on the percentage of infected kernels in an ear to identify resistant lines whiles other approach relied on kernel screening assays to develop seed-based resistance as a key in the identification of resistant germplasm (Zuber, 1983; Brown, Williams, Windham, Menkir, & Chen., 2016). Kernel screening assay and field screening contributed significantly to the identification and release of aflatoxin resistant genotypes which includes MP313E, SC54, MP420, and Tex6 in the US (Hamblin, 2000). Apart from MP313E, the expression of the resistance in these sources of germplasm tended to be highly dependent on the environment in which they were grown. The differences of some lines with respect to phenotypic performance in different environments may be because much of the resistance is highly quantitative and tends to be inherited in an additive fashion; which can lead to high general combining ability (GCA) in hybrids (Betran, Isakeit, & Odvody, (2002).

Newer breeding lines with stable resistance in reducing aflatoxin under different environments include Mp715, Mp717, GT-MAS: gk, CML176, CML269; CML322, and Tx114 (Betran et al., 2002). Additional resistant breeding lines released later includes Mp718, Mp719, Tx736, Tx739, and Tx740 (Mayfield, Betran, & Isakeit., 2012) these recent lines show much better plant type and resistance. Warburton, Williams and Windham (2013) constructed an

aflatoxin association mapping panel with 300 maize lines and concluded that 30 to 40 lines displayed good resistance in up to 7 environments. The recently released lines are currently included in an on-going joint USAID/USDA projects in some CGIAR centers, to incorporate as many of these lines into ongoing genetic studies and breeding activities as much as possible to create resistant OPV and hybrid cultivars with stable resistance to aflatoxin accumulation (Dadzie, 2019).

Breeding for resistance to *Aspergillus flavus* or its ability to produce aflatoxin plays a significant role in preventing aflatoxin infection. The genetics of resistance mechanisms for *A. flavus* and aflatoxin contamination have not been naturally elucidated yet (Umesha et al., 2017). The allelic association among more than a few sources for resistance qualities that may aid breeders to pyramid the non-allelic genes for each resistance mechanism is still unknown (Hamidou, Rathore, Waliyar, & Vadez, 2014)

**Genotype by Environment interaction**

The stability of variety traits is important because farmers cultivate the same variety under different environments with diverse management practices. Temperature and humidity cause different levels of stress to plants in an area or environment (Ouborg, Pertoldi, Loeschcke, Bijlsma, & Hedrick, 2010). Many other factors (such as pest and soil type) affect the level of aflatoxin accumulation. It is, therefore, relevant that yield stability is targeted in cultivar

development in different environments. Grain yield is a quantitative trait and exhibits genotype by environment interactions (GEI) (Badu-Apraku et al., 2020).

Genotype by environment interaction occurs when variances between genotypes are not the same in all environments within and across years. Badu-Apraku et al. (2020) defines it as the degree of difference in the performance of genotypes across environments. Moreover, Sallah et al. (2004), stated that the performance of genotypes often changes from one environment to another and this difference in response of genotype to changes in the environment is often referred to as genotype by environment interaction. This necessitate the undertaking of multi- locational trial in plant breeding to estimate yield of genotypes across diverse environments (Azuni, 2014). Failure to consider the differences in genotypic response to different environmental conditions may limit accurate yield estimation and eventually affect the identification and selection of high yielding stable genotypes (Sirman et al., 2018).

Genotype by environment interactions have long been considered important to breeding generally because the genetic architecture for traits, and thus evolutionary dynamics, differ with environmental conditions (Ouborg et al., 2010).

Duvick (2005) reported that improvements in hybrid yield gains are caused by changes in cultural practices and by contributions of plant breeding. These interactions affect yield gains as changes in cultural practices such as weed and pest control, timeliness of planting and increased efficiency of harvest equipment are dependent on changes in breeding, and vice versa. Genotype

improvement contributes about half of yield gain while, good agronomic practices accounts for the remainder (Kpotor, 2012).

Genotype by environment interaction may be due to dissimilarities in soils, rainfall patterns, seasons and years (Ewool, 2004). Environmental conditions, such as rainfall are unpredictable and difficult to estimate compared to repeatable conditions such as general climate and soil (Cooper, Woodruff, Eisemann, Brennan, & DeLacy, 1995). In several breeding programs, environments are classified based on cultivar performance and assessed in a broad range of environments, concentrating on the effects of genotype by environment interaction (Cooper *et al.*)

In an experiment conducted by Salah et al. (2002) on the potential of elite maize composites for drought tolerance in stress and non-drought environments effects, genotype by environment interaction were highly significant for grain yield, 50% silk emergence, plant height, lodging, ears per plant, and ear rating in both drought and non-drought stressed environments. From their stress environment, grain yields of the varieties ranged from 2.21 to 3.12 t $ha^{-1}$, while in the favorable environment, yields for the same varieties ranged from 4.17 to 5.96 t $ha^{-1}$.

Ewool (2004) reported high significance for genotype by environment interaction for grain yield and other agronomic characters. He also reported that genotype by environment interaction were highly significant for grain yield, days to mid silk, days to mid-anthesis, plant height, ear height, total lodging, rust,

blight, cob aspects, shelling percentage, dry stover weight, 1000 seed weight, cob length, grain depth, grain diameter, anthesis silking interval and cob diameter.

Genotype by environment interactions is of interest to plant breeders because of their influence on progress from selection (Sallah *et al*., 2002). The existence of large genotype by environment interaction poses a major problem in relating phenotypic performance to genotypic constitution and hampers effective discrimination among contending genotypes (Badu-Apraku & Fakorede, 2017). It is therefore important to know the nature of genotype by environment interaction to be able to design efficient strategies for testing and selecting superior genotypes, especially when new hybrids are to be introduced in an environment. There are at least two different concepts to determine stability of a genotype; the static and dynamic concepts (Becker & Leon, 1988), depending on the trait, being either a qualitative or quantitative.

The static concept defines a stable genotype as one having an unchanged performance irrespective of variations in the environmental conditions. This concept is particularly useful for qualitative trait where the level of performance has to be maintained at all cost. However, complex traits such as grain yield uses dynamic concept, in which trait performance may vary from environment to environment, but in a predictable way. Several methods are available for measuring the dynamic concept of phenotypic stability. All have one feature in common: their estimates of stability are derived from an analysis of genotype-environment interactions. They include regression analysis (Gauch & Zobel 1997), multivariate analysis (Westcoff, 1987), variance analysis (Perkins & Jinks,

1968) etc. The basic model for stability analysis is the same as used for the analysis of genotype by environment interactions (Heikoc, Peter, & Tigerstedt, 1998).

$Yij = \mu + gi + ej + geij + eijk$

Where μ: general mean

gi: effects of genotypes

ej: effects of environments

geij: effects of genotype x environment interactions

eijk: random error linked with observation of kth replication of ith genotype in jth environment.

The GGE biplot method provides an effective way to determine stability of genotype in different locations.

**GGE Biplot**

It is generally difficult to select superior maize genotypes evaluated in different environments due to the alterations of relative yield of genotype as a result of genotype by environment interactions. Genotypes may perform differently in different environments due to their potential performance in the different environments. Genotype by environment interaction as reported by Shiri, (2015) is responsible for the decrease in the correlation between genotype and phenotype which hampers easy selection of genotypes for subsequent improvement programmes.

GGE biplot is a version of a biplot which provides elaborate information about genotype by environment interaction and genotype main effects at the same time (Shiri, 2015) which in contrast to many other multivariate stability analysis methods which only provides information on genotype by environment interactions. Many experiments point to the fact that in various stability analysis experiments, the main effect of environment is high, while variation determined by the main effect of genotype and genotype by environment interaction that are recommendable and interpretable are low (Yan, Cornelius, Crossa & Hunt, 2001; Yan, Hunt, Sheng, & Szlavnics, 2000).

In GGE biplot method, genotype by environment interaction source of variation is used to obtain more reliable results with environment as non-controllable factors. The unique graphical display of Genotype by environment interaction effect; by GGE biplot allows plant breeders to assess the genotypic stability and combinations of genotypic stability with ease as well as yield in different environments. Again with GGE biplot the difficulty of identifying and assessing the relationship between target environments in plant breeding programs is eased (Yan et al., 2000). GGE biplot analysis has been applied to the analysis of genotype by environment interactions in crops such as wheat, maize, soybean and cotton (Blanche & Myers, 2006; Yan et al., 2001).

In breeding programmes, the GGE biplot has provided an easy model for determining and grouping of environments. Based on the performance of the genotypes in the various environments, sets of environments are generated grouping them by genotypes with the same set of performance (Shiri, 2015).

Mohammadi, Haghparast, Amri & Ceccarelli (2010) reported of using GGE biplot analysis to group environments using barley, rice and maize as test crops.

Stress related environments such as drought is associated with genotype by environment interaction hence tend to make breeding progress difficult. The difficulty could be largely due to differences in environment variations, high moisture stress and genetic variation in Anthesis and silking (Shiri, 2015). As a result, progress in breeding programmes coupled with good decision by plant breeders in repeating experiments in different locations has become easy. GGE biplot analysis uses pattern in genotype environment interaction data to produce graphs which are used to identify similarities and differences between environments, ideal environment and who won where patterns (Yan et al., 2001).

The GGE biplot method was invented by Gabriel (1971), and its use was further expanded by Kempton (1984) and Zobel, Wright and Gauch (1998). The extensive usefulness of GGE biplot has only recently been elucidated (Yan *et al*., 2000). The GGE-biplot methodology has been reported in maize (Badu-Apraku, Oyekunle, Akinwale, & Aderounmu, 2013) in genotype by environment interaction evaluation and mega-environment investigation.

The GGE biplot model equation is:

$Yij - \mu - ßj = \lambda 1 \xi i1 \eta 1j + \lambda 2 \xi i2 \eta 2j + \xi ij$

$\lambda 1$ and $\lambda 2$ are the singular values of first and second largest principal components, PC1 and PC2, respectively; the square of the singular value of a PC is the sum of squares explained by the PC; $\xi i1$ and $\xi i2$ are the eigenvectors of genotype I for

PC1 and PC2, respectively; and η1j and η2j are the eigenvectors of environment j for PC1 and PC2, respectively.

# CHAPTER THREE

# RESEARCH METHODS

The purpose of this study was to evaluate maize hybrid for resistance to aflatoxin accumulation. This chapter gives an overview of the materials used and the methods used to obtain the results for the study.

## Experimental Material

The experiment material comprised of eighteen entries of maize genotypes consisting of four local checks and 14 single cross hybrid genotypes. These materials were obtained from the CSIR-Crops Research Institute, Kumasi in the Ashanti region of Ghana. The characteristics of the varieties used are summarized in Table 2.

## Experimental Sites

The research was carried out in six locations. The locations were Ejura (7° 23' 0" North, 1° 22' 0" transition ecology, fine coarse sandy loam Oxisol), Fumesua (6°41"North, 1°28 Deciduous forest, ferric acrisols , Akumadan (7° 24' 0" North, 1° 57' 0" West), Wenchi ( $8^0$ 45'N, $2^0$ 6'W,33), Kpeve ( $3^0$ 20'N, $0^0$ 17'E –Coastal savannah, achrosols) and Ohawu both in the Volta region. The average yearly minimum and maximum temperature of the agro-ecological zones were (Fumesua 21°C min and 33°C max, Wenchi 21.2 °C min and 31°C max, Akomadan 20 °C min and 35°C max while Ejura was 24°C min and 33°C max).

**Land preparation**

The fields were disc ploughed and harrowed before planting to ensure good tillage. Glyphosphate at 1.5 l/ha was applied soon after planting for weed control.

**Establishment of crossing block**

The seeds of the hybrids for the two seasons (major and minor) were generated by planting of the male and female inbred lines in a crossing block using North Carolina II (NC2) mating design. The male inbred lines pollen was then collected and crossed with their respective female inbred lines in the crossing block. The seeds were then harvested at maturity, dried, treated and packed for planting.

**Genetic Materials Used in the Study and Their Characteristics**

The genetic materials (inbred lines) and hybrids used in the study are as presented in the Table 2 and 3 respectively below.

Table 2-*Genetic Materials (Inbred Lines) Used in the Study*

| Inbred Males | Pedigree | Source |
|---|---|---|
| CML11 | P21-C5-FS219-3-2-2-3-#-7-1-B-4-1-B | CIMMYT |
| CML247 | (G24-F119/924-F54)-6-4-1-1-B | CIMMYT |
| CML287 | (P24-F26/P27-F1)-4-1-B-1-1-B | CIMMYT |
| CML343 | LAPOSTA SEQ-C3-FS17-1-2-3-2-1-B | CIMMYT |
| CML5 | PobZ1C5HC133·1-B_B | CIMMYT |

Source: Information from, CSIR-Crops Research Institute Ghana (2019)

Table 2 - *Continued*

| Inbred Males | Pedigree | Source |
|---|---|---|
| Ki3 | Ki 3 (86329) | THAILAND |
| TZI8 | TZB x TZSR | IITA |
| FEMALES | | |
| ENTRY-5 | | CIMMYT |
| ENTRY-6 | | CIMMYT |
| ENTRY-70 | | CIMMYT |
| ENTRY-85 | | CIMMYT |
| M0826-12F* | 2-B-B:DT-SR-W-C0/1368×PAC90038-1×1368-6-07C04772B 06A11833B | IITA |
| M0826-7F* | B-B-B-B-B-B:DT-SR-W-C0/1368×PAC90038-1×1368-3-07C04754B 06A11803B | IITA |
| TZEEI-15* | TZEEI-15 WPopxLDS6(Set A) Inb.44 | IITA |
| TZEEI-6* | TZEEI – 6 WSRBC5x1368STRS7Inb.100 | IITA |

Source: Information from, CSIR-Crops Research Institute Ghana (2019)

Table 3 - *Genetic Materials (Hybrids and Checks) Used in The Study*

| Variety | Year of Release | Genotype | Source |
|---|---|---|---|
| Tintim | 2012(check) | | CRI |
| Obotantim | 2015(check) | | CRI |
| Mamaba | 1997(check) | WQPHM | CRI |
| Etubi | 2007(check) | WQPHM | CRI |
| M0826-7F×TZI-8 | - | Hybrid | CRI |
| TZEEI-15×MP-715 | - | Hybrid | CRI |
| M0826-12F×CML-176 | - | Hybrid | CRI |
| ENTRY-5 ×CML-11 | - | Hybrid | CRI |
| ENTRY-85×CML-247 | - | Hybrid | CRI |

Source: Information from, CSIR-Crops Research Institute Ghana (2019)

Table 3 - *Continued*

| Variety | Year of Release | Genotype | Source |
|---|---|---|---|
| ENTRY-5×CML-287 | - | Hybrid | CRI |
| MO826-7F×CML-11 | - | Hybrid | CRI |
| M0825-12F×CML-343 | - | Hybrid | CRI |
| M0826-7F×CML-343 | - | Hybrid | CRI |
| ENTRY-5×K1-3 | - | Hybrid | CRI |
| ENTRY-5×TZI-8 | - | Hybrid | CRI |
| TZEEI-6×CML-11 | - | Hybrid | CRI |
| ENTRY-70×CML-247 | - | Hybrid | CRI |
| M0826-7F×CML-5 | - | Hybrid | CRI |

Source: Information from, CSIR-Crops Research Institute Ghana (2019)

**Experimental Design**

A randomized complete block design was used with three replications. Each of the 18 genotypes were sown at 3 seeds per hill and thinned to two plants per hill after two weeks. Each plot consisted of two rows on a 5m long plot, 0.75m inter-rows and 0.4m intra-row spacing.

**Field Establishment**

Experimental fields were set up in all the six locations; Ejura, Fumesua, Wenchi, Akumadan, Ohawu and Kpeve both in the major and minor cropping seasons. Planting for the major season was carried out in April and May 2019 when there was appreciable rainfall. However, planting was carried out sequentially with intermittent planting dates between the various locations to ensure harvesting dates did not coincide with other locations. Planting was done

in Akumadan on 18th April 2020 and 30th April 2020 for Wenchi and Ejura. Planting was also done on the 2nd, 14th, and 15th May 2020 at Fumesua, Kpeve and Ohawu respectively.

However, planting in the minor season was carried out spatially across all the six (6) locations as was done in the major season. Planting was done on the 3rd September at Akumadan and 4th September for Ejura and Wenchi. Again, planting was carried out at Fumesua on 9th September and on 10th, 11th September at Kpeve and Ohawu respectively.

**Cultural Practices**

Application of NPK 15:15:15 was carried out at the rate of 250kg/ha and 60 kg/ha of $P_2O_5$ as basal fertilizer at two weeks after sowing and top-dressed with additional N 60kg/ha at four weeks after planting. Post emergence herbicide Atrazine at a rate of 1.5 l/ha was used to control broadleaf weeds after ploughing and sowing of the seed maize. Hoe and hand weeding were also done when necessary to control weeds during the growing period. Application of pesticide with trade name Attack was also applied at every two weeks to control fall armyworm (*Spodoptera frugiperda)* infestation and other management practices were carried out according to recommendation of the specific area.

**Inoculation Preparation**

Identified toxigenic isolate of *Aspergillus sp.* was used to prepare the inoculum as described by Windham & Williams (2002). The procedure involved

multiplication of the isolate on sterile corn cob grit in 500-ml flasks each containing 50 g of grits and 100 ml of sterile distilled water and incubated at 28°C for 3 weeks. Conidia in each flask was washed from the grits using 500 ml of sterile distilled water containing 20 drops of Tween 20 per liter and then filtered through four layers of sterile cheese-cloth.

The concentrations of conidia were determined with a hemacytometer and adjusted with sterile distilled water to $9 \times 10^7$ conidia per ml. Excess inoculum not used immediately was refrigerated at 4°C.

**Inoculation**

The side needle method was used for inoculating ears of 5 plants at random per plot 14 days after midsilk. A preparation of 3.4ml of a spore suspension of $9 \times 10^7$ conidia/ml was injected over the kernels as reported by Scott & Zammo (1994).

**Harvesting and Processing**

The crops were harvested at physiological maturity when it had completely dried and the husk had turned from green to brown. The crops were harvested plot by plot and the inoculated cobs harvested separately to avoid cross contamination and the necessary data (Fresh weight, ears harvested) taken before they were conveyed to the maize barn for processing. Cobs from each plot were harvested in separate bags labelled with the plot number. During processing, the maize was shelled plot by plot and the grain weight data were taken together with

their respective moisture contents. Samples of the grains were taken to the laboratory to determine the aflatoxin contents.

**Aflatoxin Analysis**

Aflatoxin was extracted using methods described by Sirhan et al. (2013) with modifications. Samples were milled using a Preethi Mixer Grinder into homogenized flour. Two grams (2g) of slurry was weighed into a 15 ml centrifuge tube and topped-up with a 4 ml of 60:40 (v/v) methanol acetronitrile solution. The resultant mixture was vortexed using Genie Vortex machine for 3mins. 1.32 g of anhydrous $MgSO_4$ and 0.2g of NaCl were added to the mixture and then vortexed for 1min. The tube was centrifuged for 5min at 4000rpm and the upper organic layer filtered through a 0.45µm nylon syringe prior to injection. A volume of 100µl of the filtered extract was injected into the HPLC.

A Cecil-Adept Binary Pump HPLC coupled with Shimadzu 10AxL fluorescence detector (Ex: 360nm, Em: 440nm) with Phenomenex HyperClone BDS C18 Column (150 x 4.60mm, 5um). The mobile phase used was methanol: water (40:60, v/v) at a flow rate of 1ml/min with column temperature maintained at 40 °C. To 1 liter of mobile phase were added 119 mg of potassium bromide and 350 µl of 4 M nitric acid (required for postcolumn electrochemical derivatisation with Kobra Cell, R-Biopharm Rhone). Aflatoxin Mix ($G_1$, $G_2$, $B_1$, $B_2$) standards (ng/g) were prepared from Supelco® aflatoxin standard of 2.6 ng/µL in methanol.

Concentration of $B_1$ and $G_1$ were 0.5, 1, 2, 8, 16 ng /g per 100ul injection. Concentration of $B_2$ and $G_2$ were 0.15, 0.3, 0.6, 2.4, 4.8 ng/g per 100ul injection.

Limit of Detection and Limit of quantification of total aflatoxin were established at 0.5ng/g and 1ng/g respectively. Aflatoxin concentration was estimated as, ng/g = A x (T/I) x (1/W) where A = ng of aflatoxin as eluate injected, T = final test solution eluate volume (µl), I = volume eluate injected into LC (µl), W = mass (g) of commodity represented by final extract.

**Data Collection**

The following data were recorded on all plants across the locations:

1. Days to anthesis; the number of days from the date of planting when 50% of the maize plants tassel and shed pollen.
2. Days to silking; the number of days from the date of planting to when 50% of the silk emerges.
3. Plant height; Measured with a graduated stick on five randomly selected plants and measured from the ground level to the node bearing the flag leaf.
4. Ear height; Measured by using the same plants from which the heights were taken, the height of the ear from ground level to the node bearing the uppermost ear were recorded.
5. Ear aspect; Based on a scale of 1-5 where1; clean, uniform, large and well-filled ear and 5; ears with undesirable features, plants were scored.
6. Husk cover; Data on husk cover were taken when the ears were fully developed and about three weeks to harvest using a scale of 1-5 rating 1;

husk tightly arranges and extends beyond ear tip and 5= husk poorly arrange and ear tips exposed.

7. Diseases; Diseases were scored rating 1-5. Where 1; absence of disease and 5; severe infection.

The following data was computed before the data analysis was done;

8. Anthesis-Silking interval

   The anthesis-silking interval (ASI) was obtained by the difference between the days to anthesis and days to silking.

9. Grain yield : The grain yield in kilograms were calculated for each plot at 15% grain moisture content (Rahman et al., 2007) using the formula below:

   $$\text{Field grain yield (t/ha)} = \frac{FW \times (100 - MC) \times 0.83 \times 10{,}000}{85 \times 75}$$

   Where; FW= field weight, MC= percentage moisture content, 0.83= shelling coefficient.

Other data taken during harvesting included; field weight (the weight of cobs per row measured in kilograms), Ears harvested (total number of ears harvested per row), plants harvested (total number of plants harvested per row).

**Data Analysis**

The data were analysed for variances (ANOVA) per location and across location for agronomic traits using the Genstat statistical package (Genstat, 2007). Table 4 below shows the format used for the analysis of variance.

Table 4- *Format for Analysis of Variance*

| Source | Degree of Freedom (df) | Sum of Square (SS) | Mean Square (MS) |
|---|---|---|---|
| Location (L) | L-1 | $SS_L$ | |
| Replication (R) | R-1 | $SS_R$ | |
| Genotype(G) | G-1 | $SS_E$ | $MS_G$ |
| G×L | (G-1)(L-1) | $SS_{GL}$ | $MS_{GL}$ |
| Pooled error | (q-1)(p-1) | SSe | $MS_e$ |
| Total | (pq-1) | $SS_T$ | $MS_T$ |

L-location,R-replication,G-genotype,E-error,T-total

Source: Information from Genstat Statistical Package (2020)

**Stability of the Genotypes**

The grain yield of the individual hybrids was analysed and used to determine the stability of the genotype using the GGE biplot model (Yan et al., 2001). The GGE bi-plot model equation is:

$Yij - \mu - ßj = \lambda 1 \xi i1 \eta 1j + \lambda 2 \xi i2 \eta 2j + \xi ij$

Where λ1 and λ2 are the singular values of first and second largest principal components, PC1 and PC2, respectively; the square of the singular value of a PC is the sum of squares explained by the PC; ξi1 and ξi2 are the eigenvectors of genotype I for PC1 and PC2, respectively; and η1j and η2j are the eigenvectors of environment j for PC1 and PC2, respectively.

# CHAPTER FOUR

# RESULTS AND DISCUSSION

The purpose of the study was to assess the performance of maize hybrids resistant to aflatoxin accumulation. The hybrids were evaluated across six locations in two growing seasons. The materials were planted in randomized complete block design and the data of agronomic traits were taken from which analyses of variance were computed as results. This chapter gives an overview of the results obtained from the experiments and their implications.

## Performance of 18 Maize Genotypes for Aflatoxin Resistance and Other Agronomic Traits across Locations during the Major Season of 2018

Analysis of variance (ANOVA) for aflatoxin resistance and agronomic traits among the 18 genotypes indicated that environmental effect was significant ($p < 0.05$) for all traits (Table 5). On the other hand, genotypic effect was also significant for all traits except Grain yield, anthesis silking interval, rust, blight, MSV and Ear rot. This means that the test environments were variable and that most genotypes performed differently in different environments. Genotypes by environment interactions were not significant ($P < 0.05$) for most of the traits except plant aspect, grain moisture and root lodging. Table 5 below shows the mean squares of the agronomic traits for the genotypes during the major season.

Table 5 *Mean Squares of Grain Yield, and Other Agronomic Traits of 18 Maize Genotypes across Six Environments During the Major Season*

| Sources of Variation | DF | Grain Yield (Kg/ ha) | Days to 50% Anthesis | Days to 50% Silking | ASI | Ear Aspect | Ear Harvested | Husk Cover | Plant Aspect (1-5) |
|---|---|---|---|---|---|---|---|---|---|
| ENV | 5 | 1.034E+08* | 358.73* | 245.02* | 31.75* | 26.42* | 554.3* | 11.35* | 18.23* |
| GENOTYPE | 17 | 1.182E+07ns | 67.96* | 72.37* | 1.06ns | 0.47* | 129.65* | 1.17* | 1.56* |
| GENOTYPE*ENV | 85 | 6.892E+06ns | 3.33ns | 3.54ns | 0.81ns | 0.31ns | 57.52ns | 0.48ns | 0.42* |
| REP | 2 | 1.905E+07 | 18.56 | 23.52 | 3.03 | 0.15 | 68.93 | 0.096 | 0.39ns |
| Error | 214 | 8.440E+06 | 4.17 | 4.75 | 0.77 | 0.25 | 47.97 | 0.47 | 0.28 |

*Significant at 0.05, ns not significant and DF=degree of freedom, ASI=Anthesis silking interval,

MSV=Maize streak virus

Source: Field data (2019)

Table 5 – *Continued*

| Sources of Variation | DF | Grain Moisture | Rust (1-5) | Blight (1-5) | MSV (1-5) | Ear rot (1-5) | Plant Height (cm) | Ear Height (cm) | Root Lodging | Stalk Lodging |
|---|---|---|---|---|---|---|---|---|---|---|
| ENV | 5 | 184.79* | 0.04* | 0.48* | 0.01* | 0.91* | 29902.0* | 15303.2* | 2.19* | 2.06* |
| GENOTYPE | 17 | 6.50* | 0.003ns | 0.004ns | 0.002ns | 0.003ns | 1684.6* | 968.3* | 0.12* | 0.03* |
| GENOTYPE*ENV | 85 | 2.22* | 0003ns | 0.003ns | 0.004ns | 0.004ns | 255.7ns | 193.3ns | 0.08* | 0.01ns |
| REP | 2 | 0.70 | 0.002 | 0.005 | 0.012 | 0.0004 | 1164.0 | 103.6 | 0.02 | 0.03 |
| Error | 214 | 0.83 | 0.003 | 0.002 | 0.004 | 0.004 | 302.8 | 224.00 | 0.02 | 0.01 |

* Significant at 0.05 probability level, ns not significant and DF=Degree of freedom, MSV=Maize streak virus

Source: Field data (2019)

The mean performance of the 18 genotypes evaluated during the major season showed significant (P<0.05) genotypic effect on the measured traits (Table 6). Grain yield varied from 3610 kg/ha for GH 18 (check Etubi) to 7057kg/ha for GH 05 (ENT-85×CML-247). Other traits such as days to 50% pollen and silking ranged from 45.00 to 52.72 and 47.56 to 55.83 for Obotantim and M0826-7F×CML-343, respectively. Anthesis silking interval ranged from approximately 2 to 3 days with a mean of 3 days.

The mean number of ears harvested, was approximately 35 although it ranged from 28.39 to 40.28 for some genotypes. Among the 18 genotypes studied, GH02 (TZEEI-15 X MP-715) produced the highest number of ears harvested (40.28) whilst GH 18(Check Etubi) recorded the least number of ears (28.28). The extent of genotypic variation probably accounted for the significant (P<0.05) differences observed for traits such as ear aspect, plant aspect, ear rot and husk cover, an important characteristic of aflatoxin resistance, was variable among the genotypes. It ranged from 1.94 for GH09 (MO826-7F x CML-343) to 2.89 for GH13 (ENT-70 x CML-247) with a mean of 2.38. Among the remaining genotypes, there wasn't significant variation observed in the extent of their resistance to blight, rust and Streak. The most susceptible genotype for blight disease was GH02 (TZEEI-15 X MP-715) 0.45 while GH13 (ENT-70 X CML-247) 0.39 appeared to be the most resistant. Generally, means for disease traits was approximately 1, an indication of tolerance by majority of genotypes. Plant and ear height, grain moisture, stem and root lodging were all found to be significantly (P<0.05) different among the genotypes evaluated (Table 6). The

Table 6 below shows the mean performance of the genotypes for the various traits.

Table 6- *Mean Performance of 18 Genotypes for Yield, and Other Traits across Six Environments during the Major Season of 2018*

| Genotype | DP | DS | ASI | EH | PH | YH | EA | E-HAR | HC |
|---|---|---|---|---|---|---|---|---|---|
| MO826-7F X TZ1-8 | 47.89 | 51.33 | 3.44 | 84.67 | 163.83 | 5142 | 2.44 | 36.89 | 2.06 |
| TZEEI-15XMP-715 | 47.89 | 50.72 | 2.83 | 99.61 | 175.78 | 5990 | 2.22 | 40.28 | 2.61 |
| MO826-12FXCML-176 | 50.72 | 53.44 | 2.72 | 86.28 | 178.72 | 5749 | 2.56 | 35.00 | 2.11 |
| ENT-5XCML-11 | 51.00 | 54.17 | 3.17 | 79.44 | 163.72 | 4338 | 2.28 | 30.56 | 2.22 |
| ENT-85XCML-247 | 50.11 | 53.33 | 3.22 | 92.22 | 181.17 | 7057 | 2.17 | 33.06 | 2.78 |
| ENT-5XCML-287 | 50.56 | 53.33 | 2.78 | 89.94 | 179.94 | 5051 | 2.33 | 37.94 | 2.50 |
| MO826-7FXCML-11 | 51.72 | 54.83 | 3.11 | 88.94 | 173.72 | 4568 | 2.06 | 32.61 | 2.44 |
| MO826-12FXCML-343 | 52.28 | 55.39 | 3.11 | 90.06 | 179.56 | 5054 | 2.00 | 36.22 | 2.11 |
| MO826-7FXCML-343 | 52.72 | 55.83 | 3.11 | 86.11 | 175.78 | 6061 | 2.22 | 36.00 | 1.94 |
| ENT-5XK1-3 | 49.06 | 52.11 | 3.06 | 74.78 | 161.28 | 5132 | 2.33 | 33.72 | 2.17 |
| ENT-5XT21-8 | 47.67 | 50.61 | 2.94 | 69.67 | 150.89 | 6001 | 2.28 | 34.39 | 2.28 |
| TZEE1-6XCML-11 | 47.83 | 50.94 | 3.11 | 83.50 | 169.94 | 5568 | 2.22 | 34.67 | 2.67 |

**DP**=Days to 50% pollen, **DS**=Days to 50% silking, **ASI**=Anthesis-silking interval, **EH**=Ear height, **PH**=plant height, **EA**=Ear aspect.

Source: Field data (2019)

Table 6- *Continued*

| Genotype | DP | DS | ASI | EH | PH | YH | EA | E-HAR | HC |
|---|---|---|---|---|---|---|---|---|---|
| ENT-70XCML-247 | 49.94 | 52.50 | 2.56 | 87.22 | 167.44 | 6181 | 2.22 | 35.78 | 2.89 |
| MO826-7FXCML-5 | 50.00 | 53.11 | 3.11 | 86.78 | 172.94 | 4793 | 2.44 | 34.83 | 2.33 |
| CHECK-TIM TIM | 49.22 | 52.33 | 3.11 | 78.28 | 165.83 | 5556 | 2.50 | 35.28 | 2.50 |
| CHECK-OBOTANTIM | 45.00 | 47.56 | 2.56 | 77.56 | 156.94 | 5437 | 2.50 | 33.94 | 2.39 |
| CHECK MAMABA | 47.78 | 50.89 | 3.11 | 75.89 | 153.94 | 4495 | 2.39 | 32.44 | 2.39 |
| CHECK ETUBI | 48.67 | 51.94 | 3.28 | 78.78 | 155.89 | 3610 | 2.56 | 28.39 | 2.39 |
| LSD (5%) | 0.77 | 0.83 | 0.33 | 5.68 | 6.60 | 1102.10 | 0.19 | 2.63 | 0.26 |
| MEANS | 49.45 | 52.47 | 3.05 | 83.87 | 168.19 | 5321 | 2.318 | 34.56 | 2.38 |

**DP**=Days to 50% pollen, **DS**=Days to 50% silking, **ASI**=Anthesis-silking interval, **EH**=Ear height, **PH**=plant height, **EA**=Ear aspect.

Source: Field data (2019)

Table 6 -*Continued*

| Genotype | MSV | LR | LS | RUST | BLIGHT | EAR ROT |
|---|---|---|---|---|---|---|
| MO826-7F X TZ1-8 | 0.32 | 0.39 | 0.32 | 0.33 | 0.40 | 0.29 |
| TZEEI-15XMP-715 | 0.30 | 0.41 | 0.38 | 0.35 | 0.45 | 0.27 |
| MO826-12FXCML-176 | 0.32 | 0.41 | 0.37 | 0.34 | 0.41 | 0.28 |
| ENT-5XCML-11 | 0.32 | 0.59 | 0.44 | 0.33 | 0.41 | 0.29 |
| ENT-85XCML-247 | 0.31 | 0.42 | 0.40 | 0.30 | 0.40 | 0.27 |
| ENT-5XCML-287 | 0.32 | 0.60 | 0.38 | 0.31 | 0.42 | 0.29 |
| MO826-7FXCML-11 | 0.33 | 0.59 | 0.46 | 0.30 | 0.42 | 0.29 |
| MO826-12FXCML-343 | 0.34 | 0.52 | 0.38 | 0.31 | 0.41 | 0.25 |
| MO826-7FXCML-343 | 0.32 | 0.44 | 0.35 | 0.31 | 0.42 | 0.28 |
| ENT-5XK1-3 | 0.32 | 0.46 | 0.36 | 0.32 | 0.42 | 0.26 |
| ENT-5XT21-8 | 0.32 | 0.40 | 0.32 | 0.31 | 0.41 | 0.27 |
| TZEE1-6XCML-11 | 0.32 | 0.56 | 0.40 | 0.32 | 0.41 | 0.28 |
| ENT-70XCML-247 | 0.33 | 0.41 | 0.38 | 0.31 | 0.39 | 0.26 |
| MO826-7FXCML-5 | 0.34 | 0.37 | 0.33 | 0.30 | 0.40 | 0.26 |
| CHECK-TIM TIM | 0.32 | 0.43 | 0.31 | 0.33 | 0.44 | 0.27 |
| CHECK-OBOTANTIM | 0.32 | 0.38 | 0.35 | 0.34 | 0.41 | 0.28 |
| CHECK MAMABA | 0.36 | 0.56 | 0.40 | 0.32 | 0.41 | 0.25 |
| CHECK ETUBI | 0.33 | 0.47 | 0.42 | 0.31 | 0.42 | 0.28 |
| LSD (5%) | 0.03 | 0.06 | 0.04 | 0.02 | 0.02 | 0.02 |
| MEANS | 0.33 | 0.47 | 0.37 | 0.32 | 0.42 | 0.27 |

LSD-Least significant difference, LR=Root lodging, LS=Stalk lodging, MSV=Maize streak virus

Source: Field data (2019)

**The Mean Performances of the various Traits within Each of the Six Locations during the Major Season 2018**

The Table 7 below shows the mean performance of the various traits in the six environments. The comparative performances in the various environments with regard to the traits shows that there was significant difference between yield observed in Fumesua as compared to all the other locations which suggests that Fumesua is the model environment for the highest expression of yield potential for the various genotypes.

Across the locations the yield ranges from 3136 kg/ha in Fumesua to 7442 kg/ha in Ohawu. There was no significant yield difference between Akumadan, Wenchi, Ejura and Kpeve which suggest stability of the genotypes across these environments. It could also be observed that Days to pollen in Ohawu (47.76) was significantly different from what was observed in Kpeve (54.17), however anthesis-silking interval was high, that is approximately 3 days which could account for lower yield.

Generally, husk cover, maize streak virus, rust, blight, ear rot, root lodging and stalk lodging were below 1.00 which suggests good tolerance to diseases.

Table 7- *Mean Performance of The Various Traits Within the Six Environments During the Major Season of 2018*

| LOCATION | DP | DS | ASI | Ear Height | Plant Height | kg/hectare | No. of Ear Harv |
|---|---|---|---|---|---|---|---|
| FUMESUA | 47.91 | 50.67 | 2.76 | 101.17 | 202.02 | 7442.00 | 34.65 |
| AKUMADAN | 50.74 | 53.78 | 3.04 | 85.67 | 167.30 | 5374.00 | 31.33 |
| WENCHI | 47.76 | 50.87 | 3.11 | 100.74 | 178.15 | 5036.00 | 32.69 |
| EJURA | 48.35 | 52.59 | 4.24 | 81.98 | 164.02 | 5177.00 | 32.02 |
| KPEVE | 54.17 | 56.02 | 1.85 | 77.94 | 168.26 | 5762.00 | 39.46 |
| OHAWU | 47.76 | 50.87 | 3.11 | 55.74 | 129.37 | 3136.00 | 37.19 |
| LSD (5%) | 0.76 | 0.83 | 0.33 | 5.68 | 6.60 | 1102.10 | 2.63 |

LSD-Least Significant Difference, DP=Days to 50% pollen, DS=Days to 50%Silking, ASI=Anthesis silking-interval

Source: Field data (2019)

Table 7-*Continued*

| LOCATION | EA | HC | MSV | LR | LS | RUST | BLIGHT | EAR ROT |
|---|---|---|---|---|---|---|---|---|
| FUMESUA | 2.44 | 2.37 | 0.33 | 0.40 | 0.33 | 0.33 | 0.49 | 0.33 |
| AKUMADAN | 1.00 | 1.65 | 0.33 | 0.30 | 0.48 | 0.30 | 0.30 | 0.01 |
| WENCHI | 2.96 | 2.83 | 0.34 | 0.31 | 0.32 | 0.37 | 0.50 | 0.38 |
| EJURA | 2.59 | 2.04 | 0.33 | 0.61 | 0.06 | 0.30 | 0.40 | 0.30 |
| KPEVE | 2.17 | 2.63 | 0.31 | 0.37 | 0.41 | 0.32 | 0.48 | 0.31 |
| OHAWU | 2.74 | 2.74 | 0.30 | 0.81 | 0.64 | 0.30 | 0.30 | 0.30 |
| LSD (5%) | 0.19 | 0.26 | 0.03 | 0.06 | 0.04 | 0.02 | 0.02 | 0.02 |

LSD-Least Significant Difference, EA=Ear aspect, HC=husk cover, MSV=maize streak virus, LR=Root lodging, LS=Stalk lodging.

Source: Field data (2019)

**Performance of 18 Maize Genotypes for Aflatoxin Resistance and Other Agronomic Traits across Locations during the Minor Season of 2019**

The ANOVA for the agronomic traits in the minor season showed significant ($P<0.05$) effect of environment for all traits (Table 8). Genotype was significantly different for most of the traits except Grain yield, husk cover, plant aspect, rust resistance, blight resistance, ear rot, root and stalk lodging.

Genotype by environment interaction was significant for traits such as days to 50% anthesis, days to 50% silking, ears harvested, streak disease, root lodging and stalk lodging. The mean sum of squares for the 18 genotypes during the minor season is presented in Table 8 below.

Table 8-*Mean Sum of Squares of 18 Maize Genotypes Evaluated Across Six Locations in Minor Season of 2019*

| Sources of Variation | DF | Grain Yield (kg/ ha) | Days to 50% Anthesis | Days to 50% Silking | ASI | Ear Aspect | Ear Harvested | Husk Cover | Plant Aspect (1-5) |
|---|---|---|---|---|---|---|---|---|---|
| ENV | 5 | 2.351E+07* | 428.34* | 229.17* | 36.69* | 26.42* | 644.84* | 21.41* | 28.34* |
| GENOTYPE | 17 | 4.199E+06ns | 24.94* | 29.99* | 0.85* | 0.46* | 241.82* | 0.34ns | 0.30ns |
| GENOTYPE*ENV | 85 | 2.791E+06ns | 4.88* | 5.79* | 0.52ns | 0.31ns | 67.50* | 0.29ns | 0.20ns |
| REP | 2 | 1.185E+06 | 7.26 | 4.95 | 0.31 | 0.15 | 279.75 | 0.84 | 0.70 |
| Error | 214 | 3.080E+06 | 3.60 | 4.57 | 0.45 | 0.25 | 47.45 | 0.28 | 0.27 |

*, Significant at 0.05 probability level, ns not significant

Source: Field data (2019)

Table 8 -*Continued*

| Sources of Variation | DF | Grain Moisture | Rust (1-5) | Blight (1-5) | MSV (1-5) | Ear rot (1-5) | Plant Height (cm) | Ear Height (cm) | Root Lodging | Stalk Lodging |
|---|---|---|---|---|---|---|---|---|---|---|
| ENV | 5 | 100.21* | 0.003* | 0.45* | 0.21* | 0.004* | 48693.90* | 27933.20* | 0.18* | 0.33* |
| GENOTYPE | 17 | 1.74* | 0.002ns | 0.00ns | 0.01ns | 0.001ns | 898.40* | 816.60* | 0.005ns | 0.01ns |
| GENOTYPE*ENV | 85 | 1.68* | 0.001ns | 0.00ns | 0.01* | 0.001ns | 317.8ns | 225.60ns | 0.009* | 0.01* |
| REP | 2 | 5.83 | 0.001 | 0.00 | 0.00 | 0.001 | 558.5 | 227.40 | 0.003 | 0.02 |
| Error | 214 | 1.05 | 0.001 | 0.00 | 0.01 | 0.001 | 375.5 | 209.10 | 0.006 | 0.01 |

* Significant at 0.05 probability level, ns not significant, MSV=Maize streak virus

Source: Field data (2019)

The mean performances of the 18 genotypes for agronomic traits evaluated in the minor season in all the six locations are presented in Table 9. There were significant differences ($P < 0.05$) observed among the genotypes with respect to some of the traits. Grain yield varied from 2606.00 kg/ha for GH03 (MO826-12F X CML-176) to 4642.00 kg/ ha for GH08 (MO826-12F X CML-343). Days to 50% pollen ranged from 52.94days for GH08 (MO826-12F X CML-343) to 48.00 days for GH16 (OBOTANTIM) with a mean of 51.52 days. Days to 50% silking also varied from 50.94 days for GH16 (Obotantim) to 56.28 days for GH08 (MO826-12F X CML-343) with a mean of 54.57 days. Anthesis-silking interval (ASI), which ranged from 2.61 to 3.39 days with a mean of about 3.05 days. Mean for husk cover ranged from 1.94 for GH07 (MO826-7F X CML-11) to 2.44 for GH05 (ENT-85xCML-247) (Table 9). Genotypes with large values for husk cover are more exposed to *Aspergillus* infection as it serves as easy entry points for *A. flavus* and other microbes to infect the grain and hence results in aflatoxin buildup in the grain.

Traits such as rust resistance, blight and MSVD resistance had means of 0.31, 0.42 and 0.36 respectively with the values suggesting a low susceptibility of the entire germplasm to disease. Means for ear rot, plant and ear height and root and stem logging were found to be 0.31, 166.8, 77.72, 0.37 and 0.38, respectively. The mean performance for the various agronomic traits during the minor season is presented in the table 9 below.

Table 9 - *Mean Performances of 18 Genotypes for Grain Yield, and Other Agronomic Traits across Six Environments During the Minor Season of 2019*

| Genotype | DP | DS | ASI | EH | PH | YH | E HARV |
|---|---|---|---|---|---|---|---|
| MO826-7F X TZ1-8 | 52.50 | 55.50 | 3.00 | 74.22 | 165.40 | 2693 | 26.67 |
| TZEEI-15XMP-715 | 51.33 | 54.44 | 3.11 | 95.11 | 167.80 | 2961 | 33.33 |
| MO826-12FXCML-176 | 52.22 | 55.61 | 3.39 | 87.11 | 167.20 | 2606 | 24.89 |
| ENT-5XCML-11 | 52.83 | 56.00 | 3.17 | 76.11 | 168.60 | 2646 | 23.00 |
| ENT-85XCML-247 | 51.89 | 55.06 | 3.17 | 81.61 | 169.60 | 3226 | 25.22 |
| ENT-5XCML-287 | 51.61 | 54.50 | 2.89 | 83.17 | 179.20 | 3500 | 27.22 |
| MO826-7FXCML-11 | 52.44 | 55.72 | 3.28 | 81.33 | 179.70 | 3518 | 23.33 |
| MO826-12FXCML-343 | 52.94 | 56.28 | 3.33 | 80.56 | 170.20 | 4642 | 25.33 |
| MO826-7FXCML-343 | 52.06 | 55.44 | 3.39 | 76.44 | 172.20 | 3223 | 22.50 |
| ENT-5XK1-3 | 51.33 | 54.06 | 2.72 | 74.39 | 156.90 | 2576 | 24.83 |
| ENT-5XT21-8 | 51.11 | 54.17 | 3.06 | 65.83 | 157.60 | 2965 | 26.22 |
| TZEE1-6XCML-11 | 51.00 | 53.61 | 2.61 | 78.17 | 166.90 | 2904 | 21.83 |

Source: Field data (2019)

Table 9 - *Continued*

| Genotype | DP | DS | ASI | EH | PH | YH | E HARV |
|---|---|---|---|---|---|---|---|
| ENT-70XCML-247 | 52.17 | 55.06 | 2.89 | 75.22 | 163.10 | 2956 | 22.28 |
| MO826-7FXCML-5 | 52.17 | 55.28 | 3.11 | 79.78 | 174.00 | 3253 | 26.44 |
| CHECK-TIM TIM | 51.00 | 53.89 | 2.89 | 75.33 | 166.20 | 2780 | 20.50 |
| CHECK-OBOTANTIM | 48.00 | 50.94 | 2.94 | 68.56 | 154.60 | 3346 | 15.61 |
| CHECK MAMABA | 49.89 | 52.89 | 3.00 | 72.33 | 158.30 | 2898 | 20.89 |
| CHECK ETUBI | 50.89 | 53.83 | 2.94 | 73.67 | 164.40 | 3013 | 21.89 |
| LSD (5%) | 1.25 | 1.40 | 0.44 | 9.50 | 12.73 | 1153.20 | 4.53 |
| MEANS | 51.52 | 54.57 | 3.05 | 77.72 | 166.8 | 3095 | 24.00 |

Source: Field data (2019)

Table 9-*Continued*

| Genotype | MSV | LR | LS | RUST | HC | EAR ROT | PA | BLIGHT |
|---|---|---|---|---|---|---|---|---|
| MO826-7F X TZ1-8 | 0.34 | 0.37 | 0.35 | 0.30 | 2.11 | 0.31 | 2.22 | 0.42 |
| TZEEI-15XMP-715 | 0.36 | 0.37 | 0.37 | 0.32 | 2.39 | 0.30 | 2.33 | 0.42 |
| MO826-12FXCML-176 | 0.38 | 0.34 | 0.37 | 0.30 | 2.06 | 0.31 | 2.06 | 0.42 |
| ENT-5XCML-11 | 0.36 | 0.38 | 0.43 | 0.30 | 2.17 | 0.31 | 2.22 | 0.41 |
| ENT-85XCML-247 | 0.36 | 0.36 | 0.38 | 0.30 | 2.44 | 0.31 | 2.22 | 0.42 |
| ENT-5XCML-287 | 0.38 | 0.40 | 0.40 | 0.30 | 2.17 | 0.33 | 2.17 | 0.42 |
| MO826-7FXCML-11 | 0.38 | 0.35 | 0.43 | 0.30 | 1.94 | 0.30 | 2.06 | 0.42 |
| MO826-12FXCML-343 | 0.38 | 0.37 | 0.36 | 0.31 | 2.06 | 0.30 | 2.06 | 0.43 |
| MO826-7FXCML-343 | 0.37 | 0.38 | 0.39 | 0.32 | 2.00 | 0.30 | 2.17 | 0.41 |
| ENT-5XK1-3 | 0.36 | 0.39 | 0.38 | 0.30 | 2.17 | 0.30 | 2.22 | 0.42 |
| ENT-5XT21-8 | 0.33 | 0.37 | 0.35 | 0.30 | 2.22 | 0.30 | 2.22 | 0.42 |
| TZEE1-6XCML-11 | 0.36 | 0.36 | 0.38 | 0.31 | 2.06 | 0.31 | 2.06 | 0.42 |

MSV=Maize streak virus, LR=Root lodging, LS=Stalk lodging, ASI=Anthesis-silking interval, EH=Ear height, PH=Plant height

Source: Field data (2019)

Table 9 - *Continued*

| Genotype | MSV | LR | LS | RUST | HC | EAR ROT | PA | BLIGHT |
|---|---|---|---|---|---|---|---|---|
| ENT-70XCML-247 | 0.41 | 0.34 | 0.38 | 0.30 | 2.11 | 0.30 | 1.83 | 0.42 |
| MO826-7FXCML-5 | 0.32 | 0.39 | 0.39 | 0.31 | 2.00 | 0.31 | 2.00 | 0.42 |
| CHECK-TIM TIM | 0.32 | 0.36 | 0.34 | 0.32 | 2.00 | 0.30 | 1.94 | 0.42 |
| CHECK-OBOTANTIM | 0.34 | 0.35 | 0.36 | 0.34 | 2.28 | 0.30 | 2.28 | 0.42 |
| CHECK MAMABA | 0.36 | 0.38 | 0.37 | 0.31 | 2.00 | 0.30 | 2.17 | 0.42 |
| CHECK ETUBI | 0.38 | 0.35 | 0.38 | 0.30 | 2.06 | 0.31 | 2.22 | 0.42 |
| LSD (5%) | 0.05 | 0.05 | 0.05 | 0.02 | 0.35 | 0.02 | 0.34 | 0.009 |
| MEANS | 0.36 | 0.37 | 0.38 | 0.31 | 2.12 | 0.31 | 2.14 | 0.42 |

MSV=Maize streak virus, LR=Root lodging, LS=Stalk lodging, ASI=Anthesis-silking interval, EH=Ear height, PH=Plant height

Source: Field data (2019)

**The Comparative Mean Performances of the Various Traits within Each of the Six Locations during the Minor Season 2019**

The performance of the genotypes across the six locations with regard to the traits shows that there was significant difference between yield observed in Ejura as compared to all the other locations which suggests that during the drought season Ejura is the model environment for the highest expression of yield potential for the various genotype. Fumesua which performed better during the major season performed relatively poor during the minor season with 2451 kg/ha (Table 10).

Across the locations the yield ranges from 2395 kg/ha in Kpeve to 4073.00 kg/ha in Ejura. There was significant yield difference between Akumadan, Fumesua, Ejura and Ohawu which suggest environmental influence on the genotypes across these environments. It could also be observed that Days to pollen ranges from 49.28 days in Ohawu to 56.24 days in Kpeve. Anthesis-silking interval observed in Kpeve was 4 days which is relatively higher than the other locations.

Generally, maize streak virus, rust, blight, ear rot, root lodging and stalk lodging where below 1.00 which suggest less susceptibility to diseases.

The comparative mean performance of the various traits during the minor season is presented in Table 10 below.

Table 10 - *Mean Performance of the Various Traits within the Six Environments during the Minor Season of 2019*

| LOCATION | DP | DS | ASI | EH | PH | YH | E-HAR | HC |
|---|---|---|---|---|---|---|---|---|
| FUMESUA | 51.09 | 54.17 | 3.07 | 101.54 | 201.80 | 2451.00 | 28.61 | 2.33 |
| AKUMADAN | 53.52 | 56.17 | 2.65 | 102.80 | 194.80 | 3235.00 | 23.07 | 1.09 |
| WENCHI | 49.43 | 52.89 | 3.46 | 75.20 | 162.60 | 2839.00 | 22.41 | 2.46 |
| EJURA | 49.57 | 53.65 | 4.07 | 70.09 | 164.00 | 4073.00 | 24.89 | 1.61 |
| KPEVE | 56.24 | 57.91 | 1.67 | 75.30 | 159.7 | 2395.00 | 26.37 | 2.67 |
| OHAWU | 49.28 | 52.65 | 3.37 | 41.39 | 117.6 | 3576.00 | 18.65 | 2.57 |
| LSD (5%) | 0.72 | 0.81 | 0.26 | 5.48 | 7.35 | 665.80 | 2.61 | 0.20 |

DP=Days to 50% pollen, DS=Days to 50% silking, ASI=Anthesis-silking interval, EH=Ear height, PH=Plant height, YH=Yeild/hectare, E-HARV=Ears harvested, HC=Husk cover

Source: Field data (2019)

Table 10 - C*ontinued*

| LOCATION | MSV | LR | LS | RUST | BLIGHT | EAR ROT | GRAIN MOIST | PA |
|---|---|---|---|---|---|---|---|---|
| FUMESUA | 0.37 | 0.41 | 0.31 | 0.31 | 0.48 | 0.32 | 16.94 | 2.48 |
| AKUMADAN | 0.34 | 0.46 | 0.49 | 0.30 | 0.30 | 0.30 | 13.45 | 1.00 |
| WENCHI | 0.32 | 0.30 | 0.32 | 0.31 | 0.48 | 0.30 | 14.16 | 2.65 |
| EJURA | 0.32 | 0.33 | 0.35 | 0.30 | 0.30 | 0.31 | 13.79 | 1.44 |
| KPEVE | 0.48 | 0.36 | 0.34 | 0.30 | 0.48 | 0.30 | 13.19 | 2.67 |
| OHAWU | 0.32 | 0.34 | 0.46 | 0.32 | 0.48 | 0.30 | 14.01 | 2.57 |
| LSD (5%) | 0.03 | 0.03 | 0.03 | 0.01 | 0.005 | 0.01 | 0.39 | 0.20 |

LSD= Least Significant Difference, MSV=Maize streak virus, LR=Root Lodging, LS= Stalk lodging, PA=Plant aspect

Source: Field data (2019)

**Combined Performances of 18 Genotypes for Aflatoxin Resistance and Other Agronomic Traits across Locations and Seasons (Major and Minor) of 2018/19**

The combined performances of the 18 genotypes results across the six locations in the major and minor seasons are presented in Table 11. The statistical analysis of the performances across the six environments in both seasons shows significant ($p < 0.05$) differences among genotypes with respect to some of the agronomic traits. Environment was significant ($p < 0.05$) for all the traits except Days to 50% pollen and Days to 50% silking. Genotype was significant ($p < 0.05$) for most of the traits except yield per hectare, Days to 50% pollen, days to 50% silking, ear aspect, plants aspect, ear rot and MSV.

However, genotype by environment interactions were not significant ($p < 0.05$) for most of the trait except Anthesis silking interval, ear height, root and stalk lodging. The mean of squares of the 18 genotypes during the minor and major season is presented in Table 11 below.

Table 11-*Mean Squares of 18 Maize Genotypes Evaluated across Six Environments during Both Minor and Major Season*

| Source of Variation | Df | Yield/Hectare | Days to 50% Pollen | Days to 50% Silking | ASI | Plant Height | Ear Height |
|---|---|---|---|---|---|---|---|
| Rep | 2 | 34437615.65ns | 4.59* | 4.26ns | 2.46* | 725.70ns | 36.08ns |
| GEN | 17 | 21010068.28ns | 89.39ns | 96.45ns | 1.00* | 2249.12* | 1668.53* |
| ENV | 5 | 73354066.14* | 813.49ns | 492.89ns | 65.75* | 74255.81* | 37974.49* |
| SEASON | 1 | 1093574999* | 598.96ns | 676.30ns | 0.46* | 520.93ns | 6390.78* |
| GEN:ENV | 85 | 18777185.57ns | 8.20ns | 15.26ns | 0.82* | 361.04ns | 283.48* |
| GEN:SEASON | 17 | 24421961.67ns | 13.93* | 18.81ns | 0.24* | 376.51ns | 119.34ns |
| ENV:SEASON | 5 | 104797758ns | 24.97* | 22.99ns | 1.01* | 6743.80* | 5862.83* |
| GEN:ENV:SEASON | 85 | 17017710.49ns | 7.14ns | 11.61ns | 0.28ns | 245.81ns | 136.87ns |
| Pooled Error | 430 | 18943863.66 | 7.69 | 12.61 | 0.62 | 365.43 | 220.40 |

*Significant at 0.05 probability level, ns not significant, DF=Degree of Freedom, ASI=Anthesis silking interval

Source: Field data (2019)

Table 11- *Continued*

| Source of Variation | Df | EA | Ear rot | Plant Aspect | Husk cover | Root Lodging | Stalk Lodging | Rust | MSV | Blight |
|---|---|---|---|---|---|---|---|---|---|---|
| Rep | 2 | 0.17* | 0.03ns | 1.33* | 0.56ns | 3.43ns | 1.46* | 0.06ns | 0.23ns | 0.10ns |
| GEN | 17 | 0.36ns | 0.09ns | 0.45ns | 0.97* | 12.28* | 1.62* | 0.17* | 0.25ns | 0.13* |
| ENV | 5 | 14.73* | 6.44* | 41.07* | 23.64* | 132.83* | 62.26* | 0.92* | 3.94* | 23.55* |
| SEASON | 1 | 59.28* | 0.68* | 0.04ns | 10.89* | 313.89* | 4.84* | 0.82* | 7.35* | 0.01ns |
| GEN:ENV | 85 | 0.28ns | 0.07ns | 0.33ns | 0.49ns | 9.56* | 0.58* | 0.09ns | 0.23ns | 0.09ns |
| GEN:SEASON | 17 | 0.25ns | 0.04ns | 0.50ns | 0.58ns | 10.88* | 0.41ns | 0.07ns | 0.23ns | 0.12ns |
| ENV:SEASON | 5 | 16.24* | 5.78* | 12.91* | 8.77* | 171.32* | 20.73* | 0.63* | 5.47* | 7.24* |
| GEN:ENV:SEASON | 85 | 0.33* | 0.09ns | 0.29ns | 0.29ns | 9.28* | 0.48ns | 0.06ns | 0.27* | 0.08ns |
| Pooled Error | 430 | 0.25 | 0.07 | 0.33 | 0.38 | 2.92 | 0.37 | 0.08 | 0.19 | 0.07 |

* Significant at 0.05 probability level, and ns, not significant, DF = Degree of freedom, EA= Ear Aspect, MSV= maize streak virus

Source: Field data (2019)

The mean performance of the 18 genotypes had significant (P<0.05) differences in grain yield and other traits as a result of environmental effect (Table 12). Grain yield varied from 3311.75 kg/ha for GH18 (CHECK ETUBI) to 5142 kg/ha for GH05 (ENT-85×CML-247) with a mean of 4308.00 kg/ha. Genotypic effect for the 18 genotypes showed significance (P<0.05) difference for most of the traits. Lodging stalk ranged from 1.22 for GH15 (Tintim) to 2.03 GH07 (M0826-7F×CML-11). Lodging root ranged from 1.50 for GH03 (MO826-12FxCML-176) to 3.31 for GH06 (ENT-5xCML-287). Days to 50% pollen ranged from 46 days to 52 days whilst days to 50% silking ranged between 49 and 56 days (Table 12). Anthesis silking interval ranged between 2.72 to 3.22 days whilst ear aspect ranged from 2.47 to 2.81, plant aspect ranged from 1.94 for GH08 (MO826-12FxCML-343) to 2.27 for GH18 (ETUBI).

Means observed for rust, blight and streak resistance were 1.08, 1.66 and 1.24 respectively. The tallies for ear rot ranged between 0.92 and 1.11 whilst plant height ranged between 154.25cm and 179.56cm. Ear height also varied from 73.06 cm to 97.36 cm.

The mean performance for the 18 genotypes during the major and minor season is presented in Table 12 below.

Table 12- *Mean Performances of 18 Genotypes for Grain Yield, and Other Agronomic Traits across Six Environments during the Major and Minor Season*

| Genotype | DP | DS | ASI | EH | PH | YH | PA |
|---|---|---|---|---|---|---|---|
| MO826-7F X TZ1-8 | 50.19 | 53.42 | 3.22 | 79.44 | 164.64 | 3918 | 2.11 |
| TZEEI-15XMP-715 | 49.61 | 52.58 | 2.97 | 97.36 | 171.77 | 4476 | 2.17 |
| MO826-12FXCML-176 | 51.47 | 54.53 | 3.06 | 86.69 | 172.97 | 4176 | 2.06 |
| ENT-5XCML-11 | 51.92 | 53.67 | 3.17 | 77.78 | 166.14 | 3490 | 2.22 |
| ENT-85XCML-247 | 51.00 | 54.19 | 3.19 | 86.92 | 175.39 | 5142 | 2.14 |
| ENT-5XCML-287 | 51.08 | 53.92 | 2.83 | 86.56 | 179.56 | 4276 | 2.25 |
| MO826-7FXCML-11 | 52.08 | 55.28 | 3.19 | 85.14 | 176.69 | 4043 | 2.11 |
| MO826-12FXCML-343 | 52.61 | 55.83 | 3.22 | 85.31 | 174.86 | 4848 | 1.94 |
| MO826-7FXCML-343 | 52.39 | 55.64 | 3.25 | 81.28 | 173.97 | 4642 | 1.97 |
| ENT-5XK1-3 | 50.19 | 53.08 | 2.89 | 74.58 | 159.08 | 3854 | 2.11 |
| ENT-5XT21-8 | 49.39 | 52.39 | 3.00 | 67.75 | 154.25 | 4483 | 2.17 |
| TZEE1-6XCML-11 | 48.06 | 50.86 | 2.86 | 79.69 | 165.08 | 4236 | 2.17 |

Source: Field data (2019)

Table 12- *Continued*

| Genotype | DP | DS | ASI | EH | PH | YH | PA |
|---|---|---|---|---|---|---|---|
| ENT-70XCML-247 | 51.06 | 53.78 | 2.72 | 81.22 | 165.28 | 4569 | 1.97 |
| MO826-7FXCML-5 | 51.09 | 54.19 | 3.11 | 83.28 | 173.47 | 4023 | 2.11 |
| CHECK-TINTIM | 50.11 | 53.67 | 3.00 | 76.81 | 166.00 | 4168 | 2.17 |
| CHECK-OBOTANTIM | 46.50 | 49.25 | 2.72 | 73.06 | 155.75 | 4391 | 2.17 |
| CHECK MAMABA | 48.83 | 51.89 | 3.06 | 74.11 | 156.14 | 3697 | 2.14 |
| CHECK ETUBI | 49.78 | 52.89 | 3.11 | 76.22 | 160.14 | 3312 | 2.27 |
| LSD (5%) | 1.28 | 1.65 | 0.19 | 6.88 | 8.86 | 1299.70 | 0.27 |
| MEAN | 50.49 | 53.39 | 3.03 | 80.73 | 167.29 | 4380 | 2.14 |

Source: Field data (2019)

Table 12- *Continued*

| Genotype | EA | HC | MSV | LR | LS | Ear rot | RUST | BLIGHT |
|---|---|---|---|---|---|---|---|---|
| MO826-7F X TZ1-8 | 2.67 | 2.08 | 1.17 | 1.72 | 1.28 | 1.06 | 1.08 | 1.64 |
| TZEEI-15XMP-715 | 2.56 | 2.50 | 1.22 | 1.67 | 1.53 | 0.97 | 1.25 | 1.83 |
| MO826-12FXCML-176 | 2.72 | 2.08 | 1.30 | 1.50 | 1.53 | 1.03 | 1.11 | 1.64 |
| ENT-5XCML-11 | 2.64 | 2.19 | 1.25 | 2.97 | 1.97 | 1.03 | 1.08 | 1.61 |
| ENT-85XCML-247 | 2.47 | 2.61 | 1.94 | 1.75 | 1.64 | 1.00 | 1.00 | 1.61 |
| ENT-5XCML-287 | 2.61 | 2.33 | 1.31 | 3.31 | 1.67 | 1.11 | 1.03 | 1.69 |
| MO826-7FXCML-11 | 2.50 | 2.19 | 1.33 | 3.08 | 2.03 | 1.00 | 1.00 | 1.67 |
| MO826-12FXCML-343 | 2.50 | 2.08 | 1.33 | 2.50 | 1.53 | 0.92 | 1.06 | 1.67 |
| MO826-7FXCML-343 | 2.56 | 1.97 | 1.25 | 1.86 | 1.50 | 1.00 | 1.08 | 1.67 |
| ENT-5XK1-3 | 2.67 | 2.17 | 1.22 | 1.89 | 1.53 | 0.94 | 1.06 | 1.67 |
| ENT-5XT21-8 | 2.56 | 2.25 | 1.14 | 1.67 | 1.28 | 0.97 | 1.03 | 1.64 |
| TZEE1-6XCML-11 | 2.61 | 2.30 | 1.19 | 2.56 | 1.58 | 1.03 | 1.06 | 1.58 |
| ENT-70XCML-247 | 2.56 | 2.50 | 1.42 | 1.61 | 1.58 | 0.94 | 1.03 | 1.58 |

EA=ear aspect, HC=husk cover, MSV=maize streak virus, LR=root lodging, LS=stalk lodging

Source: Field data (2019)

Table 12- *Continued*

| Genotype | EA | HC | MSV | LR | LS | Ear rot | RUST | BLIGHT |
|---|---|---|---|---|---|---|---|---|
| MO826-7FXCML-5 | 2.78 | 2.17 | 1.17 | 1.64 | 1.42 | 0.97 | 1.03 | 1.61 |
| CHECK-TIM TIM | 2.67 | 2.25 | 1.11 | 1.83 | 1.22 | 0.97 | 1.14 | 1.75 |
| CHECK-OBOTANTIM | 2.75 | 2.33 | 1.17 | 1.53 | 1.47 | 1.00 | 1.22 | 1.69 |
| CHECK MAMABA | 2.64 | 2.19 | 1.33 | 2.56 | 1.67 | 0.92 | 1.08 | 1.67 |
| CHECK ETUBI | 2.81 | 2.22 | 1.31 | 1.89 | 1.75 | 1.06 | 1.03 | 1.67 |
| LSD (5%) | 0.33 | 0.29 | 0.20 | 0.79 | 0.28 | 0.17 | 0.13 | 0.13 |
| MEAN | 2.25 | 2.25 | 1.25 | 2.09 | 1.56 | 0.95 | 1.08 | 1.67 |

EA=ear aspect, HC=husk cover, MSV=maize streak virus, LR=root lodging, LS=stalk lodging

Source: Field data (2019)

A combined ranking of the top six maize genotypes and four checks evaluated in the major and minor season across the different locations is presented in the table below (Table 13). It can be observed that GH 05 (ENT-85 X CML-247) performed appreciably better, ranking highest with yield of 5142 kg/hectare, however there was no significant difference ($P<0.05$) in yield between GH 05 (ENT-85×CML-247) and GH 08 (M0826-12F X CML-343) which ranked highest with average yield of 4848.20 yield/hectare. Aflatoxin levels were generally low for the top 10 maize genotypes ranging from 0ppb for GH11 (ENT-5 X TZ1-8), GH08 (MO826-12F X CML-343), GH06 (ENT-5 X CML-287), GH16 (Obotantim), and GH15 (TINTIM) to 30.1ppb for GH18 (Etubi) for the un-inoculated samples.

Generally, it can be observed that upon artificial inoculation, GH05 (ENT-85 X CML-247) showed high level of resistance to aflatoxin accumulation recording 3.0ppb followed by GH09 (MO826-7F X CML-343) with 9.60ppb. Highest aflatoxin accumulation was observed for GH18 (Etubi) of 29.00ppb. The local checks generally showed relatively low resistance to aflatoxin accumulation upon inoculation as compared to the resistant maize genotypes (Table 13). Etubi is observed to have showed lower yield 3311.75 kg/hectare and lower aflatoxin resistance of 30.10ppb among the top six genotypes and local checks.

The table 13 below shows the top yielding genotypes across the six locations during the major and minor season.

Table 13-*Top Six Yielding and Four Local Genotypes and Aflatoxin Levels across Six Environments in Both Major and Minor Seasons*

| GENOTYPE | YIELD kg/ha | UNINOCULATED (ppb) | INOCULATED (ppb) |
|---|---|---|---|
| GH06 (ENT-5 x CML-287) | 4276 | 0 | 26.60 |
| GH08 (MO826-12F x CML-343) | 4848 | 0 | 14.30 |
| GH05 (ENT-85 x CML-247) | 5142 | 3.6 | 3.00 |
| GH11(ENT-5 x TZ1-8) | 4483 | 0 | 11.90 |
| GH13 (ENT-70 x CML-247) | 4569 | 2.3 | 25.00 |
| GH09 (MO826-7F x CML-343) | 4642 | 17.5 | 9.60 |
| CHECK | | | |
| GH16 (TinTim) | 4391 | 0 | 27.20 |
| GH15 (Obotantim) | 4168 | 0 | 14.60 |
| GH17 (Mamaba) | 3697 | 0.2 | 25.80 |
| GH18 (Etubi) | 3312 | 30.1 | 29.00 |

Ppb=part per billion

Source: Field data (2019)

**Stability Analysis of 18 Maize Genotypes in Six Environments for Two Seasons (Major and Minor Seasons 2018/2019)**

The GGE biplot analysis of grain yield for 14 genotypes together with four checks across six environments showed that the Axis1 (principal component axis 1) explained 30.32% of the total variation whereas axis 2 explained 18.78% of the total variation in grain yield across all the environments.

Together, axis 1 and axis 2 explained 49.1% of the total variation in grain yield. The GGE biplot shows a double-arrow line which separates genotypes into two, thus those with below average means and those with above average means (Fig 2). The yield of a genotype was estimated by the projections of their representative markers on the average-tester axis while stability of the genotypes was determined by the projection length of their markers onto the average-tester coordinate y axis single-arrow line. The longer the absolute length of the projection for a genotype, the less stable it was. The presentation by the GGE biplot analysis revealed that the top five yielding maize genotypes were GH 05 (ENT-85 x CML-247), followed by GH 08 (MO826-12F x CML-343), GH09 (MO826-7F x CML-343), GH13 (ENT-70 x CML-247) and GH 11 (ENT-5 x T21-8) in that order. However, the worst performing genotypes were revealed as GH18 (Etubi) followed by GH04 (ENT-5 x CML-11), GH17 (Mamaba), GH10 (ENT-5 x K1-3) and GH01 (MO826-7F X TZ1-8). Even though, GH05(ENT-85 x CML-247) was ranked highest yielding genotype yet it was unstable due to the length of the projection. GH13 (ENT-70 x CML-247) and GH 08 (MO826-12F x CML-343) were the most stable among the top five yielding genotypes. GH 10

(ENT-5 x K1-3) was identified as poor yielding among the worst five genotypes but it was very stable among the low yielding genotypes.

The Figure 2 below shows the Mean yield and Stability biplot of grain yield of the 18 genotypes.

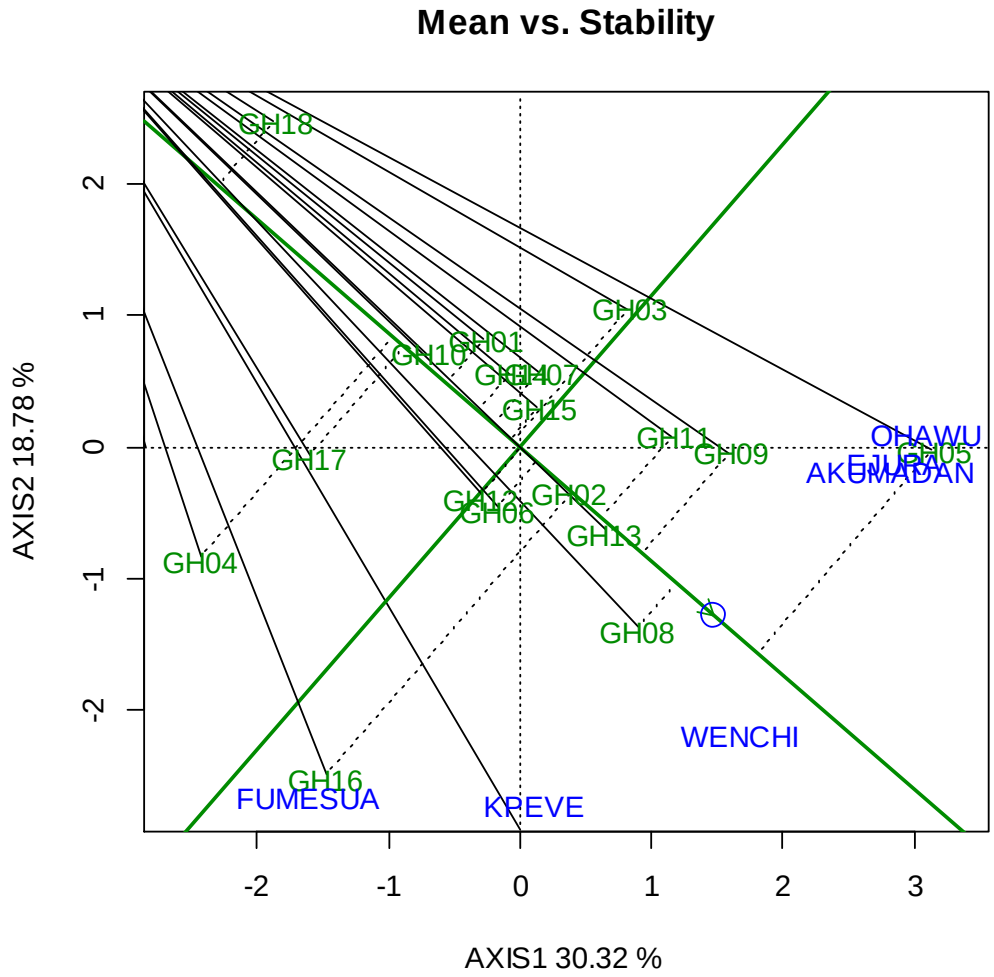


*Figure 2:* Mean yield and Stability biplot of grain yield of 18 genotypes indicating stability of high and yielding genotype across six environments.

Source: Field data (2019)

The table 14 below shows the genotypes and the codes assigned to them in the study.

Table 14-*List of Genotypes and their Respective Codes in the Study*

| Code | Genotype | Code | Genotype |
|---|---|---|---|
| GH01 | MO826-7F X TZ1-8 | GH10 | ENT-5XK1-3 |
| GH02 | TZEEI-15XMP-715 | GH11 | ENT-5XT21-8 |
| GH03 | MO826-12FXCML-176 | GH12 | TZEE1-6XCML-11 |
| GH04 | ENT-5XCML-11 | GH13 | ENT-70XCML-247 |
| GH05 | ENT-85XCML-247 | GH14 | MO826-7FXCML-5 |
| GH06 | ENT-5XCML-287 | GH15 | CHECK-TIM TIM |
| GH07 | MO826-7FXCML-11 | GH16 | CHECK-OBOTANTIM |
| GH08 | MO826-12FXCML-343 | GH17 | CHECK MAMABA |
| GH09 | MO826-7FXCML-343 | GH18 | CHECK ETUBI |

Source: Field data (2019)

Another specific feature of the GGE biplot is the ability to showcase the best performing genotypes in their respective environments as well as the low yielding ones across the six environments. Figure 3 below shows "which won where" for the various genotypes in the six environments.

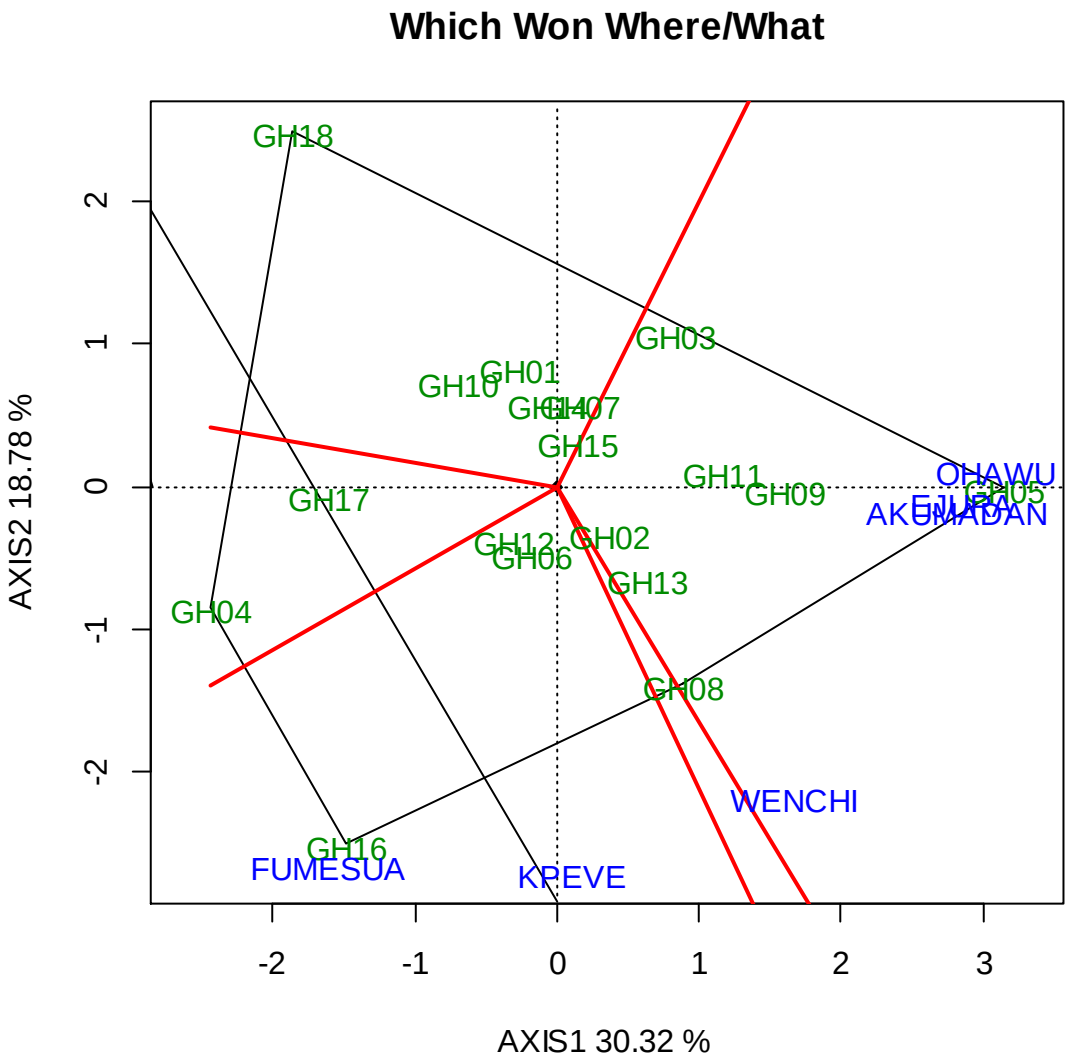


*Figure 3* -Polygon view of "which won where" GGE biplot of grain yield of 18 genotypes across six environments in two seasons (Major and Minor).

Source: Field data (2019)

The five sectors observed in the biplot created by the perpendicular line that starts from the origin of the biplot and runs perpendicular to the side of the polygon. A total of five sectors were recognized out of which three had environments within them and two sectors had no environment within them. Genotypes and environments that fell within the same sectors inferred a relationship between the genotypes with that particular environment(s). The

genotypes at the different vertices of the polygon are likely to be responsive as they are the extreme from the origin. Though, the responsive vertex genotypes can be either the top performing or the worst at one or other environments (Mohammadi *et al*., 2010).

The vertex genotype identified for environments Ohawu, Ejura and Akumadan was GH 05 (ENT-85 x CML-247) whilst GH 16 (Obotantim) was the vertex genotype for Fumesua and Kpeve. Again GH 08 (MO826-12F x CML-343) was the Vertex genotype for Wenchi environment. All other genotypes which were present in the other vertex but did not fall in any test environment were considered to be the low yielding hybrids in those particular environments. They comprised GH 04 (ENT-5XCML-11) and GH 18 (Etubi). The other genotypes which did not occupy any environment nor occupy any vertex were assumed as low yielding genotype, thus GH 04 (ENT-5 x CML-11), GH 03 (M0826-12F x CML-176), GH 11 (ENT-5 x TZI-8), GH 13 (ENT-70 x CML-247) as those which massed around the biplot origin were identified as less responsive to the environments. They comprised GH 12 (TZEEI-6 x CML-11), GH 06 (ENT-5 x CML-287), and GH 02 (TZEEI-15 x MP-715) (Figure 3).

The figure 4 below shows the 'Discriminating power and representativeness' view of GGE biplot of 18 genotypes evaluated in 6 environments for two Seasons (Major and Minor)

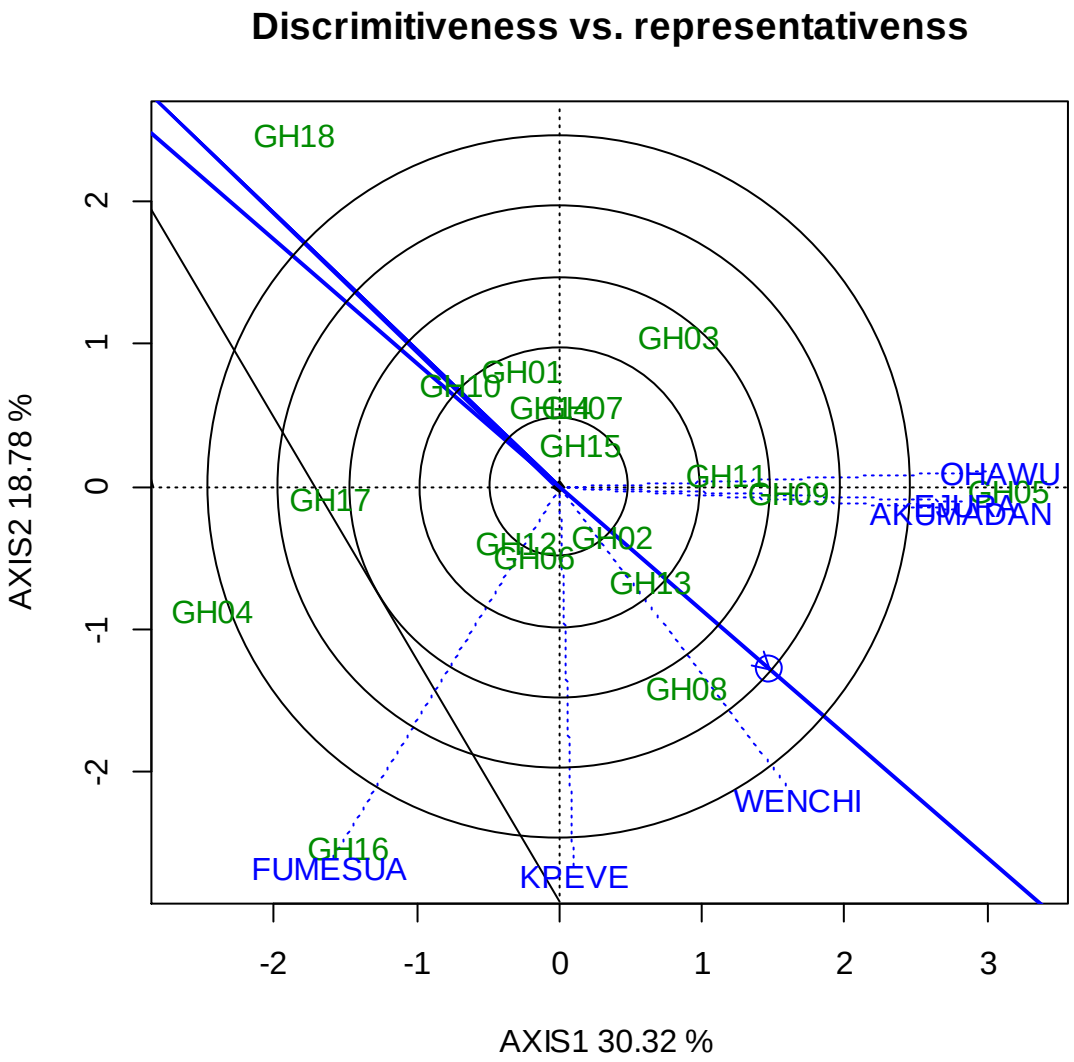


*Figure 4* -'Discriminating power and representativeness' view of GGE biplot on 18 genotypes evaluated in 6 environments for two Seasons (Major and Minor).

Source: Field data (2019)

Furthermore, the GGE Biplot identifies the representativeness and discriminating ability of the environments. The lines proceeding from the origin to the coordinates where an environment falls is the research environment vector whilst a bold straight line which passes through the origin and the average environment represents the average environment axis.

The vector length signifies the discriminating ability of the environment in evaluating genotypes in the test environments. Longer length implies, more discriminatory the environment. The angle between an environment and the average environment axis determines its representativeness, hence, shorter projection from the marker of an environment, the more representative the environment. Shorter environmental vectors implies the specific environments were not strongly interrelated with environments having longer vectors and were possibly not strongly interrelated with each other (Mohammadi *et al*., 2010). As a result, Fumesua, Kpeve, Wenchi and Ohawu were identified to be more discriminatory among genotypes as a result of their vector length whereas Akumadan and Ejura were the least discriminating environments and the most representative (Figure 4).

According to Yan & Rajcan (2002) an ideal test environment should be able to discriminate among genotypes and represent their mega-environment. The biplot identified Ejura as the ideal test environment. It can also be deduced that Ohawu and Fumesua which have long vectors and large angles can be used in removing unstable genotypes effectively but cannot be used in selecting superior genotypes (Figure 4).

GGE Biplot has a unique feature which helps to determine the relationship among tester environments, the cosine of the angle between the lines that join the environments (vectors) to the biplot origin of two environments estimates the correlation between the two environments (Mohammadi *et al*., 2010). Thus, the smaller the angle between two environments, the more highly correlated the environments are to each other. On this premise, environmental groupings, which showed groupings of

environments within the target region where tested plant materials responded similarly were determined, based on the biplot analysis and correlations. The figure 5 below shows the relationship among the 6 testing environment.

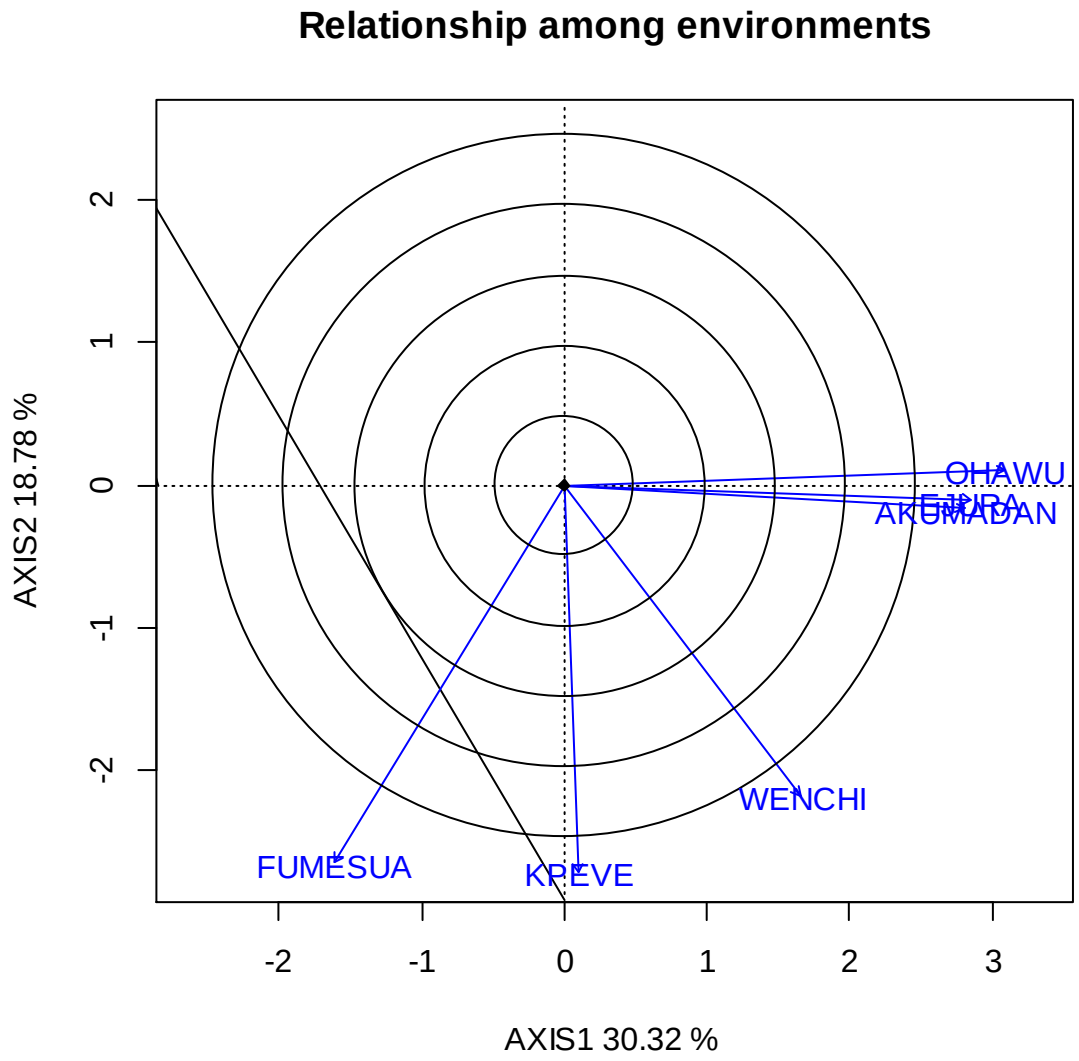


*Figure 5* GGE biplot showing relationship among the 6 testing environments based on the cosine angle between them.

Source: Field data (2019)

Ejura, Ohawu and Akumadan environments tend to show high correlation among them and are nearly identical which suggested they are

similar in their ability to discriminate among genotypes for yield performance. However, the maximum angle formed between the vectors corresponding to Fumesua, Kpeve and Wenchi is below $90^{o}$ which likewise suggest these three environments also discriminate genotype in a similar way. Again, between Ohawu, Wenchi and Fumesua formed an obtuse angle suggesting that these three environment tend to be distinctly independent.

## DISCUSSIONS

The objective of this study was to identify genotypes that are stable across environments, high yielding with good agronomic characteristics, and most importantly resistant to aflatoxin accumulation. Results from the study indicates significant variations among the 18 genotypes which suggest that they are favourable for population improvement. This is in agreement with Warburton and Williams (2014) who reported that variability among genotypes could provide novel or favourable alleles for population improvement as well as the identification of parents for the development of superior hybrids that combine high yields with resistance to aflatoxin accumulation. The observed significant phenotypic variation during the major, minor and across seasons and locations among the genotypes for aflatoxin accumulation reduction and other agronomic traits suggest that potential progress could be made in developing well adapted lines with aflatoxin accumulation resistance.

Large environmental effects detected for most of the agronomic traits indicated variability among the genotypes under different environments. The observed no significant genotype by environment interactions observed across

the seasons and environments indicates that the performance of each of the genotypes for the various traits is consistent in the same manner across all the various environments. According to Baye, Abebe and Wilke (2011), a no significant genotype by environment interactions implies that the performance of the genotypes will not vary much and that there will be no need for replication at another environment; thus one replication at one environment would be sufficient in identifying the best hybrids that the breeder could rely on.

According to Comstock and Moll (1963), genotype by environment interactions determined in multi-locational trials reduces the correlation between genotypic and phenotypic values. Abdulai, Adu, Akoma and Kena (2013) observed a no significant genotype by environmental interaction for grain yield and other agronomic traits among extra early hybrids in Ghana which is similar to findings observed in this work. However, Dadzie (2019) reported significant genotype by environment interaction when he evaluated these same hybrids across three locations in Ghana which is in contrast with the findings of this work although their work involved evaluation of larger number of genotypes. Additionally, the contrast in results could be attributed to the fewer locations within which Dadzie carried out the study.

Assessment of the agronomic traits showed a range of genotypic influence on several parameters studied. Badu-Apraku *et al.* (2010) classified maize genotypes into extra early, early, intermediate and late maturity. However, in this study the hybrids could clearly be classified into two distinct groups based on the days to 50% pollen and silking based on the combined analysis. As a result, all the materials evaluated could be classified as

intermediate variety. There was no significant difference in the corresponding Anthesis-silking interval which was all approximately three days. This observation is consistent with report by Ngugi and Ndiema (2013), where Anthesis-Silking Interval for intermediate maize varieties ranged from 3-7days. This finding suggests that the materials will be suitable for drought prone ecologies because Ngugi & Ndiema reported of Anthesis-silking interval to be associated for selection of drought resistant genotypes. Same assessments of the yield performance of the hybrids revealed that GH 11 (ENT-5 x TZ1-8) and GH06 (ENT-5 x CML-287) were among the top performing genotypes which was also found in a previous study by Dadzie.

There are scarce reports on well-defined aflatoxin hotspots in the country generally. Stable hybrids with potential for aflatoxin resistance need to be evaluated across contrasting growing areas and across seasons in the country to identify most stable ones. Stability analysis revealed that GH08 (M0826-12F x CML-343) and GH13 (ENT-70 x CML-247) as the most stable hybrids among the top five yielding hybrids whereas GH10 (ENT-5 x K1-3) was identified as low yielding yet very stable.

Due to the cost involved in aflatoxin analysis, only 6 hybrids with high grain yields and four local checks were selected for this exercise. Aflatoxin accumulation levels were determined during the minor season because, Henry *et al*. (2013) reported that aflatoxin accumulation levels increase with increase in drought and heat, this observation agrees with Dadzie *et al.* (2018) that aflatoxin levels during the major seasons in Ghana were relatively lower across environments than what was observed during the minor season. Hence testing samples during minor seasons provides an informed decision on the

actual potential aflatoxin accumulation. Genotypes such as GH 11 (ENT-5 x TZ1-8), GH08 (MO826-12F x CML-343) and GH06 (ENT-5 x CML-287) were observed to have 0ppb aflatoxin accumulation in the un-inoculated samples. However, GH11 (ENT-5 x TZ1-8), GH08 (MO826-12F x CML-343), GH05 (ENT-85 x CML-247), and GH09 (MO826-7F x CML-343) all recorded aflatoxin levels which were below the 20ppb threshold although under artificial inoculation. This observation is similar to findings from Dadzie *et al*. (2018) who reported aflatoxin levels of 17.20ppb, 21.74ppb for ENT-5 x TZ1-8 and ENT-5 x CML-287, respectively, although there is a slight difference in the figures.

# CHAPTER FIVE

# SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

## Summary

The production and consumption of maize in Ghana is particularly affected by Aflatoxins contamination which results in reduced grain quality and possess high health risk which makes grain unwholesome to be used as food or feed. Researchers into aflatoxin contamination have reported that most farmers and personnel in the value chain in Ghana do not have knowledge of what aflatoxins or causative fungi are and don't know the effects on marketing and consumption of contaminated maize products (Perrone *et al*., 2014). Even though there are serious health problems posed by aflatoxins, unsafe levels continue to be reported because the commodities market is less developed and hence there is very little enforcement of standards and grades in some parts of the country which demands urgent attention. Therefore, one of the safe measures to aflatoxin contamination in this part of the world and for most developing countries where food quality standard checks and monitoring is less developed will be the extensive use of stable high yielding resistant maize varieties by farmers.

To achieve this, promising aflatoxin resistant maize hybrids needs to be developed and evaluated across wide range of ecological zones to select prospective stable and best performing genotypes for evaluation and release to farmers for production. Therefore, this study was initiated with the following objectives; (1) to determine yield stability of aflatoxin resistant hybrids across six (6) locations in two seasons. (2) to identify stable hybrids with low level of aflatoxin contamination.

To achieve these objectives, the study evaluated 18 maize genotypes under contrasting environment for good agronomic characteristics, Stability and low aflatoxin accumulation. Genotypic effects on some traits were consistently significant across environments which meant that there was useful variation among the genotypes which could be utilized for development of resistant hybrids.

**Conclusions**

The non-significant Genotype by Environment interaction effects for grain yield suggests that promising genotype(s) can be selected in any one of these locations which will also be suitable for production in the other locations in the studied agro-ecological zones.

Environment was found to contribute greatly to the variations in performance of genotypes. This indicates that, unpredictable environmental conditions are one of the major constraints to selecting superior and widely adapted maize varieties. The use of GGE biplot analyses provided clear bases for determining stability and performance of the18 maize genotypes. Based on the analyses, GH08, GH09, GH05, GH06, GH11, GH13, were the highest yielding and GH08, GH13 was most stable hybrids. They were the closest to the ideal genotype and may be considered as the best hybrids. These two hybrids (GH 08, GH 13) have the potential for production in Ejura, Fumesua, Ohawu, Akumadan and Kpeve and other locations within the same agro-ecological zones. GH 18, GH04, GH17, GH10 and GH01 were lowest yielding but GH10 was most stable. Thus, the performance of these genotypes would be predictable in less favorable environments. GH05 was identified as

the most promising for production in Ejura, Ohawu and Akumadan, and GH16 in Fumesua and Kpeve. Again GH 08 was the Vertex genotype for Wenchi environment. Ejura located in the transition zone, was identified as the ideal testing environment for this set of genotypes.

Most of the hybrids showed low levels of aflatoxin accumulation below 20ppb despite artificial inoculation. The study revealed GH08 and GH 13 as the most stable among the top yielding hybrids with 14.30ppb and 25ppb aflatoxin levels after artificial inoculation, respectively.

**Recommendations**

Findings and conclusions from this study makes it obvious that aflatoxin accumulation is an important challenge that needs exigent action. A practical solution to this problem would offer more opportunities to farmers to increase productivity and market acceptability. It is, therefore, recommended that;

1. Good cultural practices should be encouraged among farmers to minimize aflatoxin accumulation in grains.
2. Stable and high yielding aflatoxin accumulation resistant hybrids thus MO826-12FXCML-343 and ENT-70XCML-247 should be further evaluated for release and use by farmers.
3. Stable and high yielding, aflatoxin accumulation resistant hybrids should be DNA finger-Printed for easy genetic identification.
4. Extensive research should be encouraged to determine nationwide aflatoxin hotspots areas for research and food safety purposes.

**Contribution to knowledge**

Prior research globally has shown that progress toward host resistance to Aflatoxin is cheap major food safety remedy to eliminate the challenge of aflatoxin contamination in maize. Maize hybrids that combine aflatoxin resistance with good agronomic traits could be the turning point in solving the challenge especially in Ghana where there is no release maize variety that is resistant to aflatoxin.

This research was geared towards identifying maize hybrids that could be stable, resistant to aflatoxin and has good yield. Results from the evaluation of 14 maize hybrids across six (6) different environments in two (2) seasons in this study have shown that;

1. MO826-12FXCML-343 and ENT-70XCML-247 hybrids are very stable, high yielding and very low aflatoxin accumulation, which could be extensively evaluated and released to farmers.
2. Local maize varieties (Tintim, Obotantim and Mamaba) that were used as checks for this study showed impressive aflatoxin accumulation level and could be an alternate resistant local variety.
3. There is enough variation among the set of materials used for further genetic improvement programmes.

Finally, by focusing on broad evaluation of these promising hybrids, suitable hybrids can be developed for release for farmers.

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# APPENDICES

Appendix 1- *Factorial Analysis of Variance (ANOVA) Table for Days To 50% Pollen*

| Source | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| Rep | 2 | 9.188272 | 4.594136 | 0.597638 | 0.550566 |
| GEN | 17 | 1519.6 | 89.38825 | 11.62826 | 2.3E-26 |
| ENV | 5 | 4067.452 | 813.4904 | 105.8246 | 1.22E-72 |
| SEASON | 1 | 598.9645 | 598.9645 | 77.91754 | 2.73E-17 |
| GEN:ENV | 85 | 697.0756 | 8.20089 | 1.06683 | 0.335362 |
| GEN:SEASON | 17 | 236.7855 | 13.92856 | 1.811925 | 0.024506 |
| ENV:SEASON | 5 | 124.8596 | 24.97191 | 3.248523 | 0.006848 |
| GEN:ENV:SEASON | 85 | 607.2238 | 7.143809 | 0.929317 | 0.653585 |
| Residuals | 430 | 3305.478 | 7.687159 | | |
| Grand Mean 50.4 | | | | | |
| CV 5.5 | | | | | |

Significant level (p-value = 0.05)

Source: Field data (2019)

Appendix 2- *Factorial Analysis of Variance (ANOVA) Table for Days to 50% Silking*

| Source | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| Rep | 2 | 8.530864 | 4.265432 | 0.338351 | 0.713134 |
| GEN | 17 | 1639.716 | 96.45389 | 7.651113 | 1.07E-16 |
| ENV | 5 | 2464.438 | 492.8877 | 39.09784 | 4.33E-33 |
| SEASON | 1 | 676.3025 | 676.3025 | 53.64705 | 1.19E-12 |
| GEN:ENV | 85 | 1297.284 | 15.26216 | 1.210657 | 0.115256 |
| GEN:SEASON | 17 | 319.7531 | 18.80901 | 1.492006 | 0.093144 |
| ENV:SEASON | 5 | 114.9568 | 22.99136 | 1.823768 | 0.106895 |
| GEN:ENV:SEASON | 85 | 986.6543 | 11.6077 | 0.92077 | 0.673296 |
| Residuals | 430 | 5420.802 | 12.60652 | | |
| Grand mean 53.39 | | | | | |
| CV 6.65 | | | | | |

Significant level (p-value = 0.05)

Source: Field data (2019)

Appendix 3- *Factorial Analysis of Variance (ANOVA) Table for yield per Hectare*

| Source | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| Rep | 2 | 68875231 | 34437616 | 1.817877 | 0.163616 |
| GE | 17 | 3.57E+08 | 21010068 | 1.10907 | 0.341771 |
| ENV | 5 | 3.67E+08 | 73354066 | 3.872181 | 0.001919 |
| SEASON | 1 | 1.09E+09 | 1.09E+09 | 57.72714 | 1.9E-13 |
| GEN:ENV | 85 | 1.6E+09 | 18777186 | 0.991201 | 0.505986 |
| GEN:SEASON | 17 | 4.15E+08 | 24421962 | 1.289175 | 0.194698 |
| ENV:SEASON | 5 | 5.24E+08 | 1.05E+08 | 5.532016 | 5.97E-05 |
| GEN:ENV:SEASON | 85 | 1.45E+09 | 17017710 | 0.898323 | 0.723327 |
| Residuals | 430 | 8.15E+09 | 18943864 | | |
| Grand mean 4380 | | | | | |
| CV 9.9 | | | | | |

Significant level (p-value = 0.05)

Source: Field data (2019)

Appendix 4 - *Factorial Analysis of Variance (ANOVA) Table for Plant Height*

| Source | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| Rep | 2 | 1451.392 | 725.696 | 1.985847 | 0.138521 |
| GEN | 17 | 38235.06 | 2249.121 | 6.154659 | 6.7E-13 |
| ENV | 5 | 371279.1 | 74255.81 | 203.199 | 8.13E-111 |
| SEASON | 1 | 520.9275 | 520.9275 | 1.425504 | 0.233158 |
| GEN:ENV | 85 | 30688.41 | 361.0401 | 0.987976 | 0.5137 |
| GEN:SEASON | 17 | 6400.656 | 376.5092 | 1.030307 | 0.423301 |
| ENV:SEASON | 5 | 33718.99 | 6743.798 | 18.45422 | 1.29E-16 |
| GEN:ENV:SEASON | 85 | 20893.93 | 245.8109 | 0.672655 | 0.98672 |
| Residuals | 430 | 157136.6 | 365.434 | | |
| Grand Mean | 167 | | | | |
| CV | 11.42 | | | | |

Significant level (p-value = 0.05)

Source: Field data (2019)

Appendix 5 - *Factorial Analysis of Variance (ANOVA) Table for Ear Height*

| Source | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| Rep | 2 | 72.16975 | 36.08488 | 0.163722 | 0.84903 |
| GEN | 17 | 28365.06 | 1668.533 | 7.570384 | 1.71E-16 |
| ENV | 5 | 189872.5 | 37974.49 | 172.296 | 2.7E-100 |
| SEASON | 1 | 6390.779 | 6390.779 | 28.99592 | 1.2E-07 |
| GEN:ENV | 85 | 24095.78 | 283.4798 | 1.28619 | 0.057413 |
| GEN:SEASON | 17 | 2028.804 | 119.3414 | 0.54147 | 0.93155 |
| ENV:SEASON | 5 | 29314.17 | 5862.835 | 26.60056 | 1.93E-23 |
| GEN:ENV:SEASON | 85 | 11634.41 | 136.8754 | 0.621024 | 0.995946 |
| Residuals | 430 | 94773.16 | 220.4027 | | |
| Grand mean 80.73 | | | | | |
| CV 18.38 | | | | | |

Significant level (p-value = 0.05)

Source: Field data (2019)