Chapter

Genetic Variation and Aflatoxin Accumulation Resistance among 36 Maize Genotypes Evaluated in Ghana

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Abstract

Aflatoxins are carcinogenic secondary metabolites produced predominantly by the fungi *Aspergillus flavus* and *parasiticus.* The toxin contaminate maize grains and threatens human food safety. Survey in Ghana revealed aflatoxin contamination of maize in excess of 941 ppb which is way beyond WHO and USA approved limits of 15 ppb and 20 ppb respectively. Host plant resistance is considered as the best strategy for reducing aflatoxins. This study was designed to (1) identify and select suitable maize lines that combine aflatoxin accumulation resistance and good agronomic traits under tropical conditions and (2) assess the genetic diversity among the exotic and locally adapted maize genotypes using significant morphological traits. Thirty-six maize genotypes, 19 from Mississippi State University, USA and 17 locally adapted genotypes in Ghana were evaluated for aflatoxin accumulation resistance and good agronomic characteristics across six contrasting environments using a 6x6 lattice design with three replicates. Five plants each per genotype were inoculated with a local strain of *Aspergillus flavus* inoculum at a concentration of 9 x 10^7/3.4 ml, two weeks after 50% mid silking. Total aflatoxin in the kernels were determined at harvest using HPLC method. Statistical analysis for agronomic traits and aflatoxin levels were performed using PROC GLM procedure implemented in SAS. The result indicated that genotype by environment interaction was significant ($p < 0.05$) for aflatoxin accumulation resistance and many other agronomic traits. Five genotypes (MP715, NC298, MP705, MP719, CML287 and TZEEI- 24) consistently displayed stable resistance across the environments and may serve as suitable candidates for developing aflatoxin resistant hybrids. Cluster analysis showed two distinct groups (locally adapted and exotic genotypes), an indication of re-cycled alleles per region. Broad sense heritability estimates for grain yield and aflatoxin accumulation resistance were moderately high, which could permit transfer of traits during hybrid development.

Keywords: Maize, *Aspergillus*, Aflatoxin Accumulation, Genetic Variation

1. Introduction

Adaptability and productivity of maize across a wide range of agro- ecologies makes it a suitable food security crop for most parts of the world [1]. However, a

major limitation to the contribution of maize towards food supply is the contamination of grains by aflatoxins. Aflatoxins are carcinogenic secondary metabolites produced mainly by *Aspergillus sp.* which contaminates maize grains during pre- and post- harvest seasons and renders the grains unwholesome for consumption by both humans and livestock [2]. In addition to the health risks, aflatoxin contamination is a serious challenge because the pathogen is globally widespread and causes considerable economic losses by down-grading grain quality, nutritional value and taste [3]. Due to the danger it poses to human health, several countries have set for limits to regulate aflatoxin contamination in many agricultural products including maize. Allowable limits set by Japan is 0 ppb, while the European Union and United States of America have limits of 2–4 ppb and 20 ppb respectively [4].

Approaches for the control and reduction of aflatoxin have relied on good agronomic practices, application of biocontrol preparations of atoxigenic strains of *A. flavus* (including aflasafe and aflaguard), and the use of resistant host plant germplasm [5] as well as BT varieties. Host plant resistance is seen as the method of choice since it exploits the accumulation of resistance alleles into single hybrid varieties [6] and is simple for the farmer to use.

Considerable efforts over the years have led to the development and identification of aflatoxin resistant breeding lines. However, some of these lines lack good agronomic characteristics in temperate environments [6] and may additionally lack other disease or insect resistance in tropical environments. They are useful in crosses involving elite or acceptable lines for the incorporation of novel alleles which confers aflatoxin accumulation resistance into hybrid varieties.

Studies on germplasm diversity and characterization have utilized morphological and/or molecular data for grouping of entries and breeding lines into various heterotic groups. These heterotic groupings can be used to rule out many unproductive hybrid crosses and reduce the total number of testcrosses that should be generated to ultimately find the highest yielding hybrids.

In this way, phenotypic evaluation and groupings of inbred lines could be useful in the identification of suitable inbred lines for the development of superior hybrids with high yields and aflatoxin resistance. Assessing the genetic diversity among the exotic and locally adapted maize genotypes would be useful in selecting potential parents with diverse genetic backgrounds that could be utilized in a breeding program for hybrid development.

The objectives of this study were to: (1) identify and select suitable lines that combine aflatoxin accumulation resistance and good agronomic traits under tropical environmental conditions. (2) assess the genetic diversity between exotic and local maize genotypes using significant morphological traits.

2. Materials and methods

Thirty-six genetic materials were used for the study and this included 19 exotic inbred lines developed for aflatoxin accumulation resistance by the corn host resistance plant unit (CHPPRU) in Mississippi, USA and seventeen locally adapted genotypes. Pedigree information on germplasm is presented in **Table 1**.

2.1 Field experimental sites and layout

Three locations were used for the experiments, namely Fumesua, Akomadan, and Wenchi. Fumesua is in the semi- deciduous forest zone with an altitude of 286 m above sea level and it lies within 6.712 N and 1.523 W. The mean annual rainfall is 1500 mm coupled with mean minimum and maximum temperatures of

Genotype	Pedigree	Source
ENTRY-5		CIMMYT
ENTRY-6		CIMMYT
ENTRY-70		CIMMYT
ENTRY-85		CIMMYT
GH-110		CIMMYT
ABROHEMAA		CRI
OBATANPA		CRI
HONAMPA		CRI
AHODZIN		CRI
OMANKWA		CRI
TINTIM	2-B-B: DT-SR-W-C0/1368 × PAC90038–1 × 1368–6-07C04772B 06A11833B x B-B-B-B-B-B:DT-SR-W-C0/1368 × PAC90038–1 × 1368–3-07C04754B 06A11803B	IITA
M0826-7F	B-B-B-B-B-B:DT-SR-W-C0/1368 × PAC90038–1 × 1368–3-07C04754B 06A11803B	IITA
TZEEI-4	TEE-W SR BC5 x 1368 STR S7 Inb.85	IITA
TZEEI- 24	TEE-W SR BC5 x 1368 STR S6 Inb.229B	IITA
TZEEI-15	TZEEI-15 WPopxLDS6(Set A)Inb.44	IITA
TZEEI-6	TZEEI - 6 WSRBC5x1368STRS7Inb.100	IITA
TZI8	TZB x TZSR	IITA
CML11	P21-C5-FS219–3–2-2-3-#-7–1-B-4-1-B	CIMMYT
CML158Q	EV8762SR-2-1-B-1-B	CIMMYT
CML176	(P63–12–2:1/P67–5–1-1)-1–2:e-e	CIMMYT
CML247	(G24-F119/924-F54)-6–4–1-1-B	CIMMYT
CML287	(P24-F26/P27-F1)-4–1-B-1-1-B	CIMMYT
CML322	LLMBR-17-B-5-3-1-4-B	CIMMYT
CML343	LAPOSTA SEQ-C3-FS17–1–2-3-2-1-B	CIMMYT
CML5	PobZ1C5HC133·1-B_B	CIMMYT
CML108	Pop. 44	CIMMYT
Hi27	[CM104(India)BC6 (is and MV source)	THAILAND
Ki3	Ki 3 (86329)	THAILAND
MP705	from Mp SWCB-4	MISSISSIPI
MP715	Line derived from Tuxpan	MISSISSIPI
MP719	(Mp715 x Va35) -1-3-4-2-3-1-1-1-B	MISSISSIPI
NC334	(SC76*B52); sister line of NC332	NORTH CAROLINA
NC298	PX105A.H5 x Agroc.155	NORTH CAROLINA
NC340	340 (31105)	NORTH CAROLINA
NC356	TROPHY SYN	NORTH CAROLINA

Table 1.
List of germplasm and pedigree.

21°C and 31°C, respectively. Soils around Fumesua are classified as Asuansi series, a ferric acrisol. Akomadan is situated within the forest savanna transition zone and it lies within 7.396 N and 1.973 W. It has a bimodal rainfall distribution pattern same as Fumesua. Wenchi on the other hand lies within 7.733 N and 2.100 W, a transitional savanna zone with bimodal rainfall pattern similar to the other two locations.

For the three locations, major season begins in March and usually ends in July whilst the minor season begins from September and ends in November. The experiments were conducted during the major season of 2017 and minor season of 2017/2018 in all three locations for genotype evaluation. Plantings were staggered at weekly interval between sites. Experimental design used was a 6 x 6, square - lattice with three replications. Single row plots, each 5 m long, spaced 0.70 m apart with 0.4 m spacing between plants in each row were used in all the environments. Three seeds of the lines were planted in each hole and thinned to two plants per hill at two weeks after emergence to give a population density of 66,667 plants per hectare. Weeds were controlled through the use of Atrazine and Gramozone as pre- and post- emergence herbicides at 5 liters/ha each of Primextra and praraquat and subsequently supported by manual weeding.

2.2 Data collected

Data was collected on the following specific parameters:

- DS = Number of days from planting to the time 50% silking was observed.
- DP = Number of days taken for 50% of the plants to begin to shed off pollen.
- ASI = Anthesis-silking interval (ASI) was calculated as the difference between days to 50% silking and 50% anthesis.
- PH = Plant height was determined by measuring the distance from the base of the plant to the height of the first tassel branch.
- EH = Ear height was measured as the distance from the soil surface to the node bearing the upper ear.
- RL = Root lodging (RL) was estimated as the percentage of plants leaning more than 30 degrees from the vertical.
- SL = Stalk lodging (SL) was determined as the proportion or percentage of plants with broken stalk below the ear or the stalk bending more than 45 degrees from the vertical position.
- EA = Ear aspect was estimated based on a scale of 1 to 5, where 1 = clean, uniform, large, and well-filled ears. 2 = moderately uniform and well filled, 3 = ears with mild disease/insect damage and fully-filled grains with one or two irregularities in cob size, 4 = ears with severe disease/insect damage, scanty grain filling, few ears, non-uniformity of cobs, while 5 = ears with totally undesirable features, very few or no grains.
- PA = Plant aspect was determined based on the general assessment of the plant architecture as they appear in the plot and was rated on a scale of 1–5 where, 1 = excellent overall phenotypic appeal, 2 = very good overall phenotypic appeal, 3 = good overall phenotypic appeal, 4 = poor overall phenotypic appeal and 5 = very poor overall phenotypic appeal.

- EPP = Ear number per plant was obtained by dividing the total number of ears per plot by the number of plants harvested.

- SG = Stay- green (chlorophyll concentration) was measured by randomly selecting any five plants per plot and determining chlorophyll concentration from ear leaf at approximately 4 weeks after anthesis and 2 weeks after *A. flavus* inoculation. WAA gadget with a portable SPAD meter (CCM-200 plus-opti sciences) was used to measure the chlorophyll content.

- Blight (BD) and Maize streak diseases (MSVD) were also scored on a scale of 1 to 5, where 1 = absence of disease and 5 = severe infection.

- ID = Insect damage was scored on a scale of 1–5 depending on the extent of damage caused by insects to the ear on plot by plot basis. Scale of 1 = highly resistant, 2 = resistant, 3 = moderately resistant, 4 = susceptible, 5 = highly susceptible.

- ER = Ear rot was also rated on a scale of 1 to 5. 1 = highly resistant, 2 = resistant, 3 = moderately resistant, 4 = susceptible, 5 = highly susceptible.

- HC = Husk cover or open-tip was rated on a scale of 1–5 where, 1 = very tight husk extending beyond the tip and 5 = exposed ear tip.

- Grain yield estimation = Harvested ears from each plot were shelled to determine the percentage grain moisture using moisture meter and then subsequently determine the grain yield in kg ha^{-1} from the shelled grain weight based on 80% shelling percentage and adjustment of moisture content to 15%.

Grain yield was calculated as follows:

$$GY = fwt \times \frac{(100-m)}{85} \times \frac{10000}{(8 \times \phi)} \times 0.8 \quad (1)$$

where,
GY = grain yield (kg ha^{-1}),
fwt = field weight of harvested ears per plot (kg),
m = grain moisture content at harvest.
10,000 = land area per hectare (m^2),
8 = land area per plot (0.70 m x 0.4 m),
ϕ = number of hills/plot (11) and 0.80 = 80% shelling percentage.
Broad-sense heritability (H^2) was estimated as:

$$H^2 = \sigma_G^2 / \left(\sigma_E^2 / re + \sigma_{GE}^2 / e + \sigma_G^2\right), \quad (2)$$

Where;
σ_G^2 = variation due to genotype, σ_E^2 = variation due to environment, σ_{GE}^2 = variation due to genotype by environment interactions, r = number of replications and e = number of environments.

2.3 Source of inoculum and isolation of *A. flavus*

Aflatoxin contaminated maize samples from Ejura main farms were cut into 3 mm pieces with a sterile scalpel blade, after being surface-sterilized in 1% hypochlorite for 2 minutes, then placed on Potato Dextrose Agar (PDA) and incubated at room temperature for 5 days.

After incubation, colonies of different morphology, shape, and color were observed. A pure culture of each colony was obtained through serial dilution where 1 agar plug containing mycelia was serially diluted into 9 mls of distilled water till a concentration of 1×10^5 was achieved.

One ml of the final dilution was transferred onto water agar (2% agar) and incubated at 31°C in unilluminated growth chamber. Identification slides were prepared by picking spores with isolation needle onto a slide containing a drop of distilled water. *A. flavus* was subsequently identified by observing colony characteristics, conidial morphology as described previously [7, 8].

Isolates that produced large smooth conidial surface and either an average sclerotial diameter > 400 μm or without sclerotia were identified as L-type *A. flavus* using Leica Microscope X 40. Identified isolates were subsequently maintained on potato dextrose agar (PDA) as described by Jha [9]. Maintenance of colonies were done by sub-culturing of the colony onto PDA plates and incubated at room temperature for 5 days.

2.4 Inoculum preparation

Identified toxigenic isolate was used to prepare the inoculum as described by Windham [10]. 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 cheesecloth. The concentrations of conidia was determined with a hemacytometer and adjusted with sterile distilled water to 9×10^7 conidia per ml. Excess inoculum not used immediately was refrigerated at 4°C.

2.5 Inoculation method (wounding)

The side needle technique described by Scott and Zummo [11] which utilizes an Idico tree-marking gun fitted with a 14-gauge needle was used for inoculations 14 days after mid silk. Ears were inoculated by inserting the needle under the husks on the upper 1/3 of the ear and 3.4 ml of a spore suspension of 9×10^7 conidia/ml was injected over the kernels. A total of 5 ears per genotype were used for the inoculation study.

2.6 Aflatoxin analysis

Ears that did not touch the ground were harvested from plots at maturity, approximately 60 days after mid-silk. The cobs were shelled and samples ground using a Romer mill (Romer Industries, Inc., Union, MO) according to manufacturer's instructions. Aflatoxin was extracted using the method described by Sirhan [12] with modifications. Maize samples were homogenized into suspension using a Preethi Mixer Grinder.

A weight of 2 g 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, and vortexed for

3mins. 1.32 g of anhydrous MgSO4 and 0.2 g of NaCl were added to the mixture, and vortexed for additional 1 min. The tube was centrifuged for 5 min at 4000 rpm and the upper organic layer filtered through a 0.45 μm nylon syringe prior to injection. A volume of 100 μl of the filtered extract was injected into the HPLC.

A Cecil-Adept Binary Pump HPLC coupled with Shimadzu 10 AxL fluorescence detector (Ex: 360 nm, Em: 440 nm) with Phenomenex Hyper Clone BDS C18 Column (150 x 4.60 mm, 5 μm) was used for analysis. The mobile phase used was methanol: water (40:60, v/v) at a flow rate of 1 ml/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 (G1, G2, B1, B2) standards (ng/g) were prepared from Supelco® aflatoxin standard of 2.6 ng/μL in methanol. Concentration of B1 and G1 were 0.5, 1, 2, 8, 16 ng /g per 100 μl injection of each standard.

Concentration of B2 and G2 were 0.15, 0.3, 0.6, 2.4, 4.8 ng/g per 100 μl injection of each standard. Limit of Detection and Limit of quantification of total aflatoxin were established at 0.5 ng/g and 1 ng/g respectively. The unit (ng/g is equivalent to ppb). Aflatoxin concentration was estimated as:

$$ng/g = A \times (T / I) \times (1 / W) \quad (3)$$

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.

2.6.1 Validation of HPLC method

Recovery studies were conducted to check for precision and accuracy. Blank samples were spiked at 5 (five) replicated maize samples at 13 ng/g, 26 ng/g and 104 μg/g with recoveries 91 ± 1.75%, 98 ± 1.33% and 102 ± 1.87% respectively. Blanks that were run periodically contained no detectable amount of target analyte. Trueness was further validated using a certified reference material (TR-A1000) from Triology laboratory, USA. The value obtained, 20.17 ± 1.14 μg/kg from ten replicates was within the recommended range of the certified value of 21.0 ± 2.9ug/kg. Coefficient of variation was less than 15% for replicates.

2.7 Statistical analysis

Analysis of Variance (ANOVA) was performed on plot means for grain yield and all other agronomic traits for each environment and across environments using PROC GLM procedure of SAS software, version 9.4 [13]. Data on aflatoxin contamination was transformed as Ln (y + 1) where y is the aflatoxin level whilst Ln is Log base e.

This transformation was done to reduce the heterogeneity of variance of contamination levels. Genotype or entry means were adjusted for block effects and analyzed according to lattice design [14]. Each environment was defined as season x location x *A. flavus* inoculation treatment. Effects of environment were considered as random while genotypes were classified as fixed effects. Additionally, genetic correlations between aflatoxin accumulation and selected agronomic traits were performed using the meta menus program implemented in SAS to examine the relationships among the traits and also predict strategies to enhance their improvement.

2.7.1 Clustering analysis using agro-morphological traits

Classification of genotypes was based on significant agro- morphological traits. The significant traits were standardized and used to generate Euclidean genetic distance co-efficient whiles Ward's minimum variance method implemented in SAS software version 9.4 [13] was used for the clustering.

3. Results

Environmental effect was significant ($p < 0.01$) for all agronomic and aflatoxin accumulation resistance traits except open-tip while genotypic mean squares were significant for all measured traits (**Tables 2** and **3**). Genotype by environment interactions were significant ($p < 0.05$) for all traits except days to 50% pollen and silking and streak incidence.

Broadsense heritability showed relatively low to high estimates for agronomic traits, ranging from 18.90% for open-tip to 62.70% for grain yield. For the disease traits, estimated broad sense heritability ranged from a relatively low values of 9.70% for rust incidence to 24.40% for maize streak virus disease incidence. Other traits with relatively moderate to high heritability estimates were ear (67.30%) and plant heights (78.00%).

3.1 Aflatoxin accumulation resistance and agronomic performance of germplasm

Generally, performance of the thirty-six genotypes showed significant ($p < 0.05$) differences in aflatoxin accumulation (**Table 4**). Aflatoxin accumulation ranged from a minimum of 14.85 ppb for MP705 to a maximum of 140.60 ppb for HONAMPA (local check). Grain yield varied from 565.63 kg ha^{-1} for MP715 (inbred) to 4721.03 kg ha^{-1} for AHODZEN (OPV) with a mean of 1853.22 kg ha^{-1} (**Table 5**). Days to 50% pollen ranged from 48 days to 62 days whilst days to 50% silking ranged between 50 and 65 days. Anthesis silking interval varied from 2 to 4 days with an average of 3 days.

The number of ears per plant ranged from approximately 1 to 2 whilst means for cob aspect, plant aspect and open-tip were 2.01, 1.97 and 1.49, respectively. Generally, an observed mean of 1.24 for insect damage (**Table 6**) was an indication of partial tolerance of the germplasm utilized, nonetheless, OMANKWA and NC340 appeared moderately susceptible to insect damage.

Analysis of stay-green characteristics revealed NC298 as the genotype with prolonged green pigmentation whilst CML11 had less and reduced pigmentation (**Table 6**). Means observed for rust, blight and streak resistance indicated a fairly tolerant germplasm. Mean scores obtained for ear rot showed appreciable tolerance of the germplasm whilst plant height ranged between 100.73 cm and 176.25 cm. Ear height also varied from 52.24 cm to 92.50 cm.

3.2 Location effect on aflatoxin accumulation resistance

A combined analysis of aflatoxin accumulation resistance among genotypes evaluated across the three locations in two seasons was significant ($p < 0.05$) and variable (**Table 7**). The general observation showed a relatively high aflatoxin accumulation among genotypes evaluated in Wenchi (transitional savanna zone) whilst those evaluated across Akomadan (forest transitional zone) and Fumesua (rain forest zone) recorded relatively low amount of the toxin. Aflatoxin levels

Sources of variation	DF	Grain Yield (kg/Ha)	Days to 50% pollen	Days to 50% Silking	Anthesis Silking interval (Days)	Ear Per Plant	Cob Aspect (1–5)	Plant Aspect (1–5)	Open-Tip (1–5)	Stay-green
GENOTYPE	35	27259961***	172.76***	203.57***	3.81***	0.93***	1.32***	1.38***	0.95***	348.37***
ENV*GENOTYPE	175	4510728***	40.23 ns	45.85 ns	1.28***	0.38*	0.78**	0.98**	0.52***	99.54**
REP(ENV)	12	4284472***	82.21*	90.67*	0.66 ns	0.49 ns	0.47 ns	0.75 ns	0.95***	346.31***
BLOCK(ENV*REP)	90	1072299 ns	44.77 ns	49.85 ns	1.04*	0.26 ns	0.64 ns	0.84*	0.3***	103.92**
POOLED ERROR	326	1117600	43.35	46.95	0.75	0.29	0.51	0.58	0.25	69.39
H^2		**62.70**	**28.40**	**30.40**	**28.40**	**20.20**	**25.20**	**23.40**	**18.90**	**30.60**

, **, * Significant at 0.05, 0.01 and 0.001 probability levels, respectively, and ns: not significant.*
H^2 = Broad-sense heritability

Table 2.
A combined mean squares of grain yield and agronomic traits among 36 maize genotypes evaluated across six environments.

Source of variation	DF	Aflatoxin	Insect	Rust	Blight	Streak	Ear rot	Plant height	Ear height	Root	Stalk
		ln (y + 1)	Damage (1–5)	(1–5)	(1–5)	(1–5)	(1–5)	(cm)	(cm)	Lodging (1–5)	Lodging (1–5)
ENV	5	17.73***	8.03***	1.82***	9.62***	43.76***	259.46***	80382.63***	21788.88***	27.77***	35.01***
GENOTYPE	35	4.43***	0.89***	0.32**	0.28**	1.95***	28.37***	6003.46***	2090.11***	0.34***	1.98***
ENV*GENOTYPE	175	1.06***	0.36**	0.31***	0.24***	0.63 ns	81.89**	932.37***	369.87***	0.20***	1.64**
REP(ENV)	12	0.34 ns	0.18 ns	0.25 ns	0.65***	0.98*	6.00 ns	3405.97***	1366.06***	0.24*	1.99**
BLOCK(ENV*REP)	90	0.69 ns	0.20 ns	0.34 ns	0.25*	0.81**	31.27 ns	889.42***	350.16***	0.18*	1.36**
Error	326	0.52	0.24	0.18	0.16	0.52	0.33	320.92	135.07	0.13	0.92
H^2		**61.70**	**21.80**	**9.70**	**10.00**	**24.40**	**14.80**	**78.00**	**67.30**	**15.20**	**11.80**

, **, * Significant at 0.05, 0.01 and 0.001 probability levels, respectively, and ns: not significant.*
H^2 = Broad-sense heritability

Table 3.
A combined mean squares for aflatoxin accumulation, disease and agronomic traits among 36 maize genotypes evaluated across six environments.

Genotype	Aflatoxin levels	
	ln (y + 1)	Geometric means (ppb)
ABROHENEMAA	2.73	24.60
AHODZEN	3.03	20.95
CML 108	3.70	41.62
CML11	3.05	25.83
CML158	2.79	20.16
CML176	3.29	29.65
CML247	3.28	47.33
CML287	2.87	20.17
CML322	3.05	25.59
CML343	2.99	24.19
CML5	3.35	28.92
ENTRY-5	3.20	34.86
ENTRY6	3.32	31.80
ENTRY-70	2.82	21.02
ENTRY-85	2.93	23.37
GH-110	4.04	56.83
Hi27	3.37	34.21
HONAMPA (Check)	4.93	140.60
Ki3	3.19	31.60
M0826-12F	3.42	46.12
M0826-7F	3.04	23.58
MP705	2.13	14.85
MP715	2.49	15.89
MP719	2.56	16.86
NC298	3.49	34.59
NC334	3.26	29.94
NC340	3.36	28.69
NC356	2.78	19.49
OBAATANPA	3.82	45.45
OMANKWA	4.01	55.30
TINTIM	3.28	26.95
TZEEI- 24	2.85	21.93
TZEEI- 4	3.32	28.43
TZEEI- 6	3.13	24.92
TZEEI-15	3.49	32.64
TZI8	3.25	25.87
MIN	2.13	14.85
MAX	4.92	140.60
SED	1.05	2.86

Table 4.
Mean aflatoxin accumulation levels among 36 genotypes across six environments.

Genotype	Grain yield (kg/ha)	Days to 50% Pollen	Days to 50% silking	Anthesis silking interval	Ear Per Plant	Cob Aspect (1–5)	Plant Aspect (1–5)	Open-Tip (1–5)	Insect Damage (1–5)
AHODZEN	4162.52	54	56	2	0.94	1.75	1.71	1.34	1.22
CML11	951.41	54	57	3	0.67	2.27	2.30	0.93	1.22
CML158	1022.67	57	60	3	1.26	2.11	1.81	1.22	1.17
CML176	944.15	58	62	4	0.68	2.67	2.33	1.06	1.00
CML247	1452.77	58	61	3	0.84	2.09	2.38	1.67	1.28
CML287	1024.73	58	61	3	0.60	2.51	2.07	1.68	1.11
CML322	1064.86	55	59	4	1.02	2.56	2.01	1.55	1.06
CML343	1445.98	59	62	3	1.28	1.95	1.82	1.28	1.00
CML5	1082.19	56	59	3	1.07	2.08	2.10	1.27	1.44
CML108	1930.27	54	56	2	1.05	1.68	1.61	1.63	1.33
ENTRY-5	1475.85	53	55	2	0.78	2.17	2.09	1.45	1.17
ENTRY-70	1313.41	54	57	3	0.90	1.90	2.14	1.58	1.28
ENTRY-85	1794.45	49	52	3	1.03	1.79	2.08	1.72	1.39
ENTRY- 6	1409.59	54	57	3	0.94	2.06	2.05	1.26	0.90
GH-110	4025.51	54	56	2	1.00	1.67	1.65	1.28	1.11
Hi27	1150.69	54	57	3	0.87	2.33	2.09	1.72	1.39
HONAMPA (Check)	3577.67	52	55	3	0.99	1.51	1.58	1.67	1.28
Ki3	1075.05	53	56	3	1.02	2.25	1.78	1.68	1.50
M0826-12F	4772.76	53	56	3	0.94	1.76	1.60	1.61	1.50
M0826-7F	4448.18	52	54	2	0.91	2.10	1.94	1.73	1.56
MP705	1130.63	51	55	4	0.55	2.37	2.93	1.05	1.11
MP715	565.63	62	65	3	0.76	2.60	2.56	1.39	1.33

Genotype	Grain yield (kg/ha)	Days to 50% Pollen	Days to 50% silking	Anthesis silking interval	Ear Per Plant	Cob Aspect (1–5)	Plant Aspect (1–5)	Open-Tip (1–5)	Insect Damage (1–5)
MP719	1223.68	57	61	4	0.68	2.31	2.16	1.55	1.22
NC298	690.59	52	54	2	1.62	2.02	2.42	1.09	0.89
NC340	1323.46	52	54	2	0.96	1.95	1.64	1.60	1.67
NC356	1341.91	52	55	3	0.82	2.12	2.16	0.96	0.94
NC334	718.99	52	55	3	1.95	1.77	2.03	0.95	0.89
OBAATANPA	2751.56	56	58	2	0.96	1.75	1.91	1.33	1.11
OMANKWA	4688.35	48	50	2	1.00	1.89	1.93	1.68	1.67
TINTIM	2624.39	52	54	2	1.41	1.61	1.73	1.50	1.21
TZEEI-15	1735.97	49	52	3	0.92	1.99	1.90	1.49	1.22
TZEEI- 24	1062.38	50	53	3	1.78	0.89	1.35	2.32	0.89
TZEEI-4	1382.63	48	51	3	1.15	0.93	1.56	2.60	1.28
TZEEI- 6	906.995	51	53	2	0.97	2.16	1.94	1.57	1.22
TZI8	855.43	55	58	3	0.84	2.47	1.84	1.62	1.56
MEAN	1853.22	54	57	3	1.00	2.01	1.97	1.49	1.24
MIN	565.63	48	50	2	0.55	0.89	1.34	0.94	0.88
MAX	4772.76	62	65	4	1.95	2.67	2.92	2.60	1.67
SED	909.22	2.03	2.01	0.30	0.18	0.39	0.35	0.22	0.18

Table 5.
Grain yield and agronomic performance of 36 genotypes across six environments.

Genotype	Stay-Green	RUST Incidence	BLIGHT Incidence	MSVD	Ear rot	Plant Height	Ear Height
		(1–5)	(1–5)	(1–5)	(1–5)	(cm)	(cm)
ABROHENEMAA	33.02	1.26	1.21	2.07	1.22	162.37	82.88
AHODZEN	35.32	1.18	1.09	1.43	1.06	169.90	92.22
CML11	20.77	1.04	1.03	2.34	1.06	111.76	57.27
CML158	25.85	1.29	1.42	1.90	1.11	124.12	65.27
CML176	31.90	1.53	1.43	2.61	1.50	136.93	60.45
CML247	25.02	1.16	1.16	2.43	1.33	127.34	63.52
CML287	28.31	1.10	1.13	2.19	1.22	126.15	65.64
CML322	31.28	1.08	1.42	2.28	1.50	113.03	56.65
CML343	28.19	1.14	1.44	1.92	0.94	133.30	60.77
CML5	26.05	0.99	1.10	1.72	1.33	118.77	67.20
CML108	35.06	1.12	0.99	1.58	1.17	142.35	75.60
ENTRY-5	25.50	1.12	1.16	1.82	1.06	127.47	67.17
ENTRY-70	36.31	1.51	1.36	2.32	1.06	139.52	66.30
ENTRY-85	31.37	1.22	1.16	2.09	1.22	145.64	78.10
ENTRY-6	31.46	1.38	1.31	2.79	1.21	133.66	68.08
GH-110	34.78	1.39	1.18	2.78	0.94	152.22	78.67
HI27	30.51	1.38	1.37	2.02	1.89	137.04	72.24
HONAMPA (Check)	42.58	1.34	1.23	1.87	1.11	157.23	88.38
Ki3	32.90	1.31	1.26	2.61	1.44	140.08	73.96
M0826-12F	28.77	1.29	1.40	1.94	1.06	165.72	86.19
M0826-7F	32.67	1.19	1.19	1.76	1.06	153.81	81.38

Genotype	Stay-Green	RUST Incidence	BLIGHT Incidence	MSVD	Ear rot	Plant Height	Ear Height
MP705	27.56	1.22	1.50	1.66	1.78	104.46	52.24
MP715	23.42	1.23	1.32	2.68	1.06	117.52	67.66
MP719	28.36	1.36	1.59	2.36	1.33	143.20	77.84
NC298	42.04	0.99	1.38	1.77	1.17	113.90	56.48
NC340	35.60	1.56	1.31	1.73	1.39	167.62	84.26
NC356	22.44	1.03	1.21	2.13	1.22	108.73	53.81
NC334	23.93	1.08	1.27	1.95	0.72	100.73	53.09
OBAATANPA	29.11	1.20	1.02	1.96	0.89	176.25	92.52
OMANKWA	30.82	1.27	1.15	1.88	1.28	149.15	79.97
TINTIM	34.70	1.49	1.13	1.78	1.13	164.12	85.77
TZEEI-15	31.02	1.23	1.26	1.96	1.39	140.47	70.29
TZEEI- 24	33.73	3.37	1.10	1.11	1.69	137.70	64.63
TZEEI- 4	30.73	3.07	1.38	1.23	1.77	160.21	85.91
TZEEI- 6	31.43	1.18	1.33	1.59	1.22	155.36	81.96
TZI8	30.88	1.16	1.13	1.61	1.33	129.41	64.03
MEAN	30.65	1.35	1.25	2.00	1.25	138.53	71.62
MIN	20.77	0.98	0.99	1.11	0.72	100.73	52.24
MAX	42.6	1.56	1.59	2.80	1.88	176.25	92.5
SED	5.06	0.14	0.17	0.32	0.32	13.52	7.62

Table 6.
Means of 36 genotypes for disease and agronomic traits across six environments.

Wenchi			Fumesua			Akomadan		
Genotype	**Aflatoxin Ln (y + 1)**	**Geometric Means(ppb)**	**Genotype**	**Aflatoxin Ln (y + 1)**	**Geometric Means(ppb)**	**Genotype**	**Aflatoxin Ln (y + 1)**	**Geometric means(ppb)**
Resistant			Resistant			Resistant		
MP715	(3.08)	21.79	MP715	(2.68)	14.70	NC298	(2.64)	14.02
MP719	(3.09)	22.13	MP705	(2.72)	15.20	MP715	(2.67)	14.51
MP705	(3.12)	22.71	MP719	(2.77)	15.90	MP705	(2.68)	14.73
CML287	(3.17)	23.94	CML287	(2.86)	17.40	MP719	(2.68)	14.69
CML158	(3.27)	26.39	CML158	(2.96)	19.30	CML287	(2.77)	15.95
TZEEI-24	(3.35)	28.54	TZEEI-24	(3.07)	21.70	NC356	(3.13)	22.94
TZEEI-4	(3.42)	30.78	TZEEI-4	(3.13)	22.90	TZEEI-24	(3.04)	21.09
NC298	(3.42)	30.82	ENTRY-85	(3.16)	23.70	ENTRY-85	(3.20)	24.67
ENTRY-85	(3.43)	31.05	NC356	(3.20)	24.70	CML158	(2.97)	19.47
NC356	(3.46)	32.11	CML322	(3.25)	25.90	TZEEI-4	(3.12)	22.86
CML343	(3.53)	34.25	ENTRY-70	(3.30)	27.20	CML343	(3.24)	25.63
ENTRY-70	(3.54)	34.34	CML5	(3.31)	27.50	CML322	(3.21)	24.78
CML322	(3.54)	34.43	NC298	(3.32)	27.60	CML5	(3.30)	27.23
CML5	(3.61)	37.24	CML343	(3.36)	28.90	ENTRY-70	(3.30)	27.14
CML11	(3.67)	39.41	TZEEI-6	(3.49)	32.80	CML11	(3.44)	31.28
Worst			Worst			Worst		
CML108	(4.58)	97.66	M0826-12F	(4.48)	89.00	GH-110	(4.52)	92.03
GH-110	(4.67)	107.29	GH-110	(4.52)	92.00	CML247	(4.60)	100.46
CML247	(4.67)	107.41	TZEEI-15	(4.68)	108.40	CML108	(4.65)	105.18
TZEEI-15	(4.82)	124.15	CML247	(4.89)	133.80	TZEEI-15	(4.66)	106.09

Wenchi			Fumesua			Akomadan		
Genotype	**Aflatoxin Ln (y + 1)**	**Geometric Means(ppb)**	**Genotype**	**Aflatoxin Ln (y + 1)**	**Geometric Means(ppb)**	**Genotype**	**Aflatoxin Ln (y + 1)**	**Geometric means(ppb)**
HONAMPA	(5.25)	190.03	HONAMPA	(5.19)	179.70	HONAMPA	(5.26)	193.13
Min	3.08			2.68			2.64	
Max	5.25			5.19			5.26	
CV%	21.7			22.6			24.3	
LSD(0.05)	1.42			1.40			1.57	

CV = Co-efficient of variation, Digits in parenthesis are transformed means.

Table 7.
Top 15 aflatoxin resistant and worst genotypes across six environments in three locations.

vin Wenchi ranged between 21.80 ppb for MP715 to 190 ppb for HONAMPA. In Fumesua, the accumulation level ranged between 14.70 ppb for MP715 to 179.7 ppb for HONAMPA whilst in Akomadan, it ranged between 14.51 ppb to 193.13 ppb for same genotypes.

MP715 appeared to be the most stable and resistant line across the six environments within the three locations whilst HONAMPA consistently performed poorly as the worse or most susceptible genotype (**Table 7**). The ranking order of resistance in terms of aflatoxin accumulation varied among genotypes from one location to the other. However, some particular genotypes consistently appeared in the top ten resistant genotypes irrespective of location.

3.3 Genetic correlation among selected traits

Significant and positive correlations were also observed between aflatoxin accumulation and traits such as cob and plant aspects and insect damage while stay-green, open-tip and ears per plant were not significantly correlated (**Table 8**).

	Open Tip	Cob Aspect	Stay-Green	Plant_ Aspect	Insect Damage	Ear Per Plant
Aflatoxin ln (y + 1)						
(r)	0.02	0.24	0.07	0.47	0.30	- 0.04
p (0.05)	ns	0.01	ns	0.003	0.05	ns

r = Correlation co-efficient, P = Probability.

Table 8.
Genetic correlation between aflatoxin accumulation and selected agronomic traits.

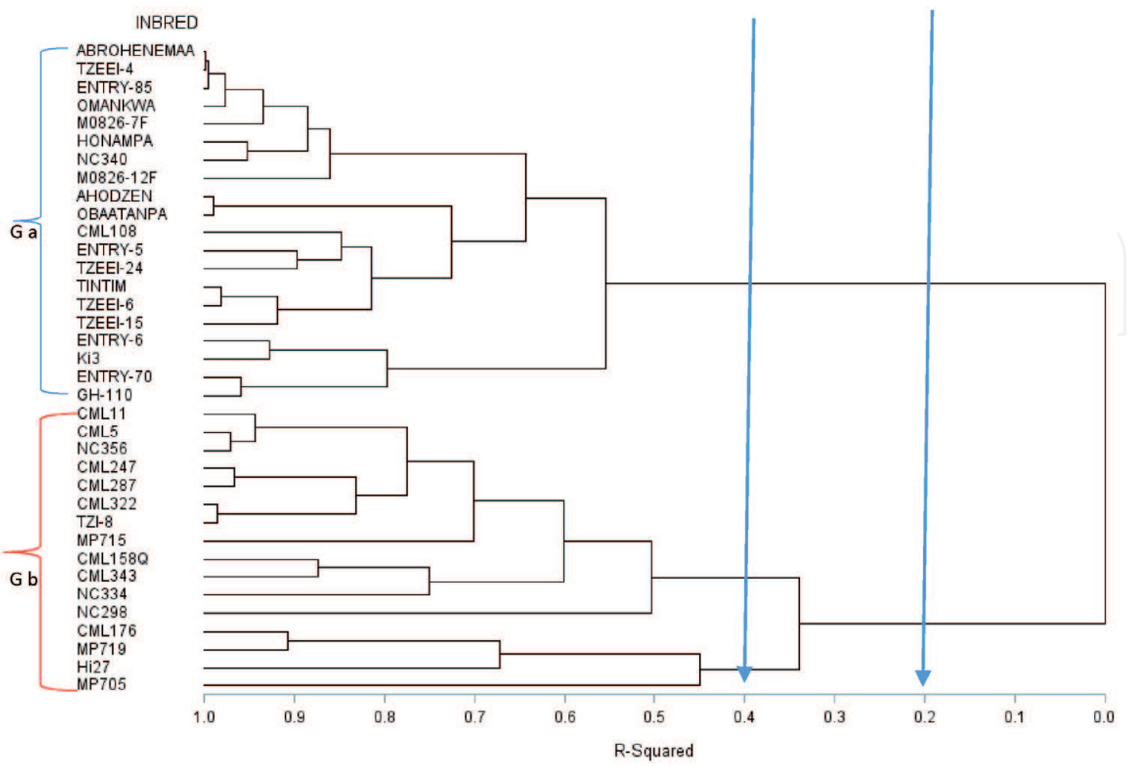

Figure 1.
Dendrogram based on agro-morphological traits showing relationships among 36 maize genotypes using the neighbor joining procedure.

3.4 Heterotic grouping based on multiple agro-morphological traits

Significant agro-morphological traits assigned genotypes into respective heterotic groups. Three groups were observed at 40% co-efficient of determination but at 20%, two major clusters (Ga and Gb) were revealed. Group Ga was made up of 20 members which were mostly local genotypes except Ki3, CML108 and NC340. All the exotic lines (16) clustered in group Gb. (**Figure 1**).

4. Discussion

High genetic variability observed for aflatoxin resistance accumulation was an indication of the presence of novel or favorable alleles for population improvement. Furtherance to this, genotypes identified with reduced aflatoxin accumulation could be exploited in the development of superior hybrids that combine resistance to aflatoxin accumulation and high yields as described previously by Warburton and Williams [6]. The observed significant phenotypic variation for the major, minor and across seasons and locations among the genotypes for aflatoxin accumulation reduction and other agronomic traits suggested that progress could be made in developing well adopted lines with good aflatoxin accumulation resistance.

The significant environmental and genotypic effects detected for aflatoxin accumulation resistance and other agronomic traits indicated variability among the genotypes under different environments. Also the significant genotype x environment interactions observed across the seasons and locations indicates the need for evaluation of genotypes across several environments in order to determine most stable genotypes for aflatoxin accumulation resistance and other agronomic traits. According to Comstock and Moll [15], genotype x environment interactions determined in multi-location trials implied reduced correlation between genotypic and phenotypic values. Zuber [16] identified significant environmental effects on aflatoxin accumulation among commercial hybrids and OPVs in the United States of America. It was not uncommon to observe G x E effects on the genotypes evaluated across contrasting environments in Ghana.

Broad sense heritability among traits ranged from moderate to very high estimates during the major season where adequate rains and less disease pressure was observed. However, significantly lower range of estimates were detected during the minor season (data not shown) confirming earlier reports by [17] who demonstrated significant environmental influence on heritability estimates in cashew. Moderate to high heritability estimates realized in this study, suggested that possible gains are achievable in hybrid maize development for high yields and aflatoxin accumulation resistance.

Further evaluation of the agronomic traits revealed a range of genotypic influence on several parameters studied. Significant among them were days to 50% pollen and silking which clearly categorized the genotypes into the three well defined and established classes of extra early, intermediate and late types as reported by Badu- Apraku [18] who categorized maize genotypes into the different maturity groups. Such information is critical and necessary to guide planting periods in breeding nurseries designed to cross among the maturity groups for trait introgression and further improvement for hybrid development.

The combined analysis showed that most genotypes belonged to the intermediate group while a few were extra early or late maturing. For instance, genotype MP715 has been reported in previous studies [19] as highly resistant to aflatoxin accumulation with delayed silking ranging between 70 and 80 days when grown in a temperate environment, however in this study it ranged between 62 and 67 days under tropical conditions in Ghana, where it is evidently adapted. On the other

hand, genotypes identified with delayed silking dates included MP719, TZI8, NC334, OBAATANPA, CML176, CML247, CML287, CML343 and CML5.

Aflatoxin accumulation levels during the major season were comparatively lower across environments although levels in Wenchi were slightly higher. Unlike the major season, the accumulation levels in the minor season were considerably higher across environments with Wenchi still ranking highest. This observation agrees with the findings of several authors [2, 6, 20, 21] who reported the existence of positive correlation of drought and heat on aflatoxin accumulation level. The Guinea savanna transition environment, which appears to be relatively drier, was conducive for aflatoxin production.

The ranking of top ten resistant genotypes across environments revealed a consistent set of genotypes (although in different ranking order of resistance per environment) during both major and minor seasons. Genotypes which consistently displayed stable resistance across the environments included MP715, NC298, MP705, MP719, CML287 and TZEEI- 24 while the rest appeared less stable. Two local extra early lines (TZEEI-24 and TZEEI-4) were identified as sources of potential resistance to aflatoxin but their level of resistance was not as good as the Mississippi lines specifically bred for resistance and that further evaluations of these two locally adapted lines may be required to confirm their levels of resistance to aflatoxin accumulation.

It was also obvious from this study that, majority of the inbred lines outperformed the OPVs and the populations' in-terms of reduced aflatoxin accumulation levels which was in agreement with the previous findings of Zuber [16] who reported superiority of hybrids (inbred combinations) over OPVs in-terms of measured aflatoxin accumulation resistance across locations and years in the United States.

The levels of total aflatoxin accumulated by the resistant genotypes in this study are comparable to the levels previously reported by William and Windham [5] and Brown [2] where a set of hybrids were evaluated for aflatoxin accumulation resistance. Information obtained from the total aflatoxin accumulation levels among the genotypes could therefore guide the selection of appropriate parental candidates for future aflatoxin resistance breeding in Ghana. Breeding for resistance involves several approaches of which trait correlations is paramount. Indirect selection of one trait simultaneously improves other traits that are significantly correlated.

Zuber [16] discovered strong significant correlation between insect damage and aflatoxin accumulation during evaluation of OPVs and released commercial hybrids in the United States of America. The correlation observed between insect damage and aflatoxin accumulation in this study was similar to that of Zuber [16] as well as the observations made by Williams [22] and Ni [23]. Significant positive correlations were also observed between aflatoxin accumulation levels and plant aspect and cob aspect whilst ears per plant showed a weak negative correlation. Stay-green and open- tip did not correlate with aflatoxin accumulation levels as recounted in other studies [24, 25].

The study of genetic relationship among genotypes which was based on significant agronomic traits assigned all genotypes into three main groups when 40% of the variation among the genotypes was explained. On the contrary, only two main groups were realized when 20% of the genotypic variation was explained by the significant traits used for the grouping. In the case of the two groups, all exotic genotypes were assigned into one group except Ki3, CML108 and NC340 which clustered together with the local genotypes. This observation was not surprising since most of the local genotypes were sourced from CIMMYT and may have similar pedigree or ancestry records.

It appears that the top resistant genotypes which also clustered in one common group perhaps originated from a common ancestry of Tuxpe˜no germplasm native to Mexico which exhibits tropical characteristics coupled with aflatoxin accumulation resistance [6].

Although the analysis of the multiple phenotypic traits assigned genotypes into distinct groups, it showed a low corroboration when compared to other molecular methods (data not shown). This is probably because the expression of most agronomic traits are influenced by the environment.

5. Conclusions

Six most stable aflatoxin accumulation resistant genotypes across six environments have been identified. They included MP715, NC298, MP705, MP719, CML287 and TZEEI- 24. Furthermore, it was evident from the study that traits such as cob and plant aspect correlated significantly and positively with aflatoxin accumulation levels whilst grain yield had significant negative correlation.

Genotypic effects on several traits were consistently significant across environments and that the environments used in this study were discriminatory enough to aid the identification and selection of consistent genotypes for aflatoxin accumulation resistance. Significant genotype by environment interaction aided in the identification of relatively stable genotypes for specific important agronomic and aflatoxin accumulation resistant traits. The Wards clustering method assigned genotypes into two main groups (exotic and local) based on the significant agromorphological traits including grain yield. Broad sense heritability estimates for grain yield and aflatoxin accumulation resistance were moderately high to enable permissible transfer of traits to progeny.

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Conflict of interest

Authors wish to state that, there is no conflict of interest in relation to the writing of this manuscript.

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