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Evaluation of maize (*Zea mays* L.) genotypes for resistance to *Aspergillus flavus* infection

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Abstract

Maize is the primary staple food in sub-Saharan Africa (SSA) accounting for up to 50% of the total calories consumed in the Eastern Africa region. *Aspergillus* ear rot is a major constraint to maize production in Kenya since the released varieties are susceptible to this condition and the post-harvest remedies are unaffordable to most small-scale farmers. Use of host-plant resistance provides a viable and sustainable management strategy for combating *Aspergillus* ear rot in Maize. Hence, the aim of this study was to develop and evaluate the performance of F_1 hybrids under *Aspergillus flavus* infection. Thirty six F_1 progenies were generated from twelve inbred lines following North Carolina II mating design. The progenies together with three checks were evaluated at the Kenya Agricultural and Livestock Research Organization (KALRO) stations in Kiboko and Katumani. Experiment was laid out in Alpha lattice design with two replications per site. The inoculation with *Aspergillus flavus* was initiated at mid-silking stage. Great genetic diversity was noted among the germplasm. The concentration of *Aspergillus flavus* in the grains ranged between 100cfu/g and 2500cfu/g. Hybrids 4, 30, 33 and 34were identified to offer better resistance to *Aspergillus flavus* with high grain yield compared with other genotypes. Crosses with parents MP 313E and NC334 produced the most resistant inbred lines. These lines could be introduced into local breeding programs for development of resistant, high yielding varieties. Good husk cover could be used as a guide during phenotypic selection of germplasm for resistance to *Aspergillus* ear rot.

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Introduction

Maize (*Zea mays* L.) is the leading and a staple crop in Kenya that provides a wide range of uses from food to income generation (Mbithi and Huylenbroeck, 2000). Despite the over reliance on the crop as evidenced by a high consumption rate of 98 kg/capita /year, postharvest losses still count for 20-30% of total maize yield production (Kang'ethe, 2011). This loss is partly due to *Aspergillus flavus*, a fungal pathogen that causes ear rot in maize. This pathogen is associated with production of the most poisonous mycotoxin, aflatoxin (Farrell and O'Keeffe, 2007) that has been reported across most maize growing regions in Kenya (Pobst *et al.*, 2007; Muthomi *et al.*, 2010).

Several strategies such as field sanitation, use of atoxigenic strains of *Aspergillus*, good agronomic practices, proper drying, packaging and storage of produce in hermetic containers have been used to manage *Aspergillus* ear rot (Turner *et al.*, 2005; Fandohan *et al.*, 2005; Strosnider *et al.*, 2006; IFPRI, 2010). However, these strategies are largely dependent on climatic conditions and are unsustainable under most small-scale farming systems (IFPRI, 2010).

Use of host plant resistance provides a viable and sustainable management strategy for combating *Aspergillus flavus* and aflatoxin accumulation in maize (Williams and Windham, 2015). Such resistant germplasm have been identified and registered (Zummo and Scott, 1989; Williams and Windham, 2006; Williams and Windham, 2012; Williams *et al.*, 2014; Williams and Windham, 2015). However, these genotypes are not adapted to the Kenyan agroecological conditions and possess poor agronomic traits like late maturity, proneness to lodging and low yield (Warburton and Williams 2014). It is therefore necessary to hybridize them with locally adapted genotypes for resistance and improved productivity (Asea *et al.*, 2012; Williams and Windham, 2015; Patial *et al.*, 2016). The objective of this study was to develop and evaluate the performance of F_1 hybrids under *Aspergillus flavus* infection.

Materials and methods

Experimental materials

The germplasm comprised of twelve maize inbred lines of diverse origin (Table 1). The male inbred lines are resistant to *Aspergillus flavus* but possess poor agronomic traits while the female inbred lines are potentially susceptible to *Aspergillus flavus* but possess superior agronomic traits.

Table 1. Pedigree and origin of inbred lines used in the study.

	Entry	Inbred line	Origin
Susceptible Female lines	1	CKL05003	Kenya
	2	P329	Kenya
	3	CKL05019	Kenya
	4	(CKL05003/La Posta Seq C7-F180-3-1-1-1-B-B - B)DH56-B-B	Kenya
	5	(CKL05003/La Posta Seq C7-F180-3-1-1-1-B-B - B)DH152-B-B	Kenya
	6	(ZM621A-10-1-1-1-2-B*8/PHG35)-B-16-2-2-B-B	Kenya
Resistant Male lines	1	CML247	Mexico
	2	Mp717	USA
	3	Mp719	USA
	4	NC334	USA
	5	Hi27	USA
	6	Mp 313E	USA

Experimental sites

The experiment was conducted at KALRO stations in Kiboko and Katumani during the 2015 and 2016 rain seasons. Kiboko and Katumani are located in Makueni and Machakos counties, respectively, in Eastern Kenya and their environmental characteristics are presented in Table 2.

Table 2. Description of experimental sites in Kiboko and Katumani.

	Location	
	Kiboko	Katumani
Characteristics		
Latitude	2° 15'S	1°31'S
Longitude	37° 45'E	37°15'E
Elevation (masl)	993	1600
Annual rainfall (mm)	560	655
Annual Max Temp (°C)	30.6	24.7
Annual Min Temp (°C)	17.4	13.7

Source: Mwacharo *et al.* (2004).

Generation of crosses

The parental inbred lines were planted in the nursery at a spacing of 20 cm by 75 cm. Crosses were generated following North Carolina II mating design whereby each female was mated by all male parental lines to generate F_1s.

Evaluation of F_1 maize hybrids for response to Aspergillus flavus

The F_1 hybrids generated together with three checks were planted at KALRO-Kiboko and KALRO-Katumani for evaluation. Planting was done at a spacing of 20 cm by 75 cm following Alpha lattice design in two replications. At mid-silking stage, the plants were artificially inoculated with *Aspergillus flavus* spores.

Inoculum preparation and inoculation

Maize kernels were cultured in Potato Dextrose Agar to obtain *Aspergillus flavus* colonies which were then grown into pure cultures. Spores of *Aspergillus flavus* were harvested then serially diluted by a factor of six to allow easy counting of cells under the haemocytometer. The suspension was then adjusted to contain 10^7 spores per milliliter using the haemocytometer (Krishnan and Damle, 1954; Hoffman, 2006). Inoculation was done by drawing 3 ml of conidial suspension using a syringe and injecting it into the top most ear of the maize plant through the silk channel technique (Zummo and Scott, 1989).

Data collection and analysis

Assessment of agronomic parameters

Days to flowering was taken by counting the number of days from planting to 50% silk emergence and pollen shedding. Plant height was taken at physiological maturity by measuring the plant from the base to the main tassel branch. Stalk lodging was taken at maturity by counting all plants that were having broken stalk below the main ear and expressed as a percentage of the total number of plants in an entry. Husk cover was taken by counting the number of plants with ears that are not completely covered by the husks and expressed as a percentage of the total for each entry.

Assessment of stem borer and ear rots

During vegetative growth, the degree of stem borer damage was recorded by counting the number of pinhole damages on the leaves and cobs of maize plants (Muturi *et al*., 2012). Ear rot was measured by counting the number of cobs with rotten ears and expressed as a percentage of total number of ears at maturity.

Assessment of yield attributes

Field weight was taken using a weighing balance by measuring the total weight of the harvested ears for each entry. Grain yield was calculated based on field weight, grain moisture and shelling percentage (80%) using the formula (Salami *et al*., 2003):

$$\text{Grain yield} = \{\text{grain weight} \times (100 - \text{grain moisture}) \times (\text{shelling percentage} \times 10000)/(100 - 12.5) \times (\text{plot area}) \quad \text{.....Equation 1}$$

Determination of level of contamination by Aspergillus flavus in grain

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

$$\text{cfu/g} = \frac{\text{Number of colonies observed}}{\text{Dilution factor}} \quad \text{.....Equation 2}$$

Statistical data analysis

i.) General analysis of variance was carried for all traits following the general linear model using PROC GLM procedure of SAS program (SAS, 2003). The means obtained were separated using Fisher's protected least significant difference (LSD) method (Frederick, 1999).

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

Results

Variation among genotypes

Significant differences were observed among the genotypes at p≤ 0.05 for all traits except ear and leaf damage, anthesis-silking interval; stalk lodging, ears per plant and plant height. Across the sites, significant differences were observed at p≤ 0.05 for all traits except leaf damage. The genotype by environment interaction (GxE) was significant for all traits except leaf and ear damage, ears per plant, anthesis-silking interval, husk cover, plant height and stem lodging (Table 4).

Table 3. Weather data for Kiboko and Katumani during the experimental period.

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

Table 4. Mean squares for *Aspergillus flavus,* grain yield and agronomic traits of maize genotypes across sites.

Source	Df	GY t/ha	ED %	LD %	ASI days	HC %	ER %	EPP No.	PH Cm	SL %	ASP cfu/g
Rep	1	0.83	8.73	6.05	2.09	45.8	9.23	0	630	467.52	0.03
Environment(E)	1	79.13*	0.94*	0.03	95.20*	4867*	401.05*	2.43*	146574*	18466*	0.41*
Genotype(G)	38	1.49*	4.84	5.81	5.13	96.62*	14.15*	0.01	982.01	434.47	0.16*
GXE	38	1.44*	4.02	7.58	4.03	62.47	14.51*	0.01	875.4	258.55	0.10*
Residual	77	0.23	3.24	4.24	2.3	32.29	2.51	0.01	489.93	206.75	0.01
Total	155										

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

Mean performance of genotypes

The checks used in this study had 536.7 cfu/g more *Aspergillus flavus* colonies than the top resistant hybrids. The top resistant hybrids (3.6 t/ha) outperformed both the bottom hybrids (2.9 t/ha) and the checks (3.2 t/ha) in terms of grain yield. They also took shorter period to flower compared to the checks. This same trend was observed in the anthesis-silking interval. The mean percentage of plants with poor husk cover was low in the top resistant hybrids (2.4%) compared to the bottom hybrids (10%) but higher than the checks (1.2%). The top resistant hybrids scored lower leaf and ear damages by stem borer than the bottom hybrids and checks. Generally, crosses involving male parents 4 and 6 produced the most resistant hybrids while those involving male parents 2 and 5 produced the most susceptible hybrids (Table 5). The mean of *Aspergillus flavus* at Kiboko was 625.9 cfu/g. Five hybrids namely 1, 4, 10, 18 and 30 had lower number of *Aspergillus flavus* colonies (<300 cfu/g) with higher grain yield (>3.5 t/ha) compared to the others. The top resistant hybrids recorded a mean of 2.18% for ear rot incidences while that of the bottom hybrids was 3.02 (Table 6). The mean of *Aspergillus flavus* at Katumani was 459.1 cfu/g. Hybrids 27, 28, 32 and 33 had low levels of *Aspergillus flavus* (<200 cfu/g) with high grain yield (>3.2 t/ha). Hybrids 5, 22 and 25 recorded low levels of *Aspergillus flavus* with low grain yields (<3 t/ha) (Table 7).

Table 5. *Aspergillus flavus* grain yield and agronomic traits of hybrids and checks across sites.

	Entry	Cross fxm	GY t/ha	AD days	ASI days	PH cm	SL %	HC %	ER %	LD %	ED %	ASP cfu/g
Top resistant hybrids	5	1x5	3.4	63.2	3.2	233.4	16.2	1.7	0.2	4.7	6.1	100
	34	6x4	3.2	62.5	2.4	231	19.2	5.8	2.6	5.2	9.1	160
	6	1x6	3.5	63.7	2.3	236.9	54.4	0.5	0.7	4.9	6.2	166.7
	1	1x1	3.5	64	2.7	232	46.2	2.9	1.7	6.4	6.8	180
	10	2x4	3.5	62.3	3.1	219.8	19.5	3.8	0.9	4.7	7	200
	30	5x6	4.3	60.9	1.6	224.1	14.3	0.7	2.1	5.2	6.6	200
	33	6x3	3.2	63.3	2.6	248	9.8	3.5	9.5	5.2	6.7	230
	4	1x4	4.3	64.1	3.2	241.1	15.7	6.1	0.2	7.2	8.7	233.3
	12	2x6	3.3	62	2	208.9	40.6	0.2	0.3	4.1	6.3	233.3
	18	3x6	3.8	61.1	1.7	232.4	42.1	0.9	0.7	3.7	7.6	266.7
	36	6x6	3.6	62	1.1	217.8	22.4	0.4	2.4	5.4	6.5	266.7
Bottom hybrids	16	3x4	3.3	61.1	3.8	240.6	19.1	0.2	0.9	6.2	9.2	1000
	23	4x5	2.4	61.5	7	220.2	12.2	17	2.5	4.2	6.1	1000
	32	6x2	2.9	62.2	4.4	218.5	31.4	10.6	1.9	6.6	6.7	1066.7
	35	6x5	3.4	60.3	3.1	219	28.5	17.4	2.1	5	7	1433.3
	8	2x2	2.7	62	2.1	220	47.8	5	0.8	7.7	6.3	2410
Checks	37		3.5	64.5	2.4	244.3	16	0.7	3.3	4.9	8.4	900
	38		3.5	64.9	2.5	237.6	13.5	1.7	0.1	4.9	6	186.7
	39		2.7	65.3	4	230.6	42.7	1.2	0.3	5.6	7.7	1133.3
	Mean		3.4	62.7	2.9	229.3	26.9	4.2	1.7	5.4	7.1	598.2
	CV (%)		20.68	2.2	55.4	6.6	81.2	62.6	22.4	30.6	19	13.9
	LSD (0.05)		1.56	3.39	2.52	40.87	21.39	10.81	4.95	3.21	2.38	20.6

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

Table 6. *Aspergillus flavus*, grain yield and agronomic traits of hybrids and checks at Kiboko.

	Entry	Cross fxm	GY t/ha	AD days	ASI days	PH cm	SL %	HC %	ER %	LD %	ED %	ASP cfu/g
Top resistant hybrids	34	6x4	2.8	57	3	212	7.1	13.3	4.2	6	9.5	160
	6	1x6	3	57	2	209	70.9	2.7	2.3	4.9	7.2	166.7
	1	1x1	3.6	56	4	215	40.9	8.9	1.9	7.8	6.8	180
	10	2x4	3.9	56	4	209	0	7.4	0	4.9	8	200
	30	5x6	4.8	55	1	216	6.2	1.3	3.3	8	7.8	200
	33	6x3	1.6	57	3	225	11.8	11	2.1	4.5	6.8	230
	4	1x4	4.6	58	3	217	7.5	14.8	1.7	4.8	8.4	233.3
	12	2x6	3.1	57	2	185	35.5	1.8	0	4.8	7.8	233.3
	18	3x6	3.6	56	1	206	38.5	4	0	4	7.2	266.7
	36	6x6	2.5	57	2	200	22.3	3.9	6.3	5.4	7.3	266.7
Bottom Hybrids	16	3x4	2.7	57	5	215	1.2	3.6	0	4.5	9.5	1000
	23	4x5	1.2	56	11	208	9.3	36.1	4.4	4	7.2	1000
	32	6x2	1.4	57	7	191	25.6	25.1	6.3	4.6	7.4	1066.7
	35	6x5	2.6	56	4	206	7.3	30.6	4.4	5.7	8.6	1433.3
	8	2x2	2.6	55	2	211	34	11.8	0	6.5	5	2410
Checks	37		3.2	59	3	213	7.2	3.6	6.8	6.1	7.8	900
	38		3	61	3	207	9.8	6	0	4.2	6.9	186.7
	39		2.1	61	7	207	9.8	3	1.9	3.9	8.4	1133.3
	Mean		2.9	57.1	3.7	208.4	19.2	10.5	2.5	5.3	7.6	625.9
	LSD (0.05)		1.4	2.5	3.9	27.6	25.4	14.7	6	3.3	2.7	20.6
	CV (%)		20.7	2.2	55.4	6.6	81.2	62.6	122.4	30.6	19	13.9

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

Table 7. *Aspergillus flavus*, grain yield and agronomic traits of hybrids and checks at Katumani.

	Entry	Cross fxm	GY t/ha	AD days	ASI days	PH cm	SL %	HC %	ER %	LD %	ED %	ASP cfu/g
Top resistant hybrids	33	6x3	3.28	71	2	264	15.8	0	0	6.1	9.1	100
	22	4x4	2.83	70	2	243	45.2	2.7	0	3.9	8.1	117
	27	5x3	3.33	68	2	249	44.3	1.4	0	4.6	8	133
	32	6x2	3.22	69	2	242	34.4	0	0	8.4	9.4	133
	5	1x5	2.73	71	2	219	15.9	0	0	3.4	9.2	160
	4	1x4	3.1	72	3	265	19.8	0	0	9.5	10.2	167
	25	5x1	2.67	69	2	206	45.1	0	0	7.5	6.9	167
	28	5x4	3.8	67	2	226	47.2	0	0	6.1	6.7	167
	30	5x6	3.15	68	2	224	20.8	0	0	2.6	8.2	167
	34	6x4	3.08	69	2	242	31.7	0	0	5.3	11.2	185
Bottom hybrids	17	3x5	2.68	64	3	233	17.2	1.2	0	6.1	10.2	767
	8	2x2	2.26	68	2	222	64.3	0	0	9.7	10.1	800
	18	3x6	3.24	67	2	260	45.9	0	0	2.9	10.6	1133
	20	4x2	2.73	68	2	240	10.6	0	0	6.1	10.2	1167
	2	1x2	2.49	72	3	253	28.2	0	0	9	10.9	1200
Checks	37		2.95	72	2	268	26.9	0	0	3.1	10	367
	38		2.44	71	2	257	18	0	0	4.3	7	700
	39		1.29	72	2	255	74.7	1.6	0	6.9	10.1	633
	Mean		2.8	69.3	2.2	242.7	33.7	0.4	0.0	5.9	9.2	459.1
	LSD (0.05)		1	3	2	58	37.8	5.7	1.3	5.1	5	6.4
	CV (%)		17.9	2	32	12	60.3	275.6	379.3	49.4	26.7	19.2

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

Correlations between Aspergillus ear rot, grain yield and agronomic traits

In this study, significant correlations were observed at p<0.05 between *Aspergillus flavus* and all traits except flowering, leaf damage, stem lodging and plant height (Table 8). There was a significant negative correlation between grain yield and anthesis-silking interval. Plant height and stalk lodging were positively correlated. *Aspergillus flavus* had a significant negative correlation with grain yield at p<0.05. Husk cover had a significant positive correlation with ear rot and *Aspergillus flavus*. Leaf damage and ear damage (stem borer) were significantly positively correlated. There was also a significant positive correlation between ear damage by stem borer and *Aspergillus flavus* at p<0.05.

Table 8. Correlations between *Aspergillus* ear rot, grain yield and agronomic traits.

	GY	AD	ASI	PH	SL	HC	ER	LD	ED	ASP
GY	---									
AD	-0.54*									
ASI	-0.24*	-0.20*								
PH	-0.22*	0.60*	-0.19*							
SL	-0.23*	0.31*	-0.19*	0.17*						
HC	0.18*	-0.44*	0.26*	-0.31*	-0.26*					
ER	0.09	-0.35*	0.02	-0.21*	-0.11*	0.27*				
LD	-0.02	0.01	0.02	0.06	0.04	-0.11	-0.02			
ED	-0.11	0.09	0.03	0.05	-0.04	-0.08	-0.03	0.25*		
ASP	-0.02*	0.15	-0.02	0.09	-0.13	0.07*	0.07*	0.07	0.53*	---

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

Discussion

The study showed large genetic variations among the genotypes for various traits, an implication that the germplasm used was genetically diverse. Similar findings were reported by Hefny *et al.* (2012), Hung and Holland (2012) and Balconi *et al.* (2014) while

studying heritability for resistance to *Fusarium* among maize genotypes. Eller *et al.* (2008), Henry *et al.* (2009) and Williams and Windham (2015) also reported variations among maize genotypes in their studies for resistance to *Aspergillus flavus* and aflatoxin accumulation. Genetic diversity is a prerequisite in breeding as it allows for genetic improvement through selection (Eller *et al.*, 2008 and Henry *et al.*, 2009).

Genotype by environment interaction (GxE) was significant for resistance to ear rot and *Aspergillus flavus*. This observation is in agreement with Betran *et al.* (2002), Warburton *et al.* (2011), Asea *et al.* (2012) and Warburton and Williams (2014) who reported significant G x E in the inheritance of resistance to *Aspergillus flavus* and aflatoxin accumulation in maize. A significant GxE interaction indicates that the trait is quantitative and hence its expression is influenced by the environment (Betran *et al.*, 2002; Warburton *et al.*, 2011; Asea *et al.*, 2012; Warburton and Williams, 2014). The influence of GxE interaction in expression of traits could be reduced by testing genotypes in multiple environments (Warburton and Williams, 2014).

Incidences of ear rot and the number of *Aspergillus flavus* colonies were noted to be high in Kiboko which had relatively high temperatures and low rainfall compared to Katumani. Such variations in the level of ear rot due to temperature and rainfall corroborate the findings of Eller *et al.* (2008) under multiple environments. The high temperature and low rainfall experienced may have induced stress on the plants providing easy access and optimum conditions for the growth of the pathogen (Payne *et al.*, 1986; Oren *et al.*, 2003 and Eller *et al.*, 2008). Drought, high temperature and relative humidity have been reported to increase ear rot and production of mycotoxins (Payne, 1998; Strosnider *et al.*, 2006; Eller *et al.*, 2008).

The mean ear rot incidence and *Aspergillus flavus* content was lower in the hybrids compared to the commercial checks. Hybrids 1, 4, 10 and 18 had relatively low levels of *Aspergillus flavus* colonies. These hybrids also had good husk cover and reported minimal damage by stem borer. Possession of traits such as good husk cover, drooping ears and resistance to insects has been reported to reduce fungal infection in crops hence are mechanisms for resistance (Betran *et al.*, 2002; IFPRI, 2010).

Correlation enables the identification of candidate traits that can be used in indirect selection for resistance to *Aspergillus* ear rot and aflatoxin accumulation. In breeding programs, simultaneous selection for a number of traits can hasten the progress in selection and ultimately hybrid development (Edwards, 2006). This can be achieved when the traits of interest are significantly correlated. In this study, significant correlations were noted between ear rot, *Aspergillus flavus* content in grains, husk cover and grain yield. Ear rot incidence was negatively correlated to grain yield while it had a positive correlation with *Aspergillus flavus*. Husk cover was positively correlated to ear rot and *Aspergillus flavus*.

The negative and significant correlation between *Aspergillus* ear rot and grain yield observed in this study suggests that high susceptibility to ear rot results to low grain yield. These findings corroborate Horne *et al.* (2016) who reported a negative correlation between grain yield and *Fusarium* ear rot in a study of recurrent selection for reduced ear rot in maize. While studying aflatoxin accumulation in maize hybrids of different maturities, Betran and Isakeit (2004) also reported low grain yield in hybrids susceptible to ear rot. The negative correlation between grain yield and ear rot could be attributed to the fact that susceptible plants fail to achieve optimum productivity (Moreno and Kang, 1999; Betr'an and Isakeit, 2004; Eller *et al.*, 2008).

The positive correlation between *Aspergillus flavus*, ear rot and husk cover could suggest that genotypes with tight husks minimize entry of fungal spores into maize kernels (Warfield and Davis, 1999; Betran *et al.*, 2002; Atehnkeng *et al.*, 2008; IFPRI, 2010).

Grain yield and days to anthesis were negatively correlated implying that early maturing hybrids were more productive than the late maturing ones. This could be as a result of late maturing hybrids tasselling at the onset of drought, hindering cob formation and kernel production (Bolaños and Edmeades, 1996 and Aslam *et al.*, 2013).

Conclusion and recommendations

Thirty six single cross hybrids were developed from twelve maize inbred lines following North Carolina II (NCII) mating design. These hybrids were evaluated in two different environments together with three checks.

There was great genetic diversity among these germplasm. The hybrids evaluated responded variably under artificial inoculation by *Aspergillus flavus*. Hybrids 4, 30, 33 and 34 were identified to offer better resistance to *Aspergillus flavus* compared with other genotypes. These hybrids also maintained high grain yield across the environments. They could be tested in multiple agroecological zones and seasons to validate their performance for release. Hybrids 5, 22 and 25 were also identified to accumulate low levels of *Aspergillus flavus*. However, they had undesirably low grain yield. These genotypes could be used as donors to improve the performance of the adapted but rather susceptible genotypes. Crosses producing the most resistant inbred lines were those involving parents MP 313E and NC334. These lines could be introduced into local breeding programs for development of varieties that are resistant and high yielding.

Important phenotypic correlations were observed between *Aspergillus flavus* and agronomic traits. Good husk cover has been identified as an important secondary trait conditioning resistance to *Aspergillus flavus* and could be used as a guide during phenotypic selection of germplasm.

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