Received: 5 June 2019 | Accepted: 20 December 2019 | Published online: 24 April 2020

DOI: 10.1002/agj2.20145

Agronomy Journal

ARTICLE

Agronomic Application of Genetic Resources

Tolerance to *Fusarium verticillioides* infection and fumonisin accumulation in maize F_1 hybrids and subsequent F_2 populations

Abigael Ouko[1] | **Sheila Okoth**[1] | **Nakisani E. l. Netshifhefhe**[2] | **Altus Viljoen**[2] | **Lindy Joy Rose**[2]

[1]School of Biological Sciences, University of Nairobi, Nairobi, Kenya

[2]Department of Plant Pathology, Stellenbosch University, Matieland, South Africa

Correspondence
Abigael Ouko, School of Biological Sciences, University of Nairobi, Nairobi, Kenya.
Email: abbykongete@uonbi.ac.ke

Funding information
International Maize and Wheat Improvement Centre (CIMMYT); National Commission for Science, Technology and Innovation; South African Maize Trust; National Research Foundation, Grant/Award Number: RPPB13102856988

Abstract

Fusarium verticillioides causes *Fusarium* ear rot (FER) in maize (*Zea mays* L.), thus reducing grain quality, yield, and contaminates grains with fumonisins. Grain infection by these fungi occurs before harvest and selection of parental lines resistant to fumonisin accumulation for breeding purposes is the most effective and environmentally friendly control strategy for *F. verticillioides*. This study intended to evaluate F_1 hybrids and F_2 breeding populations in Kenya for improved resistance to FER and fumonisin contamination. Trials were artificially inoculated and FER severity, *F. verticillioides* accumulation, and fumonisin contamination were determined. Inheritance of resistance was also determined in the F_1 hybrids. CML444 × MIRTC5, R119W × CKL05015, and CML444 × CKL05015 exhibited little to no FER and had the least fungal and fumonisin contamination, respectively. Inbred lines CML495, CKL05015, and P502 had negative, significant general combining ability (GCA) estimates for *F. verticillioides* colonization and fumonisin contamination, but positive, significant GCA estimates for 1,000-kernel weight, respectively. The genotype × environment interaction was the main source of variation observed in the F_2 populations with R119W × CKL05015 and CML444 × CKL05015 being the most tolerant to fungal and fumonisin contamination in Kiboko and MIRTC5 × CML495 the most tolerant in Katumani.

1 | INTRODUCTION

Africa is the biggest consumer of maize (*Zea mays* L.) in the world, consuming up to 30% of annual world maize production (Awika, Piironen, & Bean, 2011). The cereal serves as the main source of carbohydrates in eastern and southern Africa (Macauley, 2015), with annual consumption ranging from 90 to 180 kg per person (Awika et al., 2011; Ecker & Qaim, 2011; Shephard, Marasas, & Burger, 2007). Food security considerations in Kenya are dominated by maize crop with a per capita consumption of 98 kg per annum and accounts for about 40% of the daily calorie intake. It is consumed at a rate of 258 g person^{-1} d^{-1}. Apart from being a major component of the gruel used for weaning children, maize is also a critical constituent in animal feed, which increases its demand (Mutiga, Hoffmann, Harvey, Milgroom, & Nelson, 2014; Okoth, 2016; Okoth et al., 2012). In Kenya, maize farming is dominated by small-scale farmers who contribute 75% of the total produce

Abbreviations: AER, *Aspergillus* ear rot; FER, *Fusarium* ear rot; GCA, general combining ability; PCR, polymerase chain reaction; SCA, specific combining ability; qPCR, quantitative polymerase chain reaction; QTL, quantitative trait locus.

on 2 million hectares with an average yield of 1.2–1.6 tons per hectare (Kibe, 2015).

Maize production is constrained by ear rot fungi belonging to the genera *Aspergillus* and *Fusarium*. *Aspergillus flavus* causes *Aspergillus* ear rot (AER) and contaminates maize grain with aflatoxins while infection of maize grain with *F. verticillioides* leads to *Fusarium* ear rot (FER) and fumonisin contamination (Ouko, Okoth, Amugune, & Vesa, 2018, Okoth et al., 2017a, 2017b; Rose et al., 2017). Infection of maize grain by *F. verticillioides* and subsequent contamination with fumonisins is influenced by climate, temperature, humidity, insect infestation, and pre- and postharvest handling (Fandohan, Gnonlonfin, Hell, Marasas, & Wingfield, 2005). Infection can occur at all developmental stages, and in some cases the infection is symptomless. Contamination of grain results in yield losses, decreased nutritive value, and low grain quality in addition to contamination of the grain with fumonisins (Bii, Wanyoike, Nyande, Gituru, & Bii, 2012).

In sub-Saharan Africa, 13–70% of maize yield is lost due to ear rots. Of the mentioned mycotoxins, aflatoxin is considered a Class I carcinogen while fumonisin is classified as a Class II carcinogen. Prolonged low levels of aflatoxin consumption results in liver cancer and stunted growth in children. However, ingestion of aflatoxins at high levels (> 6,000 mg) causes liver failure and death within 1–2 wk of exposure (Obura, 2013; Okoth, 2016). In Kenya, the 2004 aflatoxicosis was one of the most severe episodes of human aflatoxin poisoning with 317 cases and 125 deaths reported following consumption of maize contaminated with *A. flavus* and aflatoxins (Probst, Njapau, & Cotty, 2007). Chronic exposure to fumonisins is associated with esophageal cancer, liver cancer, growth retardation, and immunosuppression in humans. In animals, it is known to cause diseases like leukoencephalomalacia in horses, pulmonary edema in pigs, and brain hemorrhages in rabbits (Harrison, Colvin, Green, Newman, & Cole, 1990; Howard, 2003).

Fumonisin contamination has been reported in some African countries, the United States, and China. In a random sampling study across eastern and southern Africa, Doko et al. (1996) detected fumonisins in 93% of maize samples. Fumonisin research has been extensively done in South Africa, and *F. verticilloides* is the most prevalent fungus causing ear rot (Boutigny et al., 2012). A study was conducted in Malawi to assess levels of fumonisin contamination in maize stored and consumed in rural households, and recorded fumonisin levels ranging between 1 and 7 mg kg^{-1} (Mwalwayo & Thole, 2016). Fandohan et al. (2005) reported presence of extremely high fumonisin contamination ranging from 8,240 to 16,690 mg kg^{-1} in Benin.

In the eastern part of Kenya, fumonisin contamination has been found in areas where cases of aflatoxin outbreaks have been frequently reported (Bii et al., 2012; Mwihia et al., 2008), and esophageal cancer has been present in the North Rift Valley of Western Kenya (Wakhisi, Patel, Buziba, & Ritich, 2005). In the Western part of Kenya, fumonisin levels in over half of the 985 maize samples collected were above the allowed limit by the European Union (1 mg kg^{-1}; Mutiga et al., 2014. In an assessment of locally brewed beer made from maize and millet, Kirui, Alakonya, Talam, Tohru, and Bii (2014) found fumonisin concentrations ranging from 0.2 to 4 mg kg^{-1} whereas in a different study, Alakonya, Monda, and Ajanga (2009) detected fumonisin concentrations of up to 4.4 mg kg^{-1} in symptomless grain and over 5 mg kg^{-1} in rotten grain sampled from the Western province of Kenya. Rotten grain in this region is often used in brewing, as feed for livestock, and is milled with symptomless grain into flour during maize scarcity (Alakonya, 2016).

The high levels of fumonisins detected in Kenya are concerning, and the country has become the epitome of aflatoxicosis due to recurring incidents in recent years. Knowledge and management of these fungi is, therefore, an important step in controlling human exposure to these toxins (Fandohan et al., 2005). Common agricultural practices such as application of herbicides, irrigation, use of insecticides, and fertilizers application can decrease fumonisin contamination in grain (Blandino, Reyneri, & Vanara, 2008; Cole, Sanders, Hill, & Blankenship, 1985; Jones, Duncan, Payne, & Leonard, 1980; Miller, 2001; Mukanga, Derera, Tongoona, & Laing, 2011; Munkvold, 2003). However, small-scale farmers lack enough knowledge of enhanced agricultural practices and the technical experience to reduce mycotoxin production in maize. Furthermore, subsistence farmers in developing countries barely have enough income to implement management strategies to minimize mycotoxin contamination (Olubandwa, Kathuri, Odero-Wanga, & Shivoga, 2011; Small et al., 2012). This is especially worrisome in a country such as Kenya, where small-scale farmers produce more than 70% of all the maize in the country with the produce consumed or sold locally (Government of Kenya, 2008; Guo et al., 2017; Okoth, 2016).

Breeding for resistance to mycotoxins thus remains the most affordable and practical means to reduce FER, AER, and their mycotoxins, respectively, in Kenya (Guo et al., 2017; Okoth, 2016, Ouko et al., 2018). Robertson-Hoyt et al. (2007a) identified several overlapping quantitative trait loci (QTLs) in the maize genome that affect AER, FER, aflatoxin, and fumonisin contamination, and Wisser, Balint-Kurti, and Nelson (2006) found that QTLs that account for various disease resistances in the maize genome occur in clusters. Maize hybrids accumulated lower levels of FER, *F. verticillioides*, and fumonisins when compared to their parental lines in South Africa (Netshifhefhe, Flett, Viljoen, & Rose, 2018). Hybrid response to *F. verticillioides* infection did not differ significantly from parental inbred lines, thus inbred-line response may be a strong indicator of subsequent hybrid performance (Hung & Holland, 2012; King & Scott, 1981; Netshifhefhe et al., 2018). In this study, F_1 hybrids and F_2 populations

derived from FER and fumonisin-characterized and AER-resistant inbred lines were evaluated for resistance to *F. verticillioides* and fumonisin accumulation under Kenyan climatic conditions. The inheritance of resistance to *F. verticillioides* and fumonisin accumulation was also investigated.

2 | MATERIALS AND METHODS

2.1 | Planting material and field sites

Six maize inbred lines (CML444, R119W, CKL05015, CML495, MIRTC5, and P502) previously characterized for their response to AER, FER, fumonisins and aflatoxins (Okoth et al., 2017a, 2017b; Ouko et al., 2018; Rose et al., 2016; Rose et al., 2017; Small et al., 2012) were used in this study. These germplasms originated from the International Maize and Wheat Improvement Center (CIMMYT) and Agricultural Research Council-Grain Crops Institute (ARC-GCI; Table 1). The maize inbred lines were assessed in a randomized complete block design with three replications. Each seed was planted in a single 10-m-long plot and spacing of 0.9 by 0.3 m, with each row having 33 plants. Four border rows of a commercial-maize hybrid were planted at each end of replication, and all the recommended agricultural practices observed. Standard agricultural practices such as fertilizer application, irrigation, rogueing was followed for both trials. A diallel mating system was used during cross pollination of the inbred lines and the reciprocal crosses. Subsequently, 30 F_1 hybrids were planted in Kiboko, Kenya (37°75′E, 2°15′S; 975 m asl) together with their parental inbred lines (Table 1) in 2014. The F_1 hybrids were then self-pollinated to create 27 F_2 populations that were planted with three inbred lines (CKL05015, MIRTC5, and R119W) in Katumani (1°35′S, 37°14′E, 1,600 m asl) and Kiboko during the 2014–2015 season.

2.2 | Inoculum preparation, field inoculation and disease assessment

Three isolates (K38, K48, and K58) were used in preparing inoculum. The selected isolates were preferred because they were the most virulent and were isolated from the same site of the trial (Kihara, 2013). Primary maize ears were inoculated as described in Ouko et al. (2018). Artificial inoculation was performed to ensure adequate disease pressure to discriminate between inbred lines and hybrids. Two ml of a 1×10^6 *F. verticillioides* mixed-culture spore suspension was inoculated down the silk channel using a 10-ml syringe fitted with an 18-gauge sterile needle. The ears were hand-harvested at physiological maturity and their husks removed. Ear rot severity due to *Fusarium* infection was determined according to the rating scale described by Small et al. (2012). The ears collected in each plot were shelled and bulked with the F_1 and F_2 populations bulked separately according to plot. A 250-g grain sample from each plot was then milled using a Husqvarna coarse steel grain grinder (Reliance, Sweden). Coarsely milled grain was further milled to flour using a blender (400 W, 1.75 L; Philips, Amsterdam, the Netherlands). Two sub-samples were used for fungal DNA extractions and fumonisin analysis.

2.3 | DNA extractions and quantification of *F. verticillioides* target DNA in maize grain

DNA was isolated from milled maize grain (2 g) and *F. verticillioides* mycelia as described by Boutigny et al. (2012), and the amount of *F. verticillioides* target DNA was determined by quantitative polymerase chain reaction (qPCR). The qPCR was species specific and the ability to detect and accurately quantify the strains used was validated as part of the assay. The slope of the standard curve used in this study was $m = -3.34$ while the correlation coefficient was $r^2 = .99905$. The efficiency of the reaction was 99% and the detection limit varied from 0.006 to 7.088 ng μl^{-1}. The reproducibility (inter-run variance) and repeatability (intra-run variance) of the qPCR was not investigated, as this was previously performed by Boutigny et al. (2012) under the same laboratory conditions using the same analytical equipment. The amount of *F. verticillioides* target DNA in a sample represented fungal colonization (Adejumo, Hettwer, Nutz, & Karlovsky, 2009; Janse van Rensburg, Mclaren, Flett, & Schoeman, 2015; Ncube, Flett, Waalwijk, & Viljoen, 2011; Rose et al., 2016).

2.4 | Fumonisin analysis

Fumonisin B_1, B_2 and B_3 levels in 5-g samples of milled grain were determined by liquid chromatography tandem mass spectrometry according to Rose et al. (2016) at the Central Analytical Facility, Stellenbosch University, South Africa. The detection limits were 0.02, 0.002, and 0.02 mg kg^{-1} for FB_1, FB_2, and FB_3, respectively.

2.5 | Statistical analysis

A Shapiro–Wilk test was performed on the standardized residuals from the model to verify normality (Shapiro & Wilk, 1965). The 1,000-kernel weight and *F. verticillioides* target DNA data were normally distributed. However, total fumonisins concentration data was log-transformed to achieve normality. Percentage FER severity was subjected to arcsin and log transformations. Homogeneity of locality variances were confirmed by Levene's test (Levene, 1960). The analysis of variance (ANOVA) was performed for

TABLE 1 Maize inbred lines and hybrids evaluated in Katumani and Kiboko, Kenya, during the 2014–2015 maize-growing seasons

Code	Inbred line	Description	Origin	Status
P1	CKL05015	Inbred	CIMMYT-Kenya	AER/aflatoxin-resistant
P2	CML444	Inbred	CIMMYT-Zimbabwe	FER/fumonisin-resistant
P3	CML495	Inbred	CIMMYT-Kenya	AER/aflatoxin-resistant
P4	MIRTC5	Inbred	CIMMYT-Kenya	AER/aflatoxin-resistant
P5	P502C2	Inbred	CIMMYT-Kenya	AER/aflatoxin-resistant
P6	R119W	Inbred	ARC-GCI-South Africa	FER/fumonisin-susceptible

Code	Hybrid	Trial sites		
		F_1 – Kiboko	F_2 – Katumani	F_2 – Kiboko
H1	CKL05015 × CML444	✓[a]	✓	✓
H2	CKL05015 × CML495	✓	✓	✓
H3	CKL05015 × MIRTC5	✓	✓	X
H4	CKL05015 × P502C2	✓	✓	✓
H5	CKL05015 × R119W	✓	X	✓
H6	CML444 × CKL05015	✓	✓	✓
H7	CML444 × CML495	✓	✓	✓
H8	CML444 × MIRTC5	✓	✓	✓
H9	CML444 × P502C2	✓	✓	✓
H10	CML444 × R119W	✓	X	X
H11	CML495 × CKL05015	✓	✓	✓
H12	CML495 × CML444	✓	✓	✓
H13	CML495 × MIRTC5	✓	✓	✓
H14	CML495 × P502C2	✓	✓	✓
H15	CML495 × R119W	✓	✓	✓
H16	MIRTC5 × CKL05015	✓	✓	✓
H17	MIRTC5 × CML444	✓	✓	✓
H18	MIRTC5 × CML495	✓	✓	✓
H19	MIRTC5 × P502C2	✓	✓	✓
H20	MIRTC5 × R119W	✓	✓	✓
H21	P502C2 × CKL05015	✓	✓	✓
H22	P502C2 × CML444	✓	✓	✓
H23	P502C2 × CML495	✓	✓	✓
H24	P502C2 × MIRTC5	✓	✓	✓
H25	P502C2 × R119W	✓	✓	✓
H26	R119W × CKL05015	✓	✓	✓
H27	R119W × CML495	✓	✓	✓
H28	R119W × MIRTC5	✓	✓	✓
H29	R119W × P502C2	✓	✓	✓
H30	R119W × CML444	✓	X	X

[a]✓, genotype present in the trial; X, genotype missing in the trial due to lack of seeds and some seeds also rotted.

1,000-kernel weight, FER severity, *F. verticillioides* colonization, and fumonisin contamination employing the fixed generalized linear model procedure of SAS statistical software version 9.2. (SAS Institute, Cary, NC). The experimental design was a randomized complete block design with three block replications and two trials. The treatment design was a factorial with two factors, five females, and five males. The Fisher's least significant difference (LSD) test was used at the 5% level to compare inbred lines and hybrids means for all the parameters evaluated (Ott & Longnecker, 2010). Significance was regarded to be true at a probability level of 5% ($P < .05$) for all tests. Pearson's correlation coefficients between FER severity, *F. verticillioides* colonization, and total fumonisins concentration was performed using CORR procedure in SAS.

TABLE 2 Analysis of variance for *Fusarium* ear rot (FER) severity, *Fusarium verticillioides* colonization, fumonisin concentration, and 1,000-kernel weight of the maize genotypes

	FER severity		*F. verticillioides* colonization		Fumonisins concentration		1,000-kernel weight	
	%		ng μl^{-1}		mg kg^{-1}		g 1,000 $kernels^{-1}$	
	Replications (R)	Genotype (G)	R	G	R	G	R	G
df	2	35	2	35	2	35	2	35
MS	8.0329	1.8545278	0.0004	0.0009	0.8115	1.1047	2,725.8669	2,880.7163
F-value	4.21	0.97	1.51	3.38	1.87	2.54	2.38	2.51
Pr > F	.0188	.5258	.2272	<.0001	.1624	.0005	.1003	.0005

Diallel analysis was performed using DiallelSAS05 (Zhang, Rossel, Kriegner, & Hijmans, 2004). The analysis was performed according to method 1 and model 1 of Griffing (1956), which included parental inbred lines, their F_1 offspring and reciprocal hybrids. T-tests were used to determine the significance of these estimates. Heritability of the different traits was evaluated using Griffing's (1956) equations:

$$H^2 = 2\sigma g^2 + \sigma s^2 / \left(2\sigma g^2 + \sigma s^2 + \mathrm{MSe}\right)$$

$$h^2 = 2\sigma g^2 / \left(2\sigma g^2 + \sigma s^2 + \sigma e^2\right)$$

Where H^2 is broad sense heritability, h^2 is narrow sense heritability, σg^2 is the sigma GCA square, σs^2 is the sigma specific combining ability (SCA) square, and σe^2 is the sigma error square value. The predominant gene action in the inheritance of a trait was determined by Baker (1978) calculation of the GCA/SCA ratio of the mean squares:

$$2\sigma g^2 / \left(2\sigma g^2 + \sigma s^2\right)$$

where σg^2 is the sigma GCA square and σs^2 is the sigma SCA square.

3 | RESULTS

3.1 | Performance of F_1 population trials

Significant variations occurred in *F. verticillioides* colonization ($P = < .0001$), fumonisin contamination ($P = .0005$), and 1,000-kernel weight ($P = .0005$) between genotypes in the F_1 hybrid trial, but not in FER severity ($P = .5258$). The replications had a significant effect on FER severity ($P = .0188$), but not on *F. verticillioides* colonization ($P = .2272$), fumonisin contamination ($P = .1624$), or 1,000-kernel weight ($P = .1003$; Table 2).

Of the genotypes evaluated, 22 out of 36 exhibited no FER symptoms. The mean FER severity was 0.43%, and the highest percentage FER symptoms was 2.94%. Only six genotypes exhibited FER symptoms above 1%, namely, CML495, CKL05015 × R119W, CML495 × MIRTC5, CML495 × R119W, P502C2 × CKL05015, and R119W × MIRTC5 (Table 3).

Among the inbred lines, CML444 (0.093 ng μl^{-1}) and P502C2 (0.069 ng μl^{-1}) were most colonized by *F. verticillioides*, with significantly more target DNA compared to the other genotypes. The hybrids CKL05015 × CML495 (0.007 ng μl^{-1}) and CML444 × CKL05015 (0.007 ng μl^{-1}) had the lowest fungal DNA content, which did not differ significantly from parental lines CKL05015 (0.024 ng μl^{-1}) and CML495 (0.017 ng μl^{-1}). Hybrids CKL05015 × CML495 (0.007 ng μl^{-1}) and CML444 × CKL05015 (0.007 ng μl^{-1}) had the least amount of *F. verticillioides* DNA (Table 3). The CML444 × MIRTC5 hybrid (0.083 mg kg^{-1}) accumulated least fumonisin level and differed significantly from the worst performing hybrids which included CML495 × R119W (1.172 mg kg^{-1}), MIRTC5 × CKL05015 (1.146 mg kg^{-1}), P502C2 × CKL05015 (1.163 mg kg^{-1}), P502C2 × CML444 (1.312 mg kg^{-1}), and R119W × CML444 (0. 846 mg kg^{-1}). Lines CML444 (2.370 mg kg^{-1}), P502C2 (2.546 mg kg^{-1}), and R119W (1.522 mg kg^{-1}) also accumulated significantly more fumonisins, though lines CKL05015, CML495, and MIRTC5 did not differ significantly from the best performing hybrid, CML444 × MIRTC5 (Table 3). Hybrid P502C2 × CML444 (306.70 g) and inbred line P502C2 recorded the highest kernel weight (234.53 g) while MIRTC5 × CML444 (157.73 g) and R119W (151.10 g) had the lowest kernel weight. A significant positive correlation of $r = .85$ occurred between fumonisin contamination and *F. verticillioides* colonization, however, FER severity had a low and non-significant correlation with *F. verticillioides* colonization ($r = .09$) and fumonisin contamination ($r = .18$). A negative correlation existed between FER and 1,000-kernel weight ($r = -.32$) were determined (Supplemental Table 1).

3.2 | Diallel crosses analysis

The GCA and SCA were significant for fungal colonization (GCA = < .0001; SCA = .027), fumonisin contamination (GCA = .015; SCA = < .0001), and 1,000-kernel weight

TABLE 3 Means of *Fusarium* ear rot (FER) severity, *Fusarium verticillioides* colonization, fumonisin concentration, and 1,000-kernel weight of maize genotype

Code	Genotype	FER severity	*F. verticillioides* colonization	Fumonisins Concentration	1 000-kernel weight
		%	ng μl^{-1}	mg kg^{-1}	g 1,000 $kernels^{-1}$
P1	CKL05015	0b	0.024bcd	0.255e–h	200.70d–i
P2	CML444	0b	0.093a	2.370a	224.77b–f
P3	CML495	1.52ab	0.017bcd	0.365d–h	208.83c–h
P4	MIRTC5	0.65ab	0.036b	0.553d–h	192.87e–i
P5	P502C2	0b	0.069a	2.546ab	234.53b–f
P6	R119W	0.63ab	0.034bc	1.522abc	151.10i
H1	CKL05015 × CML444	0b	0.016bcd	0.426d–h	234.50b–f
H2	CKL05015 × CML495	0b	0.007d	0.148gh	222.83b–f
H3	CKL05015 × MIRTC5	0b	0.011bcd	0.101h	218.27b–g
H4	CKL05015 × P502C2	0b	0.015bcd	0.182fgh	269.50ab
H5	CKL05015 × R119W	1.29ab	0.022bcd	0.106h	205.50d–i
H6	CML444 × CKL05015	0b	0.007d	0.097h	221.70b–f
H7	CML444 × CML495	0b	0.013bcd	0.225efh	213.37c–g
H8	CML444 × MIRTC5	0.07b	0.008 cd	0.083h	217.83b–g
H9	CML444 × P502C2	0.18b	0.012bcd	0.366d–h	262.07abc
H10	CML444 × R119W	0b	0.011bcd	0.216e–h	269.97ab
H11	CML495 × CKL05015	0b	0.010bcd	0.321e–h	244.67b–e
H12	CML495 × CML444	0b	0.008 cd	0.207e–h	213.37c–g
H13	CML495 × MIRTC5	1.25ab	0.016bcd	0.687c–h	222.20b–f
H14	CML495 × P502C2	0b	0.013bcd	0.285e–h	239.77b–f
H15	CML495 × R119W	2.38ab	0.026bcd	1.172b–e	163.43 g–i
H16	MIRTC5 × CKL05015	0b	0.026bcd	1.146 cd	242.03b–f
H17	MIRTC5 × CML444	0b	0.013bcd	0.173fgh	157.73hi
H18	MIRTC5 × CML495	0b	0.008 cd	0.128gh	213.10c–g
H19	MIRTC5 × P502C2	0b	0.014bcd	0.221e–h	187.47f–i
H20	MIRTC5 × R119W	0.36ab	0.020bcd	0.213e–h	195.07e–i
H21	P502C2 × CKL05015	2.52a	0.019bcd	1.163c–f	222.13b–f
H22	P502C2 × CML444	0.32ab	0.015bcd	1.312a–d	306.70a
H23	P502C2 × CML495	0b	0.011bcd	0.347d–h	255.63a–d
H24	P502C2 × MIRTC5	0b	0.020bcd	0.440d–h	232.27b–f
H25	P502C2 × R119W	0b	0.012bcd	0.249e–h	231.43b–f
H26	R119W × CKL05015	0b	0.009 cd	0.092h	226.40b–f
H27	R119W × CML495	0b	0.013bcd	0.191e–h	214.90b–g
H28	R119W × MIRTC5	2.94a	0.027bcd	0.515e–h	196.93e–i
H29	R119W × P502C2	0.88ab	0.014bcd	0.287e–h	235.93b–f
H30	R119W × CML444	0.62ab	0.022bcd	0.846c–g	220.51b–f
Mean		0.43	0.020	0.543	221.39

(GCA = .006, SCA = < .0001), but not for FER severity (GCA = .202; SCA = .598) and the reciprocal, maternal and nonmaternal effects were not significant for any of the variables ($P \geq .05$; Supplementary Table 2). Baker's ratio was relatively high for the 1,000-kernel weight (.87) and FER severity (.78), and moderate for the *F. verticillioides* target DNA (.47) and fumonisin contamination (.65). The broad sense heritability estimates ranged from .79 to .94, where 1,000-kernel weight was the highest and FER severity the lowest, whereas narrow sense heritability estimates ranged from .44 (*F. verticillioides* target DNA) to .82 (1,000-kernel weight; Supplementary Table 2).

Inbred line CML 495 had a significant negative GCA (−.0065) for *F. verticillioides* colonization. Line CKL05015 showed significant, negative GCA estimate (−.105) for fumonisin contamination while two inbred lines CML 444

TABLE 4 General combining ability estimates for *Fusarium* ear rot (FER) severity, *Fusarium verticillioides* colonization, fumonisin concentration, and 1,000-kernel weight of maize inbred lines

	FER severity		*F. verticillioides* colonization		Fumonisin concentration		1,000-kernel weight	
	%		ng μL^{-1}		ppm		g 1,000 $kernels^{-1}$	
Genotype	Estimate	Pr > |t|	Estimate	Pr > |t|	Estimate	Pr > |t|	Estimate	Pr > |t|
CKL05015	−0.090	.351	−0.0038	.119	−0.105	.022	4.355	.401
CML444	−0.133	.169	0.0060	.015	0.091	.046	9.217	.078
CML495	0.009	.929	−0.0065	.010	−0.070	.123	−2.978	.565
MIRTC5	0.024	.799	−0.0001	.958	−0.067	.140	−15.670	.003
P502C2	−0.042	.659	0.0038	.120	0.118	.011	21.275	<.0001
R119W	0.232	.018	0.0006	.816	0.033	.464	−16.199	.002

TABLE 5 Specific combining ability estimates for *Fusarium* ear rot (FER) severity, *Fusarium verticillioides* colonization, fumonisin concentration, and 1,000-kernel weight of F_1 maize hybrids evaluated in Kiboko in the 2014 maize-growing season

		FER Severity		*F. verticillioides* colonization		Fumonisin concentration		1,000-kernel weight	
		%		ng μl^{-1}		mg kg^{-1}		g 1,000 $kernels^{-1}$	
Code	Genotype	Estimate	Pr > |t|	Estimate	Pr > |t|	Estimate	Pr > |t|	Estimate	Pr > |t|
SCA estimates									
H1	CKL05015 × CML444	−0.038	.860	−6.862	.561	−0.0109	.054	−0.120	.246
H2	CKL05015 × CML495	−0.180	.412	10.984	.353	−0.0007	.903	0.019	.856
H3	CKL05015 × MIRTC5	−0.196	.372	20.075	.092	0.0030	.593	0.169	.104
H4	CKL05015 × P502C2	0.531	.017	−1.202	.919	−0.0028	.613	0.028	.782
H5	CKL05015 × R119W	−0.036	.870	6.405	.587	−0.0010	.862	−0.182	.080
H7	CML444 × CML495	−0.137	.532	−14.262	.229	−0.0086	.127	−0.179	.085
H8	CML444 × MIRTC5	−0.091	.677	−27.153	.024	−0.0153	.007	−0.254	.015
H9	CML444 × P502C2	0.255	.246	32.502	.007	−0.0164	.004	−0.044	.666
H10	CML444 × R119W	0.007	.975	30.832	.011	−0.0096	.087	−0.084	.413
H13	CML495 × MIRTC5	0.069	.751	14.909	.209	−0.0011	.850	0.096	.351
H14	CML495 × P502C2	−0.227	.300	8.014	.497	−0.0052	.354	−0.129	.212
H15	CML495 × R119W	−0.034	.875	−13.045	.271	0.0057	.310	0.102	.324
H19	MIRTC5 × P502C2	−0.243	.268	−17.127	.149	−0.0065	.243	−0.127	.219
H20	MIRTC5 × R119W	0.233	.287	6.480	.583	0.0033	.551	−0.053	.605
H25	P502C2 × R119W	−0.140	.522	7.219	.541	−0.0111	.048	−0.272	.010
Reciprocal estimates									
H6	CML444 × CKL05015	0.000	1.000	−6.400	.645	−0.0045	.492	−0.127	.296
H11	CML495 × CKL05015	0.000	1.000	10.917	.432	0.0015	.819	0.058	.631
H16	MIRTC5 × CKL05015	0.000	1.000	11.883	.393	0.0073	0.264	0.252	.041
H21	P502C2 × CKL05015	0.660	.012	−23.683	.091	0.0020	0.760	0.226	.065
H26	R119W × CKL05015	−0.367	.156	10.450	.452	−0.0069	0.292	−0.006	.959
H12	CML495 × CML444	0.000	1.000	0.000	1.000	−0.0027	0.684	−0.011	.927
H17	MIRTC5 × CML444	−0.061	.812	−30.050	.033	0.0023	0.731	0.038	.752
H22	P502C2 × CML444	0.098	.704	22.317	.111	0.0016	0.804	0.233	.057
H18	MIRTC5 × CML495	−0.363	.161	−4.550	.743	-0.0038	0.558	−0.189	.122
H30	R119W × CML444	0.367	.157	−24.728	.078	0.0053	0.416	0.205	.093
H23	P502C2 × CML495	0.000	1.000	7.933	.568	-0.0013	0.839	0.017	.891

(Continues)

TABLE 5 (Continued)

		FER Severity		*F. verticillioides* colonization		Fumonisin concentration		1,000-kernel weight	
		%		ng µl^{-1}		mg kg^{-1}		g 1,000 kernels^{-1}	
H27	R119W × CML495	−0.467	.073	25.733	.067	-0.0066	0.316	−0.239	.051
H24	P502C2 × MIRTC5	0.000	1.000	22.400	.110	0.0033	0.611	0.076	.530
H28	R119W × MIRTC5	0.256	.322	0.933	.946	0.0039	0.550	0.075	.537
H29	R119W × P502C2	0.310	.230	2.250	.871	0.0006	0.929	0.015	.905

TABLE 6 Genotype means of *Fusarium verticillioides* colonization and fumonisin concentration in maize evaluated in Katumani and Kiboko in the 2014–2015 maize-growing season

Code	Genotype	Katumani		Kiboko	
		F. verticillioides colonization	Fumonisins concentration	*F. verticillioides* colonization	Fumonisins concentration
		ng µl^{-1}	mg kg^{-1}	ng µl^{-1}	mg kg^{-1}
P1	CKL05015	0.021de	0.296f	0.039bcd	0.577c–i
P4	MIRTC5	0.219bc	2.840a-d	0.287a	5.107a
P6	R119W	0.075cde	1.038c–f	0.252a	3.088b
H1	CKL05015 × CML444	0.101b-e	0.870c–f	0.043bcd	0.399c–i
H2	CKL05015 × CML495	0.095b-e	1.392c–f	0.030 cd	0.253f–i
H3	CKL05015 × MIRTC5	0.081cde	1.459c–f	X	X
H4	CKL05015 × P502C2	0.179bcd	2.972a–f	0.077bc	1.218cde
H5	CKL05015 × R119W	X	X	0.035 cd	0.990cde
H6	CML444 × CKL05015	0.096b-e	2.532a–f	0.034 cd	0.054i
H7	CML444 × CML495	0.067cde	0.748c–f	0.015 cd	0.535c–i
H8	CML444 × MIRTC5	0.071cde	1.665a–f	0.045bcd	1.109cde
H9	CML444 × P502C2	0.108b-e	2.799a–e	0.039bcd	0.576c–i
H11	CML495 × CKL05015	0.106b-e	1.083c–f	0.015 cd	0.187f–i
H12	CML495 × CML444	0.039de	0.600c–f	0.028 cd	0.066i
H13	CML495 × MIRTC5	0.010e	0.372def	0.015 cd	0.125hi
H14	CML495 × P502C2	0.072cde	0.886c–f	0.062bcd	1.248c
H15	CML495 × R119W	0.080cde	1.923a–f	0.024 cd	0.331d–i
H16	MIRTC5 × CKL05015	0.057de	0.733c–f	0.011 cd	0.104hi
H17	MIRTC5 × CML444	0.270b	3.988abc	0.016 cd	0.138hi
H18	MIRTC5 × CML495	0.014e	0.184f	0.008d	0.308e–i
H19	MIRTC5 × P502C2	0.045de	0.657c–f	0.037bcd	0.382e–i
H20	MIRTC5 × R119W	0.107b-e	1.283c–f	0.017 cd	0.168hi
H21	P502C2 × CKL05015	0.249b	5.723ab	0.075bc	0.940c–f
H22	P502C2 × CML444	0.037de	0.453c–f	0.036bcd	0.792c–h
H23	P502C2 × CML495	0.073cde	1.633c–f	0.012 cd	0.217f–i
H24	P502C2 × MIRTC5	0.143b-e	1.260c–f	0.067bcd	1.107 cd
H25	P502C2 × R119W	0.140b-e	1.907c–f	0.015 cd	0.543c–i
H26	R119W × CKL05015	0.524a	9.272a	0.006d	0.034i
H27	R119W × CML495	0.180bcd	3.124a–f	0.104b	0.981c–g
H28	R119W × MIRTC5	0.072cde	5.337abc	0.039bcd	0.495c–i
H29	R119W × P502C2	0.137b–e	3.748a–e	0.013 cd	0.172ghi
Mean		0.116a	2.093a	0.050b	0.741b

and P502C2 had a significant, positive substantial GCA estimates (.091 and .118, respectively) for fumonisin contamination (Table 4). Unlike other inbred lines, P502C2 had a highly significant positive GCA estimate (21.275) for 1,000-kernel weight while lines MIRTC5 (−15.670) and R119W (−16.199) had significant GCA estimates but were not good general combiners for 1,000-kernel weight (Table 4).

Five hybrids, namely, CML495 × P502C2 (−0.227), MIRTC5 × P502C2 (−0.243), MIRTC5 × CML495 (−0.363), R119W × CKL05015 (−0.367), and R119W × CML495 (−0.467) had good SCA estimates for FER severity, but these were not significant. The worst significant SCA estimates for FER severity were observed on the hybrid CKL05015 × P502C2 (0.531) and its reciprocal hybrid (0.660). Genotypes CML444 × MIRTC5 (−0.0153), CML444 × P502C2 (−0.0164), and P502C2 × R119W (−0.0111) had significant SCA estimates and were the best combinations for *F. verticillioides* target DNA in this population. The highest positive SCA estimates for *F. verticillioides* target DNA were observed on hybrids CML495 × R119W (0.0057) and MIRTC5 × CKL05015 (0.0073), though not significant (Table 5). The best combinations for fumonisin accumulation with significant SCA estimates were CML444 × MIRTC5 (−0.254) and P502C2 × R119W (−0.272; Table 5). Of all the hybrids, MIRTC5 × CKL05015 (0.252) had the highest positive and significant SCA estimate for fumonisin contamination (Table 5). Hybrids such as P502C2 × CKL05015 (0.226) and P502C2 × CML444 (0.233) had high positive SCA estimates but were not significant for fumonisin contamination. The most desirable combinations for 1,000-kernel mass were CML444 × P502C2 (32.502) and CML444 × MIRTC5 (30.832), though CML444 × MIRTC5 (−27.15) and its reciprocal hybrid (30.05) had the largest significant and negative SCA estimates for 1,000-kernel weight. Good SCA estimates for 1,000-kernel mass were determined for CKL05015 × MIRTC5 (20.075), R119W x CML495 (25.73), and P502C2 × MIRTC5 (22.40), but these were not statistically significant (Table 5).

3.3 | Performance of F_2 populations

Infection by *F. verticillioides* ($P = < .0001$) and accumulation of fumonisins ($P = .0077$) in the F_2 trials was affected by significant genotype × environment interactions and the variation between replications at each locality was insignificant ($P = .7016$ and $P = .35179$, respectively; Supplementary Table 3). In Katumani, CML495 × MIRTC5 and its reciprocal had the lowest fungal DNA of 0.010 ng μl^{-1} and 0.014 ng μl^{-1}, respectively, which differed significantly from R119W × CKL05015 (0.524 ng μl^{-1}), the highest *F. verticillioides* target DNA in Katumani (Table 6). Two inbred lines, CKL05015 (0.021 ng μl^{-1}) and R119W (0.075 ng μl^{-1}) did not show significant variance with the best performing genotype in fungal colonization in Katumani. Hybrids R119W × CKL05015 (0.006 ng μl^{-1}) and MIRTC5 × CML495 (0.008 ng μl^{-1}) recorded the lowest fungal DNA in Kiboko, whereas lines MIRTC5 (0.287 ng μl^{-1}) and R119W (0.252 ng μl^{-1}) had the highest fungal DNA quantified (Table 6). Hybrid MIRTC5 × CML495 had the lowest fumonisin concentration of 0.184 mg kg^{-1} but did not differ significantly from inbred line CKL05015 (0.296 mg kg^{-1}) and several other F_2 populations, such as CML495 × MIRTC5 and P502C2 × CML444 in Katumani (Table 6). However, R119W × CKL05015 population (9.272 mg kg^{-1}) had the highest fumonisins level in Katumani. In Kiboko, CML444 × CKL05015 (0.054 mg kg^{-1}), CML495 × CML444 (0.066 mg kg^{-1}), and R119W × CKL05015 (0.034 mg kg^{-1}) had the lowest fumonisin levels. Inbred lines, MIRTC5 (5.107 mg kg^{-1}) and R119W (3.088 mg kg^{-1}) accumulated highest fumonisins. Two hybrids, CKL05015 × R119W and CKL05015 × MIRTC5 were missed in Katumani and Kiboko, respectively, due to crop failure (Table 6). The overall correlation between *F. verticillioides* colonization and fumonisin contamination was $r = .78$ ($P < .0001$) whereby $r = .77$ in Katumani and $r = .86$ in Kiboko ($P < .0001$).

4 | DISCUSSION

Developing maize cultivars with resistance to FER and fumonisins is complex and requires an integrated understanding of cultivar performance across environments as well as the inheritance of resistance in maize plants. In this study F_1 hybrids CML444 × MIRTC5, R119W × CKL05015, and CML444 × CKL05015 were most tolerant to FER, *F. verticillioides* colonization, and fumonisin accumulation. Tolerance to *F. verticillioides* infection did not affect grain yield as the 1,000-kernel weight of the hybrids was comparable to their parental lines. The F_2 family derived from hybrid MIRTC5 × CML495 was the most tolerant to *F. verticillioides* colonization in both localities and fumonisin accumulation in Katumani. These results indicate that improved resistance to FER and fumonisins is possible using a single cross. However, F_1 hybrid performance was not consistent with F_2 family performance in response to *F. verticillioides* infection. This highlights the need to evaluate F_2 and later generations for resistance to FER and fumonisins, and to determine the effect of resistance on yield by means of testcross evaluations with elite maize lines.

Hybrids CML444 × P502C2, MIRTC5 × CML495, R119W × CKL05015, MIRTC5 × R119W, and CML495 × MIRTC5 were least colonized by *F. verticillioides* and contaminated with fumonisins. Still, their response was not significantly better than that of their parental lines. The same result was also observed by King and Scott (1981) and Hung

and Holland (2012), and suggests that inbred line evaluations can provide breeders with a good prediction of the response of hybrids when breeding for resistance to FER and fumonisins (Hung & Holland, 2012). Mesterházy, Kovács Jr., and Kovács (2000) also stated that it is easier to predict the resistance of a hybrid if both parental lines are resistant because inbred lines with resistance to FER and fumonisins provide a good basis for a resistance-breeding program.

Parental lines CML444, CML495, CKL05015, or MIRTC5, resistant to FER and fumonisins and/or AER and aflatoxins (Okoth et al., 2017a, 2017b; Ouko et al., 2018; Rose et al., 2017), in combination with other maize lines, often produced the most tolerant F_1 hybrids. These lines exhibited significant, negative GCA estimates for *F. verticillioides* colonization and fumonisin contamination, except for CML444. The inbred line had a significant but positive GCA for *F. verticillioides* target DNA and significant, positive GCA estimate for fumonisin contamination, though CML 444 was initially classified as FER resistant. Previous studies by Rose et al., 2016 grouped CML 444 among the lines possessing resistance to aflatoxin accumulation, FER, and fumonisin accumulation and thus could be a good source for improving resistance to aflatoxin and fumonisin (Rose et al., 2016). Further still, Okoth et al., 2017a found CML 444 to be relatively resistant to *A. flavus* infection. Inbred lines resistant to AER and aflatoxin accumulation have been found to be more resistant to FER and fumonisin accumulation across localities when compared to FER- and fumonisin-resistant inbred lines. However, CML 444 has indicated inconsistent performance across environments, as supported by Rose et al., 2017, where CML 444 was the least stable inbred line in its response to *F. verticillioides* colonization, FER, and fumonisin accumulation across localities. The line however is recommended as one of the inbred lines that provide additional sources of resistance to FER and fumonisin in superlative local lines or be used for the development of hybrids with improved resistance (Rose et al., 2017).

The significant, negative GCA estimates are desirable as they indicate good combinability which is required for the inheritance of resistance. The use of such resistant lines in breeding programs could ensure comprehensive resistance to aflatoxins and fumonisins contamination in subsequent hybrids. This is of importance as the cooccurrence of mycotoxins have become more prevalent.

Previous studies portrayed either GCA or SCA effects to be more important for resistance to *F. verticillioides* colonization and fumonisin contamination (Hefny, Attaa, Bayoumi, Ammar, & Bramawy, 2012; Hung & Holland, 2012; Pádua et al., 2016; Williams & Windham, 2009). Based on SCA estimates, the best cross combinations for disease resistance were hybrid CML 444 × MIRTC5 and its reciprocal MIRTC5 × CML 444, CKL05015 × CML 444, CML 444 × CML 495, CML 444 × MIRTC5, MIRTC5 × P502C2, MIRTC5, and CML 495. The potential for resistance in these maize inbred lines could be self-determining and mainly controlled by the environment and genetic make-up of the germplasm. Therefore, there is a need for more research on resistance of the lines under different environments.

The predominant gene effects involved in resistance to *F. verticillioides* or fumonisins appear to be dependent on the population evaluated. Additive and nonadditive gene effects were almost equally important for inheritance of resistance to fungal colonization and fumonisins, whereas additive gene effects were important for the 1,000-kernel weight. This is in agreement with studies done by Srdić, Pajić, and Drinić-Mladenović (2007) and Rashmi, Haider, Chakraborty, and Sahay (2013), who also found additive gene effects to be the principal gene effect for 1,000-kernel weight. The maternal and nonmaternal effects of the genotypes evaluated in this study were not significant. Williams, Windham, and Buckley (2008) observed the same result in their diallel study of resistance to *A. flavus* and aflatoxins and proposed this was due to lack of cytoplasmic factors in the inheritance of resistance to FER and fumonisin contamination in this set of genotypes.

The environment, however, played a significant role on the amount of variation observed in fungal colonization and fumonisin contamination of the F_2 study. This was evident in the differences in the genotypes' responses between the two locations. For example, R119W × CKL05015 had an opposite response in the two test locations. In Katumani, for instance, R119W compared well to the most resistant genotypes in FER development and fumonisin contamination. However, this line performed poorly in Kiboko, where it was one of the worst performing genotypes. Also, R119W × CKL05015 was the best performing hybrid in the Kiboko F_2 hybrid trial, but it was the worst performing genotype in Katumani.

No correlations between FER severity with *F. verticillioides* colonization and fumonisin contamination was observed in the F_1 population. However, a strong association between colonization of grain with *F. verticillioides* and fumonisin occurred ($r = .86$; $P < .0001$). This makes quantification of fungal DNA on the grain a better indication of fumonisin accumulation than FER development. Different studies by Janse van Rensburg et al. (2015) and Rose et al. (2016), 2017) found analogous relationships between FER, *F. verticillioides* colonization, and fumonisin contamination. While it may be easier to evaluate maize for FER, it is important to determine the content of fumonisins when evaluating maize genotypes for their ability accumulate fumonisins.

5 | CONCLUSION

The findings of this study are useful to aid breeders in selecting maize lines with resistance to mycotoxigenic fungi

for the development of hybrids with improved tolerance to mycotoxin contamination in Kenya and other African countries. The study further supports the quantification of fungal colonization of the grain as a better indication of fumonisin accumulation levels in the grain rather than FER. Both GCA and SCA were important for hybrid resistance. Furthermore, hybrids with improved resistance to *F. verticillioides* infection and fumonisins were generated, and parental lines served as a good indicator of a hybrid's performance to infection.

ACKNOWLEDGMENTS

We acknowledge the MAIZE Competitive Grants Initiative, International Maize and Wheat Improvement Centre (CIMMYT), the National Commission for Science, Technology and Innovation (NACOSTI) of Kenya, the South African Maize Trust, and the National Research Foundation (NRF) of South Africa (South Africa–Kenya Research Partnership Program Bilateral; RPPB13102856988) for funding this research.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

ORCID

Abigael Ouko https://orcid.org/0000-0002-9883-8420

REFERENCES

Adejumo, T., Hettwer, U., Nutz, S., & Karlovsky, P. (2009). Real-time PCR and agar plating method to predict *Fusarium verticillioides* and fumonisin B1 content in Nigerian maize. *Plant Protection Research*, *49*, 399–404. https://doi.org/10.2478/v10045-009-0063-8

Alakonya, A. E., Monda, E. O., & Ajanga, S. (2009). Fumonisin B1 and aflatoxins B1 levels in Kenyan maize. *Plant Pathology*, *91*, 459–464. https://doi.org/10.4454/jpp.v91i2.978

Alakonya, A. E. (2016). *Ear rot and fumonisin B1 levels in self-selected maize varieties at different harvest intervals in Western Kenya* (Master's thesis). Kenyatta University, Nairobi, Kenya. Retrieved from http://ir-library.ku.ac.ke/handle/123456789/2038

Awika, J. M., Piironen, V., & Bean, S. (2011). *Advances in cereal science: Implications to food processing and health promotion*. Washington, D.C.: American Chemical Society.

Bii, F., Wanyoike, W., Nyande, A. B., Gituru, R. W., & Bii, C. (2012). Fumonisin contamination of maize (*Zea mays*) in aflatoxin hot zones in Eastern Province of Kenya. *African Journal Health Sciences*, *20*, 28–36.

Blandino, M., Reyneri, A., & Vanara, F. (2008). Influence of nitrogen fertilization on mycotoxin contamination of maize kernels. *Crop Protection*, *27*, 222–230. https://doi.org/10.1016/j.cropro.2007.05.008

Boutigny, A. L., Beukes, I., Small, I., Zühlke, S., Spiteller, M., Janse Van Rensburg, B. J., & Viljoen, A. (2012). Quantitative detection of *Fusarium* pathogens and their mycotoxins in South African maize. *Plant Pathology*, *61*, 522–531. https://doi.org/10.1111/j.1365-3059.2011.02544.x

Cole, R. J., Sanders, T. H., Hill, R. A., & Blankenship, P. D. (1985). Mean geocarposphere temperatures that induce preharvest aflatoxin contamination of peanuts under drought stress. *Mycopathologia*, *91*, 41–46. https://doi.org/10.1007/bf00437286

Doko, M. B., Canet, J. C., Brown, N., Sydenham, E. W., Mpuchane, S., & Siama, B. A. (1996). Natural co-occurrence of fumonisins and zearalenone in cereals and cereal based foods from Eastern and Southern Africa. *Agricultural and Food Chemistry*, *44*, 3240–3243. https://doi.org/10.1021/jf960257+

Ecker, O., & Qaim, M. (2011). Analysing nutritional impacts of policies: An empirical study for Malawi. *World Development*, *39*, 412–428. https://doi.org/10.1016/j.worlddev.2010.08.002

Government of Kenya. (2008). *Ministry of agriculture strategic plan (2008–2012)*. Nairobi, Kenya: Government Printers.

Griffing, B. (1956). Concept of general and specific combining ability in relation to diallel crossing system. *Australian Journal of Biological Sciences*, *9*, 463–493.

Guo, B., Xiangyun, J., Xinzhi, N., Jake, C., Hong, L., Hammed, K., … Brian, T. (2017). Evaluation of maize inbred lines for resistance to pre-harvest aflatoxin and fumonisin accumulation in the field. *Crop Journal*, *5*, 259–264. https://doi.org/10.1016/j.cj.2016.10.005

Harrison, L. R., Colvin, B. M., Green, J. T., Newman, L. E., & Cole, J. R. (1990). Pulmonary edema and hydrothorax in swine produced by fumonisin B1, a toxic metabolite of *Fusarium moniliforme*. *Veterinary Diagnostic Investigation*, *2*, 217–221. https://doi.org/10.1177%2F104063879000200312

Hefny, M., Attaa, S., Bayoumi, T., Ammar, S., & Bramawy, M. (2012). Breeding maize for resistance to ear rot caused by *Fusarium moniliforme*. *Pakistan Journal of Biological Sciences*, *15*, 78–84. https://doi.org/10.3923/pjbs.2012.78.84

Howard, D. H. (2003). *Pathogenic Fungi in Humans and Animals* (2nd ed.). New York, NY: Marcel Dekker.

Hung, H. Y., & Holland, J. B. (2012). Diallel analysis of resistance to *Fusarium* ear rot and fumonisin contamination in maize. *Crop Science*, *52*, 2173–2181. https://doi.org/10.2135/cropsci2012.03.0154

Fandohan, P., Gnonlonfin, B., Hell, K., Marasas, W. F. O., & Wingfield, M. J. (2005). Impact of indigenous storage systems and insect infestation on the contamination of maize with fumonisins. *African Journal of Biotechnology*, *7*, 546–552.

Janse van Rensburg, B., Mclaren, N. W., Flett, B. C., & Schoeman, A. (2015). Fumonisin producing *Fusarium* spp. and fumonisin contamination in commercial South African maize. *European Journal of Plant Pathology*, *141*, 491–504. https://doi.org/10.1007/s10658-014-0558-7

Jones, R. K., Duncan, H. E., Payne, G. A., & Leonard, K. J. (1980). Factors influencing infection by *Aspergillus* in silk-inoculated corn. *Plant Disease*, *64*, 859–863.

Kibe, N. (2015). *Occurrence of mycotoxigenic fungi in maize from food commodity markets in Kenya* (Master's thesis). Ghent University, Ghent, Belgium.

Kihara, S. N. (2013). *Occurrence of fumonisin producing Fusarium species in maize and soil in Makueni county, Kenya* (Master's Thesis). University of Nairobi, Nairobi, Kenya.

King, S. B., & Scott, G. E. (1981). Genotypic differences in maize to kernel infection by *Fusarium moniliforme*. *Phytopathology*, *71*, 1245–1247.

Kirui, M. C., Alakonya, A. E., Talam, K. K., Tohru, G., & Bii, C. C. (2014). Total aflatoxin, fumonisin and deoxynivalenol contamination of busaa in Bomet county, Kenya. *African Journal of Biotechnology*, *13*, 2675–2678. https://doi.org/10.5897/AJB2014.13754

Levene, H. (1960). Robust test in the equality of variance. In I. Olkin (Ed.), *Contributions to probability and statistics: Essays in honor of Harold Hotelling* (pp. 278–292). Palo Alto, CA: Stanford University Press.

Macauley, H. (2015). Cereal crops: Rice, maize, millet, sorghum, wheat. Retrieved from http://www.afdb.org/fileadmin/uploads/afdb/Documents/Events/DakAgri2015/Cereal_Crops-_Rice__Maize__Millet__Sorghum__Wheat.pdf.

Mesterházy, Á., Kovács Jr., G., & Kovács, K. (2000). Breeding resistance for *Fusarium* ear rot (FER) in corn. *Genetika*, *32*, 495–505.

Miller, J. D. (2001). Factors that affect the occurrence of fumonisin. *Environmental Health Perspectives*, *109*, 321–324. https://doi.org/10.1289%2Fehp.01109s2321

Mukanga, M., Derera, J., Tongoona, P., & Laing, M. D. (2011). Farmer's perceptions and management of maize ear rots and their implications for breeding for resistance. *African Journal of Agricultural Research*, *6*, 4544–4554. https://doi.org/10.5897/AJAR11.45

Munkvold, G. P. (2003). Cultural and genetic approaches to managing mycotoxins in maize. *Annual Review of Phytopathology*, *41*, 99–116. https://doi.org/10.1146/annurev.phyto.41.052002.095510

Mutiga, S. K., Hoffmann, V., Harvey, J. W., Milgroom, M. G., & Nelson, R. J. (2014). Assessment of aflatoxin and fumonisin contamination of maize in Western Kenya. *Phytopathology*, *105*, 1250–1261. https://doi.org/10.1094/PHYTO-10-14-0269-R

Mwalwayo, D., & Thole, B. (2016). Prevalence of aflatoxin and fumonisins (B_1 +B_2) in maize consumed in rural Malawi. *Toxicology Reports*, *3*, 173–179. https://doi.org/10.1016/j.toxrep.2016.01.010

Mwihia, T., Straetmans, M., Ibrahims, A., Njau, J., Muhenje, O., Guranja, A., … Lewis, L. (2008). Aflatoxins level in locally grown maize from Makueni district, Kenya. *East African Medical Journal*, *7*, 311–317. https://doi.org/10.4314/eamj.v85i7.9648

Ncube, E., Flett, B. C., Waalwijk, C., & Viljoen, A. (2011). *Fusarium* spp. and levels of fumonisins in maize produced by subsistence farmers in South Africa. *South African Journal of Science*, *107*, https://doi.org/10.4102/sajs.v107i1/2.367

Netshifhefhe, N. E., Flett, B. C., Viljoen, A., & Rose, L. J. (2018). Inheritance and genotype by environment analyses of resistance to *Fusarium verticillioides* and fumonisin contamination in maize F_1 hybrids. *European Journal of Plant Pathology*, *214*, 235. https://doi.org/10.1007/s10681-018-2310-4

Obura, A. (2013). Aflatoxins: Finding solutions for improved food safety. *Aflatoxicosis: Evidence from Kenya*. Retrieved from http://ebrary.ifpri.org/cdm/ref/collection/p15738coll2/id/127871

Olubandwa, A., Kathuri, N. J., Odero-Wanga, D., & Shivoga, W. A. (2011). Challenges facing small scale maize farmers in Western Province of Kenya in the agricultural reform era. *American Journal of Experimental Agriculture*, *1*, 466–476. https://doi.org/10.5281/zenodo.8141

Okoth, S., Nyongesa, B., Ayugi, V., Kan'gethe, E., Korhonen, H., & Vesa, J. (2012). Toxigenic potential of *Aspergillus* species occurring in maize kernels from two agro- ecological zones. *Toxins*, *4*, 491–1007. https://doi.org/10.3390/toxins4110991

Okoth, S., Rose, L. J., Ouko, A., Beukes, I., Sila, H., Mouton, M., … Viljoen, A. (2017a). Field evaluation of resistance to aflatoxin accumulation in maize inbred lines in Kenya and South Africa. *Journal of Crop Improvement*, *31*, 862–878. https://doi.org/10.1080/15427528.2017.1391915

Okoth, S., Rose, L. J., Ouko, A., Nakisani, N. E., Sila, H., & Viljoen, A. (2017b). Assessing genotype by environment interactions in *Aspergillus* ear rot and preharvest aflatoxin accumulation in maize inbred lines. *Agronomy*, *7*, 86. https://doi.org/10.3390/agronomy7040086

Okoth, S. (2016). *Improving the evidence base on aflatoxin contamination and exposure in Africa: Strengthening the Agriculture-Nutrition Nexus* (CTA Working Paper No. 16/13). Wageningen, Netherlands: Technical Centre for Agricultural and Rural Cooperation.

Ouko, A., Okoth, S., Amugune, N., & Vesa, J. (2018). Field assessment of agronomic performance resistance to aflatoxin and fumonisin accumulation in selected maize inbred lines in Kenya. *Agriculture, Forestry and Fisheries*, *7*, 94–100.

Ott, R. L., & Longnecker, M. (2010). *An introduction to statistical methods and data analysis* (6th ed.). Belmont, CA: Brooks/Cole, Cengage Learning.

Pádua, J. M. V., Das Graças Dias, K. O., Pastina, M. M., Souza, J. C. D., Queiroz, V. A. V., Costa, R. V. D., … Guimarães, L. J. M. (2016). A multi-environment trials diallel analysis provides insights on the inheritance of fumonisin contamination resistance in tropical maize. *Euphytica*, *211*, 277–285. https://doi.org/10.1007/s10681-016-1722-2

Probst, C., Njapau, H., & Cotty, P. J. (2007). Outbreak of an acute aflatoxicosis in Kenya in 2004: Identification of the causal agent. *Applied and Environmental Microbiology*, *73*, 2762–2764. https://doi.org/10.1128/AEM.02370-06

Rashmi, K., Haider, Z. A., Chakraborty, M., & Sahay, S. (2013). Diallel analysis for grain yield and its contributing traits in maize inbreds over environments. *Progressive Agriculture*, *13*, 75–83.

Robertson-Hoyt, L. A., Betrán, J., Paye, G. A., White, D. G., Isakeit, T., Maragos, C. M., … Holland, J. B. (2007a). Relationships among resistances to *Fusarium* and *Aspergillus* ear rots and contamination by fumonisin and aflatoxin in maize. *Phytopathology*, *97*, 311–317. https://doi.org/10.1094/PHYTO-97-3-0311

Robertson, L. A., Kleinschmidt, C. E., White, D. G., Payne, G. A., Maragos, C. M., & Holland, J. B. (2006). Heritabilities and correlations of *Fusarium* ear rot resistance and fumonisin contamination resistance in two maize populations. *Crop Science*, *46*, 353–361. https://doi.org/10.2135/cropsci2005.0139

Rose, L. J., Mouton, M., Beukes, I., Flett, B. C., Vyver, C. V. D., & Viljoen, A. (2016). Multi-environment evaluation of maize inbred lines for resistance to *Fusarium* ear rot and fumonisins. *Plant Disease*, *100*, 2134–2144. https://doi.org/10.1094/PDIS-11-15-1360-RE

Rose, L. J., Okoth, S., Beukes, I., Ouko, A., Mouton, M., Flett, B. C., … Viljoen, A. (2017). Determining resistance to *Fusarium verticillioides* and fumonisin accumulation in African maize inbred lines resistant to *Aspergillus flavus* and aflatoxins. *Euphytica*, *213*, 93. https://doi.org/10.1007/s10681-017-1883-7

Shapiro, S. S., & Wilk, M. B. (1965). An analysis of variance test for normality (Complete Samples). *Biometrika*, *52*, 591. https://doi.org/10.2307/2333709

Shephard, G. S., Marasas, W. F. O., & Burger, H. M. (2007). Exposure assessment for fumonisins in the former Transkei region of South Africa. *Food Additives and Contaminants*, *24*, 621–629. https://doi.org/10.1080/02652030601101136

Small, I. M., Flett, B. C., Marasas, W. F. O., McLeod, A., Stander, M. A., & Viljoen, A. (2012). Resistance in maize inbred lines to *Fusarium verticillioides* and fumonisin accumulation in South Africa. *Plant Disease*, *96*, 881–888. https://doi.org/10.1094/PDIS-08-11-0695

Srdić, J., Pajić, Z., & Drinić-Mladenović, S. (2007). Inheritance of maize grain yield components. *Maydica*, *52*, 261–264.

Wakhisi, J., Patel, K., Buziba, W., & Ritich, J. (2005). Oesophageal cancer in North Rift Valley of Western Kenya. *African Health Sciences*, *2*, 157–163.

Williams, W. P., & Windham, G. L. (2009). Diallel analysis of fumonisin accumulation in maize. *Field Crops Research*, *114*, 324–326. https://doi.org/10.1016/j.fcr.2009.08.005

Williams, W. P., Windham, G. L., & Buckley, P. M. (2008). Diallel analysis of aflatoxin accumulation in maize. *Crop Science*, *48*, 134–138. https://doi.org/10.2135/cropsci2007.05.0306

Wisser, R. J., Balint-Kurti, P. J., & Nelson, R. J. (2006). The genetic architecture of disease resistance in maize: A synthesis of published studies. *Phytopathology*, *96*, 120–129. https://doi.org/10.1094/PHYTO-96-0120

Zhang, D., Rossel, G., Kriegner, A., & Hijmans, R. (2004). AFLP assessment of diversity in sweet potato from Latin America and the Pacific region: Its implications on the dispersal of the crop. *Genetic Resources and Crop Evolution*, *51*, 115–120. https://doi.org/10.1023/B:GRES.0000020853.04508.a0

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Ouko A, Okoth S, Netshifhefhe NEl, Viljoen A, Rose LJ. Tolerance to *Fusarium verticillioides* infection and fumonisin accumulation in maize F_1 hybrids and subsequent F_2 populations. *Agronomy Journal*. 2020;112:2432–2444. https://doi.org/10.1002/agj2.20145