



ORIGINAL RESEARCH ARTICLE

Crop Breeding & Genetics

Inheritance of resistance to *Fusarium verticillioides* ear rot in maize inbred lines of southern, West and Central Africa origin

Elliot Tembo¹  | Adré Minnaar-Ontong² | Abebe Menkir³ | Gert Marais² | Cosmos Magorokosho⁴ | Maryke T. Labuschagne² 

¹Maize Breeding, Seed Co Ltd, Rattray Arnold Research Station, Harare, Zimbabwe

²Plant Sciences, Univ. of the Free State, Bloemfontein, South Africa

³Maize Breeding, International Institute of Tropical Agriculture, Oyo Rd., PMB 5320, Ibadan, Nigeria

⁴International Maize and Wheat Improvement Center (CIMMYT), Harare, Zimbabwe

Correspondence

Maryke T. Labuschagne, Plant Sciences, Univ. of the Free State, Bloemfontein, South Africa.

Email: LabuscM@ufs.ac.za

Assigned to Associate Editor Amritpal Singh.

Abstract

Maize (*Zea mays* L.) is an important crop in sub-Saharan Africa for both human consumption and livestock feed. Maize use is often limited by ear-rotting fungi, some of which produce deleterious secondary metabolites. The aim of this study was to determine the inheritance of resistance to *Fusarium verticillioides* as an indirect way of selecting for resistance to secondary metabolites such as the mycotoxin fumonisin that is produced by this fungus, under artificial inoculation and natural infection. Twelve inbred lines from Seed Co that were used as the females, were mated to 12 tester lines from the International Institute of Tropical Agriculture (IITA) that were used as males, using the North Carolina Design II. The resulting 144 F₁ hybrids and six check hybrids were evaluated in Zimbabwe. Artificial inoculation with *F. verticillioides* was done at Rattray Arnold Research Station in the first season. The general (GCA) and specific combining ability (SCA) effects for *F. verticillioides* incidence were variable across sites for the lines and the testers. Six southern African inbred lines had desirable GCA for *F. verticillioides* ear rot and can be used as resistance sources. Outstanding testers from IITA that had negative GCA for *F. verticillioides* ear rot and fumonisins were identified. Both additive and nonadditive effects were implicated in resistance to ear rot caused by *F. verticillioides* and potential lines were identified that can be used in regional breeding programs.

Abbreviations: ASI, anthesis silking interval; DMP, days to mid-pollen shed; DMS, days to mid-silk emergence; EASP, ear aspect; EC, number of ears harvested; EHT, ear height; EPO, ear position; EPP, ears per plant; GCA, general combining ability; GY, grain yield; HC, husk cover; IITA, International Institute of Tropical Agriculture; KRS, Kadoma Research Centre; PHT, plant height; QTL, quantitative trait loci; RARS, Rattray Arnold Research Station; RL, root lodging; SCA, specific combining ability; SL, stem lodging; SRC, Stapleford Research Centre.

This is an open access article under the terms of the [Creative Commons Attribution](https://creativecommons.org/licenses/by/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2022 The Authors. Crop Science published by Wiley Periodicals LLC on behalf of Crop Science Society of America.

1 | INTRODUCTION

Maize (*Zea mays* L.) plays an important role in the dietary needs of both humans and animals in Zimbabwe and most African countries. Maize suffers from both abiotic and biotic stresses, some of which give rise to secondary problems. Fusarium ear rot, caused by the fungus *Fusarium verticillioides* (Sacc.) Nirenberg, among other fungi, has been found to produce compounds such as fumonisin, which can be carcinogenic (Franceschi et al., 1990; Gelderblom et al., 1994; Osuchowski et al., 2005; Rheeder et al., 1992). Beside this,

other health challenges have been reported in human beings, rats and mice (Marasas et al., 1988; Missmer et al., 2006; Williams et al., 2010). As a result, both the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) have placed limits on provisional maximum tolerable daily intake (PMTDI) of edible maize of 2 mg per kg for fumonisin analogues B₁, B₂, and B₃, either individually, or in combination. The WHO and FAO normally set the trend for regional bodies such as the European Union (EU) and individual countries. The EU released a legal binding framework on the limits of concentration allowable for both human and animal consumption in the Commission Regulation (EC) no. 1126/2007 in September 2007. Of the several other mycotoxins, the fumonisin level limits are 4,000 and 8,000 µg per kg for human and animal consumption, respectively. For reasons such as a lack of effective regulatory frameworks, these limits are mostly not applied in sub-Saharan Africa countries (FAO, 2004; WHO, 2017). The presence of these toxins may cause serious economic losses to farmers, who have to discard contaminated grains. Feed contamination by mycotoxins also reduces livestock production (Hussein et al., 2001).

Commercially available maize cultivars in most parts of the world do not have specific resistance to *F. verticillioides* and other important mycotoxin-causing fungi such as *Aspergillus flavus*, possibly because they were developed from genetically related germplasm susceptible to such ear-rot causing fungal species. This is despite the identification of diverse sources of resistance to *A. flavus* (Brooks et al., 2005; Busboom & White, 2004; Menkir et al., 2008; Warburton et al., 2009; Williams & Windham, 2009) and to *F. verticillioides* infection (Busboom & White, 2004; Robertson et al., 2006). These known sources of resistance mainly originate from the temperate zone and are poorly adapted to production conditions in sub-Saharan Africa and most have poor combining ability for yield (Menkir et al., 2008; Warburton et al., 2009). Some sources of resistance adapted to West and southern Africa have also been identified but again with poor combining ability for yield, the most important trait considered in hybrid maize development (Menkir et al., 2008; Warburton et al., 2009). High levels of resistance found in some local sources (landraces) could be attributed to the presence of phenolic compounds such as anthocyanins and phlobaphenes that Salinas-Moreno et al. (2017) and Landoni et al. (2020) associated with low causal-fungi infection rates. Such landraces are characterized by pigmentation that results in plant tissues that are colored red, blue, or black, which are positively correlated with the presence of antioxidants and thick pericarps. Such characteristics could possibly define the pathways or mechanisms for resistance.

Breeding for resistance to fungi causing fumonisin contamination can be an effective control intervention, as it is environmentally friendly and can be applied under all socio-economic conditions (Busboom & White, 2004;

Core Ideas

- Ear-rotting fungi can produce secondary metabolites that cause diseases in humans and animals.
- Inheritance of resistance to *Fusarium verticillioides* and fumonisin was determined.
- Additive and nonadditive genetics effects were implicated in resistance to ear rot.
- Potential lines and testers were identified that can be used in regional breeding programs.

Clements et al., 2004; Gaikpa & Miedaner, 2019; Menkir et al., 2008; Warburton et al., 2009). This, therefore, suggests that even the poor people with low levels of education, which characterize most smallholder farmers in sub-Saharan Africa, can immensely benefit from cultivars that are inherently resistant. Robertson-Hoyt et al. (2007) observed a positive correlation between resistance to aflatoxins and fumonisins caused by *A. flavus* and *F. verticillioides*, respectively. As such, useful information can be derived from such a correlation as a substantial amount of research has been done in the temperate regions on *A. flavus*, while in southern Africa, a lot of research has been done on *F. verticillioides*. Only a few specific genes were found to directly confer ear rot resistance in maize. Gaikpa and Miedaner (2019) who reviewed genomics-assisted breeding for ear rot resistance and reduced mycotoxin contamination in maize, concluded that ear rot resistance was generally controlled by many small-effect quantitative trait loci (QTL). They indicated that genomic selection could be a good tool to select more resistant germplasm in a shorter time. Quantitative inheritance for resistance was also demonstrated by Maschietto et al. (2017) who used a traditional mapping approach involving two biparental Canadian maize populations and found 15 and 17 QTL for *Fusarium* ear rot and fumonisin contamination, respectively. Eight QTL were common to both traits. The presence of numerous QTL signifies quantitative inheritance.

As mycotoxin analysis costs are high, selection for resistance is often done indirectly through visual assessment of harvested ears. Specific plant characteristics can also be selected for, including good husk cover, which limits kernel infection by fungi. Genotypes that tend to have drooping ears after physiological maturity that limits accumulation of moisture in the cob; and silk characteristics that reduce penetration of the fungi into the ear, have reduced the incidence of ear rot (Bolduan et al., 2009). Mukanga et al. (2010) found high genotype × environment interaction (G × E) for the ear rot-causing fungi, indicating that genotypes respond differently to infection in different environments. Artificial inoculation allows the breeder more control when developing

resistant germplasm (Bolduan et al., 2009; Bútron et al., 2015; Maschietto et al., 2017; Windham et al., 2003). However, this does not always give the desired outcome, as the causal pathogen may not thrive in the absence of a conducive environment, a vital component of the disease triangle that also includes the reaction of the host plant to the pathogen. Success can therefore be achieved with the correct timing of the infection to coincide with the stage of the plant when it is most susceptible under the correct environmental conditions.

Diverse sources can be exploited in breeding for resistance to ear infection by *F. verticillioides* in maize, as additive and nonadditive gene effects have been associated with resistance to mycotoxin-causing fungi (Mukanga et al., 2010). Genetic variability for *F. verticillioides* under artificial inoculation was found to be significant (Bolduan et al., 2009). Ouko et al. (2020) found both nonadditive and additive gene effects playing a significant role in the inheritance of resistance to aflatoxins caused by *A. flavus*. Walker and White (2001) attributed resistance to *A. flavus* infection to additive gene effects, while nonadditive gene effects were associated with resistance to aflatoxin production. Such studies and findings are important for *F. verticillioides* which has the same pathway of resistance as that of *A. flavus*. Where dominance or overdominance is important, use of one resistant line in hybrid formation would lead to masking of the undesired alleles, while in population improvement or line development, segregating populations developed from these sources of variation could be exploited.

Commercially available maize hybrids in Africa do not have specific resistance to *F. verticillioides*, and known sources of resistance are poorly adapted to sub-Saharan Africa and combine poorly for yield and resistance to mycotoxin causing fungi. Therefore, the aim of this study was to determine the mode of inheritance of resistance to *F. verticillioides* as an indirect way of selecting for resistance to secondary metabolites such as the mycotoxin fumonisin, that are produced by this fungus, under artificial inoculation and natural infection. The understanding of the inheritance of other agronomic traits of importance is also vital, as resistance becomes useful if it exists within agronomically superior lines. This study is from the perspective of the elite lines being used within the region. These are public maize inbred lines with varying levels of resistance to aflatoxin contamination, and private sector proprietary inbred lines classified as resistant to *F. verticillioides*. The characterization was done by the respective source organizations before the study commenced.

2 | MATERIALS AND METHODS

2.1 | Inbred lines

Twelve proprietary inbred lines from southern Africa, selected based on their classification as resistant to

F. verticillioides and other ear rot-causing fungi, including *Diplodia maydis* (Berk.) Sacc. [= *Stenocarpella maydis* (Berk.) Sutton] through routine screening by artificial inoculation and selection from the Seed Co (SC) hotspot areas, and 12 maize inbred lines developed by the International Institute of Tropical Agriculture (IITA) with varying levels of resistance to ear rots and aflatoxin production, and adapted to West and Central Africa, were selected for this study (Table 1). These lines were grouped according to their known reaction to *Fusarium* ear rot and mycotoxin production, according to the data available within the respective organizations from which the lines originated, as well as their heterotic pattern and geographic origin. The 12 proprietary maize inbred lines included four lines that showed resistance to *Diplodia maydis* (Berk.) Sacc. and *F. verticillioides* (SC 1, SC 2, SC 3, and SC 4). Four lines were very susceptible to these two pathogens (SC 5, SC 6, SC 7, and SC 8), and four lines were of unknown reaction to most ear rot-causing fungi (SC 9, SC 10, SC 11, and SC 12). The IITA lines included those with high (IITA 1, IITA 2, IITA 3, and IITA 4), moderate (IITA 5, IITA 6, IITA 7, and IITA 8), and low (IITA 9, IITA 10, IITA 11, and IITA 12) levels of resistance to *F. verticillioides* and aflatoxin production. The Seed Co lines came from various heterotic groups, whereas the IITA maize inbred lines were of mixed origin but were classified based on their reaction to aflatoxin production (Table 1).

The inbred lines were crossed to make F₁ hybrids at Kadoma Research Centre (KRC, 18°20' E and 30°6' S at an altitude of 1,149 m), Rattray Arnold Research Station (RARS, 17°40' E and 31°13' S at an altitude of 1,341 m), and in the winter at a low altitude site in Muzarabani (17°40' E and 31°13' S at an altitude of 1,341 m) in Zimbabwe.

2.2 | Generation of F₁ hybrids

The three groups, each containing four lines from Seed Co, were crossed in a North Carolina II mating design (Comstock & Robinson, 1948), with each of the three groups containing four lines from IITA (Dhliwayo et al., 2009). Therefore, each group comprising of four lines from Seed Co was crossed to each group of four lines from IITA (Figure 1). The proprietary lines from Seed Co were used as female parents and the IITA lines were used as male parents to ensure that enough seed was generated as there was enough seed from the proprietary lines for as many rows as may be needed compared with the IITA lines. The 144 generated hybrids were tested in the trials (16 × 9). The seed of the parent lines were increased simultaneously to enable evaluation of lines in replicated trials alongside the F₁ trials.

TABLE 1 Maize inbred lines from southern Africa (SA) and West Africa (WA) and their known resistance levels to different ear-rot-causing fungi and mycotoxins^a

Female lines	Origin	Reaction to ear rots ^b	Male lines	Code	Reaction to aflatoxin	Origin
SC 1	SA	resistant	TZMI758	IITA 1	resistant	WA
SC 2	SA	resistant	TZMI733	IITA 2	resistant	WA
SC 3	SA	resistant	TZMI743	IITA 3	resistant	WA
SC 4	SA	resistant	TZMI407	IITA 4	resistant	WA
SC 5	SA	very susceptible	TZMI755	IITA 5	moderate	WA
SC 6	SA	susceptible	TZMI757	IITA 6	moderate	WA
SC 7	SA	very susceptible	TZMI746	IITA 7	moderate	WA
SC 8	SA	very susceptible	TZMI102	IITA 8	moderate	WA
SC 9	SA	unknown	TZMI744	IITA 9	susceptible	WA
SC 10	SA	unknown	TZMI749	IITA 10	susceptible	WA
SC 11	SA	unknown	TZMI750	IITA 11	susceptible	WA
SC 12	SA	unknown	TZMI756	IITA 12	susceptible	WA

Note. IITA, International Institute of Tropical Agriculture; SC, Seed Co.

^aSource: Seed Co Ltd. and International Institute of Tropical Agriculture (IITA).

^bModerate, moderately resistant; unknown, unknown reaction.

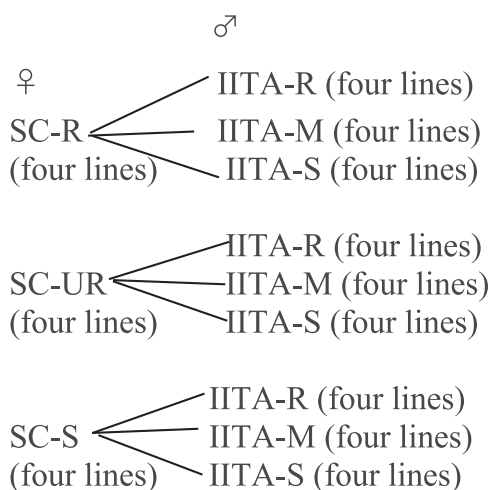


FIGURE 1 Schematic presentation of the crossing scheme between Seed Co and IITA lines. SC-R, resistant to Fusarium ear rot (FER); SC-UR, unknown reaction to FER; SC-S, susceptible to FER; IITA-R, resistant to aflatoxin; IITA-M, moderately resistant to aflatoxin; IITA-S, susceptible to aflatoxin

2.3 | Performance evaluation

The resulting 144 F₁ hybrids and the 24 parental lines were evaluated in replicated trials side by side at each site in 2 yr of testing, 2012/2013 and 2013/2014. The commercial hybrids SC 535 and SC 719 (resistant to *F. verticillioides*) and SC 537 (susceptible to *F. verticillioides*) from Seed Co in southern Africa, and three experimental hybrids from IITA, namely M0826-1, M1124-29, and M0926-8, were included in the hybrid trials to make a total of 150 entries. The experimental

design for all trials was an alpha lattice (Patterson et al., 1978). The hybrids and the parental lines were evaluated for their reaction to ear rot in two seasons at three locations in Zimbabwe. The hybrids and the parents were planted in different trials with two replications, two-row plots, 5 m long, spaced 0.75 m apart at a final population density of 53,333 plants per hectare, achieved through planting of three to four kernels per hill, followed by thinning to two plants per hill 2 wk after plant emergence. This was the practice in both summer seasons at a hot spot area at Stapleford Research Centre (17°48' S, 31°02' E and altitude, 1,470 masl) near Harare, where natural epidemics of *Fusarium* ear rots normally occur, at KRC and at RARS. At all the three locations in both years, plantings were done between 15 and 30 November.

A total of 400 kg ha⁻¹ basal application of fertilizer (7% N, 16% P₂O₅, and 8% K₂O) was done prior to planting. Nitrogen fertilizer was added approximately 4 wk after crop emergence at a rate of 138 kg ha⁻¹ in the form of ammonium nitrate with 34.5% nitrogen. Insects and weeds were controlled when necessary.

2.4 | Artificial inoculation

The hybrids and their parent lines were artificially inoculated with *F. verticillioides* at RARS only, in the 2013/2014 season. The *Fusarium* ear rot-severity scores were obtained at all the locations, including where evaluation was done under natural infection. Fumonisin concentration was determined only for the RARS location in the 2013/2014 season. Inoculum was prepared from the six isolates of *F. verticillioides* obtained

from the infected grain that was collected in Zimbabwe from the major maize-growing areas. DNA from the *F. verticillioides* isolates were extracted and sequenced and compared with DNA sequences of *Fusarium* species in Genbank (<http://www.ncbi.nlm.nih.gov/genbank>) to confirm the purity of the inoculant (Tembo, 2015), which was hence assumed to be virulent and having a high mycotoxigenic potential. Inoculation was done according to Clements et al. (2004) and Warburton et al. (2009), where the spore concentration and the sporulation potential was determined and standardized. The isolates were further increased for purification and drying, to enable easy transportation to the trial site at RARS. The isolates were grown on potato dextrose agar (PDA) (Biolab). In brief, 39 g of PDA was prepared as indicated on the container and sterilized by autoclaving at 121 °C for 20 min. The PDA plates were stored at 4–8 °C until needed.

Transfer of the fungi was done with the aid of a microscope. To avoid anticipated over growing, the lids on the petri plates were sealed with parafilm. The PDA plates were then placed in an ultraviolet light incubator for 14 d at 25 ± 2 °C. The *F. verticillioides* isolates identified from the various samples were blended in equal proportions in distilled water to form a bulk fungal blend that represented the pathotypes existing in the country (Clements et al., 2003, 2004). The increase of fungal inoculum was achieved through further propagation on sterile PDA, in petri plates. Conidia were washed from the dextrose agar using 500 ml of sterile distilled water.

At the RARS site, artificial inoculation was done in the first season using the method described by Williams and Windham (2009). In brief, 10 ml of *F. verticillioides* propagule suspension were applied through the Zummo and Scott (1989) technique through the husk of the primary ear at 7 d after the emergence of the silks on every plant within the plot (Warburton et al., 2009; Williams & Windham, 2009). The application was accomplished through the side-needle technique, where a 14-gauge needle was used with 3×10^8 conidia. Natural fungal epidemic was anticipated to further increase the disease pressure, particularly in hot-spot areas such as Stapleford Research Centre (SRC).

2.5 | Characteristics measured

Agronomic traits were recorded and derived before harvesting according to Badu-Apraku et al. (2011). Root lodging (RL) and stem lodging (SL) were derived from the proportion of plants with an inclination of 30° or more at the base of the plant and the proportion of plants at harvest with stalks broken below the ear level, respectively. Days to mid-pollen shed (DMP) and days to mid-silk emergence (DMS) were

determined as number of days from planting to when 50% of plants had started to shed pollen and showed silk emergence, respectively. The difference between the DMS and DMP was recorded as anthesis to silking interval (ASI). Plant height (PHT) and ear height (EHT) were measured as the distance between the base of a plant and the position of the first tassel branch and the position of the top ear, respectively. The other traits measured were ears per plant (EPP), ear position (EPO), and husk cover (HC), which were derived as the proportion of the number of productive ears divided by number of plants, the proportion of the height of ears harvested divided by the height of plants at harvesting time (EHT divided by PHT) and the number of plants with open tips (HC) expressed as a proportion of the total number of ears harvested (EC), respectively.

Harvesting was done post physiological maturity when grain moisture content was approximately 18%, roughly 60 d after mid-silking at most sites. *Fusarium verticillioides* ear rot was evaluated by counting the number of infected ears from each plot of the hybrid and the parent trials and were then expressed as a proportion of the total ears harvested. After drying the ears to 14% moisture, the *F. verticillioides* ear rot was further evaluated as grain disease score (GDS) at RARS, SRC, and KRC. A sample was drawn from shelled grain for all the ears harvested from each plot and given a score on a scale of 1–9, where 1 represented no visible ear rot and 9 represented complete infection of the harvested grain kernels with *F. verticillioides*. Infection severity score of 20% or less (1–2) was considered “good”, 25–30% (3–5) “intermediate”, and 35% or higher (6–9) “poor”. The ear aspect (EASP) was a score on a scale of 1–9 where 1 represented a large ear that is free of insect damage and disease and appears to be well covered with well-filled grains, while 9 represented an ear with undesirable characteristics (Badu-Apraku, 2011). Yield data were obtained following hand harvesting and weighing the actual grain yield after shelling and moisture determination with a moisture meter. Grain yield was expressed at a moisture content of 125 g kg⁻¹ (12.5%). After shelling, grain samples from each of the first season plots at RARS, where artificial inoculation was done, were randomly selected and submitted to Trilogy (www.trilogy.co) in the United States for quantification of fumonisin through high-performance liquid chromatography. Fumonisin analogues B₁, B₂, and B₃ were quantified for each hybrid and line, by Trilogy, following their company protocols.

2.6 | Data analysis

All data recorded were subjected to analysis of variance using Agrobase Generation II (Agronomix Software Inc., 2018), with replications and incomplete blocks considered random.

The analysis of variance was computed using the following linear model:

$$R_{ijk} = m + G_i + L_j + Y_k + B_r(L_j Y_k) + GL_{ij} + GY_{ik} + LY_{jk} + GLY_{ijk} + e_{ijk}$$

where m = grand mean; G_i = effect of i th genotype, L_j = effect of j th location, Y_k = effect of k th year, $B_r(L_j Y_k)$ = B replication within j th location and k th year, GL_{ij} = interaction between i th genotype and j th location, GY_{ik} = interaction between i th genotype and k th year, GLY_{ijk} = interaction between i th genotype, j th location, and k th year, e_{ijk} = the error associated with each observation.

The genotypic variance among the hybrids and the parental inbreds were considered random. The variation among hybrids was split into that caused by male inbred lines and females inbred lines. The mean squares for GCA of male lines, GCA of female lines and SCA were tested against GCA of males \times environment, GCA of females \times environment and SCA \times environment interactions, respectively. The GCA and SCA effects were computed with the Proc Mixed model using the COVTEST in SAS (SAS Institute, 2002), following the Hallauer and Miranda (1988) and Falconer (1996) model:

$$Y_{ijk} = \mu + m_1 + f_j + (m \times f)_{ij} + e_{ijk}$$

where Y_{ijk} is the k th observation on $i \times j$ th progeny, μ is the general mean, m_1 is the effect of the i th male, f_j is the effect of the j th female, $(m \times f)_{ij}$ is the interaction effect, and e_{ijk} is the error associated with each observation.

$$\begin{aligned} \text{Estimation of the GCA effects : } GCA_f &= X_f - \mu, GCA_m \\ &= X_m - \mu \end{aligned}$$

where X_f is the mean for female parent, X_m is the mean for male parent, GCA_f is the GCA for female parent, GCA_m is GCA for male parent, μ is the grand mean of the crosses.

$$\begin{aligned} \text{Estimation of the SCA effects : } SCA_x &= X_x - E(X_x) \\ &= X_x - [GCA_f + GCA_m + \mu] \end{aligned}$$

where SCA_x is SCA for a cross, X_x is the observed mean value for the cross, $E(X_x)$ is the expected value of cross based on the GCAs of its parents (GCA_f , GCA_m).

3 | RESULTS

3.1 | Reaction of hybrids to *Fusarium verticillioides*

The combined analysis of the hybrids to *F. verticillioides* infection revealed significant effects of entry with respect to

ER and GDS. All interactions with location, and interaction between location and year (meaning that locations ranked differently for the 2 yr) were also significant. The effects of year, location, and their interaction were significant for ER and GDS. Interaction between genotype and location was significant for GDS (Table 2).

Hybrids in which inbred lines SC 2, SC 5, SC 10, SC 11, IITA 1, IITA 7, and IITA 9 were parents, appeared more frequently among the top hybrids that had a mean disease incidence of 0%. Where the inbred line IITA 12 was used, higher levels of susceptibility were observed as its frequency among the most susceptible hybrids was the highest (data not shown).

The effects of location, entry, interaction of replication \times year and location \times year were significant ($P \leq .01$) for ER, GDS, GY, and ASI. Significant ($P \leq .01$) entry effects were seen for ER, GY, and ASI, year for ER and GDS, entry \times year for GDS and GY, and location \times entry \times year for GY and ASI.

3.2 | General combining ability for fumonisin

In terms of the fumonisin, measured as B_1 , B_2 , and B_3 analogues, GCA was found to be not significant for B_1 and highly significant for B_2 and B_3 ($P \leq .01$ to $P \leq .001$) (Table 3). The GCA_f estimates were highly significant for B_2 and B_3 ($P \leq .01$) and not significant for B_1 ($P > .05$). Inbred line SC 8 had the lowest, although not significant GCA_f effects for all three fumonisin analogues, suggesting that the line could confer resistance to this mycotoxin to hybrids that result from crosses where it is used as a female parent. Inbred SC 10 had the most negative GCA_f effects for B_2 and B_3 . The most positive GCA_f effects were recorded for inbred line SC 5 for B_1 , B_2 , and B_3 (Table 3) implying that hybrids made from it are generally more susceptible to fumonisins. The males showed significant differences ($P \leq .05$ to $P \leq .001$) for the fumonisins B_1 , B_2 , and B_3 , but the GCA_m effects for the males were not significant. IITA 10 had the lowest negative values for B_1 , B_2 , and B_3 . The highest positive effects for B_1 and B_3 were for IITA 12 and for B_2 , it was IITA 11 (Table 3).

3.3 | Analysis of general combining ability for *Fusarium verticillioides* ear rot

The ear rot (ER) incidence was not significant ($P > .05$) for both the female lines and the male lines (Table 3). The GCA_f effects for the ER were not significant ($P > .05$), and highly significant ($P \leq .001$) for the males lines (GCA_m). Male inbred lines IITA 1, IITA 2, IITA 3, IITA 4, IITA 6, IITA 7, IITA 10, and IITA 11 had negative values with IITA 7 having the most negative (-0.44) GCA effect for ER, while IITA 12 had the most positive effect of 0.95 (Table 3).

TABLE 2 Mean squares for measured characteristics of hybrids across locations and years in Zimbabwe

Source of variation	df	ER	GDS	GY	ASI
		%	1–5	t ha ⁻¹	days
Location (loc)	2	72.04***	1.48***	2,223.18***	1,211.95***
Entry (genotype)	149	2.56*	0.03	3.81***	3.89***
Year	1	172.24***	1.33***	0.08	4.11
Rep in loc × year	2	10.65*	1.14***	139.93***	96.3***
Loc × entry	298	2.41	0.03	1.49	2.11
Loc × year	2	271.54***	2.05***	169.40***	319.45***
Entry × year	149	2.79	0.03**	1.95**	2.53
Loc × entry × year	298	2.62	0.03	1.79*	2.22*
Error	898	2.552	0.028	1.486	1.912
Total	1,799				

Note. ASI, anthesis silking interval; df, degrees of freedom; ER, Fusarium ear rot; GDS, grain disease score; GY, grain yield.

* $P \leq .05$. ** $P \leq .01$. *** $P \leq .001$.

In general, female line SC 8 had consistently low GCA effects for all the characteristics except for ER, while SC 10 had among the lowest GCA effects for the three fumonisin analogues. Line SC 11 had the lowest GCA effects for ER and GDS. Male line IITA 10 had consistently low GCA effects for all characteristics except for ER (Table 3).

Across locations and years, of the southern Africa lines used as females, inbred line SC 3 and SC 10 had the highest negative GCA_f effects for *F. verticillioides* ear rot infection (Table 4). Despite artificial inoculation, the difference in ear rot incidence among hybrids was not significant. Inbred lines SC 1 and SC 8 had the highest positive GCA_f effects for the ER. The highest and lowest GCA_m effects for the male parents were observed for IITA 12 and IITA 1, respectively. The most negative GCA_m effects for ER were observed for inbred lines IITA 1 and IITA 7, while the highest positive values were found for IITA 4 and IITA 12.

3.4 | Analysis of specific combining ability for fumonisin and *Fusarium verticillioides* ear rot

The SCA effects for fumonisin analogues B₁, B₂ were significant ($P \leq .05$) (Table 3). The most negative SCA effects involved IITA 11 crossed to SC 5 and crossed to SC 6 (Table 5). The most positive effects were recorded for SC 5/IITA 12, SC 6/IITA 2, SC 6/IITA 9 and SC 5/IITA 6.

The SCA effects for Fusarium ear rot were highly significant ($P \leq .01$) (Table 3). Hybrid SC 7/IITA 2 had the most negative SCA effects for *F. verticillioides* ear rot across all sites in both seasons, although this was not significant. The highest effects were seen for hybrid SC 8/IITA 9 (Table 6).

The SCA effects for the ER incidence at RARS where artificial inoculation was carried out, was not significant although

the hybrids SC 12/IITA 12 and SC 10/IITA 5 had the lowest negative effects. The most positive effects were recorded for SC 10/IITA 12, SC 1/IITA 6, SC 4/IITA 5, and SC 3/IITA 11 (Table 6).

3.5 | Combining ability for agronomic traits

The GCA for the lines which were used as females (GCA_f) was highly significant for GY, DMP, DMS, ASI, and ear aspect (EASP) ($P \leq .001$) (Table 7). The female line SC 10 had the highest GCA_f effects for yield. The female inbred line SC 10 had the most positive GCA effects for GY, DMS, and ASI while inbred lines SC 4 and SC 1 had the highest GCA effects for DMP and EASP, respectively. The most negative GCA effects were recorded on inbred lines SC 3, SC 2, SC 5, SC 8, and SC 12 for GY, DMP, DMS, ASI, and EASP, respectively (Table 7). The most positive GCA effects for male lines were found for IITA 4, IITA 9, IITA 6, IITA 9, and IITA 12 for GY, DMP, DMS, ASI, and EASP, respectively. The most negative GCA effects were recorded for male inbred lines IITA 1, IITA 12, and IITA 4 for GY, DMP, and EASP, respectively. Inbred line IITA 8 had the most negative GCA effects for both DMS and ASI. The male inbred line IITA 4 had the most positive GCA effects for GY and most negative GCA for EASP which were both significant among the male lines (Table 7).

The SCA effects were not significant for GY across all the locations except for SC 11/IITA 3 that was significant ($P \leq .05$) with the most negative SCA effect (Table 8). The GCA effects for the constituting line SC 11 in this hybrid, was negative and low while that of the male IITA 3 was positive and low. The most positive SCA effect for grain yield was recorded for hybrid SC 3/IITA 1 (not significant).

TABLE 3 General combining ability (GCA) effects for female and male lines for Fusarium ear rot and fumonisin B₁, B₂, and B₃ at Rattray Arnold Research Station (RARS) and GCA effects for ear rots for grain disease score for RARS, Stapleford, and Kadoma

Lines	Code	Fusarium ear rot	Grain disease score	Fumonisin		
				B ₁	B ₂	B ₃
Female lines						
1	SC 1	0.47**	-0.05 ^a	-0.02	-0.01	-0.01
2	SC 2	-0.15	0.08	-0.07	-0.02	-0.01
3	SC 3	-0.23	0.16	0.00	-0.01	-0.01
4	SC 4	-0.25	0.00	0.15	0.05	0.01
5	SC 5	0.26	-0.05	0.38**	0.10**	0.04**
6	SC 6	0.07	0.00	0.18	0.06	0.02
7	SC 7	-0.07	0.00	-0.09	-0.03	-0.01
8	SC 8	0.36	-0.05	-0.22*	-0.06	-0.02
9	SC 9	-0.08	-0.05	-0.09	-0.02	-0.01
10	SC 10	0.18	0.00	-0.15	-0.06	-0.02
11	SC 11	-0.33**	-0.05	0.04	0.02	0.01
12	SC 12	-0.23	0.00	-0.11	-0.03	-0.01
Mean		0.96	1.05	0.27	0.06	0.02
<i>P</i> value genotype		ns	**	ns	***	**
<i>P</i> value GCA lines		ns	**	ns	**	**
<i>P</i> value SCA		**	ns	**	*	ns
Male lines						
1	IITA 1	-0.41**	0.00	-0.07	-0.01	-0.01
2	IITA 2	-0.04	-0.09	0.00	0.00	0.00
3	IITA 3	-0.09	0.00	-0.16	-0.04	-0.01
4	IITA 4	-0.40	-0.05	-0.02	-0.01	0.00
5	IITA 5	0.29**	0.04	-0.06	-0.03	-0.01
6	IITA 6	-0.01	0.00	0.06	0.00	0.00
7	IITA 7	-0.44**	0.08	-0.03	-0.02	-0.01
8	IITA 8	0.03	0.00	-0.07	-0.01	0.00
9	IITA 9	0.25**	0.08	0.10	0.03	0.01
10	IITA 10	-0.04	-0.05	-0.21	-0.06	-0.02
11	IITA 11	-0.12	0.00	0.2	0.07*	0.02
12	IITA 12	0.95	0.00	0.26*	0.06	0.03*
Mean		0.96	1.05	0.27	0.06	0.02
<i>P</i> value genotype		ns	ns	***	**	*
<i>P</i> value GCA testers		**	***	ns	ns	ns
<i>P</i> value SCA		**	ns	**	*	ns

Note. B₁, fumonisin B₁ analogue; B₂, fumonisin B₂ analogue; B₃, fumonisin B₃ analogue; IITA, International Institute of Tropical Agriculture; ns, not significant; SC, Seed Co; SCA, specific combining ability.

^aThe lowest two values in each column (more than two where the values were the same) are in bold.

* $P \leq .05$. ** $P \leq .01$. *** $P \leq .001$.

4 | DISCUSSION

This study showed that both additive and nonadditive gene effects were important in conferring resistance to both fumonisin causing fungi, *F. verticillioides* and possibly, the

fumonisin itself. Although the maternal effects were not anticipated to be important for these two traits, the differences in the magnitude of mean square values for ear rots and, loosely, the fumonisin accumulation between the female SC lines and the male IITA lines (data not shown), suggests

TABLE 4 Inbred lines and testers general combining ability (GCA) effects for *Fusarium verticillioides* ear rot across locations and 2 yr

Line	Female lines					Male lines				
	Line code	Line mean	GCA	t value	GCA rank	Line code	Line mean	GCA	t value	GCA rank
1	SC 1	0.75	0.97	1.22	12	IITA 1	0.71	-0.44	-0.78	1
2	SC 2	0.00	0.05	0.06	10	IITA 2	1.13	-0.02	-0.04	7
3	SC 3	0.25	-0.55^a	-0.69	2	IITA 3	0.79	-0.36	-0.63	4
4	SC 4	0.25	-0.04	-0.05	7	IITA 4	1.61	0.45	0.80	11
5	SC 5	0.25	-0.07	-0.09	6	IITA 5	1.51	0.36	0.63	10
6	SC 6	1.00	-0.29	-0.36	5	IITA 6	1.30	0.15	0.26	9
7	SC 7	0.42	0.36	0.45	3	IITA 7	0.74	-0.41	-0.73	2
8	SC 8	0.17	0.50	0.62	11	IITA 8	0.79	-0.36	-0.63	3
9	SC 9	0.00	-0.01	-0.01	9	IITA 9	1.09	-0.06	-0.10	6
10	SC10	0.58	-0.59	-0.74	1	IITA 10	1.04	-0.11	-0.19	5
11	SC11	0.00	-0.02	-0.03	8	IITA 11	1.13	-0.02	-0.04	8
12	SC12	0.44	-0.32	-0.40	4	IITA 12	1.98	0.83	1.45	12
		1.15	GCA SE	0.80			GCA SE	0.57		

Note. IITA, International Institute of Tropical Agriculture; SC, Seed Co; SE, standard error; t value, t test value.^aThe two values in bold indicate the best GCA for ear rot resistance

existence of maternal effects (Hallauer & Miranda, 1988), with the implication that lower ear rot infection or accumulation of the fumonisins was attainable only when certain lines were used as females or males only. As NCDII provides two measures of the GCA, it allows for the determination of maternal effects in the absence of reciprocal crosses that are achievable through other mating designs, such as the diallel (Fasahat et al., 2016). The inbred lines used in testcrosses showed varying responses in their reaction to fumonisin analogues B₁, B₂, and B₃. In terms of gene effects, this was similar to the findings by Ouko et al. (2020) who found additive and nonadditive gene effects to be almost equally important for resistance to fumonisins. There was a contradiction in terms of maternal effects, as they found no significant maternal effects. Low incidences of *F. verticillioides* were seen across sites and years, which could be attributed to the environment in terms of precipitation that is correlated with humidity at specific stage of plant development. The ear rot disease incidences were low, even under artificial inoculation at RARS, and this should, consequently, translate into low mycotoxin levels. This is despite that the 2nd year of testing was characterized by low rainfall (data not presented), which favors higher disease incidences (Cao et al., 2014). When a dry spell occurs at silking stage, natural barriers or defense mechanisms are circumvented. The period of flowering coincided with the period when there was relatively high precipitation. Links et al. (2020) observed that incidences were high when silk emergence occurred during the dry spell. Bush et al. (2004) did not find extensive ear rot in either year of their study, with an incidence of <22% for the symptoms recorded.

Among the lines for which reaction to the most frequently occurring ear rot-causing fungi were unknown, three had negative effects. The most negative GCA effects were observed for SC 10 across environments. This line has been associated with drought tolerance and wide adaptability. Negative GCA effects for a trait, such as ear rot rating, are indicative of the ability of the lines to contribute resistance. In a study conducted by Rose et al. (2016), certain maize inbred lines were found to be resistant to *F. verticillioides*, fungal target DNA and the fumonisins, and these lines included CML390, US 2540W, RO 424W, and VO 617y-2.

The GCA_m effects were significant for *F. verticillioides* ear rot, with the results showing a general trend of separation of the male lines into resistant, moderately resistant, and susceptible groups, as classified by the source institutions. Rose et al. (2016) used some lines that had previously been characterized as resistant, moderately resistant, and susceptible in their studies. The results on the GCA effects revealed that the classification of the IITA lines by their reaction to aflatoxin corresponded to their reaction to fumonisins. This is consistent with findings by Robertson-Hoyt et al. (2007) as lines resistant to aflatoxins caused by *A. flavus* were equally resistant to fumonisins. Some unexpected results were, however, observed, which would suggest existence of different pathways for resistance to the ear rot-causing fungi and the resulting mycotoxin, or other nonadditive gene effects playing a significant role. Such deviations were observed for the female line SC 8 that had been classified as susceptible to ear rots and indeed was one of the lines that had the most positive GCA effects for ear rot, while having the most negative GCA effects for the fumonisin analogues B₁, B₂, and B₃.

TABLE 5 Specific and general combining ability effects for fumonisin for hybrids at Rattray Arnold Research Station (artificially inoculated) across years

Male lines	Female lines												GCA
	SC 1	SC 2	SC 3	SC 4	SC 5	SC 6	SC 7	SC 8	SC 9	SC 10	SC 11	SC 12	
IITA 1	-0.17	0.19	-0.25	-0.48	-0.69	-0.07	-0.04	0.04	-0.15	-0.04	1.11*	0.54	-0.71
IITA 2	-0.20	0.56	-0.33	-0.56	-0.82	2.29*** ^a	-0.22	-0.05	-0.23	-0.07	-0.17	-0.19	-0.36
IITA 3	0.07	-0.02	-0.11	-0.34	-0.55	0.36	0.00	0.18	-0.01	0.50	-0.10	0.03	-0.03
IITA 4	-0.17	-0.21	-0.15	0.62	-0.84	-0.32	0.36	-0.01	-0.20	-0.04	1.01*	-0.06	-0.52
IITA 5	0.25	0.06	-0.23	-0.36	0.18	-0.41	0.08	0.06	0.02	0.23	-0.32	0.46	-0.59
IITA 6	-0.06	0.00	0.01	-0.52	2.12***	-0.47	-0.18	-0.10	-0.04	-0.08	-0.43	-0.25	0.18
IITA 7	1.41**	-0.19	0.78	-0.45	-0.76	-0.55	0.04	0.06	-0.07	0.14	-0.27	-0.13	-0.62
IITA 8	-0.23	0.58	-0.21	0.81	0.16	-0.53	-0.15	0.03	-0.06	-0.05	-0.35	-0.02	-0.30
IITA 9	-0.28	-0.37	-0.36	-0.34	0.05	1.71***	-0.15	-0.07	-0.26	-0.05	-0.40	0.53	-0.11
IITA 10	-0.01	0.15	0.01	-0.07	-0.43	-0.32	0.07	0.30	0.11	0.17	-0.08	0.10	-0.53
IITA 11	-0.14	-0.28	-0.22	1.30**	-1.16*	-0.90	0.74	-0.18	1.43**	-0.41	0.29	-0.48	-0.10
IITA 12	-0.45	-0.45	1.07*	0.39	2.73***	-0.81	-0.57	-0.25	-0.53	-0.32	-0.27	-0.54	1.85
GCA	0.49	0.15	-0.30	-0.35	0.12	-0.38	-0.16	0.53	0.29	0.32	-0.49	-0.23	

Note. GCA, general combining ability; IITA, International Institute of Tropical Agriculture; SC, Seed Co.

^aNumbers in bold indicate the most positive values* $P \leq .05$. ** $P \leq .01$. *** $P \leq .001$.

TABLE 6 Specific combining ability effects for *Fusarium verticillioides* ear rot across all locations in both seasons (natural disease occurrence) and at Rattray Arnold Research Station (artificially inoculated) in the first season

Male lines	Female lines											
	SC 1	SC 2	SC 3	SC 4	SC 5	SC 6	SC 7	SC 8	SC 9	SC 10	SC 11	SC 12
IITA 1	-0.38	0.81	-0.02	0.15	-0.31	0.46	0.08	-1.03	0.01	0.26	-0.37	0.35
IITA 2	0.57	0.53	0.46	0.52	-0.58	-0.39	-1.19	-0.65	0.55	-0.40	0.86	-0.28
IITA 3	1.98	-0.39	-0.18	-0.92	-0.25	0.67	-0.07	0.94	-0.15	-0.71	-0.60	-0.29
IITA 4	-1.08	0.00	-0.02	0.94	0.33	-0.27	0.51	-0.78	-0.32	0.03	0.24	0.42
IITA 5	-0.49	-0.59	0.75	0.19	-0.33	0.43	-0.66	-0.11	0.76	-0.02	0.24	-0.16
IITA 6	0.28	0.00	0.10	0.26	-0.08	0.59	-0.26	-1.08	-0.01	0.04	0.16	0.01
IITA 7	0.56	-1.09	0.54	0.54	-0.60	-0.39	0.09	0.18	-0.55	0.73	0.56	-0.56
IITA 8	-0.86	0.80	0.09	0.22	-0.48	-0.67	1.45	-0.79	0.34	-0.23	0.00	0.11
IITA 9	0.78	-0.59	-1.05	-0.59	1.13	0.37	-0.34	2.23	-0.34	-0.69	-0.20	-0.71
IITA 10	-0.51	0.77	-0.58	-0.47	-0.10	-0.13	0.24	-0.14	-0.39	0.57	-0.12	0.77
IITA 11	-1.14	-0.68	0.59	-0.66	0.80	-0.08	0.29	0.93	-0.55	0.13	-0.60	0.96
IITA 12	0.29	0.44	-0.71	-0.19	0.45	-0.59	-0.13	0.30	0.63	0.29	-0.16	-0.63
Rattray Arnold Research Station												
IITA 1	-1.25	-0.92	-0.46	0.23	0.47	0.96	0.10	-0.09	0.35	0.22	-0.28	0.67
IITA 2	-1.01	0.48	-0.81	0.33	-0.68	-0.09	-0.31	1.05	-0.15	-0.24	2.32	-0.89
IITA 3	-0.54	-1.65	0.65	-0.60	1.08	0.18	0.71	2.67	0.91	-1.82	-1.01	-0.57
IITA 4	-0.90	1.04	-0.65	0.54	-0.37	0.02	-0.15	-1.48	-0.69	0.03	0.78	1.83
IITA 5	0.09	-0.47	0.29	2.98	0.07	0.21	-0.21	1.25	0.25	-2.39	-0.93	-1.14
IITA 6	3.40	2.59	0.45	-0.21	-0.47	0.77	-0.90	-1.58	-0.64	-1.98	-1.17	-0.28
IITA 7	1.10	-1.01	0.05	-0.51	-0.97	0.17	-0.10	-0.73	1.61	-1.18	1.58	-0.03
IITA 8	0.23	0.42	-0.82	-0.13	0.91	-0.80	-0.42	0.75	0.44	-0.95	-0.69	1.05
IITA 9	-0.71	0.43	-0.41	-1.02	0.97	-0.29	-1.21	-0.05	-1.65	2.31	-0.88	2.51
IITA 10	-0.19	-1.10	-0.65	0.55	-1.07	0.08	0.51	0.47	-0.54	1.38	0.69	-0.12
IITA 11	-1.32	-0.38	2.93	-0.38	0.06	-1.00	1.38	-1.20	-1.11	0.65	0.31	0.05
IITA 12	1.08	0.57	-0.57	-1.78	0.01	-0.20	0.58	-1.05	1.24	3.95	-0.74	-3.10

Note. IITA, International Institute of Tropical Agriculture; SC, Seed Co. The bold type indicates the highest positive value.

The public IITA lines used in this study as male lines had been carefully selected for their reaction to aflatoxins, unlike the proprietary lines used as females, which were identified by the source organization as being resistant to *F. verticillioides* and other ear rot-causing fungi, without any information on their reaction to mycotoxins. Some negative correlations between reaction to *F. verticillioides* ear rot and the degree of accumulation of the fumonisin analogues were intriguing. Inbred line IITA 11 had negative GCA effects for ear rot while the GCA_m for all the fumonisins analogues were positive. Inversely, inbred IITA 5 and IITA 8 had positive GCA effects for ear rot, while the effects for the fumonisin analogues were negative. Such results are contrary to the observations by Butrón et al. (2015) who reported a correlation of 0.97 between *Fusarium* ear rot and fumonisin contamination. Fumonisin may be present in asymptomatic kernels, albeit at low levels (Bútron et al., 2015). Hybridization by the source organizations usually involves use of adapted germplasm, which at times include some landraces.

These landraces usually have different kernel colors that are further selected against to attain the desired kernel color. Such colored grains have been shown to be inherently rich in some phenolic compounds, such as phlobaphenes (Landoni et al., 2020; Salinas-Moreno et al., 2017) that are positively correlated with resistance to the ear rot-causing fungus *F. verticillioides*. Hence it is not surprising to see some IITA lines having inherent resistance to *F. verticillioides*, and it can further explain the resistance to aflatoxins associated with them. Use of temperate germplasm in most maize-breeding programs is a common practice as excellent alleles accumulated in advanced programs can be exploited to enhance yield. While focusing on the yield, genes for resistance to mycotoxin-causing fungi can be incorporated into the tropical germplasm.

Hybrids SC 5/IITA 12, SC 6/IITA 2, and SC 5/IITA 6 recorded the highest positive effects, hence significant differences were observed for the SCA for the fumonisin analogues, which indicated that they were most susceptible. Male line

TABLE 7 General combining ability (GCA_f) effects across locations in two seasons for female and male lines for yield and other agronomic traits

Entry	Code	GY	DMP	DMS	ASI	EASP
1	SC 1	-0.20	-0.64	-0.19	0.46	0.51*
2	SC 2	0.08	-1.45**	-1.25*	0.19	0.29
3	SC 3	-0.22	-0.03	0.00	0.03	-0.05
4	SC 4	0.03	1.21*	1.05*	-0.16	-0.23
5	SC 5	0.28	-1.25*	-1.33*	-0.09	-0.24
6	SC 6	-0.06	0.53	0.49	-0.04	0.03
7	SC 7	-0.17	0.85	1.189*	0.34	-0.09
8	SC 8	0.24	0.49	-0.04	-0.53	0.08
9	SC 9	-0.27	0.07	0.18	0.11	-0.05
10	SC 10	0.41	0.79	1.25*	0.47	-0.29
11	SC 11	-0.08	-0.83	-1.20*	-0.37	0.34
12	SC 12	-0.03	0.26	-0.16	-0.41	-0.30
1	IITA 1	-0.56	0.59	0.74	0.15	0.33
2	IITA 2	0.35	0.38	0.93	-0.05	0.19
3	IITA 3	0.03	-0.75	-0.66	-0.20	0.16
4	IITA 4	0.71*	0.20	0.00	0.15	-0.71***
5	IITA 5	-0.02	0.70	0.17	0.23	-0.18
6	IITA 6	0.00	-0.56	1.6**	-0.10	0.05
7	IITA 7	0.13	0.21	-0.39	-0.22	-0.32
8	IITA 8	0.12	0.73	-1.16*	-0.56	0.31
9	IITA 9	-0.29	1.05*	-0.97	0.57	0.17
10	IITA 10	-0.08	-0.73	0.33	0.34	0.04
11	IITA 11	-0.05	-0.89*	-0.95	-0.25	-0.59**
12	IITA 12	-0.36	-0.92*	0.36	-0.07	0.53**
Mean		4.75	67.08	68.17	1.09	4.88
GCA SE		0.34	0.48	0.51	0.32	0.25
P value GCA_f		***	***	***	***	***

Note. ASI, anthesis to silking interval; DMP, days to mid-pollen shedding; DMS, days to silking; EASP, ear aspect; GCA, general combining ability; GY, grain yield; IITA, International Institute of Tropical Agriculture; SC, Seed Co; SCA, specific combining ability.

* $P \leq .05$. ** $P \leq .01$. *** $P \leq .001$.

IITA 11 and female line SC 1 were involved in combinations resulting in low accumulation of fumonisins, which is consistent with the observation that their susceptibility was low, as GCA was negative or close to zero. Maupin et al. (2003) found dominance playing a major role in conferring ear rot resistance. In a different report on resistance to *A. flavus* ear rot and aflatoxin production, Walker and White (2001) attributed resistance to additive gene effects.

Molecular studies have confirmed additive genetic effects for inheritance of ear rot resistance and reduced mycotoxin accumulation. Butrón et al. (2015) studied the genetic control of *F. verticillioides* in maize lines from different genetic backgrounds under various environments and found both additive and dominance effects important, with little epistatic effects. Paul et al. (2003) studied QTL using the resistant inbred Tex6 and susceptible B73 and identified loci from these two par-

ents that contributed to resistance. Chromosomes 3, 4, 5, and 10 were associated with the resistance QTL, suggesting polygenic inheritance of resistance, although large environmental effects contributed towards making most QTL significant in 1 yr only. In the current study, the environmental effects were equally important, as they largely contributed to variation. High $G \times E$ interaction for the ear rot causing fungi was reported (Okoth et al., 2017) that necessitates the use of artificial inoculation and possibly manipulation of the environment by ensuring high humidity through interventions such as use of misting.

Inbred line SC 10, used as female, had the highest GCA_f for yield and generally exhibited negative GCA_f for *F. verticillioides* ear rot across sites, and possibly fumonisins, as observed from the 1-yr data obtained. As with female lines SC 10 and SC 5, positive GCA_f across all the sites for grain

TABLE 8 Grain yield general combining ability of the female and male lines and specific combining ability effects for grain yield across all sites in two seasons

Male lines	Female lines											
	SC 1	SC 2	SC 3	SC 4	SC 5	SC 6	SC 7	SC 8	SC 9	SC 10	SC 11	SC 12
IITA 1	-0.44	0.32	0.74	0.00	0.07	-0.31	0.04	-0.69	0.05	0.27	0.17	-0.22
IITA 2	0.26	-0.01	-0.57	0.09	0.07	0.16	0.02	0.10	0.27	0.30	-0.77	0.08
IITA 3	0.07	-0.23	-0.19	-0.09	0.30	0.21	-0.17	0.25	-0.13	0.35	-1.06*	0.70
IITA 4	0.26	-0.34	0.28	-0.01	0.26	0.28	0.31	-0.47	-0.04	0.09	-0.53	-0.09
IITA 5	0.18	0.30	0.17	0.14	-0.19	-0.24	0.16	-0.23	-0.12	-0.01	0.42	-0.58
IITA 6	-0.20	0.20	-0.23	0.27	-0.21	0.41	0.30	-0.46	-0.52	0.36	0.44	-0.36
IITA 7	0.20	-0.25	0.10	-0.41	-0.01	0.52	-0.38	0.01	-0.08	-0.45	0.40	0.36
IITA 8	-0.22	0.19	-0.43	-0.04	0.19	-0.38	-0.10	0.19	0.71	0.16	0.24	-0.51
IITA 9	-0.21	0.60	0.03	-0.05	-0.17	-0.18	-0.33	0.31	0.19	-0.19	0.33	-0.31
IITA 10	-0.64	-0.04	-0.12	0.08	0.16	0.20	-0.05	0.49	-0.67	-0.23	0.39	0.43
IITA 11	0.52	-0.70	0.36	-0.11	0.14	-0.14	0.07	0.02	0.53	-0.90	-0.05	0.26
IITA 12	0.22	-0.03	-0.15	0.13	-0.62	-0.52	0.11	0.50	-0.19	0.25	0.04	0.26

Note. IITA, International Institute of Tropical Agriculture; SC, Seed Co.

*Significantly different from zero at $P \leq .05$.

yield was observed. Positive GCA for yield is desirable as it indicates that the line increases yield. This is not surprising, especially for the inbred line SC 5, as several hybrids with this parent exist in southern Africa, which all have wide adaptability. Inbred line SC 10, in a separate study on genetic diversity involving all the lines in this study, exhibited wide diversity from the two major clusters of female and the male lines (Tembo, 2015).

Grain yield GCA for both the female and male lines was significantly different across environments for the lines (GCA_f) and as well as for the testers (GCA_m). This implies high contribution to yield by additive gene effects. The opposite is true for the SCA where there were no significant differences across sites. Observations of both GCA and SCA that respectively explain additive and nonadditive gene effects, have been reported by Long et al. (2004) who found SCA effects to be more important than GCA effects for yield.

The mean squares for height-related traits, PHT and EHT, were variable between sites for both GCA_f and GCA_m . Although these have no bearing on the trait under study, it is important to know how the lines under study can stand as good disease resistance alone may not be useful. Taller plants may be desirable under certain circumstances, where stover is important, if they do not lodge. On the other hand, short-statured plants may be important in increasing plant density. For leaf disease ratings of gray leaf spot and rust, the mean squares for the GCA_f were either always higher than or equal to those of GCA_m , which would indicate marginal or lack of maternal effects for such traits. Negative GCA effects for ear rots shown by lines and SCA effects exhibited by hybrids are obviously desirable.

Yield is generally a function of both additive and nonadditive effects. For traits associated with flowering, the SCA effects for DMP, DMS, and ASI across all sites in both seasons were significant, indicating existence of nonadditive gene effects controlling duration of flowering. This is consistent with Dhliwayo et al. (2009) who observed significant differences for days to anthesis in different environments. Flowering has a bearing on the ultimate infection by *F. verticillioides*. Date of flowering determines the length of silk channel as silking may coincide with the period when inoculum is most abundant. Length of the silk channel, maturity that is a function of DMS and DMP, and husk cover, have been associated with ear rot resistance (Butrón et al., 2006; Warfield & Davis, 1996). Bolduan et al. (2009), however, found a negative correlation between silk channel length and both Fusarium ear rot and fumonisin accumulation. There was no evidence of maternal effects for traits such as GY, DMS, DMP, ASI, PH, and EHT.

In conclusion, maize production suffers from abiotic and biotic stress factors, of which some contribute to production of secondary metabolites that are detrimental to consumers worldwide. Both additive and nonadditive effects were

important in conferring resistance to fumonisins and ear rot. The SCA is associated with nonadditive gene effects such as dominance while GCA is associated with additive effects. This was found relevant to *F. verticillioides* and the fumonisin accumulation in this study.

Ear rot resistance in this study could be attributed to additive gene effects, more importantly, as the GCA_f and GCA_m effects were significant, although some nonadditive effects were also found to play a role, albeit making a smaller contribution. These findings are consistent with those from several molecular studies elsewhere, where even different sets of genotypes and different environments were used, hence it can be universally accepted to apply in any part of the world.

It has been demonstrated that sources of resistance to Fusarium ear rot and possibly the metabolites produced by the pathogens exist, which include those screened and adapted to Central and West African tropical mid-altitude and southern African mid-altitude areas. These sources include those that have previously been screened for resistance to aflatoxin production. Such lines were developed by public and private entities, respectively. While private breeding programs are more focused on yield and its attributes, with other characteristics being secondary, public institutions focus on issues that affect the small-holder farmer who practices farming as both livelihood and business and include traits that are of less importance to private companies. As such, landraces form part of the pedigree of the populations from which lines are derived. Such landraces are inherently rich in some characteristics that are important but may not contain alleles that confer high yields such as the B73 type of germplasm commonly used in some developed temperate regions. The contrast between the two types of germplasm is the inherent poor yields in the landraces and the susceptibility to ear rots and mycotoxins in the B73. The use of public and private proprietary lines has been demonstrated to be beneficial for breeding towards resistance to ear rot-causing fungi. The approach capitalizes on the use of resistance to diseases and possibly the secondary metabolites exuded by such fungi, and the high yields associated with private sector bred maize inbred lines.

ACKNOWLEDGMENT

SEED CO is acknowledged for funding the project.

AUTHOR CONTRIBUTIONS

Elliot Tembo: Formal analysis; Investigation; Methodology. Adré Minnaar-Ontong: Supervision. Abebe Menkir: Conceptualization; Methodology; Supervision. Gert Marais: Formal analysis; Methodology; Supervision. Cosmos Magorokosho: Methodology; Project administration. Maryke T. Labuschagne: Data curation; Formal analysis; Methodology; Supervision; Writing – review & editing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ORCID

Elliot Tembo  <https://orcid.org/0000-0002-5472-9775>
Maryke T. Labuschagne  <https://orcid.org/0000-0003-0593-2678>

REFERENCES

- Agrobase (2018). *Agrobase generation II user's manual* (Version II, revised ed.). Agronomix Software.
- Badu-Apraku, B., Fontem, L. A., Akinwale, R. O., & Oyekunle, M. (2011). Biplot analysis of diallel crosses of early maturing tropical early maize inbreds in stress and nonstress environments. *Crop Science*, *51*, 173–188. <https://doi.org/10.2135/cropsci2010.06.0366>
- Bolduan, C., Miedaner, T., Schipprack, W., Dhillon, B. S., & Melchinger, A. E. (2009). Genetic variation for resistance to ear rots and mycotoxins contamination in early European maize inbred lines. *Crop Science*, *49*, 2019–2028. <https://doi.org/10.2135/cropsci2008.12.0701>
- Brooks, T. D., Williams, W. P., Windham, G. L., Willcox, M. C., & Abbas, H. K. (2005). Quantitative trait loci contributing resistance to aflatoxins accumulation in the maize inbred line Mp313E. *Crop Science*, *45*, 171–174. <https://doi.org/10.2135/cropsci2005.0171a>
- Busboom, K. N., & White, D. G. (2004). Inheritance of resistance to aflatoxin production and Aspergillus ear rot of corn from the cross of inbreds B73 and Oh516. *Phytopathology*, *94*, 1107–1115. <https://doi.org/10.1094/PHYTO.2004.94.10.1107>
- Bush, B. J., Carson, M. L., Cubeta, M. A., Hagler, W. M., & Payne, G. A. (2004). Infection and fumonisin production by *Fusarium verticillioides* in developing maize kernels. *Phytopathology*, *94*, 88–93. <https://doi.org/10.1094/PHYTO.2004.94.1.88>
- Butrón, A., Reid, L. M., Santiago, R., Cao, A., & Malvar, R. A. (2015). Inheritance of maize resistance to Gibberella and Fusarium ear rots and kernel contamination with deoxynivalenol and fumonisins. *Plant Pathology*, *64*, 1053–1060. <https://doi.org/10.1111/ppa.12351>
- Butrón, A., Santiago, R., Mansilla, P., Pintos-Varela, C., Ordás, A., & Malvar, R. A. (2006). Maize (*Zea mays* L.) genetic factors for preventing fumonisin contamination. *Journal of Agricultural and Food Chemistry*, *54*, 6113–6117. <https://doi.org/10.1021/jf0611163>
- Cao, A., Santiago, R., Ramos, A. J., Souto, X. C., Aguín, O., Malvar, R. A., & Butrón, A. (2014). Critical environmental and genotypic factors for *Fusarium verticillioides* infection, fungal growth and fumonisin contamination in maize grown in north-western Spain. *International Journal of Food Microbiology*, *177*, 63–71. <https://doi.org/10.1016/j.ijfoodmicro.2014.02.004>
- Clements, M. J., Kleinschmidt, C. E., Maragos, C. M., Pataky, J. K., & White, D. G. (2003). Evaluation of inoculation techniques for *Fusarium* ear rot and fumonisin contamination in corn. *Plant Disease*, *87*, 147–153. <https://doi.org/10.1094/PDIS.2003.87.2.147>
- Clements, M. J., Maragos, C. M., Pataky, J. K., & White, D. G. (2004). Sources of resistance to fumonisin accumulation in grain and *Fusarium* ear and kernel rot in corn. *Phytopathology*, *94*, 251–260. <https://doi.org/10.1094/PHYTO.2004.94.3.251>
- Comstock, R. E., & Robinson, H. F. (1948). The component of genetic variance of populations of biparental progenies and their use in estimating the average degree of dominance. *Biometrics*, *4*, 254–266. <https://doi.org/10.2307/3001412>

- Dhliwayo, T., Pixley, K., Menkir, A., & Warburton, M. (2009). Combining ability, genetic distances, and heterosis among elite CIMMYT and IITA tropical maize inbred lines. *Crop Science*, *49*, 1201–1210. <https://doi.org/10.2135/cropsci2008.06.0354>
- Falconer, D. S., & Mackay, T. F. C. (1996). *Introduction to quantitative genetics* (4th ed.). Longman.
- FAO. (2004). *Worldwide regulations for mycotoxins in food and feed in 2003* (FAO Food and Nutrition Papers. Version 81). FAO.
- Fasahat, P., Rajabi, A., Rad, J. M., & Derera, J. (2016). Principles and utilization of combining ability in plant breeding. *Biometrics & Biostatistics International Journal*, *4*, 1–22. <https://doi.org/10.15406/bbij.2016.04.0008>
- Franceschi, S., Bidoli, E., Baron, A. E., & La Vecchia, C. (1990). Maize and risks cancer of oral cavity, pharynx and esophagus in northern Italy. *Journal of the National Cancer Institute*, *82*, 1407–1411. <https://doi.org/10.1093/jnci/82.17.1407>
- Gaikpa, D. S., & Miedaner, T. (2019). Genomics-assisted breeding for ear rot resistances and reduced mycotoxin contamination in maize: Methods, advances and prospects. *Theoretical and Applied Genetics*, *132*, 2721–2739. <https://doi.org/10.1007/s00122-019-03412-2>
- Gelderblom, W. C. A., Cawood, M. E., Snyman, S. D., & Marasas, W. F. O. (1994). Fumonisin B₁ dosimetry in relation to cancer initiation in rat-liver. *Carcinogenesis*, *15*, 209–214. <https://doi.org/10.1093/carcin/15.2.209>
- Hallauer, A. R., & Miranda, F. O. (1988). *Quantitative genetics in maize breeding* (2nd ed.). Iowa State University Press.
- Hussein, H. (2001). Toxicity, metabolism, and impact of mycotoxins on humans and animals. *Toxicology*, *167*, 101–134. [https://doi.org/10.1016/S0300-483X\(01\)00471-1](https://doi.org/10.1016/S0300-483X(01)00471-1)
- Landoni, M., Puglisi, D., Cassani, E., Borlini, G., Brunoldi, G., Comaschi, C., & Pilu, R. (2020). Phlobaphenes modify pericarp thickness in maize and accumulation of the fumonisin mycotoxins. *Scientific Reports*, *10*, 1417. <https://doi.org/10.1038/s41598-020-58341-8>
- Links, S., Van Zyl, K., Cassiem, A., Flett, B. C., Viljoen, A., & Rose, L. J. (2020). The association of maize characteristics with resistance to *Fusarium verticillioides* and fumonisin accumulation in commercial maize cultivars. *World Mycotoxin Journal*, *13*, 367–379. <https://doi.org/10.3920/WMJ2019.2537>
- Long, J. K., Bänziger, M., & Smith, M. E. (2004). Diallel analysis of grain iron and zinc density in Southern African-adapted maize inbreds. *Crop Science*, *44*, 2019–2026. <https://doi.org/10.2135/cropsci2004.2019>
- Marasas, W. F. O., Kellerman, T. S., Gelderblom, W. C. A., Coetzer, J. A. W., Thiel, P. G., & van der Lugt, J. J. (1988). Leucoencephalomalacia in a horse induced by B₁ isolated from *Fusarium moniliforme*. *Onderstepoort Journal of Veterinary Research*, *55*, 197–203.
- Maschietto, V., Colombi, C., Pirona, R., Pea, G., Strozzi, F., Marocco, A., Rossini, L., & Lanubile, A. (2017). QTL mapping and candidate genes for resistance to *Fusarium* ear rot and fumonisin contamination in maize. *BMC Plant Biology*, *17*, 1–21. <https://doi.org/10.1186/s12870-017-0970-1>
- Maupin, L. M., Clements, M. J., & White, D. G. (2003). Evaluation of the M182 corn line as a source of resistance to aflatoxin in grain and use of BGYF as a selection tool. *Plant Disease*, *87*, 1059–1066. <https://doi.org/10.1094/PDIS.2003.87.9.1059>
- Menkir, A., Brown, R. L., Bandyopadhyay, R., Chen, Z. Y., & Cleveland, T. E. (2008). Breeding maize for resistance to aflatoxins at IITA. In J. F. Leslie, R. Bandyopadhyay, & A. Visconte (Eds.), *Mycotoxins: Detection methods, management, public health and agricultural trade* (pp. 277–286). Cromwell Press.
- Missmer, S. A., Suarez, L., Felkner, M., Wang, E., Merrill, A. H., Rothman, K. J., & Hendricks, K. A. (2006). Exposure to fumonisins and occurrence to neural tube defects along the Texas-Mexico border. *Environment Health Perspectives*, *114*, 237–241. <https://doi.org/10.1289/ehp.8221>
- Mukanga, M., Derera, J., & Tongoona, P. (2010). Gene action and reciprocal effects for ear rot resistance in crosses derived from five tropical maize populations. *Euphytica*, *174*, 219–231. <https://doi.org/10.1007/s10681-010-0178-z>
- Okoth, S., Rose, L., Ouko, A., Netshifhefhe, N., Sila, H., & Viljoen, A. (2017). Assessing genotype-by-environment interactions in *Aspergillus* ear rot and pre-harvest aflatoxin accumulation in maize inbred lines. *Agronomy*, *7*, 86. <https://doi.org/10.3390/agronomy7040086>
- Osuchowski, M. F., Edwards, G. L., & Sharma, R. P. (2005). Fumonisin B₁-Induced neurodegeneration in mice after intracerebroventricular infusion is concurrent with disruption of sphingolipid metabolism and activation of proinflammatory signaling. *Neuro Toxicology*, *26*, 211–221. <https://doi.org/10.1016/j.neuro.2004.10.001>
- Ouko, A., Okoth, S., Netshifhefhe, N. E. L., Viljoen, A., & Rose, L. J. (2020). Tolerance to *Fusarium verticillioides* infection and fumonisin accumulation in maize F1 hybrids and subsequent F2 populations. *Agronomy Journal*, *112*, 2432–2444. <https://doi.org/10.1002/agj2.20145>
- Patterson, H. D., Williams, E. R., & Hunter, E. A. (1978). Block designs for variety trials. *Journal of Agricultural Science*, *19*, 395–400. <https://doi.org/10.1017/S0021859600055507>
- Paul, C., Naidoo, G., Forbes, A., Mikkilineni, V., White, D., & Rocheford, T. (2003). Quantitative trait loci for low aflatoxin production in two related maize populations. *Theoretical and Applied Genetics*, *107*, 263–270. <https://doi.org/10.1007/s00122-003-1241-0>
- Rheeder, J. P. (1992). *Fusarium moniliforme* and fumonisin in corn in relation to human esophageal cancer in Transkei. *Phytopathology*, *82*, 353–357. <https://doi.org/10.1094/Phyto-82-353>
- 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*, 53–361. <https://doi.org/10.2135/cropsci2005.0139>
- Robertson-Hoyt, L. A., Betrán, J., Payne, G. A., White, D. G., Isakeit, T., Maragos, C. M., Molnár, T. L., & Holland, J. B. (2007). Relationship 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/PHTO-97-3-0311>
- Rose, L. J., Mouton, M., Beukes, I., Flett, B. C., Van Der Vyver, C., & 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>
- Salinas-Moreno, Y., García-Salinas, C., Ramírez-Díaz, J. L., & Alemán-de la Torre, I. (2017). Phenolic compounds in maize grains and its nixtamalized products. In M. Soto-Hernández (Ed.), *Phenolic compounds-natural sources, importance and applications*. InTech.
- SAS Institute (2002). *SAS statistical software*. SAS Institute.
- Tembo, E. (2015). *Mycotoxigenic fungi associated with ear-rots in Zimbabwe: Identification and inheritance of resistance in southern and*

- West African maize inbred lines* [PhD thesis, University of the Free State]. www.academia.edu/es/63530728
- Walker, R. D., & White, D. G. (2001). Inheritance of resistance to *Aspergillus* ear rot and aflatoxins production from C12. *Plant Disease*, 85, 322–327. <https://doi.org/10.1094/PDIS.2001.85.3.322>
- Warburton, M. L., Brooks, T. D., Krakowsky, M. D., Shan, X., Windham, G. L., & Williams, W. P. (2009). Identification and mapping of new sources of resistance to aflatoxin accumulation in maize. *Crop Science*, 49, 1403–1408. <https://doi.org/10.2135/cropsci2008.12.0696>
- Warfield, C. Y. (1996). Importance of the husk covering on the susceptibility of corn hybrids to *Fusarium* ear rot. *Plant Disease*, 80, 208–210. <https://doi.org/10.1094/PD-80-0208>
- World Health Organization (WHO). (2017). *Evaluation of certain contaminants in food* (Eighty-third report of the Joint FAO/WHO Expert Committee on Food Additives, WHO Technical Report Series: WHO Technical Report Series, no.1002). World Health Organization.
- Williams, J. H., Grubb, J. A., Davis, J. W., Wang, J. - S., Jolly, P. E., Ankrah, N. - A., Ellis, W. O., Afriyie-Gyawu, E., Johnson, N. M., Robinson, A. G., & Phillips, T. D. (2010). HIV and hepatocellular and esophageal carcinomas related to consumption of mycotoxin-prone foods in sub-Saharan Africa. *American Journal of Clinical Nutrition*, 92, 154–160. <https://doi.org/10.3945/ajcn.2009.28761>
- 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>
- Windham, G. L., Williams, W. P., Buckley, P. M., & Abbas, H. K. (2003). Inoculation techniques used to quantify aflatoxin resistance in corn. *Journal of Toxicology-Toxin Reviews*, 22, 313–325. <https://doi.org/10.1081/TXR-120024096>
- Zummo, N. (1989). Evaluation of field inoculation techniques for screening maize genotypes against kernel infection by *Aspergillus flavus* in Mississippi. *Plant Disease*, 73, 313–316. <https://doi.org/10.1094/pd-73-0313>

How to cite this article: Tembo, E., Minnaar-Ontong, A., Menkir, A., Marais, G., Magorokosho, C., & Labuschagne, M. T. (2022). Inheritance of resistance to *Fusarium verticillioides* ear rot in maize inbred lines of southern, West and Central Africa origin. *Crop Science*, 62, 1818–1833. <https://doi.org/10.1002/csc2.20776>