

ORIGINAL RESEARCH ARTICLE

Crop Breeding & Genetics

Genetic analysis of grain yield and resistance of extra-early-maturing maize inbreds to northern corn leaf blight

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Abstract

Maize (*Zea mays* L.) is a food security crop in sub-Saharan Africa (SSA). Incidence of northern corn leaf blight (NCLB), caused by *Exserohilum turcicum*, in lowlands of SSA during the past decade has caused 30–70% reduction in maize yield. This study (a) examined the combining abilities of extra-early maize (EEM) inbreds and classified them into heterotic groups; (b) elucidated gene action controlling resistance to NCLB; (c) assessed grain yield (GYLD) and yield stability of EEM hybrids under NCLB infection; and (d) identified testers. One hundred and fifty EEM hybrids, obtained by crossing 15 inbreds each of white- and yellow-endosperm maize using the North Carolina Design II, plus six checks, were evaluated in nine environments, six of which were inoculated with an isolate of *E. turcicum* and three of which were non-inoculated in 2018 and 2019. The white and yellow inbreds were placed in three heterotic groups using the heterotic grouping based on general combining ability (GCA) of multiple traits and stability of GYLD using the genotype plus genotype × environment (GGE) biplot analysis. The GCA, specific combining ability (SCA) and genotype × environment (G×E) interactions were significant for GYLD, disease severity, and other measured traits. The GCA effects were more important than the SCA effects for GYLD and NCLB severity scores across environments, implying that recurrent selection could facilitate improvement for GYLD and NCLB resistance. Three inbred testers and four single-cross testers were identified for developing high-yielding NCLB-resistant hybrids. Four white and five yellow single-cross hybrids were identified for on-farm testing and possible commercialization.

Abbreviations: AEC, average-environment coordination; ASI, anthesis–silking interval; BI, base index; DA, days to 50% anthesis; DS, days to 50% silking; EASP, ear aspect; EEM, extra-early-maturing; EHT, ear height; EPP, number of ears per plant; G×E, genotype × environment; GCA, general combining ability; GGE, genotype plus genotype × environment; GYLD, grain yield; HGCAMT, heterotic grouping based on GCA of multiple traits; IITA, International Institute of Tropical Agriculture; MIP, Maize Improvement Program; NCD II, North Carolina Design II; NCLB, northern corn leaf blight; PASP, plant aspect; PC, principal component; PCA, principal component analysis; SCA, specific combining ability; SS, sum of squares; SSA, sub-Saharan Africa; TURC, average disease severity score; TURC2WAI, disease score 2 wk after inoculation; TURC6WAI, disease score 6 wk after inoculation; WCA, West and Central Africa.

1 | INTRODUCTION

Maize (*Zea mays* L.) is of great importance in sub-Saharan Africa (SSA). It plays an important role as a staple food crop in combating food insecurity and malnutrition problems confronting the subregion (Badu-Apraku et al., 2015). Researchers in SSA have dedicated efforts and resources to increase production and productivity of the crop; however, diverse abiotic and biotic factors have constrained these efforts (Sibiya, Tongoona, Derera, & Makanda, 2013). Of the numerous diseases that reduce production and productivity of maize in the subregion, foliar diseases cause significant losses, of which northern corn leaf blight (NCLB), incited by the fungus *Exserohilum turcicum* (Pass.) Leonard and Suggs, is of utmost importance (Pratt & Gordon, 2010; Vivek et al., 2010). The fungus thrives in areas characterized by relatively low temperatures (17–28 °C) and high humidity (80–100%) (Carson, 2016; Hooda et al., 2017), including the mid-altitudes of the tropics. In recent years, however, the emergence of NCLB in areas traditionally free of the disease has been a major concern in West and Central Africa (WCA) (Akinwale & Oyelakin, 2018). Northern corn leaf blight causes grain yield (GYLD) losses ranging from 50% when infection occurs at the grain-filling stage (Human et al., 2016), to 100% when infected at the seedling stage (Hooda et al., 2017). These losses have serious economic implications for farmers in SSA.

Host-plant resistance has been identified as the most reliable and economically viable among several control options to mitigate plant diseases (Ayiga-Aluba et al., 2015; White, 2016; Wiesner-Hanks & Nelson, 2016). Welz and Geiger (2000) have identified both qualitative and quantitative gene actions governing resistance and tolerance to NCLB. However, novel *E. turcicum* races can overcome preexisting, qualitative resistance in some genotypes, and this requires a continuous search for new sources of stable and durable resistance (quantitative) to manage the disease (Sibiya, Tongoona, & Derera, 2013). In addition, it is necessary to identify resistant germplasm with high GYLD potential. Therefore, maize genotypes that combine stable resistance to NCLB with desirable agronomic traits have become one of the research priorities of maize breeders in SSA.

Hybrids have been identified as the best alternative for improving yield and stability of maize across environments in SSA (Oyekunle & Badu-Apraku, 2014). The availability of extra-early-maturing (EEM) maize hybrids, which reach physiological maturity between 80 and 85 d after planting, has received major attention of the Maize Improvement Program of the International Institute of Tropical Agriculture (IITA-MIP). Commercialization of EEM maize cultivars has led to recent expansion and spread of the crop to several regions across WCA. The EEM maize cultivars have been found to use fertilizer more efficiently with the shorter life cycle. Thus,

Core Ideas

- Grain yield and *turcicum* resistance in extra-early maize is controlled by additive gene action.
- Potential exists for enhancing grain yield and *turcicum* resistance in extra-early maize inbreds.
- Three heterotic groups were identified for planned hybrid crosses involving extra-early inbreds.
- Inbred and single-cross testers for enhanced yield and *turcicum* resistance were identified.
- High-yielding and stable extra-early hybrids with resistance to *turcicum* have been developed.

farmers find EEM cultivars valuable, as they could be produced faster than other cereals.

Climatic changes and recurrent dry spells that occur between November and March in WCA make EEM cultivars indispensable in bridging the hunger gap during dry-spell periods (Badu-Apraku et al., 2013, 2018). Understanding the underlying principles governing the mode of inheritance of resistance to NCLB, combining ability, and heterotic patterns among EEM maize inbred lines tested in contrasting environments would be invaluable to the development of EEM hybrids with stable resistance to NCLB for use in the lowland tropics of WCA.

Genetic studies have demonstrated the major role played by additive gene action in the inheritance of resistance to NCLB (Sibiya, Tongoona, & Derera, 2013; Vivek et al., 2010). However, significant dominance effects have also been shown to be important (Sibiya, Tongoona, & Derera, 2013). It would, therefore, be desirable to study the gene action and combining abilities for resistance to NCLB in germplasm of interest. In addition, identification of testers has been useful for the development of different types of hybrids. Pswarayi and Vivek (2008) described the criteria for the identification and selection of an inbred or single-cross hybrid as a tester, including high per se GYLD, positive general combining ability (GCA) effects, and classification into heterotic group. Presently, no testers have been identified for developing EEM maize hybrids with improved GYLD under NCLB infection.

Although the EEM hybrids have been evaluated for yield and stability under multiple stresses including drought, combined heat and drought, low N, and *Striga* infestation, the yield and stability of EEM hybrids under NCLB infection have not been studied. Additionally, information on the gene action conditioning resistance to NCLB in EEM inbred lines, as well as the combining abilities of these inbred lines, are completely lacking. This information is a prerequisite for increased and rapid adoption of appropriate breeding strategies to

accelerate selection gains and determine parents for superior hybrid development. The present study was conducted to generate additional information that will allow development of high-yielding, NCLB-resistant EEM maize hybrids for commercialization in WCA. Specifically, the study was designed (a) to determine the combining abilities of white and yellow EEM maize inbred lines and place them in distinct heterotic groups, (b) to examine the type of gene action controlling resistance to NCLB of each grain-color group, (c) to investigate the GYLD and stability of EEM hybrids under NCLB infection, and (d) to identify testers for genetic enhancement of EEM in SSA.

2 | MATERIALS & METHODS

Thirty EEM inbred lines of maize, consisting of 15 yellow- and 15 white-endosperm grain color, were selected and crossed using the North Carolina Design II (NCD II) of Comstock and Robinson (1948). The inbred lines were grouped into sets (three sets for each grain color), with five inbred lines in each set. Inbreds within each grain color were crossed, with one set used as female and another set as male. A total of 150 single-cross hybrids (75 for each grain color) were generated. Seventy-five EEM single-cross hybrids plus six checks for each grain color were evaluated under artificial *E. turcicum* inoculation during the growing season of 2018 and 2019 at three locations in Nigeria—namely, Ikenne (6°53' N, 3°42' E), Ile-Ife (7°18' N, 4°33' E), and Zaria (11°7' N, 7°45' E), and natural (non-inoculated) conditions in 2019 at the same locations. A nine-by-nine lattice design with two replications for each grain color was used. Each hybrid (entry) was planted in 4-m single-row plots, spaced 0.75 × 0.40 m, with two plants per hill, resulting in 22 plants per plot. We applied N–P–K 15–15–15 and urea fertilizer at 3 and 5 wk after planting (WAP), respectively. *Exserohilum turcicum* inoculum used for the artificially inoculated trials was prepared by seeding preconditioned sorghum [*Sorghum bicolor* (L.) Moench] grains (100 g per Erlenmeyer flask) with a 4-ml suspension (10⁵ spores ml⁻¹) of a virulent *E. turcicum* isolate (NGIB16-13). The isolate was characterized in previous research efforts (Badu-Apraku et al., 2021). Prior to inoculation, the sorghum grains were soaked overnight, thoroughly washed in three changes of clean water, decanted, and sterilized in an autoclave (121 °C, 1 h). The inoculated grains were incubated at room temperature for 4 d (Badu-Apraku et al., 2021). At 4 WAP, all maize plants within each plot of the artificially inoculated trials were inoculated by placing *E. turcicum* colonized sorghum grains into the maize whorl at 40 kg ha⁻¹ using a calibrated sterile scoop (~15 grains). The non-inoculated trials were treated with 0.4 ml l⁻¹ fungicide (a combination of pyraclostrobin and dimethomorph) emulsified concentrate at 2-wk intervals until 10 WAP to prevent the spread of natural *E. turcicum*

populations to the non-inoculated trials. Each plot was visually scored twice for disease severity: (a) 2 wk after inoculation (TURC2WAI, 42 d after planting [DAP]), and (b) 6 wk after inoculation (TURC6WAI, 70 DAP). The scale of 1–9 proposed by Badu-Apraku et al. (2021) was adopted for the disease severity ratings.

Data were collected on days to anthesis (DA) and silking (DS), anthesis–silking interval (ASI, d), plant (PLHT, cm) and ear height (EHT, cm), percentage root and stem lodging, ears per plant (EPP), plant (PASP) and ear (EASP) aspect, husk cover, field weight (kg), and GYLD (kg ha⁻¹). The GYLD was calculated based on 80% shelling percentage and expressed at a moisture content of 150 g kg⁻¹ (Badu-Apraku & Fakorede, 2017). In 2019, the experiment was conducted under natural conditions, in addition to the artificial inoculations.

2.1 | Statistical analysis

The mixed model of the SAS package version 9.13 (SAS Institute) was used for the combined ANOVA, where blocks nested within replication by environments, and replications within environments were considered random factors and the genotypes were fixed. Location–year combinations were regarded as test environments and ANOVA was computed for each test environment to obtain entry means adjusted for block effects according to the lattice design (Cochran & Cox, 1960). A RANDOM statement with the TEST option was employed with the PROC GLM statement. The statistical model used was

$$Y_{ijk} = \mu + E_i + R_{j(i)} + B_{k(ij)} + G_g + EG_{ig} + \varepsilon_{ijk}$$

where Y_{ijk} is the observed measurement for the g th genotype in environment i , in the k th block in replicate j ; μ is the grand mean; E_i is the main effect of environment; $R_{j(i)}$ is the effect of replication nested within environment; $B_{k(ij)}$ is the effect of k th block nested within replicate j in environment i ; G_g is the effect of the genotype; EG_{ig} is the interaction effect between genotype and environment, and ε_{ijk} is the error term.

Analysis of variance for NCD II was pooled across test-environments and sets, and the mean square for the hybrid component was partitioned into the male (sets), female (sets), and female × male (sets). The main effects of male (sets) and female (sets) was considered as the GCA effect, whereas the female × male (sets) interaction was regarded as the specific combining ability (SCA) effect (Hallauer et al., 2010).

Classification of the inbred lines into heterotic groups was done using the heterotic grouping of GCA of multiple traits (HGCAMT) (Badu-Apraku et al., 2013), and the base index (BI) was adopted to classify hybrids either as resistant or

susceptible to *E. turcicum*. The BI was calculated as follows (Badu-Apraku et al., 2020):

$$BI = GYLD - 0.6(PASP) - 0.6(EASP) - 0.5(TURC) + 0.5(EPP)$$

The parameters of the BI were each standardized ($\mu = 0$, $\sigma = 1$) before using them so as to reduce the effects of the different scales of measurement. A positive BI value indicated resistance to *E. turcicum* infection, whereas negative BI values indicated susceptibility. The higher the BI, the higher the level of resistance or susceptibility, respectively. Based on the BI, 35 (best 20, middle 5, and the worst 10 genotypes) each of white and yellow single-cross hybrids were selected for stability analysis. Stability of GYLD of the hybrids was determined by genotype plus genotype \times environment (GGE) biplots (Yan & Tinker, 2005). For GGE biplot, the principal component analysis (PCA) is divided into two principal components (PCs): PC1 that identifies outstanding genotypes for average GYLD across test environments, and PC2 that describes the stability of GYLD. An ideal hybrid in terms of GYLD is, therefore, expected to have a high PC1 value that corresponds to high mean yield, whereas its PC2 value should be close to zero.

3 | RESULTS

3.1 | Performance of the hybrids under artificial *E. turcicum* inoculation and natural conditions

The GYLD (under *E. turcicum* artificial inoculation and natural conditions), percentage yield reduction, and the BI values of the white and yellow EEM single-cross hybrids are presented in Tables 1 and 2. The average GYLD for white EEM single-cross hybrids under artificial *E. turcicum* inoculation ranged from 2,985 kg ha⁻¹ for TZEEI 6 \times TZEEI 36 to 7,119 kg ha⁻¹ for TZEEI 51 \times TZEEI 8 whereas the same hybrids had higher GYLD of 5,178 and 8,054 kg ha⁻¹, respectively, under natural conditions. TZEEI 51 \times TZEEI 8 outyielded the best-performing white check TZEEI 29 \times TZEEI 21, which had GYLD of 5,517 kg ha⁻¹ under artificial *E. turcicum* inoculation. TZEEI 8 \times TZEEI 46 had GYLD of 8,513 kg ha⁻¹ under natural conditions but 25% GYLD reduction when artificially inoculated with *E. turcicum*. The highest GYLD reduction of 42% was recorded for TZEEI 6 \times TZEEI 36, which also had the lowest GYLD under *E. turcicum* artificial inoculation (Table 1). Average GYLD for yellow kernel EEM single-cross hybrids under artificial inoculations ranged from 1,029 kg ha⁻¹ for TZEEI 75 \times TZEEI 63 to 6,153 kg ha⁻¹ for TZEEI 78 \times TZEEI 157, whereas the same hybrids had higher average GYLD of 2,227 and 6,992 kg ha⁻¹, respectively, under natural conditions (Table 2). Additionally,

TZEEI 78 \times TZEEI 157 outyielded the best yellow check TZEEI 9 \times TZEEI 7, which had GYLD of 5,460 kg ha⁻¹ under artificial *E. turcicum* inoculation. The GYLD reduction due to artificial inoculation ranged from 1 to 54% in the EEM yellow hybrids (Table 2). Generally, the white EEM hybrids had higher GYLD than the yellow hybrids under artificially inoculated and natural conditions. Higher BI values were also recorded for the white EEM hybrids compared with the yellow counterpart. Based on the BI, 57 white EEM hybrids were resistant, whereas 18 were susceptible to the disease. In contrast, only 34 of the yellow EEM hybrids were resistant, whereas 41 were susceptible.

The GGE biplots for GYLD of selected (best 20, middle 5, and the worst 10) white and yellow EEM single-cross hybrids artificially inoculated with *E. turcicum* at the test locations based on the BI are presented in Figures 1 and 2. The PCA (PC1 = 81.6% and PC2 = 5.7%) explained 87.3% of the total variation (Figures 1). The environments used in the present study belong to a single mega-environment for GYLD, indicating the absence of significant crossover interaction. Based on the “which-won-where” view of the biplot, hybrids falling on the vertex of the sector were the highest yielding in that environment. Thus, Entry 72 (TZEEI 51 \times TZEEI 8) was the highest yielding across three environments (E1, E4, and E5), all artificially inoculated. Entry 14 (TZEEI 6 \times TZEEI 36) was the lowest yielding and was not adapted to any of the test environments. There was no hybrid with outstanding performance in environments E2 and E3 (Figure 1a). Presented in Figure 1b is the average-environment coordination (AEC) view of the GGE biplot. Entry 72 (TZEEI 51 \times TZEEI 8) had the highest yield, followed by Entry 22 (TZEEI 13 \times TZEEI 8) and then Entry 17 (TZEEI 8 \times TZEEI 20), whereas Entry 14 (TZEEI 6 \times TZEEI 36) was the lowest yielding. TZEEI 8 \times TZEEI 46, TZEEI 51 \times TZEEI 8, TZEEI 32 \times TZEEI 51, TZEEI 1 \times TZEEI 51, and TZEEI 15 \times TZEEI 32 were the highest yielding and stable EEM white hybrids across test environments (Figure 1b). In addition, Entry 72 (TZEEI 51 \times TZEEI 8) was the highest yielding hybrid with relatively high stability across the environments, whereas Entries 17 (TZEEI 8 \times TZEEI 20) and 22 (TZEEI 13 \times TZEEI 8), which were also high yielding, were less stable.

The GGE biplot of the yellow hybrids revealed a pattern different from the white hybrids. The PCA explained 87.3% of the total variation (PC1 = 79.6% and PC2 = 7.7%; Figure 2a). With respect to the “which-won-where” view of the biplot, Entries 115 (TZEEI 78 \times TZEEI 157) and 135 (TZEEI 157 \times TZEEI 179) had the highest GYLD at E2, and Entries 139 (TZEEI 158 \times TZEEI 86), 150 (TZEEI 179 \times TZEEI 158), and 145 (TZEEI 172 \times TZEEI 158) had the highest GYLD at E5, E3, and E1, respectively. Entry 101 (TZEEI 75 \times TZEEI 63) was the lowest yielding hybrid and was not adapted to any environment (Figure 2a). Presented in Figure 2b is the AEC view of the GGE biplot for the yellow hybrids. The

TABLE 1 Mean grain yield and percentage yield reduction of selected (best 20, middle 5, and worst 10 based on grain yield) white extra-early-maturing single-cross maize hybrids artificially inoculated with *Exserohilum turcicum* and under natural conditions at Ikenne, Ile-Ife, and Zaria in 2018 and 2019

Entry	Pedigree	GYLD ^a artificially inoculated	GYLD ^a natural conditions	Reduction ^b	BI ^c
		kg ha ⁻¹		%	
72	TZEEI 51 × TZEEI 8	7,119	8,054	11.6	7
22	TZEEI 13 × TZEEI 8	6,896	7,526	8.4	6
17	TZEEI 8 × TZEEI 20	6,424	6,476	1.0	5
20	TZEEI 8 × TZEEI 46	6,421	8,513	25.0	5
2	TZEEI 1 × TZEEI 13	6,328	6,741	6.1	5
5	TZEEI 1 × TZEEI 51	6,454	6,721	4.0	4
65	TZEEI 46 × TZEEI 51	6,273	7,501	16.4	4
45	TZEEI 32 × TZEEI 51	5,759	6,237	7.7	3
71	TZEEI 51 × TZEEI 3	6,199	7,644	18.9	3
73	TZEEI 51 × TZEEI 15	6,609	7,001	5.6	3
3	TZEEI 1 × TZEEI 21	5,468	6,505	15.9	3
28	TZEEI 15 × TZEEI 32	5,432	6,167	11.9	3
60	TZEEI 45 × TZEEI 51	5,486	5,783	5.1	3
23	TZEEI 13 × TZEEI 15	5,411	5,790	6.5	2
76	TZEEI 29 × TZEEI 21	5,517	5,627	2.0	3
37	TZEEI 21 × TZEEI 8	4,623	6,240	25.9	2
24	TZEEI 13 × TZEEI 36	5,437	6,277	13.4	2
52	TZEEI 38 × TZEEI 8	4,668	5,492	15.0	2
16	TZEEI 8 × TZEEI 1	4,665	5,280	11.6	1
68	TZEEI 49 × TZEEI 32	4,607	5,360	14.0	1
7	TZEEI 3 × TZEEI 20	4,598	4,960	7.3	0
70	TZEEI 49 × TZEEI 46	3,630	4,568	20.5	-1
15	TZEEI 6 × TZEEI 49	3,550	5,151	31.1	-2
40	TZEEI 21 × TZEEI 49	3,247	4,547	28.6	-2
63	TZEEI 46 × TZEEI 21	3,404	4,332	21.4	-2
38	TZEEI 21 × TZEEI 15	3,118	3,388	8.0	-3
62	TZEEI 46 × TZEEI 13	3,120	4,275	27.0	-3
11	TZEEI 6 × TZEEI 3	3,144	3,684	14.7	-3
32	TZEEI 20 × TZEEI 13	3,029	3,673	17.5	-3
30	TZEEI 15 × TZEEI 46	3,228	4,755	32.1	-3
14	TZEEI 6 × TZEEI 36	2,985	5,178	42.4	-4
LSD _{0.05}		674	1,168		

^aGYLD, grain yield.

^bPercentage GYLD reduction = (GYLD artificially infected/GYLD natural condition) × 100.

^cBI, base index.

hybrids TZEEI 78 × TZEEI 157 and TZEEI 157 × TZEEI 179 had the highest yield and were most stable across environments. In contrast, Entries 139 (TZEEI 158 × TZEEI 86), 150 (TZEEI 179 × TZEEI 158), and 145 (TZEEI 172 × TZEEI 158) had high yield but were less stable across the environments based on the absolute length of their projections from the AEC abscissa.

3.2 | Analysis of variance and combining abilities of GYLD and NCLB disease severity scores of EEM inbred lines under artificial *E. turcicum* inoculation

The research environments where the present study was conducted are described in Table 3. The environments were

TABLE 2 Mean grain yield (kg ha⁻¹) and percent yield reduction of selected (best 20, middle 5, and worst 10 based on grain yield) yellow kernel extra-early maturing single-cross maize hybrids artificially inoculated with *Exserohilum turcicum* and under natural conditions at Ikenne, Ile-Ife, and Zaria in 2018 and 2019

Entry	Pedigree	GYLD ^a artificially inoculated	GYLD ^a natural conditions	Reduction ^b	BI ^c
		kg ha ⁻¹		%	
115	TZEEI 78 × TZEEI 157	6,153	6,992	12.0	4
135	TZEEI 157 × TZEEI 179	6,025	6,461	6.8	4
125	TZEEI 86 × TZEEI 179	5,374	6,684	19.6	4
134	TZEEI 157 × TZEEI 172	5,570	5,968	6.7	3
149	TZEEI 179 × TZEEI 79	5,328	6,981	23.7	3
139	TZEEI 158 × TZEEI 86	5,702	6,385	10.7	3
144	TZEEI 172 × TZEEI 79	5,340	6,244	14.5	3
150	TZEEI 179 × TZEEI 158	5,590	6,516	14.2	3
143	TZEEI 172 × TZEEI 78	5,283	5,944	11.1	2
90	TZEEI 67 × TZEEI 157	5,662	5,917	4.3	2
114	TZEEI 78 × TZEEI 86	5,301	5,506	3.7	2
119	TZEEI 79 × TZEEI 86	5,598	5,965	6.1	2
100	TZEEI 73 × TZEEI 179	5,235	5,426	3.5	2
145	TZEEI 172 × TZEEI 158	5,466	5,515	1.0	2
156	TZdEEI 9 × TZEEI 79	5,460	6,528	16.3	2
130	TZEEI 88 × TZEEI 158	5,200	5,674	8.4	1
91	TZEEI 69 × TZEEI 67	4,240	5,057	16.2	0
84	TZEEI 63 × TZEEI 79	4,357	4,409	1.2	-1
123	TZEEI 86 × TZEEI 88	4,319	4,909	12.0	-1
93	TZEEI 69 × TZEEI 78	4,259	5,077	16.1	-1
88	TZEEI 67 × TZEEI 75	4,319	6,070	28.8	-1
78	TZEEI 59 × TZEEI 88	3,082	4,374	29.5	-4
96	TZEEI 73 × TZEEI 63	3,054	4,309	29.1	-4
76	TZEEI 59 × TZEEI 63	2,676	3,023	11.5	-5
89	TZEEI 67 × TZEEI 86	2,832	4,126	31.4	-5
107	TZEEI 76 × TZEEI 73	3,047	4,464	31.7	-5
106	TZEEI 76 × TZEEI 59	2,746	4,789	42.7	-6
108	TZEEI 76 × TZEEI 75	2,563	5,087	49.6	-6
82	TZEEI 63 × TZEEI 76	2,354	4,875	51.7	-6
140	TZEEI 158 × TZEEI 157	1,558	1,923	19.0	-7
101	TZEEI 75 × TZEEI 63	1,029	2,227	53.8	-11
LSD0.05		602	1,175		

^aGYLD, grain yield.

^bPercentage GYLD reduction = (GYLD artificially infected/GYLD natural condition) × 100.

^cBI, base index.

suitable for the study and recent outbreak of the disease have been experienced in the surrounding environments. These sites also matched the target test environments for the IITA-MIP. For the white hybrids, highly significant environment (ENV) variances occurred for GYLD, *E. turcicum* infection ratings, and all other measured traits (Table 4). Variances for SET and ENV × SET were not significant for GYLD but were significant for most other

measured traits. Similarly, variances for replication (REP; ENV × SET) were not significant for GYLD and all other measured traits except ASI (Table 4). Particularly important were the variances for MALE(SET), FEMALE(SET), and FEMALE × MALE(SET) interaction, all of which, in addition to BLOCK (ENV × REP), displayed highly significant mean squares for all traits except in a few cases (Table 4). In other words, GCA-male (MALE/SET), GCA-female

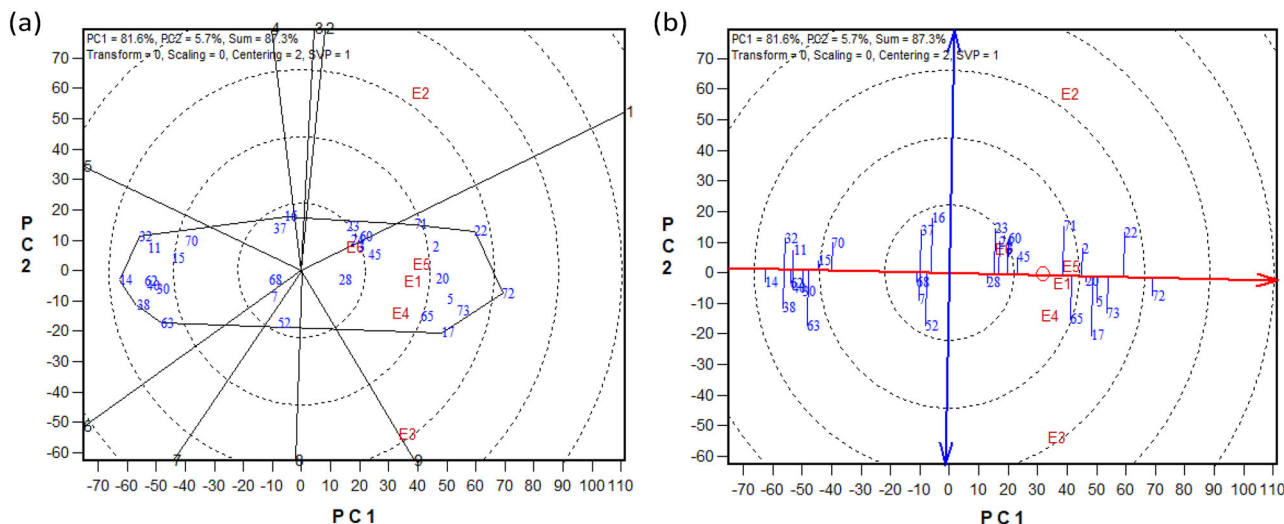


FIGURE 1 (a) Which-won-where view of the genotype plus genotype × environment (GGE) biplot to show which white extra-early-maturing hybrids performed best in which environments under artificial *Exserohilum turcicum* inoculation. (b) The “mean vs. stability” view of the GGE biplot based on genotype × environment grain yield (GYLD) data of white extra-early-maturing hybrids under artificial *Exserohilum turcicum* infection. PC, principal component; SVP, singular value partitioning

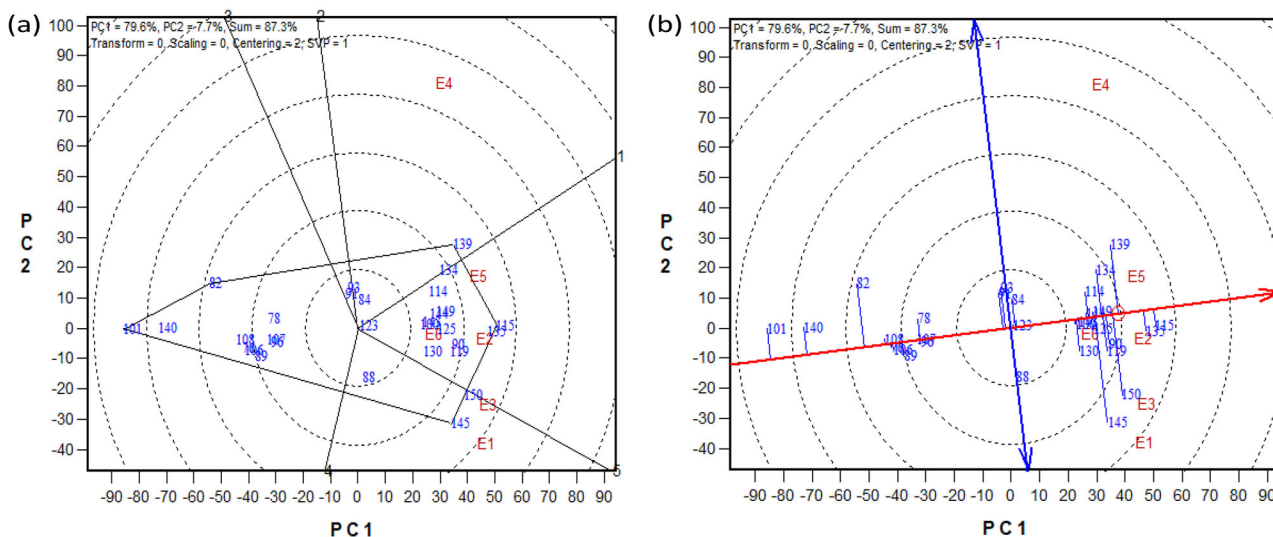


FIGURE 2 (a) Which-won-where view of the genotype plus genotype × environment (GGE) biplot to show which white extra-early-maturing hybrids performed best in which environments under artificial *Exserohilum turcicum* inoculation. (b) The “mean vs. stability” view of the GGE biplot based on genotype × environment grain yield (GYLD) data of yellow extra-early-maturing hybrids under artificial *Exserohilum turcicum* infection. PC, principal component; SVP, singular value partitioning

TABLE 3 Characteristics of locations used to evaluate extra-early-maturing maize hybrids artificially inoculated with *Exserohilum turcicum* during 2018 and 2019. The same locations were used to evaluate the hybrids under non-inoculated (natural) conditions in 2019

Location	Coordinates	Agroecological zone	Elevation	Avg. humidity	Annual rainfall	Avg. temperature
			m	%	mm	°C
Ikenne	6°53' N, 3°42' E	Rain forest	60	81	1,800	26
Ile-Ife	7°18' N, 4°33' E	Rain forest	244	81	1,600	25
Zaria	11°7' N, 7°45' E	Northern Guinea savanna	640	68	1,500	27

TABLE 4 Mean squares derived from the ANVOA for grain yield, *Exserohilum turcicum* infection ratings, and other agronomic traits for white extra-early-maturing, single-cross hybrids artificially inoculated with *E. turcicum*

Source of variation ^a	df	Grain yield kg ha ⁻¹	Days to anthesis	Days to silk	Anthesis-silking interval	Plant height	Ear height	Plant aspect ^b	Ear aspect ^c	TURC6WAI ^e		Ears per plot
										TURC2WAI ^d	TURC	
ENV	5	178,833,302.2**	500.3**	651.9**	23.5**	36,650.4**	20,835.1**	40.1**	11.1**	44.1**	8.1**	13.3**
SET	2	645,228.9	13.8**	16.1**	0.2	3,456.1**	940.5**	0.4	0.2	1.5**	5.2**	3.0**
ENV × SET	10	1,111,526.2	3.5**	3.5**	0.3	348.7**	111.3	0.4	0.6	0.5**	0.9	0.4
Rep (ENV × Set)	12	336,124.9	1.2	1.4	0.7**	77.4	93.1	0.4	0.4	0.1	0.5	0.2
Block (ENV × Rep)	96	1,259,856.6**	3.4**	3.7**	0.4	274.8**	150.1**	0.5**	0.7**	0.3**	0.9**	0.4**
MALE (SET)	12	12,493,839.9**	56.7**	52.8**	3.7**	1,376.0**	951.9**	3.4**	4.1**	1.6**	5.6**	2.9**
FEMALE (SET)	12	19,500,563.0**	82.1**	80.1**	2.3**	1,789.8**	1,135.8**	4.5**	4.9**	1.5**	3.8**	2.2**
FEMALE × MALE (SET)	48	4,829,490.6**	5.8**	6.8**	0.5	407.6**	184.2**	1.2**	1.6**	0.4**	1.3**	0.6**
ENV × MALE (SET)	60	872,222.2	2.0	2.9**	0.6**	133.7	107.4*	0.5**	0.4	0.3**	0.6	0.3
ENV × FEMALE (SET)	60	1,266,009.9**	2.8**	3.1**	0.5*	207.4**	93.0	0.4	0.5	0.4**	0.9**	0.4**
ENV × FEMALE × MALE (SET)	240	991,273.5**	1.9*	2.0**	0.4	131.8	84.0	0.4	0.4	0.2**	0.7**	0.3**
Error	336	579,777.0	1.3	1.6	0.4	104.7	69.1	0.4	3.3	0.2	0.4	0.2
R ²		90.4	92.8	93.5	79.6	89.7	89.0	84.3	80.6	86.2	79.2	83.3
CV, %		17.9	2.4	2.3	83.1	6.4	10.7	12.8	13.4	17.8	19.5	14.8
GM ^g		4704.0	51.6	52.2	0.7	172.6	81.4	4.6	4.6	2.6	3.8	3.2

^aENV, environment; Rep, replication.

^bPlant aspect, plant aspect recorded on a scale of 1–9 based on plant type, where 1 = excellent and 9 = poor.

^cEar aspect, ear aspect rated on a scale of 1–9, where 1 = clean, uniform, large, and well-filled ears and 9 = ears with undesirable features.

^dTURC2WAI, disease score 2 wk after inoculation.

^eTURC6WAI, disease score 6 wk after inoculation were rated on a scale of 1–9, where 1 = complete resistance, no visible northern corn leaf blight symptoms and 9 = abundant and coalescing lesions on all leaves covering >80% of the leaf surface with some plants prematurely dead.

^fTURC, average disease severity score.

^gGM, grand mean.

*Significant at the .05 probability level.

**Significant at the .01 probability level.

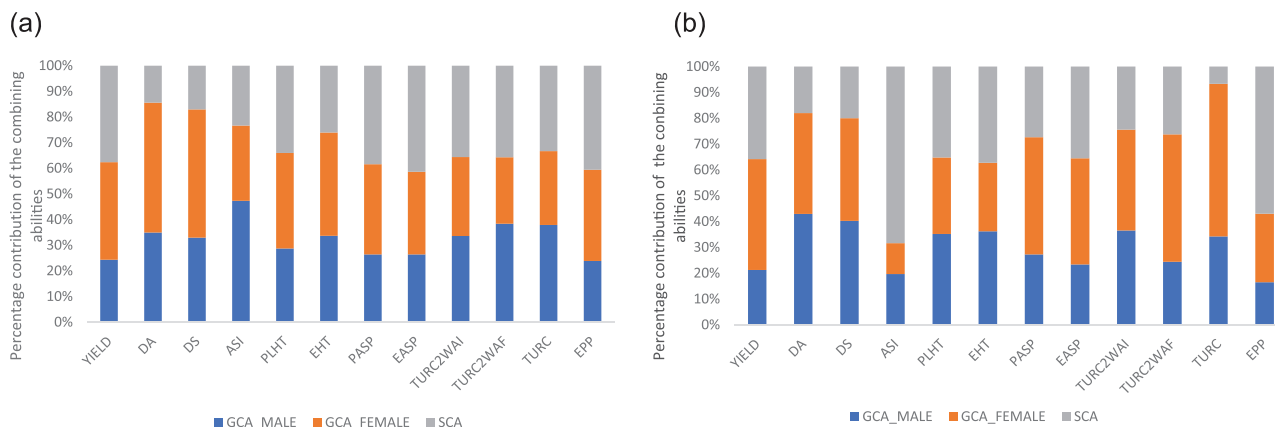


FIGURE 3 (a) Chart showing the percentage contribution of the combining ability effects of white extra-early inbred lines for grain yield, *Exserohilum turcicum* infection ratings, and other agronomic traits under artificial inoculation. (b) Chart showing the percentage contribution of the combining ability effects of yellow extra-early inbred lines for grain yield, *Exserohilum turcicum* infection ratings and other agronomic traits under artificial inoculation. GCA, general combining ability; SCA, specific combining ability; DA, days to 50% anthesis; DS, days to 50% silking; ASI, anthesis–silking interval; PLHT, plant height; EHT, ear height; PASP, plant aspect; EASP, ear aspect; TURC2WAI, disease score 2 wk after inoculation; TURC6WAI, disease score 6 wk after inoculation; TURC, average disease severity score; EPP, ears per plant

(FEMALE/SET), and SCA (MALE×FEMALE/SET) effects were detected for all measured traits except for ASI for the white EEM single-cross hybrids artificially inoculated with *E. turcicum*. Mean squares of the interaction of ENV with MALE(SET), FEMALE(SET), and FEMALE × MALE(SET) were all highly significant for DS and the three TURC measurements—that is, GCA-male, GCA-female, and SCA for the four traits interacted with the ENV. For the yellow hybrids, similar results to the white hybrids were obtained for the mean squares of ENV, BLOCK (ENV × REP), MALE(SET), FEMALE(SET), and FEMALE × MALE(SET) interaction for all measured traits except in a few cases (Table 5). Also mean squares for SET and ENV × SET interaction for GYLD and most other traits were highly significant. Mean squares of the interaction of ENV with MALE(SET), FEMALE(SET), and FEMALE × MALE(SET) were all highly significant for GYLD and most other measured traits—that is, GCA-male, GCA-female, and SCA for nearly all of the traits interacted with the ENV (Table 5).

Two white inbred lines, TZEEI 8 and TZEEI 51, showed the combining ability in the desirable direction (Table 6). They had statistically significant positive GCA-female and GCA-male for GYLD with significant negative effects for the two GCAs for TURC6WAI. The GCA effects were quite close in each case. TZEEI 1 and TZEEI 13 had significant positive GCA-female for GYLD and negative GCA-female for TURC6WAI. Inbred line TZEEI 15 showed negative GCA-female and GCA-male for TURC6WAI but nonsignificant effects for GYLD thus making it undesirable. Similarly, TZEEI 38 had significant positive GCA-female and GCA-male but nonsignificant GCAs for GYLD, thus rendering it undesirable. The worst white inbred lines are TZEEI 6 and TZEEI 49, which had negative GCA-female

and GCA-male for GYLD and positive GCA-female and GCA-male for TURC6WAI, suggesting that they are susceptible to *E. turcicum* infection and poor combiners for GYLD. Five yellow inbred lines are quite desirable in this study. Inbred lines TZEEI 86, TZEEI 157, TZEEI 79, TZEEI 172, and TZEEI 179 all had positive GCA-female and GCA-male for GYLD and negative GCAs for TURC6WAI, suggesting they are resistant and good general combiners. TZEEI 78 had positive GCA-female for GYLD and negative GCA-female for TURC6WAI, whereas TZEEI 75, TZEEI 76, and TZEEI 63 had negative GCAs for GYLD and positive GCAs for TURC6WAI, indicating their undesirability.

The proportion of the sum of squares (SS) of GCA (female and male) and SCA for the yellow and white EEM hybrids under artificial inoculation and natural conditions are presented in Figures 3 and 4. Percentage of the total variation due to GCA (male and female) for measured traits under artificial inoculation was larger (58–83%) than that for SCA (14–41%). Similarly, GCA-female was larger than GCA-male for GYLD, DA, DS, PLHT, EASP, EHT, and EPP, whereas GCA-male SS was higher for the NCLB disease severity scores. Under natural conditions, the SCA SS was larger for GYLD, PLHT, PASP, EASP, EPP, and NCLB disease severity scores, whereas the GCA-female SS was higher for DA, DS, ASI, and EHT (Figures 3). For the EEM yellow hybrids, the proportions of the GCA (male and female) SS accounted for 32–93%, whereas the SCA accounted for 7–68% of the total variation under artificial *E. turcicum* inoculation. As was observed for EEM white hybrids, the GCA-female SS was greater than the GCA-male SS for GYLD, PASP, and EASP but contrasted the NCLB disease severity scores of the yellow EEM hybrids. Similar to the observations for the white EEM hybrids, the SCA SS was larger for GYLD, PASP, EASP, EPP, and NCLB

TABLE 5 Mean squares derived from the analysis of variance for grain yield, *Exserohilum turcicum* infection, and other agronomic traits of yellow extra-early-maturing (EEM) single-cross hybrids artificially inoculated with *E. turcicum* and under natural conditions

Source ^a	df	Grain yield kg ha ⁻¹	Days to		Anthesis–		Plant height cm	Ear height cm	Plant aspect ^b	Ear aspect ^c	TURC6WAI ^e		Ears per plot
			anthesis	silking	silking	interval					TURC2WAI ^d	TURC	
ENV	5	140,610,424.4**	461.3**	641.6**	38.5**	37,188.8**	16,804.3**	19.2**	21.7**	91.0**	43.0**	42.0**	0.5**
SET	2	10,757,663.7**	20.8**	16.1**	0.5	1,390.8**	1,504.4**	7.3**	24.0**	0.8*	13.7**	5.2**	0.01
ENV × SET	10	1,324,737.8**	3.4**	5.9**	0.9*	176.0	135.5*	0.5	5.5	0.7**	1.5**	0.7**	0.03
Rep (ENV × Set)	12	439,902.1	1.5	1.8	0.7	90.7	77.5	0.5	1.1	0.2	0.3	0.2	0.02
Block (ENV × Rep)	96	1,416,946.2**	4.1**	4.6**	0.6**	281.2**	144.0**	0.7**	3.3	0.5**	0.9**	0.5**	0.03**
MALE(SET)	12	10,198,920.0**	53.0**	53.0**	0.8*	1,931.1**	792.9**	4.4**	10.3**	2.6**	5.4**	3.7**	0.04*
FEMALE(SET)	12	22,322,922.5**	52.3**	56.6**	0.5	1,765.6**	628.9**	7.9**	19.6**	3.1**	11.8**	6.3**	0.1**
FEMALE × MALE(SET)	48	4,659,473.6**	6.0**	7.1**	0.7**	523.4**	221.2**	1.2**	4.2	0.5**	1.6**	0.7**	0.04**
ENV × MALE(SET)	60	1,702,434.2**	2.0**	2.5**	0.5	136.6	95.1*	0.7**	3.4	1.2**	1.3**	1.0**	0.04**
ENV × FEMALE(SET)	60	1,623,369.8**	1.7**	1.8	0.7*	186.3**	108.3**	0.8**	4.5	1.0**	1.4**	0.8**	0.03*
ENV × FEMALE × MALE(SET)	240	935,108.8**	2.3**	2.5**	0.5	130.1*	81.8	0.5**	3.1	0.3**	0.7**	0.4**	0.02
Error	334	579,777.0	1.3	1.6	0.4	104.7	69.1	0.4	3.3	0.2	0.4	0.2	0.02
R ² , %		92.0	93.1	93.2	78.8	91.5	88.3	84.5	70.1	92.4	88.9	91.6	76.3
CV, %		18.1	2.3	2.4	76.1	6.0	10.8	12.7	37.8	15.1	14.1	11.5	14.9
GM ^g		4,210.4	50.7	51.6	0.8	170.6	76.8	5.0	4.8	3.1	4.6	3.9	0.9

^aENV, environment; Rep, replication.

^bPlant aspect recorded on a scale of 1–9 based on plant type, where 1 = excellent and 9 = poor.

^cEar aspect, ear aspect rated on a scale of 1–9, where 1 = clean, uniform, large, and well-filled ears and 9 = ears with undesirable features.

^dTURC2WAI, disease score 2 wk after inoculation.

^eTURC6WAI, disease score 6 wk after inoculation were rated on a scale of 1–9, where 1 = complete resistance, no visible northern corn leaf blight symptoms and 9 = abundant and coalescing lesions on all leaves covering >80% of the leaf surface with some plants prematurely dead.

^fTURC, average disease severity score.

^gGM, grand mean.

*Significant at the .05 probability level.

**Significant at the .01 probability level.

TABLE 6 Estimates of general combining ability effects for grain yield and disease severity of white and yellow extra-early-maturing maize inbred lines artificially inoculated with *Exserohilum turcicum*

White Inbred lines				
Genotype	Grain yield		TURC6WAI ^a	
	GCA-female	GCA-male	GCA-female	GCA-male
kg ha ⁻¹				
White inbred lines				
TZEEI 1	637.3**	-123.0	-0.2*	0.04
TZEEI 20	-394.4*	212.6	0.2	-0.14
TZEEI 32	75.2	-38.4	-0.02	0.08
TZEEI 45	108.8	-2.8	0.0	0.05
TZEEI 46	-426.8*	-48.4	0.1	-0.03
TZEEI 3	-272.2	-166.9	0.03	-0.13
TZEEI 8	817.4**	806.0**	-0.3**	-0.31*
TZEEI 15	-134.5	209.5	-0.3**	-0.22*
TZEEI 36	-177.9	-143.3	0.3**	0.34**
TZEEI 49	-232.8	-704.9**	0.3**	0.32*
TZEEI 6	-966.7**	-598.1*	0.4**	0.56**
TZEEI 13	737.6**	-136.9	-0.4**	-0.34**
TZEEI 21	-743.1**	-300.7*	-0.04	-0.45**
TZEEI 38	-189.5	-124.7	0.4**	0.54**
TZEEI 51	1,161.7**	1,160.5**	-0.3**	-0.31*
SE	107.8	129.9	0.1	0.11
Yellow inbred lines				
TZEEI 59	-693.8*	-485.0*	-0.07	0.05
TZEEI 73	22.0	-19.7	-0.04	0.09
TZEEI 75	-943.6**	-404.9*	0.90**	0.25*
TZEEI 86	740.2**	526.8*	-0.34**	-0.28*
TZEEI 157	875.1**	382.8*	-0.45**	-0.12
TZEEI 67	-145.5	7.7	-0.09	-0.29*
TZEEI 76	-867.0**	-675.0	0.74**	0.85**
TZEEI 78	603.9*	113.6	-0.59**	-0.16
TZEEI 79	585.1*	339.6*	-0.27*	-0.44**
TZEEI 158	-176.5	214.1	0.20	0.03
TZEEI 63	-838.6**	-914.0**	0.43**	0.42**
TZEEI 69	-262.4	-180.4	0.41**	0.16
TZEEI 88	132.9	-74.3	-0.30**	0.02
TZEEI 172	494.3*	439.4*	-0.42**	-0.30**
TZEEI 179	473.8*	729.3**	-0.12	-0.31**
SE	150.7	147.1	0.1	0.1

^aTURC6WAI, disease score 6 wk after inoculation rated on a scale of 1–9, where 1 = complete resistance, no visible northern corn leaf blight symptoms and 9 = abundant and coalescing lesions on all leaves covering >80% of the leaf surface with some plants prematurely dead.

*Significant at the .05 probability level.

**Significant at the .01 probability level.

disease severity scores under natural conditions, unlike DA, DS, ASI, PLHT, and EHT for which total GCA SS were higher than the SCA SS (Figure 4). Comparing the trends observed in the contributions of the components of GCA (i.e., GCA-female and GCA-male for the EM and EEM inbred lines),

GCA-male SS had higher contribution to the performance of the inbred lines when crossed in hybrid combinations for GYLD, TURC6WAI, and other agronomic traits. However, the GCA-female had higher contributions to the performance of the yellow EEM inbred lines for these traits.

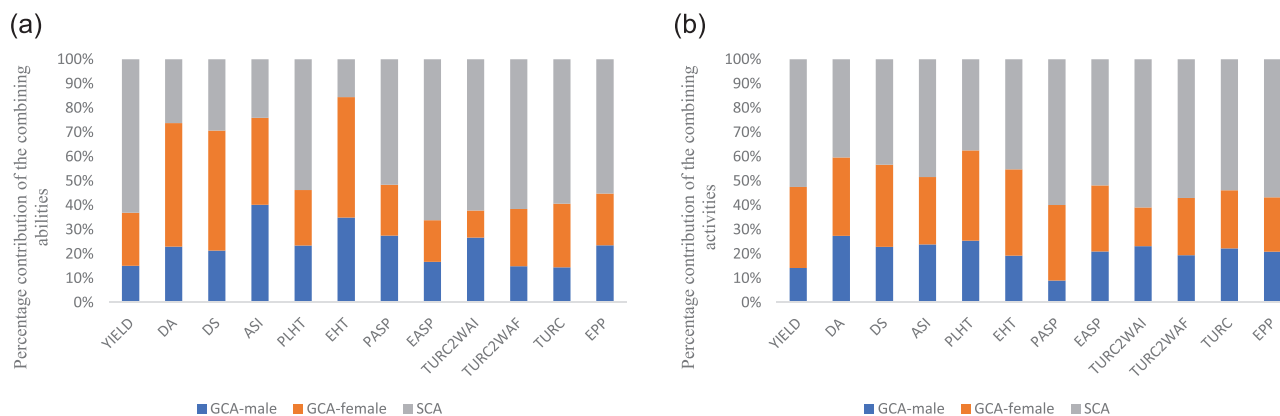


FIGURE 4 (a) Chart showing the percentage contribution of the combining ability effects of white extra-early inbred lines for grain yield, *Exserohilum turcicum* infection ratings and other agronomic traits under natural conditions. (b) Chart showing the percentage contribution of the combining ability effects of yellow extra-early inbred lines for grain yield, *Exserohilum turcicum* infection ratings and other agronomic traits under natural conditions. GCA, general combining ability; SCA, specific combining ability; DA, days to 50% anthesis; DS, days to 50% silking; ASI, anthesis–silking interval; PLHT, plant height; EHT, ear height; PASP, plant aspect; EASP, ear aspect; TURC2WAI, disease score 2 wk after inoculation; TURC6WAI, disease score 6 wk after inoculation; TURC, average disease severity score; EPP, ears per plant

3.3 | Identification of testers and heterotic groups

Three distinct heterotic groups were obtained for the white EEM maize inbred lines at 45% level of dissimilarities (R^2 , Figure 5). Seven inbred lines (TZEEI 1, TZEEI 32, TZEEI 13, TZEEI 20, TZEEI 21, TZEEI 15, and TZEEI 3) were classified into Group 1, six (TZEEI 45, TZEEI 36, TZEEI 38, TZEEI 46, TZEEI 49, and TZEEI 6) were placed in the second group, and two (TZEEI 8 and TZEEI 51) were placed in the third group. Using the criteria proposed by Pswarayi and Vivek (2008) for identification of a good tester (namely high and positive GCA effects, placement into heterotic groups, and per se GYLD), TZEEI 13 in Group 1 had significant positive GCA-female effect as well as significant negative GCA-effects (female and male) for NCLB disease severity score. In addition, this line had high GYLD under artificial *E. turcicum* inoculation (Table 7) and was identified as the tester for Group 1. However, none of the lines in Group 2 satisfied the criteria for selection of a good tester. In contrast, TZEEI 51 (Group 3) combined significant positive GCA (male and female) effects for GYLD and significant negative GCA (male and female) effects for NCLB disease severity scores. Furthermore, TZEEI 8 had significant positive GCA effects for GYLD and significant negative GCA effects for NCLB disease severity scores. However, TZEEI 51 had significantly higher per se GYLD (Table 7), thus satisfying the criteria for an inbred tester. Therefore, TZEEI 51 was selected as the white inbred tester for heterotic Group 3. The combination of both inbreds as single-cross hybrid produced the highest GYLD, as well as the highest BI (Table 1). TZEEI 51 × TZEEI 8 was therefore identified as the single-cross tester in this study.

Similarly, three distinct heterotic groups were identified for the yellow kernel EEM inbred lines at 50% level of dissimilarity (R^2 , Figure 6). Six inbred lines (TZEEI 59, TZEEI 67, TZEEI 88, TZEEI 73, TZEEI 158, and TZEEI 69) were classified into Group 1, six (TZEEI 86, TZEEI 79, TZEEI 179, TZEEI 172, TZEEI 157, and TZEEI 78) were assigned to Group 2, and three (TZEEI 75, TZEEI 63, and TZEEI 76) were assigned into Group 3. None of the inbred lines in Group 1 satisfied the criteria for a good tester, although Group 2 comprised the inbred lines that combined significant and positive GCA (male and female) effects for GYLD with significant and negative GCA for NCLB disease severity scores. The hybrid combination of most inbred lines in this group also produced the highest GYLD across the research environments. Hybrids TZEEI 78 × TZEEI 157, TZEEI 157 × TZEEI 179, and TZEEI 86 × TZEEI 179, belonging to the same heterotic group, were ranked as first, second, and third in terms of GYLD (Table 2). These could also serve as single-cross hybrid testers. TZEEI 79 satisfied the criteria for an inbred tester and was identified as the tester for Group 2. Of the inbred lines assigned to heterotic Group 3, none satisfied the selection criteria for a tester.

4 | DISCUSSION

The need for significant increase in maize production and productivity in SSA is inevitable due to the increasing need for food for the burgeoning population and raw materials for existing and emerging feed and brewing industries. Yield reduction (as reported in the present study) due to emerging NCLB disease in regions that were known to be free from this

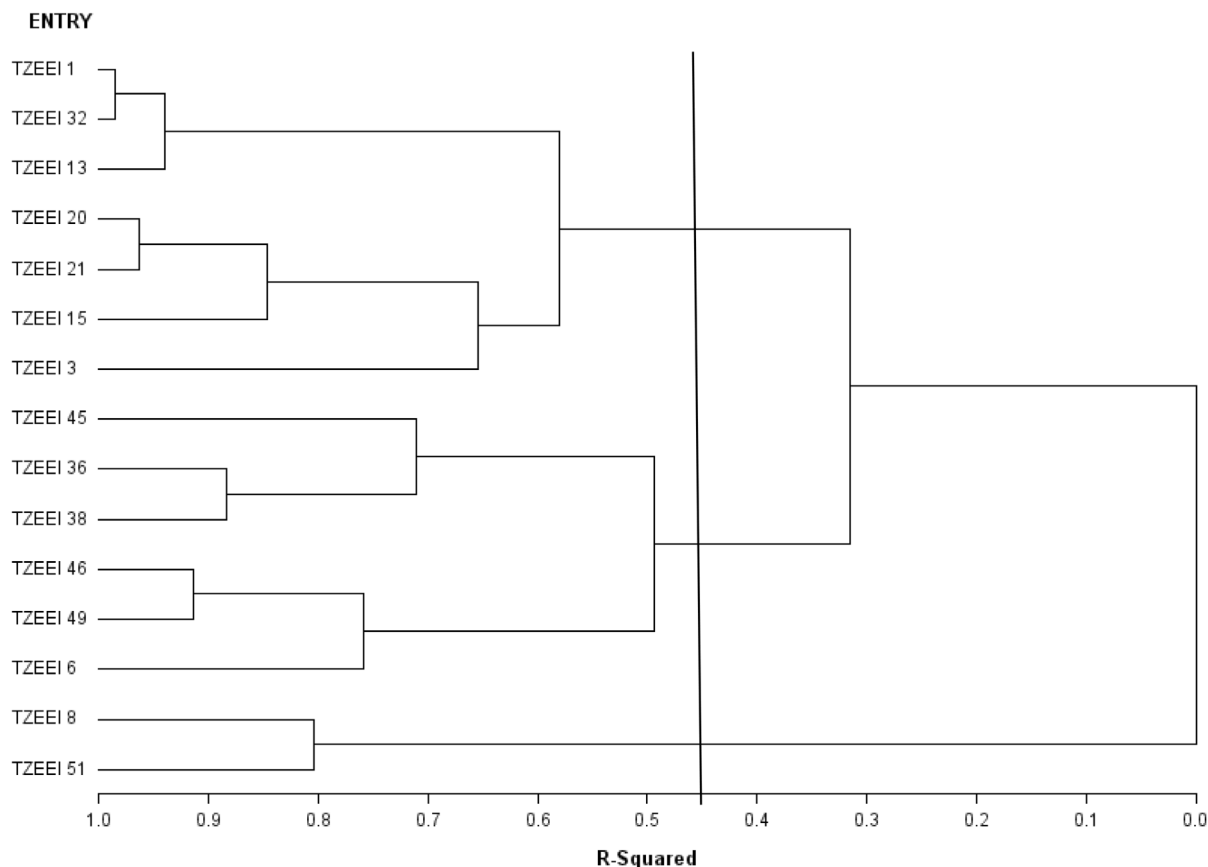


FIGURE 5 Heterotic grouping for white extra-early-maturing inbreds identified by the general combining ability of multiple traits (HGCAMT) under artificial *Exserohilum turcicum* inoculation

disease is a major challenge that needs rapid response by seeking appropriate management strategies (Human et al., 2016; Hooda et al., 2017). White and yellow EEM maize hybrids responded differently to NCLB infection across the contrasting environments where the experiments were conducted as revealed by significant entry (genotype), environment, and the genotype \times environment ($G \times E$, partitioned into GCA-male and GCA-female) interaction for almost all measured traits, particularly GYLD and NCLB disease severity. This indicated that the hybrids performed differently under contrasting environmental conditions and that resistance to NCLB also varied across environments. These observations are similar to those of Pswarayi and Vivek (2008) and Vivek et al. (2010).

Elucidating the patterns of inheritance of resistance to NCLB, combining ability and the heterotic patterns among EEM maize inbred lines tested under diverse environments yielded good results valuable for developing maize hybrids with stable resistance to NCLB. Significant GCA (female and male) and SCA effects for GYLD and NCLB disease severity scores brought to the fore the role of GCA and SCA (dominant) genetic effects in conditioning these traits under *E. turcicum* inoculation. Sibiyá, Tongoona, and Derera (2013), reported significant additive and nonadditive genetic effects

for these traits under NCLB disease pressure. Additive gene effects had higher contributions than the nonadditive genetic effects for most measured characters, particularly GYLD and NCLB disease severity scores. This indicated the predominance of the additive over the nonadditive genetic effects and implied that the GCA effects were responsible for the variations observed among the parents of the hybrids evaluated in the present study. Vivek et al. (2010) and Sibiyá, Tongoona, and Derera (2013), reported similar results on the role of additive gene effects in conferring NCLB resistance. Nonetheless, the contributions of the nonadditive genetic effects reported in the present study need to be exploited to develop single-cross hybrids with resistance to NCLB. Inbred lines with higher GCA-female effects could serve as good female parents, whereas those with higher GCA-male effects could be used as male parents in crosses for improving resistance to NCLB. Additionally, significant $SCA \times ENV$ interactions for GYLD and the disease severity scores for some hybrids suggested that those EEM hybrids showed differential performance with respect to GYLD and resistance to NCLB across environments. Results of earlier studies have indicated that evaluating single-cross hybrids in several locations with contrasting environmental conditions was necessary to validate

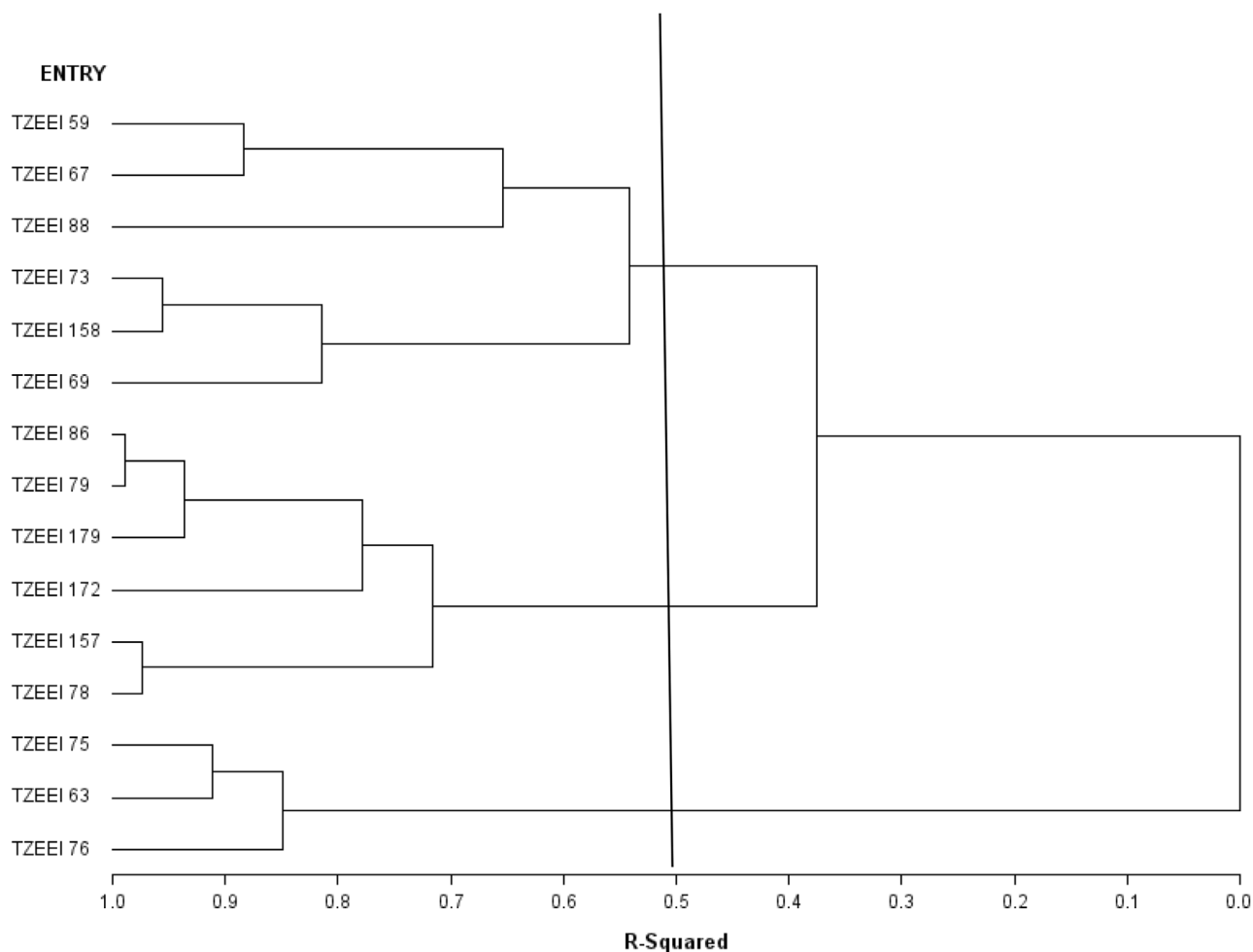


FIGURE 6 Heterotic grouping for yellow extra-early-maturing inbreds identified by the general combining ability of multiple traits (HGCAMT) under artificial *Exserohilum turcicum* inoculation

their performance, as well as identify reliable testers because of the sensitivity of those hybrids to environmental changes (Akinwale et al., 2014; Hallauer et al., 2010).

The combining abilities of the EEM inbred lines, especially the GCA effects for most measured traits in the present study, revealed that certain inbred lines combined significant and positive GCA (male and female) effects for GYLD with significant and negative GCA (male and female) effects for NCLB disease severity scores. This is important for simultaneously breeding for both resistance to NCLB and increased GYLD. The inbred lines with these desirable features were identified as good combiners and would be invaluable for developing NCLB resistance in high-yielding EEM hybrids.

Classifying inbred lines into appropriate heterotic groups and identifying testers are of crucial importance in any breeding program (Badu-Apraku et al., 2013). These would assist the development of hybrids, synthetics, and heterotic populations. Northern corn leaf blight-resistant inbred lines could be extracted from such populations for development of high-yielding, resistant hybrids. Fan et al. (2009) reported that an

efficient heterotic grouping method should facilitate identification of groups which permit inter-heterotic group crosses to display higher heterosis than intra-heterotic group crosses. The HGCAMT method used in the present study classified the white and yellow inbred lines with positive GCA effects for GYLD and negative GCA effects for NCLB disease severity scores into the same group, whereas inbred lines with negative GCA effects for GYLD and positive GCA effects for NCLB scores were assigned to a different group. In this study, we relied on the criteria described by Psarayi and Vivek (2008) to select outstanding inbred and single-cross testers. Use of inbred testers TZEEI 51, TZEEI 13, and TZEEI 79 and single-cross testers TZEEI 51 \times TZEEI 8, TZEEI 78 \times TZEEI 157, TZEEI 157 \times TZEEI 179, and TZEEI 86 \times TZEEI 179 would enhance the development of hybrids with resistance to NCLB, as well as increase GYLD.

Yan et al. (2007) reported that when different research environments belong to different sectors of the polygon view, different high-yielding hybrids (resistant or susceptible) are present in those sectors. This is an indication of

TABLE 7 Per se grain yield and disease severity score 6 wk after inoculation of extra-early-maturing maize inbred lines evaluated under artificial *Exserohilum turcicum* inoculation

Genotype	Grain yield kg ha ⁻¹	TURC6WAI ^a
White inbred lines		
TZEEI 1	2,899	4.3
TZEEI 3	2,059	4.5
TZEEI 6	1,750	6.2
TZEEI 8	1,732	3.5
TZEEI 13	2,220	4.3
TZEEI 15	2,327	3.3
TZEEI 20	1,934	4.0
TZEEI 21	2,327	4.3
TZEEI 32	1,981	3.5
TZEEI 36	1,420	5.5
TZEEI 38	1,773	4.9
TZEEI 45	2,118	3.5
TZEEI 46	1,513	5.2
TZEEI 49	1,593	5.4
TZEEI 51	2,296	4.3
LSD _{0.05}	439	0.7
Yellow inbred lines		
TZEEI 59	1,308	4.7
TZEEI 63	1,290	5.9
TZEEI 67	1,733	4.2
TZEEI 69	1,422	5.4
TZEEI 73	1,554	5.0
TZEEI 75	1,079	5.9
TZEEI 76	1,226	5.3
TZEEI 78	1,877	4.1
TZEEI 79	1,188	3.9
TZEEI 86	1,068	5.2
TZEEI 88	1,344	5.4
TZEEI 157	1,692	4.7
TZEEI 158	1,808	4.4
TZEEI 172	1,518	3.7
TZEEI 179	1,089	4.6
LSD	439	0.7

^aTURC6WAI, disease score 6 wk after inoculation rated on a scale of 1–9, where 1 = complete resistance, no visible northern corn leaf blight symptoms and 9 = abundant and coalescing lesions on all leaves covering >80% of the leaf surface with some plants prematurely dead.

crossover G×E interaction, and it suggests that the test environments could be partitioned into mega-environments. In the present study, the test environments fell within one sector of the polygon view (Figures 1a and 2a) indicating the absence of crossover interactions and that there was a single mega-environment. Vivek et al. (2010) evaluated 66 hybrids

obtained from a diallel cross of 12 elite inbred lines for combining ability of those inbred lines for GYLD and resistance to seven diseases in six environments. The authors reported no significant correlations between GCA effects for disease scores across the test environments and, therefore, recommended the need for screening for resistance to prevailing diseases using artificial inoculation or reliable hot spots. In the present study, artificial inoculation of *E. turcicum* was carried out in each test environment.

Based on the criteria described by Yan et al. (2000) and Yan and Tinker (2005) on the relevance of GGE biplots in elucidating the genotype and G×E interactions, the hybrids that fell on the vertex of the “which-won-where” polygon were the best performing in those environments. However, the performance of the hybrids depended on the relative position either on the positive (good performance) or negative (poor performance) axis of the PC1. Hybrids that fell within the polygon were less responsive compared with the vertex hybrids. The AEC abscissa was the single-arrowed line, which pointed to higher mean yield across the test environments. Also, the double-arrowed line (AEC ordinate) separated entries with below-average GYLD from those with above-average GYLD. The longer the absolute length of the projection of a hybrid from the AEC abscissa, the lower the stability (Yan & Tinker, 2005). Based on these criteria, EEM white hybrids TZEEI 51 × TZEEI 8, TZEEI 8 × TZEEI 46, TZEEI 32 × TZEEI 51, TZEEI 1 × TZEEI 51, and TZEEI 15 × TZEEI 32 and the yellow EEM single-cross hybrids TZEEI 78 × TZEEI 157, TZEEI 157 × TZEEI 179, TZEEI 86 × TZEEI 179, TZEEI 179 × TZEEI 79, and TZEEI 79 × TZEEI 86 had stable performance across the test environments. These EEM hybrids should be subjected to extensive testing on-farm to affirm their stability for future commercialization in SSA. In any case, our results are preliminary and the 25 most high-yielding, stable, and NCLB-resistant hybrids of the present study are presently being evaluated in multilocation on-station trials in NCLB endemic zones in Nigeria to examine the consistency of the performance of the hybrids before on-farm trials and commercialization in Nigeria.

5 | CONCLUSION

In the present study, NCLB accounted for up to 54% GYLD reduction, hence the need to develop resistant cultivars (hybrids) as a reliable management option. The predominance of the GCA (male and female) effects over the SCA effects for GYLD and NCLB disease severity scores across the test environments implied the predominance of additive gene action in the inheritance of GYLD and resistance to NCLB in white- and yellow-endosperm EEM maize hybrids. Each of the white and yellow EEM maize inbred lines was classified into three heterotic groups. TZEEI 13 and TZEEI 51 were identified as

inbred testers, whereas TZEEI 51 × TZEEI 8 was identified as a single-cross tester for the white kernel EEM hybrids. Similarly, TZEEI 78 × TZEEI 157, TZEEI 157 × TZEEI 179, and TZEEI 86 × TZEEI 179 were identified as single-cross testers, whereas TZEEI 79 was identified as an inbred tester for the yellow EEM hybrids. In terms of the hybrid yield and stability, TZEEI 8 × TZEEI 46, TZEEI 32 × TZEEI 51, TZEEI 1 × TZEEI 51, and TZEEI 15 × TZEEI 32 were the most stable and high-yielding white EEM hybrids, whereas TZEEI 51 × TZEEI 8 was the highest yielding white EEM maize hybrid and was relatively stable across test environments. TZEEI 78 × TZEEI 157, TZEEI 157 × TZEEI 179, TZEEI 86 × TZEEI 179, TZEEI 179 × TZEEI 79, and TZEEI 79 × TZEEI 86 were the highest yielding and most stable yellow EEM hybrids across test environments under artificial inoculations. The testers identified in this study will be invaluable for developing hybrids with resistance to NCLB, whereas the hybrids with stable GYLD as well as resistance to NCLB should be subjected to further testing across multiple stress environments prior to commercial release. When used at scale, EEM hybrids with good yield and resistance to NCLB will contribute immensely to achieving increased production and productivity of maize and guaranteed food security in SSA.

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AUTHOR CONTRIBUTIONS

Baffour Badu-Apraku: Conceptualization; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Writing-review & editing. **Faith A. Bankole:** Formal analysis; Investigation; Methodology; Software; Writing-original draft; Writing-review & editing. **Morakinyo A. B. Fakorede:** Writing-review & editing. **Olalekan Ayinde:** Methodology. **Alejandro Ortega-Beltran:** Methodology; Supervision; Writing-review & editing.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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