Genetic of resistance to ear rot causal agent (*Fusarium moniliforme*) in quality protein maize (QPM) using line×tester analysis

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Abstract

Breeding for QPM ear rot resistant cultivars could offer a reliable environmental and economic control of mycotoxins especially for the resource-poor communities that require inexpensive protein diets. This research aims at evaluating a testcross of QPM inbreds with ear rot resistant cultivars to develop resistant topcrosses with high grain protein quality and yield. Seven QPM inbreds (lines) and two open pollinated ear rot resistant varieties (testers) were crossed in a line×tester method (2×7). The 14 F_1 topcrosses, 9 parents and 2 commercial hybrids (checks) were evaluated at the Lower Niger River Basin Authority, Oke-Oyi, Nigeria in 2014 and 2015 cropping seasons. The ear rot disease ratings in all topcrosses were low (<3.0), relative to the two controls of 3.4. K^2GCA/ K^2SCA values were higher than unity for grain yield, ear rot rating, husk cover, tryptophan and lysine characters, signifying that additive effects were controlling the inheritance of the traits. The three topcross hybrids (TZEQI 76×AMA TZBR YCF, TZEQI 74×AMA TZBR YCF, and TZEQI 81×TZEI 25) that possessed consistently low ear rot severity infection across years with TZEQI 76×AMA TZBR YCF, TZEQI 74×AMA TZBR YCF, and TZEQI 81×TZEI 25 outstanding for grain yield and quality protein are recommended for further evaluation in several years and locations before being released for commercial use.

Key words: Additive, Grain Yield, Lysine, Topcross, Tryptophan.

INTRODUCTION

Improvement and espousal of quality protein maize (QPM) that has approximately twice lysine and tryptophan levels, as well as comparable maize grain yield with the recent normal commercial hybrids, might enrich the nutritional value of feed and food maize products in Sub-saharan Africa (Mbuya *et al*., 2010; Bello *et al*., 2012; 2013; Bello 2017). QPM is easily obtainable and an inexpensive protein source for the resource-poor setting especially among babies, lactating mothers, and the adults. The QPM could also reduce malnutrition and other health challenges such as underweight, kwashiorkor and weakened intelligent

(Mbuya *et al.*, 2011).

The major li+ the ear via the silks, and the kernel infected. Infections that commenced at the vegetative phase can stretch all over the plant affecting the kernels and the losses in maize grain yield and quality could range between 0 and 70% (Reid *et al.*, 2009). The severity of infections depends on the genetic makeup of the cultivars and the growth stage in which infection occurs (Bua and Chelimo 2010). The use of host- maize plants resistant cultivars and early plantings could evade the severe injury (Field Guide for Integrated Pest Management, 2015). However, maize husk cover is an important trait to be evaluated for ear rot disease. A very long and tight husk covering the tip with several husk leaves extending over the maize ear could reduce insect infestations and microbes infections in the field and storage. Therefore, cognisant of good husk cover is sacrosanct in the development of maize ear rot resistant cultivars.

Diallelic crosses were found to be the superior methods of inheritance diversity, heterotic classification, the manifestation of quantitative characters and parental selection in progeny performance (Bello and Olaoye 2009; Zarea *et al.*, 2011; Bello and Olawuyi, 2015). The major drawback of diallel crosses, however, is that the numbers of crosses to be evaluated upsurge extensively as additional parents are involved. To overwhelm the challenges of diallel, several researchers proposed the topcross mating design which enabled a lesser number of cultivars to be evaluated than those involved in complete diallel crosses (Ferreira *et al.*, 2009; Rovaris *et al.*, 2014).

It has been established theoretically that first generation of topcrosses normally produces greater grain yield than open pollinated varieties (OPVs) (Meseka and Ishaaq, 2012). Breeding of non-conventional hybrids with a minimum of one non-inbred line are less responsive to inbreeding depression, easier and cheaper to produce. Ferreira *et al.* (2009) and Rovaris *et al.* (2014) also stated that the topcross approach is by crossing a group (Lines) with another group (Testers) in order to jettison worthless genotypes and increase efficiency of hybrid development. The authors suggested that this approach can be adopted in maize improvement programs as it provides the preliminary data assessment of the tested genotypes by obtaining combining ability effects. Meanwhile, Correjado and Magulama (2008) reported a 17% grain yield increase of topcross hybrids above their OPVs with a highly significant difference in the topcrosses for grain yield ranging between 3.8 and 6.2 t ha^{-1}. Therefore, maize breeders in the region where recycling of seeds by the farmers is practiced should employ a topcross mating system which is less

receptive to inbreeding depression and enhanced yield productivity and stability (Meseka and Ishaaq, 2012).

Against this backdrop, breeding improved QPM ear rot resistantcultivars could offer farmers the reliable environmental and economic control of mycotoxins, especially in West Africa where limited fungicides are used. Development of host plant resistant to ear rot, however, would be the best valuable strategy in controlling the menace of the disease (Mbuya *et al.*, 2011; Bello *et al.*, 2015; Bello, 2017). Resistance to conventional maize ear rot was adjudged to be heritable (Olowe *et al.*, 2015), but highly QPM resistant cultivars had not been identified. Adaptation of QPM resistant genotypes is therefore urgent in West Africa especially for the resource-poor communities that require inexpensive protein diets. This research aims at evaluating a testcross of QPM lines for resistance to *Fusarium* ear rot using a line×tester analysis. These were intended for obtaining information on the combining ability of the parents and identify auspicious topcross hybrids for the tropics.

MATERIALS AND METHODS

Genotypes used

Seven QPM inbreds (lines) and two open pollinated ear rot resistant varieties (AMA TZBR YCF and TZEI 25) designated as testers were used as male in a line×tester method (2×7). The obtained 14 F$_1$ topcrosses, 9 parents and 2 commercial hybrids (OBA SUPER 5 and OBA 98) were used as checks and evaluated at the Lower Niger River Basin Authority, Oke-Oyi, Nigeria (80° 30'N and 8° 36'E) in 2014 and 2015 cropping seasons. Description and pedigree of seven QPM inbreds, two ear rot resistant varieties and two commercial checks are presented in Table 1.

Experimental design and cultural practices

Based on the agronomic standard of cultivating maize in the savanna zone of Nigeria, the experimental field was plowed, harrowed and ridged with 0.75 m spacing between ridges. Each plot comprised of four 5 m rows and 50 cm intra-row spacing that gave 53,333 plants ha^{-1} as plant density. Pre-emergence (a.i 3 kg/l Metolachlor and 170 g/l Atrazine ha^{-1}) and post-emergence (a.i. 3 kg/l Paraquat ha^{-1}) herbicides were applied after land preparation for weed control. The 14 F$_1$ hybrids, their respective parents, and the two checks were evaluated on 24th and 10th July 2014 and 2015, respectively, under artificial infection, laid out in a randomized complete block design (RCBD) with four replications. Three maize seeds were sown per hole and later thinned to two at 2 weeks after planting. Supplemental hand weeding was conducted 4 weeks

Table 1. Description and pedigree of seven QPM inbreds, two ear rot resistant cultivars and two commercial checks.

S/N	QPM inbreds	Pedigree
1	TZEQI 76	TZE COMP5-Y C6S6 Inb 25 × Pool 18 SR QPM BC1S6 11-39-2-2-2-8
2	TZEQI 79	TZE COMP5-Y C6S6 Inb 25 × Pool 18 SR QPM BC1S6 11-39-2-2-8-8
3	TZEQI 74	TZE COMP5-Y C6S6 Inb 10 × Pool 18 SR QPM BC1S6 2-2-1-1
4	TZEQI 87	TZE COMP5-Y C6S6 Inb 31B × Pool 18 SR QPM BC1S6 7-45-2-3-4-7
5	TZEQI 91	TZE-Y Pop STR C0 S6 Inb 142 × Pool 18 SR QPM BC1S6 4-35-5-8-4-8
6	TZEQI 89	TZE-Y Pop STR C0 S6 Inb 16 2-3 × Pool 18 SR QPM BC1S6 2-4-1-1-2-8
7	TZEQI 81	TZE COMP5-Y C6S6 Inb 25 × Pool 18 SR QPM BC1S6 2-3-1-1-4-6
	Testers	Description
8	AMA TZBR YCF	Open pollinated ear rot resistant cultivar
9	TZEI 25	Open pollinated ear rot resistant cultivar
	Commercial Control	Description
10	OBA SUPER 5	Conventional commercial hybrids
11	OBA 98	QPM commercial hybrids

after planting before fertilizer was applied. Fertilizers were applied at the rate of 40 kg TZEQI 79O_5 ha^{-1} (single superphosphate), 40 kg K_2O ha^{-1} (muriate of potash) and 80 kg N ha^{-1} (urea).

Fusarium verticillioides inoculation

The assessed plots were inoculated artificially as described by Eller *et al*. (2010). In the two cropping seasons, *F. verticillioides* strains were isolated from infected maize plants and were cultured on potato dextrose agar with the use of hostile isolate of the pathogen. The cultures were washed with distilled water to amass the ascopores, and the ascopores brushed with a paintbrush. Thereafter, conidia suspension was strained through the cheesecloth and diluted to about 2.106 mL^{-1} in H_2O. Two injections were carried out at 7-day intervals to lessen seepages and reproduce natural infection. The silk channels were inoculated when 100% of the plants protruded silks and reapplied the next 7 days. On the 10 plants per plot, the primary ear was injected at the mid-whorl phase of each ear using 5 mL of 2.106 ascopores suspensions with injection 16-gauge needle syringe of 5-mL. To every liter of the suspension, a drip of concentrated Tween-20 was applied to disintegrate the suspension. Each inoculated ear was wrapped with shoot bags of waxed paper after inoculation for two days, for ensuring maintenance of high humidity and protection of the inoculum from being dried by extreme heat or drained by the rainfall. Meanwhile, the same concentration was applied during the two years of experimentation, and the injections were effected at the same growth stage.

Ear rot symptom rating

The symptoms of *F. verticillioides* ear rot are cottony white to pink mold commencing from any location on the ear at the split kernels or insect damage points. Normally, the whole ears are not affected, but infected kernels are spread throughout the ear. The infected kernels are mostly brown, tan or with white streaks. In stern infections, the kernels fasten to the husks with the mycelia. As described by Badu-Apraku *et al*. (2012), the ear rot score was measured at harvest based on the proportion of ears showing rot, where 1=no infection, 2=1 to 3% infection, 3=4 to 10% infection, 4=11 to 25% infection, 5=26 to 50% infection, 6=51 to 75% infection, 7=>75% infection.

Husk cover and grain yield measurements

Husk cover was rated visually on a range of 1 - 5 where 1=deep green appearance with uniform covered husk and 5=yellowish and opened husk. For grain yield, all the cobs harvested from every plot shelled separately and percentage moisture at harvest was calculated. Grain yield (t ha^{-1}) was determined after 12.5% adjustment of the moisture content.

Proximate analysis of tryptophan and lysine

A single step papain hydrolysis method described by Hornandez *et al*. (1969) was employed for protein solubilization. Iron ions oxidized acetic acid to glyoxylic acid with the application of sulphuric acid. The indole ring of free tryptophan which bound in the soluble proteins reacted with glyoxylic acid and a violet-purple compound was generated. The intensity of the violet-purple color was measured at 545 nm with a model 6305 spectrophotometer. The drawing of optical density standard curve against tryptophan concentration, the percentage of tryptophan in the sample was recorded as follows:

$$(1) \quad \% \text{ tryptophan in protein} = \frac{\% \text{ tryptophan in sample}}{\% \text{ protein in sample}}$$

Table 2. Combined analysis of variance for five investigated characters of fourteen topcrosses and two checks evaluated at Ilorin, Nigeria in 2014 and 2015.

Source of variation	df	Mean squares				
		Husk cover	Grain yield	Ear rot	Tryptophan	Lysine
Year	1	67.22*	69.53*	50.76*	11.09	23.78
Rep (Year)	4	20.58	11.64	9.34	2.75	10.12
Crosses	13	73,75*	134,71**	88,92**	15,35	9,82
Lines	6	81.90*	64.88*	64.55*	9.12	10.67
Testers	1	12.45	11.09	8.51	14.75	15.11
Lines×Testers	6	76.44*	67.84*	81.04*	7.01	12.4
Crosses×Year	13	72.25*	56.75*	67.11*	76.42*	68.33*
Lines×Year	6	70.17*	73.92*	69.67*	16.78	18.17
Testers×Year	1	57.59*	79.15*	61.80*	11.06	15.53
Lines×Testers×Year	6	125.93**	249.62**	183.57**	67.82*	83.27*
Pooled error	468	15.67	11.74	9.01	7.52	11.34

* and **, significant at 5 and 1% of the probability, respectively.

Tryptophan was utilized as the factor for protein quality assessment, while obtained value was multiplied by 4 to attain lysine value (Sentayehu 2008).

Statistical analyses

Analysis of variance was computed for each year and later combined as a result of homogeneity of errors described by Steel and Torrie (1980), with the application of SAS software program version 9.2 (SAS, 2012). The genotypic effects were considered to be fixed, while year effects were assumed to be random. According to Bello (2017), the combined analysis of the parents and testcrosses (genotypes) across two years of experimentation for the five characters (grain yield, tryptophan, lysine, husk cover and ear rot ratings) were as follows:

(2) $$Yijkl = \mu + \text{genotype } i + \text{y}j + (\text{genotype} \times \text{y}ij) + \text{rep(y)}jk + \text{block(rep} \times \text{y)}jkl + \varepsilon ijkl$$

Where μ=overall mean, genotype i=ith genotype effect, year j=jth year effect, genotype×year ij=effect of the interaction of the ith genotype and the jth year, rep (year)jk=kth replication effect within the jth year, block(rep×year)jkl=lth incomplete block effect within the kth replication and within the jth year, and ε$ijkl$= residual variance.

The method of line×tester analysis proposed by Kempthorne (1957) was applied for the estimation of general (GCA) and specific combining ability (SCA) variances and effects. As described by Baker (1978) and Cisar *et al.* (1982), the progeny performances might be predicted using the ratio of the component of combining ability variances. The closer the ratio to unity, the higher the predictability on GCA effects only.

Percentage coefficient of variation ($p < 0.05$) however, was used to calculate the degree of variation. Standard errors (SE) for all the effects were computed exploiting error mean squares from hybrids for the untransformed data. Differences in character means were also measured using the least significant difference (LSD) test.

RESULTS AND DISCUSSION

Combined analysis of variance

The significant mean squares of 23 entries across the two years revealed significant differences (P<0.05) among the genotypes for husk cover, grain yield and ear rot rating (Table 2). This corroborates with the report of Rovaris *et al.* (2014). Replication, however, had no significant effect on any of the tested traits. Similarly, mean squares of crosses were highly significant ($p<0.001$) for husk cover, ear rot disease, and grain yield delved into, denoting that the parental accessions applied in this research extensively varied. However, decidedly differences were obtained among lines, crosses, line×tester, line×year and tester×year for all the investigated characters, except for grain quality traits, tryptophan, and lysine. This indicates that these genotypes varied in genic frequencies distribution and the effects were also governed by the environmental conditions across years. Again, lines×testers×year and crosses×year mean squares markedly differed for all characters, showing that there was interference from the weather conditions across the two years of field evaluation for the expression of these traits. Similar results were observed by many researchers which validate the idea that topcrosses of narrow genetic base

Table 3. Mean values for five studied traits of fourteen topcrosses and two checks evaluated at Ilorin, Nigeria between 2014 and 2015.

Varietal checks	Husk cover (No.)	Grain yield (t ha^{-1})	Ear rot (No.)	Tryptophan (%)	Lysine (%)
OBA SUPER 5	3.39	5.45	3.25	2.68	0.63
OBA 98	3.49	5.68	3.33	3.89	0.92
Mean	3.44	5.57	3.29	3.29	0.78
Topcross hybrids					
TZEQI 76 × AMA TZBR YCF	2.14	6.94	2.31	3.78	0.93
TZEQI 79 × AMA TZBR YCF	2.22	6.55	2.36	3.67	0.88
TZEQI 74 × AMA TZBR YCF	2.53	6.43	2.42	3.89	0.93
TZEQI 87 × AMA TZBR YCF	2.37	6.11	2.49	3.88	1.01
TZEQI 91 × AMA TZBR YCF	2.85	6.01	2.93	3.71	0.90
TZEQI 89 × AMA TZBR YCF	2.17	6.28	2.87	3.12	0.91
TZEQI 81 × AMA TZBR YCF	2.34	6.74	2.79	3.51	0.94
TZEQI 76 × TZEI 25	2.72	6.63	2.98	3.96	1.02
TZEQI 79 × TZEI 25	2.11	6.88	2.88	3.87	1.00
TZEQI 74 × TZEI 25	2.63	6.67	2.76	3.44	0.92
TZEQI 87 × TZEI 25	2.81	6.31	2.41	3.11	0.92
TZEQI 91 × TZEI 25	2.19	6.18	2.96	3.35	0.88
TZEQI 89 × TZEI 25	2.02	6.97	2.64	3.02	0.97
TZEQI 81 × TZEI 25	2.99	6.63	2.89	3.83	0.93
Mean	2.45	6.52	2.69	3.58	0.94
Tester cross					
AMA TZBR YCF × TZEI 25	1.20	4.61	1.33	2.11	0.62
SE+	0.447	0.979	0.851	0.521	0.756
LSD (0.05)	0.48	0.48	6.78	0.54	0.09
Coefficient of variation (%)	10.55	10.55	6.78	3.92	0.93

usually possess higher crosses×year interaction than the populations of a broad genetic base (Rovaris *et al*., 2014; Bankole *et al*., 2017).

Mean performances

A significant difference of maize grain yield was obtained for the topcross hybrids, while the varietal checks did not differ markedly across the two years of study (Table 3). The topcross hybrids exhibited superior performance on grain yield with an overall mean of 6.52 t ha^{-1} representing 14.6 and 28.4% increase compared with the varietal checks and the ear rot resistant varietal cross (tester-cross), respectively. Three topcross hybrids TZEQI 89×TZEI 25, TZEQI 76×AMA TZBR YCF and TZEQI 79×TZEI 25 ha^{-1} expressed highest grain yield of 6.97, 6.94 and 6.88 ha^{-1}, while the tester-cross AMA TZBR YCF×TZEI 25 had the least (4.61 t ha^{-1}) with 33.9% below the most outstanding yielding TZEQI 89×TZEI 25. It was also obvious that the resultant progeny (AMA TZBR YCF×TZEI 25) of the two resistant cultivars that were utilized as testers had the best rating of husk cover and ear rot scores, but very low in grain yield, lysine and tryptophan contents. Nonetheless, most of

the genotypes revealed moderate husk cover and ear rot infection scores (<3.5). This corroborates with the report of Afolabi *et al*. (2007). On the yield qualities, however, the topcrosses with QPM combinations and OBA 98 (a quality protein commercial hybrid) were very remarkable for both lysine and tryptophan contents in relation to OBA SUPER 5, a normal commercial hybrid with abysmal performance. The three topcrosses (TZEQI 89×TZEI 25, TZEQI 76×AMA TZBR YCF and TZEQI 79×TZEI 25) that had highest grain yield also possessed better grain qualities, husk cover and ear rot scores.

Genetic components

Based on the diallelic analysis, the estimates of genetic parameters for the five examined characters over two years are pointed out in Table 4. The K^{2}SCA and K^{2}GCA mean squares were greatly different for the characters considered, signifying that non-additive and additive nature were imperative in the inheritance of these characters. The results showed that K^{2}GCA$_L$ was greater than K^{2}GCA$_T$ for all the characters. K^{2}GCA/ K^{2}SCA values were higher than unity except for husk cover, showing that the genetic additive nature was

Table 4. Genetic components of five examined traits at Ilorin, Nigeria between 2014 and 2015.

Genetic components	Husk cover (No.)	Grain yield (t ha^{-1})	Ear rot (No.)	Tryptophan (%)	Lysine (%)
K^{2}GCA$_L$	17.241	10.564	16.189	18.017	17.874
K^{2}GCA$_T$	10.284	7.421	12.754	13.183	12.752
K^{2}GCA	18.117	18.746	19.248	17.956	16.172
K^2 SCA	12.064	5.615	14.157	11.732	12.093
K^2 GCA/ K^2 SCA	1.663	1.273	1.564	1.986	1.113
K^{2}GCA$_L$×Year	6.529	5.126	7.879	4.381	3.231
K^{2}GCA$_T$×Year	4.382	-0.079	3.162	5.004	4.431
K^{2}GCA×Year	9.629	6.473	8.371	7.175	5.274
K^{2}SCA×Year	6.836	4,155	5.038	5.119	4.511

All the negative values were regarded as zero (Robinson *et al.*, 1955).

regulating the inheritance of these characters, Similar deductions have been depicted from various genetic studies undertaken previously (Gabr *et al*., 2008; Aliu *et al*., 2016; Emmanuel *et al*., 2017). This result also corroborates that of Fan *et al*., (2016). K^{2}GCA effects create a vital contribution to the topcross differences for ear rot infection. Taking a broad view, Abd El-Mottalb *et al*. (2013) and Izhar and Chakraborty (2013) posited that the relative amount of variation in single crosses is the total SCA variance and twice GCA variance. The authors further stressed that the proportion of the sum is influenced by GCA variation, which demonstrates that the predictability of the crosses performance is attributed to GCA only. The closer ratio to one indicates that the SCA is trivial and that crosses performance could be precisely predicted due to the mean of the parental GCA effects**.** K^{2}GCA accounted for more than half of the topcross deviation in these characters, indicating that ear rot resistance screen could be utilized efficiently using multiple testers. Thus, in the applied breeding schemes where topcross hybrids are generated only with those between heterotic groups, employment of a few single testers would be effective for the initial screening of topcross exercise. It showed that selection for resistance to ear rot could be efficiently and simply integrated into topcross QPM hybrid breeding scheme. The non-additive gene effects of K^{2}SCA exhibited a significant function in the inheritance of the husk cover. Furthermore, K^{2}GCA$_L$×Year interaction was greater than K^{2}GCA$_T$×Year interaction for all characters except tryptophan and lysine, depicting that the K^{2}GCA for the lines was decidedly influenced by the environmental conditions than the testers. This also shows that efficient selection process would necessitate assessments in several years. Similar findings were obtained from Amin *et al*. (2014), who discovered that the interaction between the two types of genetic effects and environment was vastly significant. K^{2}GCA×Year

interaction that was higher than K^{2}SCA×Year for all studied characters denotes that additive genetic effect is affected by the environmental conditions more than the non-additive genetic effect.

General combining ability effects

Significant positive GCA estimates are needed for disease resistance and grain yield where the aspirations are for higher resistance and greater yield. Nonetheless, significant negative GCA effects are important for disease resistance character whenever the objectives are set for resistance to diseases. This research clarified four lines, TZEQI 76, TZEQI 79, TZEQI 74, and TZEQI 81 as well as the two testers, AMA TZBR YCF and TZEI 25 possessing desirable positive and significant GCA effects for husk cover (Table 5). This reveals that GCA is a helpful guide to cross performances and assessment of a single tester and is adequate for initial topcross selections. This is in conformity with earlier reports of many researchers (Hung and Holland, 2012 and Tembo *et al*. (2013). Similarly, four lines TZEQI 76, TZEQI 74, TZEQI 87, and TZEQI 81 along with the two testers exhibited suitable significant GCA estimates for grain yield. Nonetheless, three lines (TZEQI 76, TZEQI 74, and TZEQI 81) besides the two testers demonstrated appropriate negative significant GCA effects for ear rot disease. The two ear rot resistant cultivars had auspicious GCA resistance estimates, implying that resistance to ear rot leans towards additive genetic effects. Therefore, optimum resistance in topcrosses would involve crossing QPM inbreds that both contain resistance genes. This finding is comparable with the reports of Mukanga *et al*. (2010), Loffler *et al*. (2011) and Mesterházy *et al*. (2012).

The estimation of the two ear rot resistant cultivars used as testers showed that they are good combiners with QPM inbred lines. This signifies that these ear rot

Table 5. General combining ability effects of seven inbred lines and two testers evaluated at Ilorin, Nigeria between 2014 and 2015.

Lines and Testers	Husk cover (No.)	Grain yield t ha^{-1}	Ear rot (No.)	Tryptophan (%)	Lysine (%)
Lines					
TZEQI 76	33.13*	43.27**	-1.47*	37.12*	41.23*
TZEQI 79	36.67*	7.24	1.28	43.74*	52.11*
TZEQI 74	27.38*	51.11**	-9.53*	34.12*	56.98*
TZEQI 87	-1.69	28.56*	0.37	37.88*	41.18*
TZEQI 91	11.87	13.38	-1.67	51.74*	50.35*
TZEQI 89	0.99	-1.66**	0.84	56.21*	62.27*
TZEQI 81	73.07**	76.11**	-2.53*	33.78*	39.56*
S.E. (gi)	3.11	1.76	2.04	0.99	1.07
S.E. (gi-gj)	1.43	0.83	4.23	0.27	0.97
Testers					
AMA TZBR YCF	35.34*	27.25*	-13.11*	-6.26*	-5.97*
TZEI 25	47.56*	30.66*	-14.27*	-6.78*	-9.45*
S.E. (gi)	5.45	8.11	7.09	1.01	2.11
S.E. (gi-gj)	1.47	2.64	3.86	4.24	3.27

* and **, significant at 5 and 1% of the probability, respectively.

resistant cultivars offer the breeders a better allusion for selecting relevant tester. The association between ear rot resistant cultivars and their topcrosses are similar to the report of Loffler *et al*. (2011) but higher than the Gibberella ear rot found by Bolduan *et al*. (2010) in European maize accessions. This is beneficial, as it allows breeders to decrease the size of the population by selecting proven line generations prior to breeding for topcross seeds. Higher susceptibility of lines to ear rot than the topcrosses facilitates higher disparity for resistance, and this might enable direct selection of the line generations to be very effective than indirect selection among the topcrosses. Contrarily, Loffler *et al*. (2011) and Tembo *et al*. (2016) envisaged that indirect selection in the lines for resistance to ear rot in the topcrosses was more efficient than direct selection. The effective application of lines and resistant topcross screening prospectively require practical skills. These include the collection of promising seeds, the outlay of creating and assessing topcrosses, which could be different from one accession to the other. It is obvious that no two susceptible QPM inbreds accounted for a topcross with better resistance to ear rot. It is also clear that all the QPM lines showed the right significant positive GCA for both lysine and tryptophan contents, while the two testers contained undesirable negative significant GCA effects.

Specific combining ability effects
The estimation of SCA variances was usually found whenever noticeable SCA values occurred. The results established eight topcrosses (TZEQI 76×AMA TZBR YCF, TZEQI 79 ×AMA TZBR YCF, TZEQI 74 ×AMA TZBR YCF, TZEQI 87×AMA TZBR YCF, TZEQI 81×AMA TZBR YCF, TZEQI 74×TZEI 25, TZEQI 87×TZEI 25, and TZEQI 91×TZEI 25) displaying required positive and significant SCA effects for husk cover (Table 6). Five topcrosses (TZEQI 76×AMA TZBR YCF, TZEQI 74×AMA TZBR YCF, TZEQI 87×AMA TZBR YCF, TZEQI 76×TZEI 25, and TZEQI 81×TZEI 25) expressed notable positive significant SCA effects towards prolificacy and high grain yield. Likewise, five topcrosses (TZEQI 76×AMA TZBR YCF, TZEQI 74×AMA TZBR YCF, TZEQI 81×AMA TZBR YCF, TZEQI 74×TZEI 25, and TZEQI 81×TZEI 25) exhibited remarkable negative and remarkable SCA variances for ear rot infection. This result might ascribe to the elevated echelon of resistance performance accomplished with all the hybrids by the two ear rot resistant varieties possessing prominent dissimilarity among them. These results possibly symbolize the usage of these topcross hybrids in maize improvement program. Regarding lysine and tryptophan contents, it is noticeable that six topcrosses (TZEQI 76×AMA TZBR YCF, TZEQI 74×AMA TZBR YCF, TZEQI 87×AMA TZBR YCF, TZEQI 76×TZEI 25, TZEQI 87×TZEI 25, and TZEQI 81×TZEI 25) were prominent for positive significant SCA effects for the grain quality. The five topcrosses (TZEQI 76×AMA TZBR YCF, TZEQI 74×AMA TZBR YCF, TZEQI 87×AMA TZBR YCF, TZEQI 76×TZEI 25, and TZEQI 81×TZEI 25) hybrids that were high grain yielding and quality could be recommended for direct release as novel commercial hybrids or for further breeding in maize production program.

Table 6. Specific combining ability effects of seven QPM lines and two testers evaluated at Ilorin, Nigeria between 2014 and 2015.

Topcrosses	Husk cover	Grain yield	Ear rot	Tryptophan	Lysine
TZEQI 76 × AMA TZBR YCF	54.13*	44.78**	-5.55*	17.13*	20.33*
TZEQI 79 × AMA TZBR YCF	45.32*	2.82	6.17	5.25	9.90
TZEQI 74 × AMA TZBR YCF	63.67*	31.54*	-7.22*	44.12*	39.33*
TZEQI 87 × AMA TZBR YCF	36.63*	15.23*	5.52	54.37*	43.42*
TZEQI 91 × AMA TZBR YCF	-1.69	1.58	5.33	1.99	8.11
TZEQI 89 × AMA TZBR YCF	1.75	1.88*	3.95	7.56	9.43
TZEQI 81 × AMA TZBR YCF	44.87*	6.56	-6.12*	1.54	1.73
TZEQI 76 × TZEI 25	4.11	34.52**	7.73	13.22*	19.47*
TZEQI 79 × TZEI 25	3.54	9.67	2.90	4.87	9.67
TZEQI 74 × TZEI 25	65.77*	2.11	-6.23*	7.11	9.99
TZEQI 87 × TZEI 25	39.34*	4.72	6.11	41.25*	57.83*
TZEQI 91 × TZEI 25	57.88*	2.39	1.42	6.99	0.38
TZEQI 89 × TZEI 25	2.33	2.56	6.67	7.35	9.77
TZEQI 81 × TZEI 25	4.51	36.22*	-5.98*	15.49*	10.67*
SE (Sij)	3.13	4.78	7.13	7.56	10.84
SE (Sij-Skl)	4.63	3.87	5.11	2.41	4.09

* and **, significant at 5 and 1% of the probability, respectively.

Where SCA is important, multiple tester evaluations should be followed because there are vital parameters of hybrid resistance which could not be predicted from the effects of another tester. While non-additive genetic action was noticed as crucial in the grain yield inheritance by some researchers (Singh and Roy 2007; Abdallah and Hassan 2009; Osman *et al.*, 2012), additive genetic action was reported by others to be more significant than the non-additive for high grain yielding (Derera *et al.*, 2008; Vivek *et al.*, 2010; Sibiya *et al.*, 2011; Ibrahim 2012). Since SCA and GCA are important for ear rot resistance and grain yield, assessment of multiple testers and topcrosses should be at the last phase in the breeding for resistance programs. The indications from this research will be worthwhile for the improvement of ear rot disease resistant and high grain yielding cultivars. Pre-eminence of good testers in single crosses were earlier noticed by many researchers (Mosa 2010; Mousa and Aly 2012; Izhar and Chakraborty 2013).

CONCLUSION

The ear rot disease ratings in all topcrosses were low (<3.0), relative to the two controls of 3.4. K^2GCA/K^2SCA values were higher than unity for grain yield, ear rot rating, husk cover, tryptophan and lysine characters, signifying that additive effects were controlling the inheritance of the lines. The three topcross hybrids (TZEQI 76×AMA TZBR YCF, TZEQI 74×AMA TZBR YCF, and TZEQI 81×TZEI 25) that were outstanding

for grain yield, quality protein, and ear rot resistance are recommended for direct release as novel commercial hybrids or for further maize breeding program.

Compliance with ethical standards

This study has no involvement of animals and human as participants.

Author contribution statement

Drs. Mahamood J. and Ige, S. A. supervised the land preparation, layout of the experiments and record the field data. Mr. Lawal M. and Mr. Azeez, A. H. analyzed the data. Drs. Suleiman Y. A. and Afolabi, M. S. Kioko. J. I., and Agbolade, J. O. wrote the manuscript while Dr. Bello, O. B., the the corresponding author provided scientific cutting-egde and novel approach to manuscript write-up.

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Declaration of interest

The authors proclaim not any conflict of interest. The authors are also accountable for the subject matter and write-up of the article.

REFERENCES

Abd El-Mottalb A. A., Mostafa M. A., and Gamea A. A.

(2013). Combining ability for yield and some agronomic traits of seven new white maize inbred lines. *Egyptian Journal of Plant Breeding,* 17: 13–22.

Afolabi C. G., Ojiambo P. S., Ekpo E. J. A., Menkir A., and Bandyopadhyay R. (2007). Evaluation of maize inbred lines for resistance to Fusarium ear rot and fumonisin accumulation in grain in tropical Africa. *Plant Diseases,* 91: 279–286.

Aliu S., Rusinovci I., Fetahu S., and Rozman L. (2016). The combining ability of maize (*Zea mays* L.) inbred lines for grain yield and yield components. *Agriculture* and *Forestry*, 62: 295-303.

Amin M. N., Amiruzzaman M., Ahmed A., and Ali M. R. (2014). Evaluation of inbred lines of maize (Zea mays L.) through line×tester method. *Bangladesh Journal Agricultural of Research,* 39(4): 675-683.

Badu-Apraku B., Fakorede M. A. B., Menkir A., and Sanogo D. (2012). Conduct and management of maize field trials. International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, 26–28.

Baker R. J. (1978). Issues in diallel analysis. *Crop Science,* 18: 533–536.

Bankole F., Menkir A., Olaoye G., Crossa J., Hearne S., Unachukwu N., and Gedil M. (2017). Genetic gains in yield and yield related traits under drought stress and favorable environments in a maize population improved using marker assisted recurrent selection. *Frontiers Plant Science,* 8: 808.

Bello O. B., and Olaoye G. (2009). Combining ability for maize grain yield and other agronomic characters in a typical southern guinea savanna ecology of Nigeria. *African Journal of Biotechnology,* 8(11): 2518–2522.

Bello O. B., Olawuyi O. J., Azeez M. A., Lawal M. Abdulmaliq S. Y., Afolabi M., Ige, S.A., and Mahamood J. (2012). Genotypic variation in protein, lysine and tryptophan contents of extra early maize genotypes and their quality protein versions in nitrogen stress and non-stress environments. *Journal of Research (Science),* 23(1–4): 27–48.

Bello, O. B., Mahamood J., Afolabi M. S., Azeez M. A., Ige S. A., and Abdulmaliq, S. Y. (2013). Evaluation of biochemical and yield attributes of quality protein maize (*Zea mays* L.) in Nigeria. *Tropical Agriculture,* 90(4): 160–176.

Bello O. B., Olawuyi O. J., Ige S. A., Mahamood J., Afolabi M. S., Ganiyu O. T., Azeez M. A., and Abdulmaliq, S. Y. (2015). Interactive effects of genotype×year on disease reactions, grain yield and other agronomic traits of newly developed quality protein maize in Nigeria. *Jordan Journal of Agricultural Sciences*, 11(2): 399–412.

Bello O. B., and Olawuyi O. J. (2015). Gene action, heterosis, correlation and regression estimates in developing hybrid cultivars in maize. *Tropical Agriculture*, 92(2): 102–117.

Bello O. B. (2017). Diallelic analysis of maize streak virus resistance in quality protein maize topcrosses. *Euphytica*, 213: 270–279.

Bolduan C., Miedaner T., Utz H. F., Dhillon B. S., and Melchinger A. E. (2010). Genetic variation in testcrosses and relationship between line per se and testcross performance for resistance to Gibberella ear rot. *Crop Science*, 50: 1691–1696.

Bua B., and Chelimo B. M. (2010). The reaction of maize genotypes to maize streak virus disease in central Uganda. Second RUFORUM Biennial Meeting, 20–24 September, Entebbe, Uganda, 293–297.

Cisar C., Brown C. M., and Jedlinski H. (1982) Diallel analyses for tolerance in winter wheat to the barley yellow dwarf virus. *Crop Science*, 22: 328–333.

Derera J., Tongoona P., Pixly K.V., Vivek B., Laing M. D., and Van Rij N. C. (2008). Gene action controlling gray leaf spot resistance in southern African maize germplasm. *Crop Science*, 48: 93–98.

Eller M. S., Payne G. A., and Holland J. B. (2010). Selection for reduced Fusarium ear rot and fumonisin content in advanced backcross maize lines and their topcross hybrids. *Crop Science*, 50: 2249–2260.

Emmanuel G., Vah E.G., Ndebeh J., Akromah R., and Obeng-Antwi K. (2017). Evaluation of maize top cross hybrids for grain yield and associated traits in three agro-ecological zones in Ghana. *International Journal of Environment, Agriculture and Biotechnology*, 2(4):2076-2087.

Fan X., Yin X., Zhang Y., Bi Y., Liu L., Chen H., and Kang M. S., (2016). Combining ability estimation for grain yield of maize exotic germplasm using testers from three heterotic groups. *Crop Science,* 56: 2527–2535.

Ferreira E. A., Paterniani, M. E. A. G. Z., Duarte A. P, Gallo, P. B., Sawazaki E., Azevedo F. J. A., and Guimarães P. S. (2009). Desempenho de híbridos top crosses de linhagens S3 de milho em três locais do estado de São Paulo. *Bragantia,* 68(2): 319–327.

Field Guide for Integrated Pest Management (2015). In Hops, 3ed. Retrieved from http://msue.anr.msu.edu/ uploads/234/71503/Hop_Field_Guide_Third_Edition. pdf.

Gichuru L., Njoroge K., Ininda J., and Peter, L. (2011). Combining ability of grain yield and agronomic traits in diverse maize lines with maize streak virus resistance for Eastern Africa region. *Agriculture and Biology Journal of North America*, 2: 432–439.

Hornandez H., and Bates L. S. (1969). A modified method for rapid tryptophan analysis of maize. *CIMMYT Research Bulletin*, 13, Retrieved from http://libcatalog. cimmyt.org/Download/cim/1857.pdf.

Hung H., and Holland J. B. (2012). Diallel analysis of resistance to Fusarium ear rot and fumonisin contamination in maize. *Crop Science,* 52: 2173–2181.

Izhar T., and Chakraborty M. (2013). Combining ability and heterosis for grain yield and its components in maize inbreds over environments (*Zea mays* L). *African Journal of Agricultural Research,* 8: 3276–3280.

Kempthorne O. (1957). *An introduction to Genetic Statistical*. John Wiley and Sons Inc., New York, U.S.A. Retrieved from https://www.cabdirect.org/cabdirect/ abstract/19580101090.

Kurosawa R. N. F., Junior A. T. A., Vivas J. M. S., Guimarães A. G., Miranda S. B., Dias V. M., and Scapim C. A. (2017). Potential of popcorn germplasm as a source of resistance to ear rot. *Bragantia Campinas,* 76: 378-385.

Lanubile A., Luca P., and Adriano M. (2010). Differential gene expression in kernels and silks of maize lines with contrasting levels of ear rot resistance after *Fusarium verticillioides* infection. *Journal of Plant Physiology*, 167: 1398–1406.

Loffler M., Kessel B., Ouzunova M., and Miedaner T. (2011). Covariation between line and testcross performance for

reduced mycotoxin concentrations in European maize after silk channel inoculation of two *Fusarium* species. *Theoretical and Applied Genetics*, 122: 925–934.

Mbuya K., Nkongolo K. K., and Kalonji-Mbuyi A. (2011). Nutritional analysis of quality protein maize varieties selected for agronomic characteristics in a breeding program. *International Journal of Plant Breeding and Genetics,* 5: 317-327.

Meseka S., and Ishaaq, J. (2012). Combining ability analysis among Sudanese and IITA maize germplasm at Gezira Research Station. *Journal of Applied Biosciences*, 57: 4198–4207.

Mesterházy A., Lemmens M., and Reid. L. M. (2012). Breeding for resistance to ear rots caused by *Fusarium spp*. in maize - A review. *Plant Breeding,* 131(1): 1–19.

Mukanga M., Derera J., and Tongoona P. (2010). Gene action and reciprocal effects for ear rot resistance in crosses derived from five tropical maize populations. *Euphytica*, 174: 293–301.

Olowe O., Olawuyi O., and Odebode A. (2015). Response of maize genotypes to *Fusarium verticillioides* strains from two agro-ecological zones in southwest Nigeria. *International Journal of Pure and Applied Science and Technology, 27*: 77–86.

Pereira G. S., Camargos R. B., Balestre M., Von Pinho R. G., and Melo, W. M. C. (2015). Indirect selection for resistance to ear rot and leaf diseases in maize lines using biplots. *Genetics and Molecular Research*, 14 (3): 11052–11062.

Pinto R., Kvitschal M. C., Scapim M. V., Fracaro C. A., Bignotto M., and Souza Neto L. S. (2007). Análise dialélica parcial de linhagens de milho-pipoca. *Revista Brasileira de Milho e Sorgo*, 6(3): 325–337.

Reid L. M., Zhu C. X., Parker C. A., and Yan C. W. (2009). Increased resistance to *Ustilago zeae* and *Fusarium verticillioides* in maize inbred lines bred for *Fusarium graminearum* resistance. *Euphytica*, 165: 567–578.

Robinson J. O., Comstock R. E., and Harvey P. H. (1955). Genetic variance in open pollinated varieties of corn. *Genetics*, 40: 45–60.

Rovaris S. R. S., Paterniani M. E. Z., and Sawazaki E. (2014). Combining ability of white corn genotypes with two commercial hybrids. *Maydica*, 59: 96–103.

SAS (2009). The statistical application software (SAS), the statistics system for windows release version 9.2. SAS Institute, Inc, Cary. NC, USA.

Sentayehu A. (2008). Protein, tryptophan and lysine contents in quality protein maize. *North Indian Ethiopian Journal of Health Sciences*, 18(2): 9–15.

Sibiya J., Tongoona P., Derera J., and Van Rij N. (2011). Genetic analysis and genotype×environment (G×E) for grey leaf spot disease resistance in elite African maize (*Zea mays* L.) germplasm. *Euphytica*, 179(1): 312–325.

Singh P. K. and Roy A. K. (2007). Diallel analysis of inbred lines in maize (*Zea mays* L.). *International Journal of Agricultural Sciences*, 3(1): 213–216.

Steel R. G. D., and Torrie J. H. (1980). Principle and Procedures of Statistics. A biometric Approach. 2nd Ed. McGraw Hill, N.Y, USA.

Tembo L., Asea G., Gibson P. T., and Okori P. (2013). Resistance breeding strategy for *Stenocarpella maydis* and *Fusarium graminearum* cob rots in tropical maize. *Plant Breeding,* 132 (1): 83-89.

Tembo L., Asea G., Gibson P. T., and Okori P. (2016). Indirect selection for resistance to *Stenocarpella maydis* and *Fusarium graminearum* and the prospects of selecting for high-yielding and resistant maize hybrids. *Plant Breeding*, 135(4): 446–451.

Vivek B. S., Odongo O., Njuguna J., Imanywoha J., Bigirwa G., Diallo A., and Pixley K. (2010). Diallel analysis of grain yield and resistance to seven diseases of 12 African maize (*Zea mays* L.) inbred lines. *Euphytica,* 172: 329–340.

Zarea M., Choukan R., Bihamta M. R., Majidi H. E., and Kamelmanesh M. M. (2011).Gene action for some agronomic traits in maize (*Zea mays* L.). *Crop Breeding Journal*, 1(2): 133-141.