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Genic resistance mechanisms of *Turcicum* leaf blight in early provitamin A quality protein maize

Mecanismos genéticos de resistencia al tizón de la hoja (*Turcicum*) en el maíz tempranero de calidad con provitamina A

Bashir Omolaran BELLO[1*]; Sunday Ayodele IGE[2] and Micheal Segun AFOLABI[3]

[1] University of Abuja, Department of Crop Science, Nigeria.
[2] Landmark University, Department of Crop Science, Omuaran, Kwara State, Nigeria.
[3] Osun State University, Department of Agronomy, Ejigbo Campus, Osun State, Nigeria.

*Corresponding author: obbello2002@yahoo.com
https://orcid.org/0000-0001-8183-9884

Abstract

Provitamin A quality protein maize (PVA-QPM), as a cost-effective staple maize with improved nutritional quality, has the potential to address malnutrition in rural communities in sub-Saharan Africa. The objective was to identify early maturing *Turcicum* leaf blight (TLB)-resistant PVA-QPM hybrids and lines with promising grain quality and yield. There are significant yield discrepancies among the selected PVA-QPM hybrids, with five hybrids - TZEIORQ 11 × TZEIORQ 20, TZEIORQ 11 × TZEIORQ 24, TZEIORQ 20 × TZEIORQ 24, TZEIORQ 22× TZEIORQ 42 and TZEIORQ 24 × TZEIORQ 42 - standing out with an average yield of 6.91 t.ha^{-1}. The inbreds TZEQI 82 and TZEIORQ 69 exhibited notably low disease scores. The inbreds TZEIORQ 2, TZEIORQ 11, TZEIORQ 20, and TZEIORQ 70 displayed exceptional characteristics. There were significant mean squares of specific combining ability (SCA) and general combining ability (GCA) for all traits suggest that the genetic variations governing these traits are primarily influenced by additive effects. The genetic studies conducted on various traits, except TLB resistance, have indicated a positive and significant impact on GCA. There were high SCA variances for all the traits across the selected twenty-two crosses. These findings confirm that traditional breeding methods can increase maize resistance to TLB disease and develop new cultivars with high disease resistance, grain quality, and yield.

Keywords: *combining ability, malnutrition, grain yield, tryptophan, disease-resistance, provitamin A.*

Resumen

El maíz proteico de calidad con provitamina A (PVA-QPM), como maíz básico rentable con capacidad nutricional mejorada, tiene el potencial de hacer frente a la malnutrición en las comunidades rurales del África Sub-Sahariana. Por ello, se tuvo como objetivo identificar híbridos y líneas de PVA-QPM de maduración temprana resistentes al tizón de la hoja del Turcicum (TLB) y con una calidad del grano y un rendimiento prometedores. Entre los híbridos de PVA-QPM seleccionados existen importantes diferencias, destacando cinco híbridos -TZEIORQ 11 × TZEIORQ 20, TZEIORQ 11 × TZEIORQ 24, TZEIORQ 20 × TZEIORQ 24, TZEIORQ 22× TZEIORQ 42 y TZEIORQ 24 × TZEIORQ 42- con un rendimiento medio de 6.91 t.ha^{-1}. Las razas TZEQI 82 y TZEIORQ 69 mostraron unos índices de enfermedad notablemente bajos. Las razas TZEIORQ 2, TZEIORQ 11, TZEIORQ 20 y TZEIORQ 70 presentaron características excepcionales. La existencia de cuadrados medios significativos en la aptitud de combinación específica (ACE) y de la habilidad

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combinatoria general (HCG) para todos los caracteres sugiere que las variaciones genéticas que rigen estos caracteres están influidas principalmente por efectos aditivos. Los estudios genéticos realizados sobre diversos caracteres, excepto la resistencia al TLB, han indicado un impacto positivo y significativo sobre la HCG. En los veintidós cruces seleccionados se observaron elevadas varianzas ACE para todos los caracteres. Estos resultados confirman que los métodos tradicionales de mejora genética pueden aumentar la resistencia del maíz a la enfermedad TLB y desarrollar nuevos cultivares con alta resistencia a la enfermedad, calidad del grano y rendimiento.

Palabras clave: *Habilidad combinatoria, desnutrición, rendimiento de grano, triptófano, resistencia a enfermedades, provitamina A.*

Introduction

Many individuals in West and Central Africa rely on traditional maize varieties that lack essential nutrients such as provitamin A, tryptophan, and lysine amino acids (Lea et al., 2016). It leads to widespread food insecurity and malnutrition in these regions. Vitamin A is crucial for the immune system and good eyesight, but the human body cannot produce it, so it is from external sources. Provitamin A (PVA) maize contains 15 $\mu g.g^{-1}$ dry weight (DW) of PVA, whereas normal-endosperm yellow maize only contains 2 $\mu g.g^{-1}$ DW (Pixley et al., 2013). Additionally, traditional maize lacks sufficient tryptophan and lysine, with around 0.46 amino acids compared to the 0.73 content in quality protein maize (QPM) (Badu-Apraku & Fakorede, 2017). Consuming maize deficient in these essential nutrients, especially without additional sources of protein for a balanced diet, can lead to stunted child growth, conditions such as kwashiorkor, weakened immune systems, and even death (Ige et al., 2023). PVA-QPM, as a cost-effective staple maize with improved nutritional quality, has the potential to address malnutrition in rural communities in sub-Saharan Africa.

Maize breeders are making relentless efforts to boost and enhance productivity. However, given the current biotic and abiotic stressors, the sustainability of maize production is encouraging. *Turcicum* Leaf Blight (TLB), denoted as northern corn leaf blight is a severe maize foliar fungal pathogen, *Setosphaeria turcica* anamorph *Exserohilum turcicum* [Pass] (Leonard and Suggs) ravaging globally, particularly Africa, Asia, America and Europe (Bankole et al., 2022; Bankole et al., 2023; Zhu et al., 2023).

Spores formation requires a dew temperature between 20 °C and 25 °C for 14 hours (Kutawa et al., 2017). Meanwhile, wetness periods higher than 6 hours with a temperature between 18 °C and 27 °C are favourable to the disease (Badu-Apraku et al., 2020; Badu-Apraku et al., 2021; Iseghohi et al., 2023), while susceptible lesions appear within 12 days (Mueller et al., 2016). The disease symptom appears on the leaf surface and spreads with resultant necrotic lesions. The lesions commenced at the lower leaves and elongated to the entire leaves and husks (Akinwale & Oyelakin, 2018). For a complete disease cycle, susceptible and resistant lesions will form in 14 days and 20 days, respectively (Salgado et al., 2016). The symptom of TLB emerged on the surface of the leaves as tiny greyish-green elliptical lesions with narrowed ends spreading from the leaves to the husks, resulting in leaf necrosis and premature foliage death (Nwanosike et al., 2015; Kutawa et al., 2017). The borders differ in colour based on the type of maize varieties (Zhang et al., 2020). Severe infection can result in a decrease in seed germination and grain quality by lessening the sugar contents. The severity can also cause defoliation of plant stands at the grain-filling stage during and after flowering, thereby predisposing stalk rot disease (Muiru et al., 2015). Under heavy infection, TLB causes about 70 % and 91 % grain yield and silage losses, respectively (Human et al., 2016; Abdelsalam et al., 2022).

Typically, the disease is pandemic in areas where maize cultivation is predominant and highly significant based on geographic distribution and capacity to increase yield losses. When the disease showed before crop flower intrusion, more than 50 % yield losses occurred. It is devastating in the mid-altitude ecology. It varies from moderate to high dew, rainfall and humidity with temperatures of 17 °C to 28 °C (Badu-Apraku & Fakorede, 2017). Quantitative and qualitative genetic effects conditioned tolerance and resistance to TLB, but quantitative inheritance is most important. The factors associated with the infection epidemic are host susceptibility, new emergence races, alternative hosts, host-pathogen interactions and disease breakdown due to quantitative resistance to the disease (Hooda et al., 2017; Bello et al., 2019). TLB occurrence has been ravaging disease-free areas in the lowland regions of Cameroon and Nigeria (Akinwale & Oyelakin, 2018). Oke-Oyi is in the southern Guinea savannah of Nigeria and pandemic to TLB. It is 457 m.a.s.l with an annual temperature between 20 °C and 45 °C and mean relative humidity and rainfall of 1002 mm and 84 %, respectively. However, TLB control is challenging in Savannah agroecology due to erratic rainfall, low temperature and high humidity enhancing its prevalence and damage (Badu-Apraku et al., 2021). However, new races of *E. turcicum* can overwhelm previous resistance among some maize cultivars, and this necessitates continual searching for novel, robust and stable resistance cultivars to control the disease (Sibiya et al., 2013). Further, there is a need to identify cultivars which combine improved grain yield, quality and stable resistance with agronomic traits to mitigate undernourishment and food insecurity in WCA.

Many disease management approaches by application of fungicide, fertilization, residue elimination, seed treatment, crop rotation, adequate spacing and plant density could lessen yield losses (Bello, 2017). Host-plant resistance breeding is the optimal and enduring strategy for effectively managing TLB disease. Its sustainability, cost-effectiveness, and environmental compatibility make it the most advantageous approach (Ayiga-Aluba et al., 2015; Wiesner-Hanks & Nelson, 2016). However, appropriate selection methods of promising parents are important in breeding for TLB resistance. Breeders usually use various mating designs to select potential, favourable and applicable breeding materials to achieve their goals. The diallel cross was employed to evaluate maize breeding parents among the notable mating designs (Bello & Olawuyi, 2015). Combining ability studies using diallel cross is an essential assessment in a hybrid improvement programme. It allows breeders to estimate specific combining ability (SCA) and general combining ability (GCA). It offers information concerning the gene action governing characteristics of inbred lines. It also helps to select favourable inbreds that can serve as parents of maize hybrids in hybrid breeding (Bello & Olawuyi, 2015). Oyekunle & Badu-Apraku (2014) have identified derived diallel cross hybrids as the notable option for enhancing stable and enhanced maize yield at SSA. Thus, the study of the genetic action of PVA-QPM lines for TLB resistance to ascertain outstanding cultivars in formulating a desirable maize varietal development in WCA.

Studies on genic resistance mechanisms revealed epistatic, dominant and additive genetic effects controlling the TLB inheritance in maize (Sibiya et al., 2013). However, additive effects were most important, signifying the recurrent selection approaches in improving TLB resistance would be worthwhile (Bucheyeki et al., 2017). However, GCA for TLB resistance was area-specific and influenced by environmental conditions. It shows that TLB resistance differs across locations except when monogenic before breaking down. Bucheyeki et al. (2017) further reported non-significant maternal and cytoplasmic effects conditioning TLB resistance in maize.

Ample information on the genetics of TLB resistance mechanism and stability of causal disease factors are crucial in breeding for resistance in maize. There are many refined studies on the genetic effects of turcicum-resistant maize genotypes in Nigeria (Sibiya et al., 2013; Abera et al., 2016; Bucheyeki et al., 2017; Bello et al., 2019; Badu-Apraku et al., 2021). Currently, information on the genetic basis of TLB resistance in PVA-QPM genotypes is lacking. Based on this premise, the research was carried out to:

i. Compare the novel early maturing PVA-QPM lines and their derived F_1 hybrids TLB resistance using artificial field infection.

ii. Conduct differential analyses to examine the preponderance of general (GCA) and specific combining ability (SCA) to the TLB resistance.

iii. Identify early maturing TLB-resistant PVA-QPM hybrids and lines with promising grain quality and yield for further stability testing before commercialization in SSA.

This study could contribute to the breeding thrust on elevated nutrition and food security among rural poor who relied on maize products in SSA.

Materials and methods

The test sites, genetic materials and derived hybrids

Ten new early-maturing PVA-QPM lines were procured from the International Institute of Tropical Agriculture (IITA) Ibadan at the Maize Improvement Programme. These lines were drought and nitrogen-tolerant. They also exhibit high grain yield, provitamin, and quality protein contents suitable for Sub-Saharan Africa (Obeng-Bio et al., 2019). Additionally, two newly created commercial maize hybrids, Oba Super 6 and Oba 98, were used as controls (Table 1). The ten PVA-QPM lines were crossed in a partial-diallel design without reciprocals [n(n-1)/2] (n = number of parents). A total of 57 genotypes, including two commercial hybrids, ten inbreds, and 45 F_1-derived hybrids, were subjected to field artificial TLB inoculation at the Lower Niger River Basin Development Authority, Oke-Oyi, Nigeria (80° 30’ N and 8° 36’ E) during the 2022 and 2023 rain-fed seasons. Oke-Oyi, located in the Nigeria southern Guinea savannah, is recognized as a hotspot for TLB. It has an elevation of 457 meters above sea level. The area experiences an annual temperature range of 20 °C to 45 °C. The average relative humidity and rainfall measured were 84 % and 1002 mm, respectively.

Field test and management

At the experimental site, ploughing, harrowing, and ridging were conducted. Three maize seeds were planted per hill and thinned to two at 0.75 m × 0.5 m spacing of a 4 row 5 m plot. The experimental design used was Randomized Complete Block Design. The plant density was around 53 333 plants ha^{-1}. The NPK (15: 15: 15) fertilizer was supplied two weeks after planting (WAP) at the rate of 60 $kg.ha^{-1}$ of N. Further, 30 $kg.ha^{-1}$ of N was applied as top dressing at 4 WAP. Pre-emergence herbicides (3 kg. L^{-1} Metolachlor and 170 $g.L^{-1}$ Atrazine ha^{-1} active ingredients) were sprayed to kill the weeds after land preparation.

Cross-pollination procedures

According to the Standard Procedures described by Bello et al. (2019), maize seed planting was staggered twice at seven days intervals to catch the niche of flowering date variation. Pollen grains shedding and ear shoot protrusion were observed daily before cross-pollination. Before silks’ emergence, ear shoots were cut and bounded with transparent shoot bags to dissuade

Table 1 Description of ten early-maturing PVA-QPM inbreds and two commercial hybrids as control

Genotypes	Pedigree
TZEIORQ 2	2009-TZE OR2 DT STR-QPM S6 inb 2-2/3-1/3-1/3-1/2-1/1-
TZEIORQ 11	2009-TZE OR2 DT STR QPM S6 inb 7-1/3-1/2-1/2-4/4-1/1
TZEIORQ 20	2009-TZE OR2 DT STR QPM S6 inb 26-1/1-1/2-1/6-1/2-1/1
TZEIORQ 24	2009-TZE OR2 DT STR-QPM S6 inb 26-1/1-1/2-4/6-2/3-1/1
TZEIORQ 26	2009-TZE OR2 DT STR-QPM S6 inb 26-1/1-1/2-6/6-2/3-1/1
TZEIORQ 42	2009-TZE OR2 DT STR QPM S6 inb 35-2/3-3/3-2/4-2/2-1/1
TZEIORQ 59	2009-TZE OR2 DT STR QPM S6 inb 50-2/2-1/3-2/3-2/2-1/1
TZEIORQ 69	2009-TZE OR2 DT STR QPM S6 inb 57-2/2-2/2-1/1-1/2-1/1
TZEIORQ 70	2009-TZE OR2 DT STR-QPM S6 inb 60-2/2-1/2-1/3-1/4-1/1
TZEQI 82	TZE-COMP5-Y C6S6 Inb 25 × Pool 18 SR QPM BC1S6 2-3-1-1-6-6
Varietal Control	
OBA SUPER 6	Commercial Provitamin A maize hybrid
OBA 98	Commercial quality protein maize hybrid

undesirable pollination. The shoot bags were enclosed cautiously with the stalks of maize plant stand (female) to enable a uniform silk protuberance and disallow external pollination either through rainfall drips or by wind. Adequate spacing was ensured during the fastening of the shoot bags to allow for suitable silk growth. Sequentially, the paper tassel bag was buttoned to the tassels with clips to permit clean pollens from a designated male line. When adequate silks protruded and were receptive, preferred pollens were used to pollinate the selected plant silks by jiggling the tassel bags carefully and then unclasping before spreading the pollens against the silks of the preferred ear. Lastly, the pollinated ears were wrapped with tassel bags till harvesting to disallow undesirable pollination. The maize grain hybrids developed were stored independently in sacks for further testing.

Preparation of inoculum of TLB

For effective inoculum preparation, TLB-diseased lesion was collected from leaves of maize grown naturally on the field during the harvesting stage. The disease samples were poured unto the moist chamber for spores to be form in between 2 and 3 days. The sterilized needle was used to scrape off the spore suspensions into potato dextrose agar (PDA) medium (potato extract: 4 $g.L^{-1}$, dextrose 20 $g.L^{-1}$, agar 15 $g.L^{-1}$) with the addition of sterilized distilled water for ten days at 25 °C ± 2 °C after using sterilized cheesecloth and incubated. Separate colonies of TLB are sub-cultured on different clean PDA plates. The sub-cultured inoculums were deposited on sorghum kernels inside the inoculation autoclave for ten days to ensure efficient colony formation. The sorghum kernels were then dried in the air and stored in an oven for 48 hours at 40 °C. Spore suspension was deposited on APDA plates and incubated at 25 °C for five days. The recovered spores were saved as cultured agar plugs in 4 mL suspension comprising two ml sterilized water and stored at 4 °C.

Inoculation of TLB

For uniform TLB infection, artificial inoculation was conducted as described by Nwanosike et al. (2015). Pure cultured TLB isolates on storage for twenty days were prepared by washing the spores with distilled water. About 3×105 spore per milliliter was measured using a haemocytometer. The same spore concentration volumes of separate conidia were mixed and sprayed using an atomizer. Spore suspensions were mixed and sprayed using an atomizer between 3 and 4 maize leaf phases in the evening with regular application of water to facilitate adequate infection. Two complementary inoculations were subsequently repeated between 1 and 2 weeks intervals for a suitable coverage of inoculations.

TLB disease evaluation

Disease indices assessments were conducted on the field two weeks after inoculum application by rating each plant based on the size, number and necrotic lesion positions. Twenty plants were selected at random and a score of 0 to 5 was used to evaluate disease severity on the infected surface of the leaf using a 0-5 disease rating scale where 0 reveals slight infection and 5 signifies high susceptibility as follows: ($0 \leq 1$ %; 1 = 1 % to 20 %; 2 = 21 % to 40 %; 3 = 41 % to 60%; 4 = 61 % to 80 % and $5 \geq 80$ %).

Measurement of grain yield

After the maize maturation of maize ears, harvesting and weighing were done for each plot separately. Then, grain yield in kg ha^{-1} was calculated as described by Bello et al. (2012):

$$\text{Grain yield } (kg.ha^{-1}) = \frac{(100-MC) \times 10{,}000\ m^2 \times \text{Fresh Weight}}{7.5\ m^2 \times (100 - 12.5)}$$

MC is the moisture content. The fresh weight shows the weight of the cob per plot, 7.5 m^2 indicates the plot area harvested (5 m × 0.75 m × 92 m), 10 000 m^2 signifies the plot ha^{-1}, and the 12.5 % reveals grain moisture content. With the weight of the ears per plot, grain yield was calculated with an 80 % shelling percentage assumption. Later, the grain yield transformed to tonnes per hectare (Bello et al., 2012). To minimize the border effects in the field study, we removed the plants along the edges on both sides before harvesting the ears.

Proximate analysis of tryptophan content

The maize grain sample of every genotype was ground and defatted using the Kjeldahl device. For protein solubilization, a solitary papain hydrolysis method was applied. When sulfuric acid was introduced, a chemical reaction occurred in which iron ions oxidized acetic acid, leading to the formation of glyoxylic acid. The chemical reaction of glyoxylic acid with protein-free tryptophan indole ring-produced violet-purple products was evaluated with the 560 nm spectrophotometer. As described by Teklewold et al. (2015), the spectrophotometer was used to read the tryptophan optical density standard curve of concentration and converted to tryptophan percentages viz:

$$\text{Factor} = \frac{\text{Hydrolysate volume}}{\text{Weight sample} \times \text{Slope of the standard curve}} \quad (1)$$

The Optical density = Optical density 560-nm sample − Optical density 560-nm papain blanks mean % Tryptophan = Factor × Corrected optical density at 560 nm

For individual genotypes, a recording of 2 separate readings was done. Since tryptophan is associated with lysine, analysis of tryptophan only was carried out ($r > 0.9$) (Nurit et al., 2009). Farther, the tryptophan determination is cheaper in comparison with lysine.

Proximate analyses of carotenoid contents

For dried maize endosperm analysis, effective liquid chromatography was utilized to analyze the carotenoids (Obeng-Bio et al., 2020). From each cross-pollinated ear (hybrids), thirty maize grains were randomly collected from 10 g grains and dry-freezing at -80 °C. Carotenoid analysis was conducted on the collected sample by grounding it into finite powders (0.5 µm). The carotenoids α-carotene (αC), zeaxanthin (ZEA), lutein (LUT), (cis and trans isomers), β-cryptoxanthin (βCX) and β-carotene (βC) contents were measured (Nurit et al., 2009). The overall sum of carotenoid contents was computed as the total concentrations of αC, ZEA, LUT, βC and βCX. For each sample, PVA content = βC + ½(αC + βCX) = βCX and αC (assuming 50 % PVA activity of βC) (Institute of Medicine (US) Panel on Micronutrients [U.S. IMFNB], 2001). Two replications of each trial were carried out on all sampled carotenoids and tryptophan contents.

Statistical analyses

According to Griffing (1956), diallel analysis of Model I (Fixed model) and Method 2 (parents and hybrids without reciprocal crosses) were used. SAS program (SAS, 2018) was employed to compute general combining ability (GCA) and specific combining ability (SCA) mean squares and error variances from the analysis of variance. Combined analysis of PVA-QPM of lines and derived hybrids for the two rain-fed seasons of assessment with nine determined traits were determined as described by Griffing (1956) using SAS program (SAS, 2018). Standard residuals for measured traits were used with the hybrid's mean squares error for untransformed data. The least significant difference test was exploited to quantify the variations among each mean trait. The percentage coefficient of variation (PCV) was used to calculate the different summations.

Results and discussion

Grain yield

The performance of several PVA-QPM hybrids for grain yield exceeded that of the inbreds and the commercial hybrids, as indicated in Table 2. There was a notable variance in grain yield among the PVA-QPM inbreds. Inbreds TZEIORQ 24, TZEIORQ 42, TZEIORQ 20, and TZEIORQ 11 exhibited impressive grain yields of 5.98 $t.ha^{-1}$, 5.90 $t.ha^{-1}$, 5.88 $t.ha^{-1}$, and 5.87 $t.ha^{-1}$, respectively. Additionally, there are significant yield discrepancies among the selected PVA-QPM hybrids, with five hybrids - TZEIORQ 11 × TZEIORQ 20, TZEIORQ 11 × TZEIORQ 24, TZEIORQ 20 × TZEIORQ 24, TZEIORQ 22× TZEIORQ 42 and TZEIORQ 24 × TZEIORQ 42 - standing out with an average yield of 6.91 $t.ha^{-1}$. Particularly, the hybrid TZEIORQ 11 × TZEIORQ 24 demonstrated an increase in yield percentage compared to the best inbreds (TZEIORQ 24) and the check (OBA 98) by 15 % and 16 %, respectively. The four inbreds were more productive for grain yield and their derived hybrids.

Table 2: Mean grain yield, tryptophan and carotenoid contents of early PVA-QPM inbreds, derived hybrids and commercial checks, artificially inoculated with TLB in 2022 and 2023 rainfed seasons

Genotypes	**Grain yield (t.ha^{-1})**	**TLB (N°)**	**Tryptophan (%)**	Carotenoids (µg.g^{-1} DW)					
				PVA	**βCX**	**βC**	**αC**	**ZEA**	**LUT**
TZEIORQ 2	5.31	3.61	4.02	4.04	3.02	2.67	0.50	7.88	11.35
TZEIORQ 11	5.87	3.19	3.71	3.98	3.01	2.63	0.54	7.69	12.30
TZEIORQ 20	5.89	3.04	3.67	3.88	2.78	2.54	0.49	7.91	12.54
TZEIORQ 24	5.98	3.30	3.88	3.91	2.34	2.36	0.52	7.75	12.59
TZEIORQ 26	5.17	3.44	3.92	3.54	2.87	2.61	0.61	7.88	12.53
TZEIORQ 42	5.90	3.02	3.31	3.77	2.89	2.11	0.58	7.96	11.33
TZEIORQ 59	5.12	3.16	3.44	3.54	2.79	2.69	0.51	7.73	12.61
TZEIORQ 69	5.05	2.11	3.32	3.78	2.91	2.11	0.54	7.96	12.45
TZEIORQ 70	5.13	2.82	3.11	3.98	2.87	2.56	0.46	7.83	12.78
TZEQI 82	5.03	1.77	3.34	3.22	2.11	2.04	0.33	6.11	12.49
Selected derived hybrids									
TZEIORQ 2 × TZEIORQ 11	6.78	4.22	3.88	4.35	3.69	2.94	0.71	7.54	12.98
TZEIORQ 2 × TZEIORQ 20	6.82	4.26	3.91	4.11	3.58	2.89	0.66	7.92	12.78
TZEIORQ 2 × TZEIORQ 24	6.84	4.14	3.97	4.63	3.47	2.71	0.82	7.45	12.89
TZEIORQ 2 × TZEIORQ 42	6.80	4.36	3.89	4.78	3.66	2.98	0.61	7.91	12.92
TZEIORQ 2 × TZEQI 82	6.54	4.51	3.54	4.83	3.34	2.94	0.45	7.56	12.77
TZEIORQ 2 × TZEIORQ 70	6.11	4.32	3.61	4.91	3.56	2.87	0.71	7.82	12.65
TZEIORQ 11 × TZEIORQ 20	6.94	3.27	3.45	4.64	3.37	2.71	0.49	7.74	12.79
TZEIORQ 11 × TZEIORQ 24	6.96	3.55	3.55	4.53	3.44	2.88	0.54	7.90	12.51
TZEIORQ 20 × TZEIORQ 24	6.93	3.43	3.71	4.92	3.57	2.91	0.63	7.74	12.58
TZEIORQ 22 × TZEIORQ 42	6.92	4.14	3.65	4.67	3.54	2.65	0.76	7.89	12.71
TZEIORQ 22 × TZEQI 82	6.77	4.34	3.55	4.22	3.61	2.78	0.89	7.67	12.88
TZEIORQ 24 × TZEIORQ 26	6.80	4.56	3.67	4.63	3.78	2.53	0.67	7.92	11.56
TZEIORQ 24 × TZEIORQ 42	6.93	4.32	3.88	4.39	3.22	2.62	0.44	7.90	12.73
TZEIORQ 24 × TZEIORQ 59	6.80	4.39	3.29	4.34	3.36	2.89	0.79	7.77	12.90
TZEIORQ 24 × TZEIORQ 69	6.73	4.50	3.76	4.69	3.59	2.72	0.88	7.97	12.97
TZEIORQ 26 × TZEIORQ 42	6.12	3.44	3.53	4.57	3.37	2.75	0.91	7.84	12.94
TZEIORQ 26 × TZEIORQ 59	6.39	3.23	3.67	4.11	3.51	2.98	0.54	7.60	12.91
TZEIORQ 26 × TZEIORQ 69	6.33	3.45	3.58	4.84	3.59	2.765	0.72	7.88	12.88
TZEIORQ 26 × TZEIORQ 70	6.43	3.61	3.66	4.35	3.33	2.60	0.59	7.79	12.74
TZEIORQ 26 × TZEQI 82	5.58	3.46	3.52	4.23	3.57	2.77	0.61	7.67	11.89
TZEIORQ 42 × TZEIORQ 59	6.84	3.33	3.90	4.67	3.41	2.89	0.79	7.80	12.87
TZEIORQ 42 × TZEIORQ 69	6.85	3.46	3.68	4.35	3.56	2.54	0.63	7.79	12.91
TZEIORQ 42 × TZEIORQ 70	6.83	3.22	3.52	4.53	3.76	2.61	0.85	7.90	12.78
TZEIORQ 42 × TZEQI 82	6.79	3.67	3.67	4.61	3.56	2.79	0.90	7.89	12.93
TZEIORQ 59 × TZEIORQ 69	6.54	3.62	3.44	4.67	3.44	2.88	0.67	7.61	12.85
TZEIORQ 59 × TZEIORQ 70	6.22	3.09	3.34	4.23	3.69	2.70	0.34	7.93	12.90
TZEIORQ 69 × TZEIORQ 70	6.34	3.33	3.69	4.11	3.56	2.53	0.69	7.72	12.93
TZEIORQ 69 ×TZEQI 82	6.53	2.02	3.45	4.54	3.88	2.79	0.71	7.54	11.89
Commercial hybrid checks									
OBA SUPER 6	5.71	2.11	2.34	3.67	2.76	2.01	0.21	6.61	11.83
OBA 98	5.83	2.14	2.36	3.54	2.69	2.03	0.23	6.73	11.90
SE	0.032	0.04529	0.062	0.043	0.040	0.051	0.033	0.045	0.032
CV (%)	7.54	6.33	9.67	7.01	8.77	10.11	7.63	10.66	5.63
LSD (0.05)	0.54	0.69	0.71	0.88	0.41	0.64	0.87	0.52	0.83

TLB, Turcicum leaf blight: PVA, total provitamin A; βCX, β-cryptoxanthin; ZEA, zeaxanthin; LUT, lutein; βC = β-carotene; αC, α-carotene

TLB disease scoring

The inbreds TZEQI 82 and TZEIORQ 69 exhibited notably low disease scores, indicating high tolerance to TLB. These particular inbreds exhibited superior hybrid traits in TLB tolerance, with a disease score of 2.02, outperforming the values of commercial hybrid checks. Despite TLB symptoms on the maize crops in the field, their productivity remained largely unaffected. The susceptibility of other hybrids to TLB underlines the potent virulence of the pathogen and the effectiveness of virus inoculum application in the field (Nwanosike et al., 2015).

Grain-quality protein

The inbred maize lines TZEIORQ 2, TZEIORQ 24, and TZEIORQ 26 exhibited significantly higher levels of Tryptophan compared to the control group, with an increase of approximately 50 %. These particular inbred lines demonstrated compatibility with their respective hybrid offspring and other inbred lines. Furthermore, they appear to possess alleles that confer resistance to TLB and showed promise for enhancing the quality of protein traits in other maize genotypes (Ige et al., 2023).

Carotenoids

The inbreds TZEIORQ 2, TZEIORQ 11, TZEIORQ 20, and TZEIORQ 70 displayed exceptional characteristics. They exhibited high and comparable values for various carotenoids, indicating their potential to yield high-quality grain with attributes resistant to TLB and containing justifiable protein suitable for the savanna region (Ige et al., 2023). Consequently, these inbreds have the potential to serve as parents in the development of new hybrids and could play a significant role in advancing future improvement strategies (Ige et al., 2023). The hybrids derived from these inbreds, and those combined with them, are recommended for direct cultivation for commercial production within this agroecology. However, further evaluation over multiple years and locations to confirm their promising performance is necessary.

Combined analysis of variance

During the two growing seasons, the analysis of variance for virus resistance scoring, carotenoids, grain quality protein, grain yield, TLB-resistance cultivars and PVA-QPM inbreds was conducted (Table 3). The results revealed significant differences in cross mean squares among the studied traits, indicating the use of diverse parents and varied contributions from genotypes in the topcrosses. It reflects the apparent genetic diversity in the improvement scheme. Significant mean squares of Specific Combining Ability (SCA) and General Combining Ability (GCA) for all traits suggest that the genetic variations governing these traits are primarily influenced by additive effects (Badu-Apraku et al., 2021).

Table 3. Combined ANOVA mean squares for grain yield, tryptophan and carotenoid contents of early PVA-QPM inbreds and derived hybrids, artificially inoculated with TLB in 2022 and 2023 rainfed seasons

Source of variation	Df	Grain yield ($t.ha^{-1}$)	TLB (N°)	Tryptophan (%)	Carotenoids ($\mu g.g^{-1}$ DW)					
					PVA	βCX	βC	αC	ZEA	LUT
Year	1	8.71	5.55	9.42	8.90	5.63	7.51	4.43	11.30	7.66
Rep (Year)	6	5.67	8.41	10.93	7.60	6.66	8.67	9.32	6.57	9.11
Hybrids	45	66.42**	93.11**	96.33**	76.20**	74.62**	88.12**	90.54**	67.76**	93.51**
Hybrids × Year	45	8.61	10.56	7.62	10.53	6.11	9.98	6.33	10.18	8.27
GCA	9	67.55*	89.23**	91.11**	87.59**	76.23**	96.54**	87.99**	72.44**	91.52**
SCA	35	51.22*	49.99*	47.74*	51.22*	64.11*	53.11*	61.45*	53.13*	64.01*
Year × GCA	6	11.94	8.22	5.89	7.53	9.87	8.66	7.34	11.01	8.78
Year × SCA	35	11.42	9.63	7.99	8.13	11.54	10.421	9.67	11.58	6.80
Pool error	329	8.66	7.56	923	9.46	6.89	8.42	952	8.55	6.54
GCA/SCA (Baker ratio) %		1.23	0.91	0.77	0.94	0.88	0.63	0.89	0.45	0.72
CV %		10.56	9.45	6.89	11.45	10.54	8.66	9.11	10.23	0.43

** and * significant at 0.01 and probability levels, respectively.

Additionally, the study identified a varied genetic frequency distribution in the genotypes, highlighting the potential for evolving divergent parents for hybrid improvement and serving as unique sources of alleles in modifying the genic base of adapted genotypes. The reported findings not only emphasize the possibility of improving hybrids but also indicate their potential as sources of unique alleles that can modify the genetic base of adapted genotypes. The substantial genetic diversity observed in this study may offer valuable resources for future breeding programs aimed at enhancing the performance of maize cultivars. The mean squares results showed a non-significant interaction between years and both SCA and GCA, suggesting comparable gene actions (non-additive and additive) across the two years. This consistency in gene actions across years may have significant implications for breeding programs and the development of resilient cultivars (Badu-Apraku et al., 2021). The study's findings underscore the importance of considering additive and non-additive genetic effects in maize breeding programs to achieve improved and stable cultivars across different environmental conditions (Abera et al., 2016).

General combining ability estimates

The genetic studies conducted on various traits, except TLB resistance, have indicated positive and significant effects on general combining ability (GCA). It suggests a potential for improving disease resistance, carotenoid content, grain quality protein, and overall yield in maize varieties (Table 4). The findings also point towards the additive genetic action influencing grain yield, implying that recurrent selection could be a promising approach in developing maize varieties with enhanced TLB resistance (Abdelsalam et al., 2022). Additionally, the study on PVA-QPM inbreds showed that certain combinations of inbreds have similar levels of TLB resistance, indicating the potential for creating hybrids with consistent TLB resistance. The evaluation of leaf diseases also revealed negative GCA, indicating genetic expressions of disease resistance. The research underscored the economic importance of host-plant TLB resistance varieties in combating TLB disease pandemics. The study findings also highlighted potential genetic markers for carotenoids and grain quality protein that could aid in selecting desirable genotypes in crop breeding programs (Badu-Apraku et al., 2021). The study has suggested that efficient breeding methods, particularly those involving improved genes in homozygous form and linkage block breaking, could substantially reduce maize yield losses associated with TLB resistance.

Specific combining ability estimates

Assessment of SCA variances is crucial in cases where significant SCA effects are observed (Badu-Apraku et al., 2020). There were high SCA variances for all the traits across the selected twenty-two crosses, suggesting the presence of dominant effect loci controlling grain yield (Table 5), which is consistent with previous research

Table 4. GCA of early PVA-QPM lines for grain yield, tryptophan and carotenoid contents, artificially inoculated with TLB in 2022 and 2023 rainfed seasons

Genotypes	Grain yield ($t.a^{-1}$)	TLB (N°)	Tryptophan (%)	Carotenoids ($\mu g.g^{-1}$ DW)					
				PVA	βCX	βC	αC	ZEA	LUT
TZEIORQ 2	0.88*	0.32	112.76**	96.55**	99.75**	100.44**	92.66**	93.55**	92.55**
TZEIORQ 11	1.34**	0.61	100.22**	93.74**	90.26**	87.23**	98.56**	89.63**	89.33**
TZEIORQ 20	1.32**	0.31	108.34**	82.54**	100.23**	84.78**	81.77**	90.54**	90.82**
TZEIORQ 24	1.78**	0.54	112.63**	98.11**	82.11**	91.44**	71.56**	89.18**	89.80**
TZEIORQ 26	0.93*	0.62	90.35**	83.13**	93.55**	89.00**	91.78**	76.52**	87.11**
TZEIORQ 42	1.87**	0.41	99.11**	79.22**	87.43**	94.55**	80.32**	99.45**	99.56**
TZEIORQ 59	0.91*	0.78	97.09**	95.66**	87.11**	95.70**	92.22**	88.63**	89.00**
TZEIORQ 69	1.94**	0.54	111.52**	89.14**	98.21**	89.11**	88.71**	91.73**	95.89**
TZEIORQ 70	1.89**	0.23	97.22**	94.56**	94.77**	91.59**	89.49**	90.55**	94.61**
TZEQI 82	0.90*	0.54	91.45**	87.33**	111.48**	83.74**	99.28**	89.39**	98.55**

** and * significant at 0.01 and 0.05 probability levels, respectively.

Table 5. SCA of selected early PVA-QPM derived hybrids for grain yield, tryptophan and carotenoid contents, artificially inoculated with TLB in 2022 and 2023 rainfed seasons

Genotypes	Grain yield ($t.ha^{-1}$)	TLB (N°)	Tryptophan (%)	Carotenoids ($\mu g.g^{-1}$ DW)					
				PVA	βCX	βC	αC	ZEA	LUT
TZEIORQ 2 TZEIORQ 11	1.56**	0.66	100.33**	96.34**	83.11**	99.45**	87.76**	92.26**	111.23**
TZEIORQ 2 TZEIORQ 24	1.01**	0.78	98.53**	98.89**	97.34**	92.11**	76.13**	90.99**	72.51**
TZEIORQ 2 TZEIORQ 26	1.63**	0.70	89.67**	79.11**	89.55**	89.89**	80.99**	80.53**	99.53**
TZEIORQ 2 TZEIORQ 59	0.87**	0.52	72.51**	98.24**	93.43**	70.78**	82.23**	79.22**	88.65**
TZEIORQ 2 TZEQI 82	1.35**	0.87	100.52**	90.82**	90.67**	92.53**	88.62**	93.53**	70.11**
TZEIORQ 2 TZEIORQ 70	1.62**	0.69	96.80**	79.58**	89.11**	81.22**	100.35**	89.21**	96.23**
TZEIORQ 11 TZEIORQ 69	2.32**	0.98	91.44**	79.57**	93.94**	79.84**	85.11**	88.77**	80.65**
TZEIORQ 11 TZEIORQ 70	1.49**	0.72	79.67**	99.24**	88.45**	95.99**	112.81**	99.32**	70.54**
TZEIORQ 20 TZEIORQ 42	1.88**	0.84	70.55**	79.11**	89.89**	89.67**	81.77**	111.47**	90.58**
TZEIORQ 20 TZEIORQ 70	1.99**	0.95	92.83**	89.25**	79.23**	78.45**	87.34**	99.76**	89.68**
TZEIORQ 20 TZEQI 82	1.54**	0.71	101.79**	92.89**	98.51**	97.73**	80.54**	90.18**	77.42**
TZEIORQ 24 TZEIORQ 26	2.44**	0.79	79.66**	70.77**	85.67**	89.25**	86.22**	76.11**	75.21**
TZEIORQ 24 TZEIORQ 42	1.42**	0.88	90.54**	79.27**	78.32**	78.33**	87.56**	77.23**	98.89**
TZEIORQ 24 TZEIORQ 59	1.22**	0.87	78.72**	99.71**	96.89**	98.54**	92.56**	90.34**	77.32**
TZEIORQ 24 TZEIORQ 69	1.40**	0.55	79.89**	78.11**	80.67**	89.77**	101.44**	89.53**	97.52**
TZEIORQ 26 TZEIORQ 42	0.89**	0.98	91.55**	74.23**	79.99**	70.62**	80.59**	100.62**	99.67**
TZEIORQ 26 TZEIORQ 59	1.32**	0.73	89.72**	92.67**	92.74**	98.55**	86.99**	90.64**	101.33**
TZEIORQ 26 TZEIORQ 69	1.45**	0.67	79.67**	79.17**	80.29**	88.32**	85.67**	89.77**	99.54**
TZEIORQ 26 TZEIORQ 70	1.78**	0.97	90.58**	78.61**	73.71**	99.11**	97.92**	79.99**	92.77**
TZEIORQ 26 TZEQI 82	1.56**	0.72	87.74**	90.71**	99.92**	90.84**	81.78**	92.54**	79.67**
TZEIORQ 42 TZEIORQ 59	0.98**	0.72	79.66**	79.66**	89.67**	89.67**	99.32**	88.63**	78.45**
TZEIORQ 42 TZEIORQ 69	0.87**	0.97	78.54**	76.54**	93.66**	79.33**	77.16**	79.58**	96.21**
TZEIORQ 42 TZEIORQ 70	1.67**	0.85	90.89**	90.99**	97.65**	97.78**	112.52**	90.17**	80.89**
TZEIORQ 42 TZEQI 82	2.11**	0.69	79.11**	79.31**	81.88**	80.11**	87.67**	67.22**	77.45**
TZEIORQ 59 TZEIORQ 69	0.67**	0.70	99.44**	78.69**	79.34**	99.89**	81.11**	98.67**	91.77**
TZEIORQ 59 TZEIORQ 70	1.31**	0.72	111.28**	99.72**	98.27**	93.94**	86.12**	75.45**	97.69**
TZEIORQ 69 TZEIORQ 70	1.47**	0.83	79.55**	78.59**	88.29**	89.33**	89.24**	98.63**	89.63**
TZEIORQ 70 TZEQI 82	0.90**	0.69	90.37**	99.63**	79.62**	93.40**	87.58**	78.51**	78.50**

** and * significant at 0.01 and 0.05 probability levels, respectively.

(Badu-Apraku et al., 2021). It is worth noting that using breeding synthetic and composite varieties is a reasonable approach to developing enhanced TLB resistance, quality protein, and carotenoid levels. These findings confirm that traditional breeding methods can increase maize resistance to TLB disease and develop new cultivars with high disease resistance, grain quality, and yield (Abera et al., 2016).

Conclusion

There are significant yield discrepancies among the selected PVA-QPM hybrids, with five hybrids - TZEIORQ 11 × TZEIORQ 20, TZEIORQ 11 × TZEIORQ 24, TZEIORQ 20 × TZEIORQ 24 TZEIORQ 22× TZEIORQ 42 and TZEIORQ 24 × TZEIORQ 42 - standing out with an average yield of 6.91 $t.ha^{-1}$ The inbreds TZEQI 82 and TZEIORQ 69 exhibited notably low disease scores, indicating high tolerance to TLB. The inbreds TZEIORQ 2, TZEIORQ 11, TZEIORQ 20, and TZEIORQ 70 displayed exceptional characteristics. Significant mean squares of SCA and GCA for all traits suggest that the genetic variations governing these traits are primarily influenced by additive effects. The genetic studies conducted on various traits, except TLB resistance, have indicated positive and significant effects on general combining ability (GCA). It suggests a potential for improving disease resistance, carotenoid content, grain quality protein, and overall yield in maize varieties. There were high SCA variances for all the traits across the selected twenty-two crosses, suggesting the presence of dominant effect loci controlling grain yield. These findings confirm that traditional breeding methods can increase maize resistance to TLB disease and develop new cultivars with high disease resistance, grain quality, and yield.

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Authors' contributions

BOB: initiated the idea of the study and conducted the field trial, ISA: recorded the raw data AMS: carried out the Statistical Analysis. All contributed to the written manuscript.

Conflict of interest

The authors declared no conflict of interest of this research.

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ORCID and E-mail

Bashir Omolaran Bello obbello2002@yahoo.com
https://orcid.org/0000-0001-8183-9884

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