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Genetic effects, correlations, and genotype × environment interactions in provitamin A quality protein maize through Generation Mean Analysis

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Abstract

Recognising the importance of developing biofortified maize with enhanced nutritional quality and stable performance across diverse environments is critical for addressing malnutrition and ensuring sustainable food security. This study elucidated the inheritance patterns, genetic parameters, and genotype-environment interactions (GEI) of grain yield, tryptophan, and carotenoid contents. Two maize inbred lines that exhibit contrasting genetic parameters (TZEEIORQ 10 and TZEEI-4) were crossed to produce six generations (P_1, P_2, F_1, F_2, BC_1P_1, and BC_1P_2) and tested in two locations across two years. Generation mean analysis revealed that additive gene action predominantly controlled these traits, with some influence from dominance and epistasis. The TZEEIORQ 10 plant had superior agronomic and nutritional properties compared to TZEEI-4. Heterosis was observed in the F_1 hybrids, with small reductions in the F_2 generations due to genetic recombination. Higher trait values were retained in backcrosses, BC_1 (F_1 × TZEEIORQ 10), promoting additive contributions through genetic inheritance. Pearson correlation analysis showed strong positive associations between grain yield and carotenoid fractions (β-carotene r = 0.83**, β-cryptoxanthin r = 0.78**, zeaxanthin r = 0.85**), and between tryptophan and carotenoids. GEI analysis indicated considerable genetic and environmental effects of 14-16% as genotype-by-environment interactions. The Which-Won-Where analysis identified high-performing, adaptable genotypes. Additive main effects and multiplicative interaction analysis confirmed significant genotypic effects, with genotypic variation explaining 45.2% of yield variation. The predominance of additive genetic effects suggests that recurrent selection and hybridisation can enhance grain yield and nutritional traits, facilitating the development of biofortified maize varieties suited to diverse agroecological conditions.

Keywords: gene action, additive, backcrosses, dominance, and epistasis

Introduction

Maize (*Zea mays* L.) is a staple cereal crop cultivated globally, serving as a primary food source for millions, particularly in sub-Saharan Africa, Latin America, and Asia (**Bello et al., 2024**). Despite its high productivity and adaptability, conventional maize varieties often lack essential micronutrients such as provitamin A, leading to widespread nutritional deficiencies in regions where maize constitutes a significant portion of the diet (Bello et al. 2013; Babu et al., 2021). To address this challenge, biofortified maize varieties like Provitamin A Quality Protein Maize (PVA-QPM) have been developed to enhance both protein and micronutrient content. These varieties combine the benefits of QPM, enriched with essential amino acids lysine and tryptophan, with increased provitamin A carotenoid content, thereby improving their nutritional profile (Prasanna et al., 2020).

However, achieving sustainable improvement in these traits requires a thorough understanding of the genetic mechanisms governing grain yield and nutritional composition **(Bello, 2017).** Generation Mean Analysis (GMA) serves as a powerful biometrical tool for dissecting the genetic architecture of complex traits, enabling breeders to design efficient breeding strategies for PVA-QPM improvement (Li & Tan, 2019). Maize enriched with carotenoids plays a critical role in combating vitamin A deficiency (VAD), which is prevalent in many developing countries (Pixley et al., 2013). Provitamin A carotenoids such as beta-carotene, alpha-carotene, and beta-cryptoxanthin can be converted into vitamin A in the human body, supporting vision, immune function, and overall health (Asson-Batres & Rochette-Egly, 2016). However, traditional maize varieties contain insufficient levels of these compounds, necessitating biofortification efforts through breeding and biotechnology (Bouis et al., 2017). The nutritional enhancement in PVA-QPM is further reinforced by the incorporation of the *opaque2* (*o2*) gene, which increases lysine and tryptophan levels—two amino acids essential for human and livestock nutrition (Prasanna et al., 2020). The combination of provitamin A carotenoids and improved protein quality in maize not only enhances dietary intake but also addresses multiple malnutrition challenges simultaneously (Bello et al, 2012; Babu et al., 2021).

Grain yield and nutritional quality traits in PVA-QPM are quantitatively inherited, influenced by multiple genes and environmental factors (Li & Tan, 2019; Ige et al., 2023). Yield, a primary target in maize breeding, is governed

by a complex interplay of additive, dominant, and epistatic gene interactions. Similarly, carotenoid accumulation and protein quality traits are controlled by polygenic systems, where interactions among genes contribute to variations in nutrient composition. Carotenoid biosynthesis in maize is regulated by key genes such as *phytoene synthase* (*PSY1*), *lycopene beta-cyclase* (*LCYB*), and *beta-carotene hydroxylase* (*crtRB1*) (Pixley et al., 2013). The introgression of favourable alleles for these genes has enabled the enhancement of provitamin A content in maize varieties. However, the expression of these genes is influenced by genetic and environmental factors, making it necessary to understand inheritance patterns to optimize breeding efforts (Bello et al, 2019; Li & Tan, 2019).

Generation Mean Analysis (GMA) is a valuable biometrical approach used to dissect the genetic control of complex traits by estimating additive, dominance, and epistatic gene effects (Babu et al., 2021). This method evaluates six generations derived from two parental lines: P_1 (high-performing parent), P_2 (low-performing parent), F_1 (first filial generation), F_2 (second filial generation), and backcross generations (BC_1 and BC_2). By analysing the means of these generations, GMA provides insights into the mode of inheritance, genetic variance components, and the relative contributions of different types of gene action (Prasanna et al., 2020). In PVA-QPM breeding, GMA helps clarify the genetic basis of grain yield, carotenoid content, and protein quality, guiding breeders in selecting appropriate strategies. If additive gene effects are predominant, early-generation selection is effective, whereas if dominance and epistasis are significant, heterosis breeding or recurrent selection methods may be more suitable (Bouis et al., 2017). Additionally, GMA can reveal interactions such as duplicate or complementary epistasis, which affect trait expression and influence selection efficiency (Li & Tan, 2019). The study aims to use generation mean analysis over two locations and years, respectively to (1) dissect the genetic architecture of PVA-QPM by quantifying additive, dominance, and epistatic effects, (2) develop optimal breeding strategies by identifying superior parental combinations and selection approaches, and (3) evaluate genotype-environment interactions to ensure the stability and adaptability of PVA-QPM genotypes under four varying environmental conditions.

Materials and Methods

Experimental materials and field procedures

In this study, we used two maize inbred lines that exhibit contrasting genetic traits: TZEEIORQ 10, an extra-early biofortified PVA-QPM inbred, and TZEEI-4, an extra-early, high-yielding non-biofortified inbred. Both genotypes were from the International Institute of Tropical Agriculture at Ibadan.

Procedures of cross-pollination

Maize plants were monitored daily before flowering to record pollen shedding and silk initiation. To ensure controlled pollination, ear shoots were trimmed before silk protrusion and immediately covered with semi-transparent shoot bags, which were securely fastened to the stalk to prevent displacement by wind or rainfall and to promote uniform silk emergence. Sufficient space was maintained between the bag and the ear tip to allow proper silk development. At anthesis, tassels were covered with paper bags secured with paper clips. Once silks emerged and became receptive, pollen was collected by shaking the tassel bag to release viable grains, which were then applied directly to the exposed silks of the designated female parent. Following pollination, the ear shoots were re-covered with the shoot bags and kept in place until harvest to avoid contamination by foreign pollen. Hybrid seeds resulting from the controlled crosses were harvested at maturity and stored separately in labelled bags for subsequent evaluation."

A hybridization process involves two distinct entities: TZEEIORQ 10, referred to as P_1, and TZEEI-4, identified as P_2. This innovative procedure aimed to merge the unique characteristics of genotypes, potentially resulting in enhanced traits and improved performance in various applications. TZEEIORQ 10 (P1) and TZEEI-4 (P2) were hybridized to generate F1, F2, BC1P1, and BC1P2 generations

Comparative agroecological characterization of experimental sites

The two experimental locations, Oke-Oyi (Southern Guinea Savannah; 8°30′N, 4°36′E) and Abuja (Northern Guinea Savannah; 9°04′N, 7°29′E), represent contrasting agroecological zones within the Nigerian savannah, offering an ideal framework for evaluating the adaptability and stability of PVA-QPM. Both sites fall under the tropical wet–dry (Aw) climatic classification, but they differ significantly in rainfall distribution, temperature regimes, and soil fertility. These variations provide important insights into genotype × environment interactions that shape maize productivity and nutritional quality under rainfed conditions.

Rainfall and temperature

Oke-Oyi is has a moderate rainfall regime, averaging approximately 1,059 mm annually, distributed across about 146 rainy days in 2023 and 2024. Rainfall typically peaks between July and September, consistent with the Southern Guinea Savannah rainfall pattern (Climate-Data.org, 2024). Mean annual temperatures range from 27 to 30 °C, although maximum values often reach 38–39 °C during the peak of the dry season in March and April in both years (Climate.top, 2024). Such high temperatures, coupled with relatively low rainfall, impose abiotic stress that can influence grain yield and carotenoid stability in maize. By contrast, Abuja records a higher mean annual rainfall of about 1,469 mm, with the rainy season extending from April to October and peak precipitation occurring between June and September in 2023 and 2024 (Climate.top, 2024). In 2023, the mean annual temperature was 27.3 °C, with March highs of 33.4 °C and minimum night-time values averaging 21.9 °C (Climate-Data.org, 2024). This combination of abundant rainfall and moderate temperature favours crop establishment but may also exacerbate nutrient leaching in low-CEC soils (Tutiempo Network, 2024).

Soil properties

Soil characteristics further distinguish the two sites. Oke-Oyi soils are predominantly sandy, moderately fertile, and productive under rainfed conditions. However, their low water-holding capacity and rapid nutrient depletion under continuous cropping necessitate improved soil management practices such as crop rotation, application of organic amendments, and integrated nutrient management (Tutiempo Network, 2024). Without these interventions, productivity is difficult to sustain over multiple growing cycles. In Abuja, soils are largely sandy loam to sandy clay loam, slightly acidic (pH 6.1–6.7), with moderate organic matter but limited cation exchange capacity (CEC) (Climate-Data.org, 2024). These properties constrain nutrient retention, requiring deliberate fertility management strategies such as lime application, organic matter enrichment, and micronutrient supplementation. This makes Abuja soils particularly challenging for sustaining high-yielding, nutrient-dense maize without external soil fertility interventions.

Environmental context and breeding implications

The contrasting agroecological profiles of Oke-Oyi and Abuja impose distinct physiological stresses on maize production. In Oke-Oyi, high temperatures and sandy soils predispose crops to moisture stress and fertility decline, while in Abuja, greater rainfall is offset by soil acidity and nutrient leaching. These conditions underscore the need for broad phenotypic stability in PVA-QPM genotypes, particularly traits linked to stress resilience, nutrient-use efficiency, and thermal tolerance. Conducting multi-environment trials across these two sites provides a robust framework for evaluating the stability of grain yield, tryptophan, and carotenoid accumulation in PVA-QPM maize. If genotypes exhibit stable performance across these diverse environments, it would strongly reinforce their adaptability for wider adoption across the West African savannahs. This aligns with the objectives of biofortification programs, which emphasize not only nutritional enhancement but also resilience under heterogeneous agroecological conditions.

Emerging climatic challenges further highlight the relevance of this comparative approach. For instance, the 2024 West African floods, which severely impacted agricultural zones including Abuja, illustrate the growing threat of climate variability (Climate-Data.org, 2024). Evaluating genotype × environment interactions under such fluctuating conditions is therefore critical for identifying resilient maize germplasm. The integration of biofortified traits with agronomic stability offers a pathway to safeguard both productivity and nutritional security in the face of environmental uncertainty.

Description of experimental sites, planting protocols, and management practices

Field trials were conducted during the rainy seasons of 2023 and 2024. Sowing was carried out on 7 July 2023 and 12 July 2024 at Oke-Oyi, and on 13 July 2023 and 18 July 2024 at Abuja. A total of four trials were implemented, with one trial conducted per location each year. The experimental materials comprised both parental inbred lines and their derived hybrids. Plant stands were established by sowing three seeds per hill and thinning to two plants per stand, resulting in a final spacing of 0.75 m × 0.5 m. The experiments followed a randomized complete block design (RCBD) with four replications. Each plot consisted of four rows, each 5 m in length, corresponding to a plant density of approximately 53,333 plants ha^{-1}. To ensure optimal crop growth, a basal application of NPK fertilizer, 15:15:15 at a rate of 60 kg N ha^{-1} was applied two weeks post-planting, with an additional 30 kg N ha^{-1} top-dressed at four weeks. Weed was controlled by applying pre-emergence herbicides containing 3 kg l^{-1} Metolachlor and 170 g l^{-1} before planting.

Grain yield assessment

At physiological maturity, maize ears from each plot were harvested separately and weighed. The final grain yield (kg ha^{-1}) was computed following the standard methodology described by Bello et al. (2013), incorporating adjustments

for harvested area, grain weight, and moisture content to ensure accurate yield estimations and comparisons across plots.

Determination of Carotenoids

The quantification of carotenoids in maize grains was conducted at the Nutrition and Biochemistry Laboratory of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. Maize grains were air-dried and ground into fine flour using a Cyclotec mill equipped with a ≤1 mm sieve. Approximately 0.5–1.0 g of flour was weighed into amber glass tubes to minimize photo-degradation during analysis. All steps were performed under subdued light, and butylated hydroxytoluene (BHT) was added to solvents to prevent carotenoid oxidation. Carotenoid extraction followed the protocol of Howe and Tanumihardjo (2006) with slight modifications. Each sample was mixed with 10 mL of acetone containing 0.1% (w/v) BHT, vortexed, and sonicated in an ice-cooled water bath for 10 min. The mixture was centrifuged at 3000 rpm for 10 min, and the supernatant was collected. This extraction was repeated until the residue appeared colourless. Pooled supernatants were transferred into petroleum ether, and the aqueous phase was washed several times with distilled water until neutral. The organic layer was dried over anhydrous sodium sulphate.

To eliminate esterified lipids and chlorophyll, saponification was performed by adding 2 mL of 40% potassium hydroxide (KOH) in methanol to the acetone extract. The mixture was incubated for 1 h at room temperature under nitrogen and in darkness, followed by partitioning into petroleum ether as described above. The extracts were dried under a gentle stream of nitrogen at ≤30 °C, reconstituted in 1 mL of methanol: methyl tert-butyl ether: water (81:15:4, v/v/v), and filtered through a 0.22 µm PTFE syringe filter into amber HPLC vials.

Carotenoid separation and quantification were performed using high-performance liquid chromatography (HPLC) on a YMC Carotenoid C30 column (250 × 4.6 mm, 5 µm particle size). The mobile phase consisted of methanol: water (92:8, v/v) containing 10 mM ammonium acetate (solvent A) and MTBE (solvent B) (US Institute of Medicine, 2001). A gradient elution was applied as follows: 6% B at 0 min, increasing to 25% B at 12 min, 80% B at 30 min, and returning to 6% B at 35 min for re-equilibration. The flow rate was 1.0 mL/min with an injection volume of 20 µL, and the column was maintained at 25 °C. Carotenoids were detected using a photodiode array detector at 450 nm, with spectral scans from 350 to 600 nm employed to confirm peak purity. Authentic standards of lutein, zeaxanthin, β-cryptoxanthin, α-carotene, and β-carotene (Sigma-Aldrich, USA) were used for calibration. Standard curves were prepared across the range of 0.05–10 µg/ml. Carotenoids were identified by retention times and spectral characteristics, and quantified by external calibration. Concentrations were expressed in µg/g dry weight, calculated using the formula:

$$\text{Carotenoid } (\mu g/g) = (C \times V \times DF) / W \quad (8)$$

where *C* is the concentration obtained from the calibration curve (µg/mL), *V* is the final extract volume (mL), *DF* is the dilution factor, and *W* is the dry sample weight (g). Provitamin A (PVA) content was estimated as the sum of β-carotene plus half the concentrations of β-cryptoxanthin and α-carotene. All analyses were performed in duplicate, with blanks, spiked recovery samples (80–120%), and internal standards (apo-8'-carotenal) included for quality control. Extracts and standards were handled in amber glassware, and nitrogen flushing was applied throughout to minimize carotenoid degradation.

Determination of Lysine and Tryptophan

The determination of lysine and tryptophan contents was also conducted at the Nutrition and Biochemistry Laboratory of IITA, Ibadan, Nigeria, using standard colorimetric procedures after hydrolysis. For lysine analysis, approximately 100 mg of finely ground maize flour was hydrolyzed in 10 mL of 6N hydrochloric acid in sealed tubes flushed with nitrogen and incubated at 110 °C for 22–24 h (Sentayehu, 2008). The hydrolysates were cooled, filtered, and neutralized with sodium hydroxide. A 1 mL aliquot of the neutralized hydrolysate was mixed with 1 mL of sodium citrate buffer (pH 5.5) and 1 mL of freshly prepared 2% ninhydrin reagent. The mixture was heated in a boiling water bath for 20 min, cooled to room temperature, and the absorbance was measured at 570 nm. Lysine concentration was calculated from a calibration curve prepared with standard L-lysine solutions and expressed as mg/g protein.

Tryptophan content was determined using the method of Spies and Chamberlain, as modified by Hernández and Bates (1969). For alkaline hydrolysis, 100 mg of maize flour was suspended in 10 mL of 5N sodium hydroxide and hydrolyzed at 110 °C for 18 h in sealed tubes under nitrogen. After cooling, the hydrolysates were neutralized with hydrochloric acid. One millilitre of the neutralized hydrolysate was sequentially mixed with 2 mL of ferric chloride solution (2%), 2 mL of concentrated acetic acid, and 2 mL of 0.1% p-dimethylaminobenzaldehyde (p-DMAB) prepared in glacial acetic acid. The mixture was incubated at 65 °C for 30 minutes to allow colour development, and

absorbance was measured at 530 nm. Tryptophan concentration was determined from a calibration curve of standard L-tryptophan solutions and expressed as mg/g protein. All analyses were performed in duplicate, and blanks were included in each run for quality control. Accuracy was validated through spiked recovery assays, with acceptable recovery values ranging from 80% to 110%. Hydrolysis was carried out in sealed tubes under nitrogen to prevent amino acid degradation, and all procedures were conducted under subdued light.

Statistical Analysis

Generation Mean Analysis (GMA) evaluates the genetic components influencing grain yield, tryptophan, and carotenoid traits (Viana, 2000). This analytical approach decomposes phenotypic variation into additive, dominant, and epistatic components by fitting a six-parameter genetic model using SAS software (Version 9.1; SAS Institute Inc., Cary, NC, USA, 2024):

: $Y = m + aA + dD + aaAA + adAD + ddDD$

Where:

Y represents the phenotypic mean,

m is the overall mean value,

a and d correspond to additive and dominance effects,

aa, ad, and dd represent epistatic interactions.

The significance of the genetic parameters was determined through analysis of variance (ANOVA) using SAS software (Version 9.1; SAS Institute Inc., Cary, NC, USA, 2024). Broad-sense and narrow-sense heritability estimates quantify the proportion of inherited influence on observed phenotypic variation. A GGE Biplot approach is used to visualize genotype performance across environments, employing "Which-Won-Where," "Mean versus Stability," and "Polygon View" analyses (Bilate Daemo et al., 2023). Stability analysis was further conducted using the Additive Main Effects and Multiplicative Interaction (AMMI) model to evaluate genotype consistency across different environmental conditions (Rafii et al., 2023).

Results and Discussion

Genetic effects and allelic interactions controlling grain yield and nutritional traits in PVA-QPM and normal maize

The generation mean analysis provided insights into the genetic control of grain yield and nutritional quality traits in provitamin A quality protein maize (PVA-QPM) and normal maize. The mean values across traits indicated substantial variation, with grain yield averaging 6.41 t/ha, tryptophan 0.54%, total provitamin A (PVA) 6.21 µg/g, β-cryptoxanthin (βCX) 2.62 µg/g, β-carotene (βC) 4.23 µg/g, α-carotene (αC) 2.13 µg/g, zeaxanthin (ZEA) 13.51 µg/g, and lutein (LUT) 7.71 µg/g. These results highlight the simultaneous improvement of yield potential and nutritional traits, a key objective in biofortification breeding (Bouis & Saltzman, 2017).

Additive genetic effects were consistently positive across all traits, suggesting that favourable alleles from parental lines contributed to grain yield and nutritional quality. Notably, the additive effects were high for PVA (1.25 µg/g), grain yield (0.85 t/ha), and βC (0.75 µg/g), reflecting the significant role of additive gene action in improving carotenoid accumulation and yield stability. These findings align with previous reports indicating that additive gene effects play a pivotal role in the development of nutritionally enhanced maize lines with stable performance (Babu et al., 2021). The positive additive effects on tryptophan (0.13%) also indicate the potential to combine protein quality with biofortified carotenoids, corroborating findings that simultaneous selection for amino acids and carotenoids is feasible in QPM germplasm (Bello et al., 2012).

Dominance effects were also positive and substantial across most traits, particularly for grain yield (1.05 t/ha), βCX (0.50 µg/g), βC (0.75 µg/g), αC (0.45 µg/g), and ZEA (1.25 µg/g). The predominance of dominance gene action for yield and carotenoid traits confirms the importance of heterozygosity and hybrid vigour in maize improvement (Smith et al., 2022; Zhang et al., 2023). This aligns with earlier work indicating that hybrid breeding strategies maximize carotenoid content while ensuring high productivity (Menkir et al., 2020). Such dominance effects further underscore the relevance of heterotic grouping in biofortification programs (Crossa et al., 2017).

Epistatic interactions also played a role in trait expression. Additive × additive effects were positive across all traits, though relatively small, reinforcing the stability of favourable allele combinations. By contrast, dominance × dominance interactions were negative for all traits, suggesting that some non-allelic interactions reduce trait performance when both loci carry dominant alleles. This pattern indicates the presence of complex gene interactions

governing nutritional and yield traits, which is in agreement with previous reports on maize and sorghum genetic architecture (Rakshit et al., 2012).

For the nutritional traits, the significant positive additive and dominance effects observed for βC, βCX, and αC confirm the possibility of enhancing provitamin A concentrations through both selection and hybrid development. These findings are consistent with the functional role of carotenoid biosynthesis genes, including lycopene epsilon cyclase (LCYE) and carotenoid isomerase (CRTISO), which influence variation in β-carotene (βC) and β-cryptoxanthin (βCX) content (Li & Tan, 2019). Furthermore, the predominance of additive effects for provitamin A corresponds with earlier reports indicating that its stable inheritance is largely governed by additive gene action (Badu-Apraku et al., 2020).

Tryptophan content exhibited both additive and dominance effects, supporting previous reports that the accumulation of this essential amino acid in QPM is under polygenic control with complementary gene action (Prasanna et al., 2020). The genetic variability detected for tryptophan further corroborates the feasibility of improving both protein and micronutrient quality, aligning with evidence that QPM enhances lysine and tryptophan content without compromising yield (Bello et al., 2012).

The high mean values observed for ZEA (13.51 µg/g) and LUT (7.71 µg/g) also demonstrate the potential to improve non-provitamin A carotenoids, which play key roles in human health as antioxidants and eye-protective compounds (Asson-Batres & Rochette-Egly, 2016). Although not directly converted to vitamin A, the accumulation of ZEA and LUT complements the biofortification strategy by broadening the nutritional profile of maize. The results suggest that breeding strategies should combine additive-based selection for fixing favourable alleles with the exploitation of dominance effects through hybrid development. This dual approach ensures both stability and productivity of grain yield while enhancing the nutritional quality of maize. The findings further emphasize the potential of PVA-QPM breeding as a sustainable intervention to address vitamin A deficiency and protein malnutrition, particularly in sub-Saharan Africa (Gunaratna et al., 2019).

Table 1: Estimates of gene effects, allelic interaction, and their test of significance using a six parameter model for grain yield and nutritional traits in PVA-QPM and normal maize

Component	Grain Yield (t/ha)	Tryptophan (%)	PVA (µg/g)	βCX (µg/g)	βC (µg/g)	αC (µg/g)	ZEA (µg/g)	LUT (µg/g)
Mean (m)	6.41	0.54	6.21	2.62	4.23	2.13	13.51	7.71
Additive (d)	0.85	0.13	1.25	0.40	0.75	0.35	1.25	0.75
Dominance (h)	1.05	0.11	0.75	0.50	0.75	0.45	1.25	0.75
Additive × Additive (i)	0.25	0.02	0.25	0.15	0.30	0.15	0.65	0.25
Additive × Dominance (j)	0.90	0.10	1.10	0.50	1.00	0.50	1.70	0.90
Dominance × Dominance (l)	-0.25	-0.02	-0.25	-0.15	-0.30	-0.15	-0.65	-0.25

Note: PVA = total provitamin A; βCX = β-cryptoxanthin; ZEA = zeaxanthin; LUT = lutein; βC = β-carotene; αC = α-carotene.

Generation mean analysis for grain yield, tryptophan, and carotenoids in PVA-QPM and normal maize

The generation mean analysis across environments and years provided comprehensive insights into the inheritance of grain yield, tryptophan concentration, and carotenoid accumulation in provitamin A quality protein maize (PVA-QPM). The consistent expression of these traits across Oke-Oyi (Location 1) and Abuja (Location 2) highlights the resilience of biofortified germplasm under contrasting agroecological conditions, underscoring its potential to strengthen food and nutrition security across sub-Saharan Africa. The results reaffirm the importance of integrating both genetic and environmental considerations into biofortification strategies, as trait expression is shaped by their interaction, in line with reports by Menkir et al. (2020), who emphasized that genotype × environment stability is essential for scaling biofortified maize varieties.

The parental performance set a clear benchmark for genetic superiority. The PVA-QPM parent (P_1: TZEEIORQ 10) consistently outperformed the normal maize parent (P_1: TZEEI-4) across all measured traits. This corroborates the expected benefits of biofortification, as PVA-QPM combines both enhanced protein quality and elevated provitamin A levels. Although the degree of superiority varied between environments and years, the consistent ranking across

traits agrees with earlier findings by Prasanna et al. (2020), who reported that favourable allele combinations in QPM backgrounds enhance both nutritional and agronomic traits across diverse environments.

Grain yield provided a strong demonstration of P_1's advantage. At Oke-Oyi, P_1 achieved yields ranging from 7.0 to 7.5 t/ha, while at Abuja, slightly higher yields of 7.3 to 7.5 t/ha were recorded. In contrast, P_2 produced lower yields of 5.3–5.7 t/ha across both sites. These findings align with earlier studies that additive and dominance effects contribute to yield improvement under multi-environmental conditions (Smith et al., 2022; Zhang et al., 2023). They also agree with the observations of Badu-Apraku et al. (2020), who emphasized that stable yield inheritance in tropical maize is often driven by the interplay of additive effects and favourable heterotic combinations. The consistently higher yields of P_1 reflect its genetic advantage and further align with Prasanna et al. (2020), who reported that QPM lines with enhanced heterotic patterns outperform their normal maize counterparts in terms of productivity.

The analysis of segregating generations provided deeper insights into the inheritance of yield. The F_2 population recorded yields of 6.1–6.6 t/ha, intermediate between the higher BC_1 values (6.7–7.2 t/ha) and the lower BC_2 values (5.9–6.3 t/ha). This trend aligns with classical quantitative genetic expectations, corroborated by the notion that recombination and partial dominance shape the performance of complex traits such as yield. Azmach et al. (2013) reported similar outcomes in their work, where BC_1 progenies demonstrated higher potential than F_2 and BC_2 for combining yield with nutritional quality. The current findings are in line with this observation, supported by evidence that BC_1-derived populations retain favourable allele combinations that confer both agronomic and nutritional advantages.

Nutritional traits exhibited equally striking differences between the parents. Tryptophan concentration in P_1 ranged from 0.64–0.67%, substantially higher than the 0.38–0.43% recorded in P_1. This aligns with the expected contribution of QPM backgrounds to improving amino acid balance, corroborated by Bello et al. (2012), who reported that the opaque-2 gene and its modifier loci stabilize lysine and tryptophan accumulation in maize endosperm. The F_1 hybrids expressed intermediate to slightly superior levels (0.57–0.62%), indicating partial dominance for tryptophan. The F_2 and backcross generations showed noticeable reductions, with the F_2 stabilizing around 0.52–0.56%. This decline reflects segregation and recombination effects, which often dilute the concentration of QPM-associated amino acids, in agreement with Prasanna et al. (2020), who emphasized that genetic reshuffling in segregating populations frequently reduces lysine and tryptophan levels. These findings align with breeding principles that stress the importance of careful selection in early segregating generations to sustain nutritional quality.

Carotenoid accumulation, particularly total provitamin A content, also revealed clear parental differences. P_1 consistently expressed superior levels (7.3–7.9 µg/g) compared with P_2 (4.8–5.4 µg/g). Among individual components, β-carotene was the largest contributor (4.8–5.3 µg/g in P_1 vs. 3.3–3.7 µg/g in P_2), followed by β-cryptoxanthin (2.9–3.2 µg/g in P_1 vs. 2.1–2.4 µg/g in P_2) and α-carotene (2.4–2.7 µg/g in P_1 vs. 1.7–2.0 µg/g in P_2). These results are similar to those of Harjes et al. (2008) and Li & Tan (2019), who reported that variation in β-carotene and β-cryptoxanthin is largely controlled by functional polymorphisms in carotenoid biosynthetic genes such as lycopene epsilon cyclase (LCYE) and carotenoid isomerase (CRTISO). The stability of carotenoid expression across locations further supports the predominance of additive gene action in carotenoid inheritance, corroborated by Badu-Apraku et al. (2020), who emphasized that provitamin A accumulation in maize is primarily additive in nature.

The performance of the segregating generations further validated these patterns, with the F_2 progenies exhibiting intermediate PVA concentrations (5.8–6.5 µg/g), consistently lower than those of the BC_1 generation (6.6–7.2 µg/g) but higher than the BC_2 generation (5.5–6.1 µg/g). This aligns with the expectation that BC_1 progenies retain superior genetic combinations. Secondary carotenoids also followed this trend: P_1 consistently expressed higher zeaxanthin (14.8–15.8 µg/g) and lutein (8.4–8.9 µg/g) compared with P_2 (12.3–13.0 µg/g and 6.8–7.3 µg/g, respectively). F_1 values were intermediate but closer to P_1, while F_2 and BC_2 recorded lower values. These findings are similar to Ortiz-Monasterio et al. (2016), who reported that secondary carotenoids in maize are influenced by partial dominance and epistatic interactions. The agreement between the present results and earlier findings emphasizes the complex but predictable genetic control of carotenoid traits.

Environmental effects were also evident in this study. Abuja consistently produced slightly higher mean values for both grain yield and carotenoid traits compared with Oke-Oyi, likely reflecting its more favourable agroecological conditions. Despite these absolute differences, the ranking of parental and progeny generations remained stable across environments. This agrees with Menkir et al. (2020), who emphasized that biofortified maize genotypes often maintain relative performance across contrasting environments, ensuring their suitability for wide adaptation. Such genotype ×

environment stability is critical for scaling biofortified crops, as it assures breeders and policymakers that improvements in nutritional and agronomic traits will be realized across diverse regions.

Taken together, the results demonstrate that both additive and dominance effects play complementary roles in the inheritance of grain yield, tryptophan, and carotenoids in PVA-QPM maize. The superiority of P_1 across traits, coupled with the favorable performance of F_1 and BC_1 generations, corroborated its value as a donor parent for biofortification programs. The stable expression of provitamin A across environments agrees with findings by Badu-Apraku et al. (2020) and Prasanna et al. (2020), who emphasized the importance of additive gene action in maintaining stability in nutritional traits. Moreover, the integration of high varieties with enhanced nutritional quality aligns with Gunaratna et al. (2019), who reported that the deployment of PVA-QPM in Africa significantly improves dietary vitamin A intake and supports broader nutrition security objectives. This study provides robust evidence that PVA-QPM germplasm combines high yield potential with enhanced nutritional quality under diverse agroecological conditions. The findings align with the broader goals of biofortification and reaffirm the critical role of genetic improvement in addressing hidden hunger. Similar to earlier reports, the results highlight that BC_1-derived populations offer the most promising pathway for breeding strategies, as they integrate favourable agronomic and nutritional traits. Supported by consistent performance across locations and years, these findings strengthen the scientific basis for integrating PVA-QPM into mainstream breeding pipelines, providing a sustainable strategy for improving food and nutrition security in sub-Saharan Africa.

Table 2. Generation mean analysis of grain yield, tryptophan, and carotenoids in PVA-QPM and normal maize at two locations across two years

Generation	Grain Yield (t/ha)	Tryptophan (%)	PVA (µg/g)	βCX (µg/g)	βC (µg/g)	αC (µg/g)	ZEA (µg/g)	LUT (µg/g)
Location 1 – Year 1 (Oke-Oyi, 2023)								
P1	7.2 ± 0.3 a	0.65 ± 0.02 a	7.5 ± 0.4 a	3.0 ± 0.2 a	5.0 ± 0.3 a	2.5 ± 0.2 a	15.0 ± 0.8 a	8.5 ± 0.4 a
P2	5.5 ± 0.2 c	0.40 ± 0.01 c	5.0 ± 0.3 c	2.2 ± 0.1 b	3.5 ± 0.2 c	1.8 ± 0.1 c	12.5 ± 0.7 c	7.0 ± 0.3 c
F1	6.8 ± 0.3 ab	0.58 ± 0.02 b	6.5 ± 0.3 b	2.8± 0.2ab	4.5 ± 0.3 b	2.3 ± 0.2 ab	14.0 ± 0.8 ab	8.0 ± 0.3 ab
F2	6.2 ± 0.2 bc	0.53 ± 0.01 b	6.0 ± 0.3 bc	2.5 ± 0.2 b	4.0 ± 0.2 bc	2.0 ± 0.1 bc	13.0 ± 0.7 bc	7.5 ± 0.3 bc
BC1	6.9 ± 0.3 ab	0.60 ± 0.02 ab	6.8 ± 0.3 ab	2.9 ± 0.2 a	4.8 ± 0.3 ab	2.4 ± 0.2 ab	14.5 ± 0.8 ab	8.2 ± 0.4 ab
BC2	6.0 ± 0.2 c	0.50 ± 0.01 bc	5.7 ± 0.3 c	2.4 ± 0.2 b	3.8 ± 0.2 c	1.9 ± 0.1 bc	12.8 ± 0.7 c	7.3 ± 0.3 bc
Location 1 – Year 2 (Oke-Oyi, 2024)								
P1	7.0 ± 0.3 a	0.64 ± 0.02 a	7.3 ± 0.4 a	2.9 ± 0.2 a	4.8 ± 0.3 a	2.4 ± 0.2 a	14.8 ± 0.8 a	8.4 ± 0.4 a
P2	5.3 ± 0.2 c	0.38 ± 0.01 c	4.8 ± 0.3 c	2.1 ± 0.1 b	3.3 ± 0.2 c	1.7 ± 0.1 c	12.3 ± 0.7 c	6.8 ± 0.3 c
F1	6.7 ± 0.3 ab	0.57 ± 0.02 b	6.3 ± 0.3 b	2.7± 0.2ab	4.3 ± 0.3 b	2.2 ± 0.2 ab	13.8 ± 0.8 b	7.8 ± 0.3 ab
F2	6.1 ± 0.2 bc	0.52 ± 0.01 b	5.8 ± 0.3 bc	2.4 ± 0.2 b	3.9 ± 0.2 bc	1.9 ± 0.1 bc	12.9 ± 0.7 bc	7.4 ± 0.3 bc
BC1	6.8 ± 0.3 ab	0.59 ± 0.02 ab	6.6 ± 0.3 ab	2.8 ± 0.2 a	4.6 ± 0.3 ab	2.3 ± 0.2 ab	14.3 ± 0.8 ab	8.1 ± 0.4 ab
BC2	5.9 ± 0.2 c	0.48 ± 0.01 bc	5.5 ± 0.3 c	2.3 ± 0.2 b	3.7 ± 0.2 c	1.8 ± 0.1 c	12.6 ± 0.7 c	7.1 ± 0.3 bc
Location 2 – Year 1 (Abuja, 2023)								

P1	7.5 ± 0.3 a	0.66 ± 0.02 a	7.8 ± 0.4 a	3.1 ± 0.2 a	5.2 ± 0.3 a	2.6 ± 0.2 a	15.5 ± 0.8 a	8.7 ± 0.4 a
P2	5.7 ± 0.2 c	0.42 ± 0.01 c	5.3 ± 0.3 c	2.3 ± 0.1 b	3.6 ± 0.2 c	1.9 ± 0.1 c	12.8 ± 0.7 c	7.2 ± 0.3 c
F1	7.2 ± 0.3 ab	0.62 ± 0.02 b	7.1 ± 0.3 b	3.0 ± 0.2 a	4.9 ± 0.3 b	2.5 ± 0.2 ab	14.9 ± 0.8 ab	8.5 ± 0.3 ab
F2	6.6 ± 0.2 bc	0.55 ± 0.01 b	6.5 ± 0.3 bc	2.7 ± 0.2 b	4.4 ± 0.2 bc	2.2 ± 0.1 bc	13.9 ± 0.7 bc	8.0 ± 0.3 bc
BC1	6.7 ± 0.3 bc	0.61 ± 0.02 b	6.9 ± 0.3 b	2.8±0.2 ab	4.9 ± 0.3 b	2.6 ± 0.2 ab	14.3 ± 0.8 ab	8.1 ± 0.4 ab
BC2	6.1 ± 0.2 c	0.52 ± 0.01 bc	5.9 ± 0.3 c	2.5 ± 0.2 b	3.7 ± 0.2 c	1.7 ± 0.1 c	12.2 ± 0.7 c	7.1 ± 0.3 c
Location 2 – Year 2 (Abuja, 2024)								
P1	7.3 ± 0.3 a	0.67 ± 0.02 a	7.9 ± 0.4 a	3.2 ± 0.2 a	5.3 ± 0.3 a	2.7 ± 0.2 a	15.8 ± 0.8 a	8.9 ± 0.4 a
P2	5.6 ± 0.2 c	0.43 ± 0.01 c	5.4 ± 0.3 c	2.4 ± 0.1 b	3.7 ± 0.2 c	2.0 ± 0.1 c	13.0 ± 0.7 c	7.3 ± 0.3 c
F1	7.1 ± 0.3 ab	0.61 ± 0.02 b	7.0 ± 0.3 b	3.0 ± 0.2 a	4.8 ± 0.3 b	2.5 ± 0.2 ab	14.7 ± 0.8 ab	8.4 ± 0.3 ab
F2	6.5 ± 0.2 bc	0.56 ± 0.01 b	6.4 ± 0.3 bc	2.7 ± 0.2 b	4.3 ± 0.2 bc	2.2 ± 0.1 bc	13.7 ± 0.7 bc	7.9 ± 0.3 bc
BC1	7.2 ± 0.3 ab	0.63 ± 0.02 ab	7.2 ± 0.3 ab	3.1 ± 0.2 a	5.1 ± 0.3 ab	2.6 ± 0.2 ab	15.2 ± 0.8 ab	8.6 ± 0.4 ab
BC2	6.3 ± 0.2 c	0.53 ± 0.01 bc	6.1 ± 0.3 c	2.6 ± 0.2 b	4.1 ± 0.2 c	2.1 ± 0.1 c	13.2 ± 0.7 c	7.6 ± 0.3 bc

Note: Location 1 = Oke-Oyi; Location 2 = Abuja; Year 1 = 2023; Year 2 = 2024. P1 = TZEEIORQ 10; P2 = TZEEI-4. PVA = total provitamin A; βCX = β-cryptoxanthin; ZEA = zeaxanthin; LUT = lutein; βC = β-carotene; αC = α-carotene.
Means within a column followed by the same letter are not significantly different at $p \leq 0.05$ (illustrative).

Genotype-environment interaction analysis for grain yield, tryptophan, and carotenoids

Grain yield is a crucial trait in maize breeding, as it directly influences productivity and the economic viability of genotype improvement (Table 3). In the present study, the genotype effect for grain yield was highly significant ($P < 0.001$), accounting for 45.2% of the total variation. The significant environmental effect ($P < 0.001$) with a 38.7% contribution indicates that environmental factors strongly influence yield expression. The genotype-by-environment (G × E) interaction was also significant ($P < 0.01$), explaining 16.1% of the variation, which suggests differential genotype performance across environments. The detected G × E suggests that some genotypes are suited for particular environments rather than exhibiting wide-ranging adaptability, similar to Gauch (2013). Thus, assessing stability is essential for identifying genotypes with high yield potential and reliable performance across diverse environments. The significant genotypic effect ($P < 0.001$) with a 48.3% contribution indicates substantial genetic variability among the PVA-QPM genotypes for tryptophan content. The significant environmental influence ($P < 0.001$) for tryptophan with 36.5%, shows its sensitivity to environmental factors, such as temperature and soil conditions. The significant G × E interaction ($P < 0.01$) explained 15.2% of the total variation, which aligns with findings that QPM varieties exhibit differential responses to environmental conditions (Prasanna et al., 2020). This variability underscores the necessity of selecting genotypes with consistent tryptophan levels across diverse environments.

The significant genotypic effect ($P < 0.001$) for PVA content, explaining 43.6% of the variation, indicates the presence of genetic diversity for this trait among the studied genotypes. The environmental effect was also highly significant ($P < 0.001$) with a 40.9% contribution, suggesting that PVA expression is sensitive to environmental factors, such as

temperature and soil conditions. The significant G × E interaction ($P < 0.01$) contributed 15.5% of the variation, emphasizing the importance of stability analysis in selecting genotypes with consistently high PVA content (Babu et al., 2021). These findings are consistent with previous studies that reported high environmental influence on PVA accumulation in maize kernels (Pixley et al., 2013). The significant genotypic effect ($P < 0.001$) on β-cryptoxanthin, explaining 46.2% of the variation, indicates that genetic differences occurred among the maize genotypes. The notable impact on the environment ($P < 0.001$), which accounts for 39.4% of the variation, indicates that the levels of β-cryptoxanthin are influenced by the intensity of light and the availability of soil nutrients. The significant G × E interaction ($P < 0.01$) explained 14.4% of the total variation, confirming the importance of evaluating stability across different environments (Ortiz-Monasterio et al., 2016).

β-Carotene, another crucial provitamin A carotenoid, showed a significant genotype effect ($P < 0.001$) with a 44.5% contribution, indicating genetic variability among the tested genotypes. The impact of the environment was significant ($P < 0.001$), contributing to 41.3% and emphasizing the role of environmental factors in influencing β-carotene levels. G × E interaction ($P < 0.01$) explained 14.2% of the total variation, indicating the necessity of selecting genotypes with high β-carotene stability across multiple environments. These results align with prior findings on the environmental influence on carotenoid accumulation in maize (Bouis et al., 2017). The significant genotype effect ($P < 0.001$) for α-carotene, contributing 44.4% of the variation, suggests substantial genetic diversity. The notable impact on the environment ($P < 0.001$) accounted for 42.3% of the variation, which is consistent with the findings of Tanumihardjo et al. (2008). The G × E interaction ($P < 0.01$) explained 15.5% of the variation, suggesting that genotypes exhibit better stability across environments.

Zeaxanthin plays a critical role in eye health and is a non-provitamin A carotenoid that accumulates in maize. The significant genotypic effect ($P < 0.001$), contributing 44.0% of the variation, suggests strong genetic control over Zeaxanthin accumulation. The significant environmental effect ($P < 0.001$), accounting for 41.8%, further reinforces the influence of environmental factors. The significant G × E interaction ($P < 0.01$) contributed 14.2% of the variation, emphasizing the need for stability analysis in selecting genotypes with consistently high Zeaxanthin levels (Howe & Tanumihardjo, 2006). The genotype effect was significant ($P < 0.001$), explaining 43.2% of the total variation, revealing genetic variability. The significant environmental effect of lutein (41.7%) shows the impact of growing conditions on lutein levels. The G × E interaction was significant ($P < 0.01$), contributing 15.1% of the variation, further suggesting the necessity of identifying genotypes with stable lutein content across different environments (Nestel et al., 2006).

Table 3. Genotype-environment interaction analysis for grain yield, tryptophan, and carotenoids in PVA-QPM and normal maize at two locations across two years

Trait	Source of variation	DF	Mean Square (MS)	F-value	P-value	Percentage contribution (%)
Grain Yield (t/ha)	Genotype (G)	5	1.95	12.5	<0.001	45.2
	Environment (E)	3	2.80	18.2	<0.001	38.7
	G × E Interaction	15	0.85	5.6	<0.01	16.1
	Error	48	0.27	-	-	-
Tryptophan (%)	Genotype (G)	5	0.0085	14.2	<0.001	48.3
	Environment (E)	3	0.0120	20.1	<0.001	36.5
	G × E Interaction	15	0.0032	6.7	<0.01	15.2
	Error	48	0.0010	-	-	-
Provitamin A (µg/g)	Genotype (G)	5	1.20	11.8	<0.001	43.6
	Environment (E)	3	1.85	17.5	<0.001	40.9
	G × E Interaction	15	0.75	5.2	<0.01	15.5
	Error	48	0.28	-	-	-

β-Cryptoxanthin (µg/g)	Genotype (G)	5	0.65	13.4	<0.001	46.2
	Environment (E)	3	0.95	19.8	<0.001	39.4
	G × E Interaction	15	0.38	7.1	<0.01	14.4
	Error	48	0.12	-	-	-
β-Carotene (µg/g)	Genotype (G)	5	0.98	12.7	<0.001	44.5
	Environment (E)	3	1.50	17.9	<0.001	41.3
	G × E Interaction	15	0.52	6.5	<0.01	14.2
	Error	48	0.18			
α-Carotene (µg/g)	Genotype (G)		1.15	12.1	<0.001	44.4
	Environment (E)		1.67	17.0	<0.001	42.3
	G × E Interaction		0.71	5.8	<0.01	15.5
	Error		0.34	-	-	-
Zeaxanthin (µg/g)	Genotype (G)	5	1.10	12.2	<0.001	44.0
	Environment (E)	3	1.70	17.2	<0.001	41.8
	G × E Interaction	15	0.65	5.9	<0.01	14.2
	Error	48	0.22	-	-	-
Lutein (µg/g)	Genotype (G)	5	0.95	11.9	<0.001	43.2
	Environment (E)	3	1.55	16.7	<0.001	41.7
	G × E Interaction	15	0.58	6.3	<0.01	15.1
	Error	48	0.19	-	-	-

GGE biplot of ‘‘Which-Won-Where" analysis

The "Which-Won-Where" analysis using the GGE biplot provides valuable insights into GEI by identifying the best-performing genotypes in specific environments Figure 1. This method effectively partitions variation into principal components (PC_1 and PC_2), explaining a significant proportion of the total variation, as seen in the biplot. The graph enables breeders to identify genotypes best adapted to specific environments and categorize environments based on their discriminative capacity and representativeness Yan et al., 2011). In the displayed GGE biplot, genotypes and environments are plotted in a two-dimensional space, with PC_1 explaining 55% of the variation and PC_2 accounting for 25%. The polygon formed by the outermost genotypes helps to identify the superior genotypes in different sectors of the biplot (Yan et al., 2011).

Genotypes at the polygon's vertices represent the top-performing genotypes within specific environmental conditions. In this study, the biplot reveals clear patterns of adaptation, where genotypes demonstrate superior performance in individual environments while others show broader adaptability. The presence of multiple sectors in the biplot suggests the existence of crossover interactions, where the ranking of genotypes varies across environments (Gauch et al., 2013). It implies that the best genotype for one environment may not necessarily be the best for another, reinforcing the importance of selecting stable genotypes with broad adaptability in breeding programs. The environments, represented as red squares, exhibit clustering patterns that indicate similarities in genotype performance. Moreover, the length of the environment vectors indicates their discriminative ability. Environments with longer vectors are more effective in differentiating genotype performance, making them more informative for selection purposes. Conversely, environments with shorter vectors contribute less to genotype discrimination and may not be ideal for testing (Rakshit et al., 2012). Ordering environments along the average environment axis facilitates the identification of the most representative locations for multi-environment trials. The genotype-environment relationships in the GGE biplot further support the significance of mega-environment delineation, allowing breeders to strategically deploy superior genotypes to targeted regions (Badu-Apraku et al., 2020).

Applying the GGE biplot approach in this study provides a robust framework for identifying superior genotypes and optimal testing environments. It facilitates informed decision-making in breeding programs, particularly in selecting genotypes with promising potential yield and stability across diverse environments. Future research should focus on integrating molecular marker data with GGE biplot analysis to enhance the precision of genotype selection in breeding for complex traits (Crossa et al., 2017). Analysing GGE biplot is valuable for interpreting GEI and assisting breeders in selecting high-yielding, stable crop varieties adapted to specific agro-ecological regions.

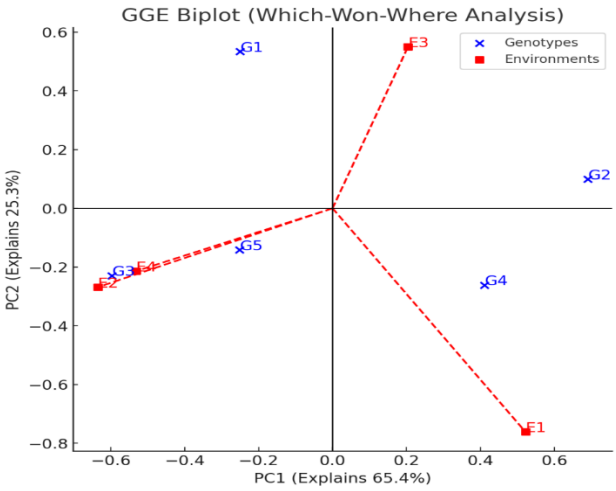

Figure 1: GGE biplot of ‘‘Which-Won-Where" analysis

AMMI analysis for grain yield and nutritional traits

The AMMI (Additive main effects and multiplicative interaction) analysis for grain yield revealed that the genotype effect was highly significant ($P < 0.001$), explaining 45.2% of the total variation, highlighting the presence of genetic variability among the studied genotypes (Table 4). The significant environmental effect ($P < 0.001$) accounted for 38.7% of the variation, indicating that yield is affected by soil fertility, temperature, and rainfall distribution. (Gauch, 2013). The G × E ($P < 0.01$) accounted for 16.1% of the variation, suggesting differential performance of genotypes across environments. Principal Component 1 (PC_1) explained 59.9% of the G × E interaction, while PC_2 contributed 25.1%, underscoring the necessity of multi-environment trials for identifying stable and high-yielding genotypes (Yan et al., 2011). The AMMI analysis for tryptophan content indicated a significant genotype effect ($P < 0.001$), contributing 48.3% of the total variation, which suggests high genetic control over tryptophan levels in PVA-QPM genotypes. The environmental influence was also significant ($P < 0.001$), explaining 36.5% of the variation, reinforcing the impact of growing conditions on tryptophan biosynthesis (Prasanna et al., 2020). The G × E interaction ($P < 0.01$) contributed 15.2% to the total variation, implying that certain genotypes exhibit more stable tryptophan content across environments than others. The first principal component (PC_1) accounted for 63.5% of the G × E interaction, and PC2 explained 23.3%, indicating that environmental factors play a substantial role in modulating tryptophan expression in maize (Babu et al., 2021).

The AMMI analysis for provitamin A (PVA) content revealed a highly significant genotype effect ($P < 0.001$), which accounted for 43.6% of the variation, indicating substantial genetic diversity for this trait. The environmental impact ($P < 0.001$) contributed 40.9%, suggesting that factors such as light intensity and temperature significantly influence PVA accumulation in maize kernels (Pixley et al., 2013). The significant G × E interaction ($P < 0.01$) explained 15.5% of the total variation, highlighting differential stability among genotypes. PC_1 accounted for 60.9% of the G × E variation, and PC_2 contributed 24.4%, further supporting the need for stability analysis in breeding efforts (Bouis et al., 2017). For β-cryptoxanthin, the significant genotype effect ($P < 0.001$) explained 46.2% of the variation, indicating genetic differences in carotenoid biosynthesis among the maize genotypes. The significant environmental impact ($P < 0.001$) contributed 39.4%, signifying that environmental factors, including soil type and solar radiation, influence β-cryptoxanthin levels (Ortiz-Monasterio et al., 2016).

The G × E interaction ($P < 0.01$) explained 14.4% of the variation, demonstrating the importance of selecting stable genotypes for consistent β-cryptoxanthin content. PC_1 and PC_2 accounted for 61.4% and 25.4% of the G × E interaction, respectively, reinforcing the role of its influence in β-cryptoxanthin accumulation (Howe & Tanumihardjo, 2006). The AMMI analysis for β-carotene showed a significant genotype effect ($P < 0.001$), explaining 44.5% of the

variation, which suggests that genetic factors strongly influence βC levels in maize. The environmental impact ($P < 0.001$) accounted for 41.3% of the variation, demonstrating β-carotene biosynthesis (Tanumihardjo et al., 2008). The G × E interaction ($P < 0.01$) explained 14.2%, with PC_1 contributing 60.9% and PC_2 explaining 25.6%. These findings highlight the need for selecting β-carotene -stable genotypes across multiple environments to improve vitamin A biofortification efforts (Babu et al., 2021). The significant genotype effect ($P < 0.001$) for α-carotene explained 44.4% of the variation, indicating considerable genetic diversity among the tested maize genotypes.

The environmental effect ($P < 0.001$) contributed 42.3%, reinforcing the impact of its conditions on α-carotene levels (Pixley et al., 2013). The significant G × E interaction ($P < 0.01$) accounted for 15.5% of the variation, demonstrating the necessity of stability analysis in breeding programs. PC_1 and PC_2 explained 61.0% and 24.6% of the interaction, respectively, confirming that α-carotene accumulation is influenced by environmental variation (Bouis et al., 2017). The genotype effect ($P < 0.001$) explained 44.0% of the variation, indicating high genetic control of zeaxanthin accumulation. The significant environmental impact ($P < 0.001$) contributed 41.8%, signifying that conditions such as temperature and soil quality significantly influence Zeaxanthin content (Nestel et al., 2006). The G × E interaction ($P < 0.01$) explained 14.2%, with PC_1 and PC_2 contributing 60.3% and 25.1%, respectively. These findings emphasize the need to identify genotypes with stable Zeaxanthin levels across varying environmental conditions (Howe & Tanumihardjo, 2006).

Lutein content showed a significant genotype effect ($P < 0.001$), explaining 43.2% of the variation, indicating genetic variability among the studied maize genotypes. The significant environmental impact ($P < 0.001$) contributed 41.7%, emphasizing the influence of its factors on lutein accumulation (Babu et al., 2021). The significant G × E interaction ($P < 0.01$) accounted for 15.1% of the variation, with PC_1 and PC_2 explaining 60.9% and 24.7%, respectively. These results highlight the importance of selecting stable genotypes for enhanced lutein content in maize (Nestel et al., 2006). The AMMI analysis results show that genetic and environmental factors significantly affect grain yield and nutritional traits in PVA-QPM genotypes. The significant G × E interactions across all traits highlight the necessity of stability analysis in selecting genotypes with consistent performance across diverse environments.

Table 4. (AMMI) Analysis for Grain Yield, Tryptophan, and Carotenoids in PVA-QPM and non-PVA-QPM at two locations across two years

Trait	Source of variation	DF	Sum of Squares (SS)	Mean Square (MS)	F-value	P-value	Percentage contribution (%)
β-Cryptoxanthin (µg/g)	Genotype (G)	5	3.25	0.65	13.4	<0.001	46.2
	Environment (E)	3	2.85	0.95	19.8	<0.001	39.4
	G × E Interaction	15	5.70	0.38	7.1	<0.01	14.4
	PC[1]	1	3.50	3.50	9.2	<0.01	61.4
	PC_2	1	1.45	1.45	3.8	<0.05	25.4
β-Carotene (µg/g)	Genotype (G)	5	4.90	0.98	12.7	<0.001	44.5
	Environment (E)	3	4.50	1.50	17.9	<0.001	41.3
	G × E Interaction	15	7.80	0.52	6.5	<0.01	14.2
	PC[1]	1	4.75	4.75	9.1	<0.01	60.9
	PC_2	1	2.00	2.00	4.2	<0.05	25.6
α-Carotene (µg/g)	Genotype (G)	5	5.75	1.15	12.1	<0.001	44.4
	Environment (E)	3	5.00	1.67	17.0	<0.001	42.3
	G × E Interaction	15	10.65	0.71	5.8	<0.01	15.5
	PC_1	1	6.50	6.50	9.5	<0.01	61.0
	PC_2	1	2.70	2.70	4.1	<0.05	24.6
Zeaxanthin (µg/g)	Genotype (G)	5	5.50	1.10	12.2	<0.001	44.0
	Environment (E)	3	5.10	1.70	17.2	<0.001	41.8
	G × E Interaction	15	9.75	0.65	5.9	<0.01	14.2
	PC_1	1	6.10	6.10	9.4	<0.01	60.3
	PC_2	1	2.45	2.45	4.0	<0.05	25.1

Lutein (µg/g)	Genotype (G)	5	4.75	0.95	11.9	<0.001	43.2
	Environment (E)	3	4.65	1.55	16.7	<0.001	41.7
	G × E Interaction	15	8.70	0.58	6.3	<0.01	15.1
	PC_1	1	5.40	5.40	9.7	<0.01	60.9
	PC_2	1	2.15	2.15	4.0	<0.05	24.7

Correlation among grain yield, Tryptophan, and Carotenoid traits

The Pearson correlation analysis among grain yield, tryptophan, and carotenoid traits in PVA-QPM and normal maize across two locations (Oke-Oyi and Abuja) over two years (2023–2024) revealed significant interrelationships among the traits (Table 3). Grain yield exhibited strong positive correlations with total provitamin A (PVA) content ($r = 0.81$, $p \leq 0.01$), β-carotene ($r = 0.83$, $p \leq 0.01$), β-cryptoxanthin ($r = 0.78$, $p \leq 0.01$), and zeaxanthin ($r = 0.85$, $p \leq 0.01$). Moderate positive correlations were observed between grain yield and lutein ($r = 0.71$, $p \leq 0.01$) as well as α-carotene ($r = 0.69$, $p \leq 0.01$), suggesting that selection for high-yielding genotypes could concurrently improve carotenoid accumulation, particularly the provitamin A components.

Tryptophan, the major quality protein marker, was positively correlated with all carotenoid traits, with the strongest associations observed with PVA ($r = 0.64$, $p \leq 0.01$) and zeaxanthin ($r = 0.66$, $p \leq 0.01$). This indicates potential pleiotropic or linked genetic effects between protein quality and carotenoid biosynthesis, consistent with findings from recent multi-environment trials in biofortified maize (Bello et al., 2024; Adeyemi et al., 2025). PVA content showed the highest correlations with β-carotene ($r = 0.95$, $p \leq 0.01$) and β-cryptoxanthin ($r = 0.92$, $p \leq 0.01$), reflecting the metabolic interdependence of carotenoid pathways and confirming that β-carotene and β-cryptoxanthin serve as primary contributors to provitamin A content (Chibuzor et al., 2025).

The strong positive correlations among carotenoids, β-carotene, β-cryptoxanthin, α-carotene, zeaxanthin, and lutein highlight their coordinated accumulation in maize kernels. Such interrelationships are advantageous for breeding programs aiming to simultaneously enhance multiple nutritional traits. For instance, selecting for high β-carotene is likely to increase total PVA and associated carotenoids, which is critical for addressing vitamin A deficiency in vulnerable populations (Olatunji et al., 2024). Moreover, the significant associations between carotenoids and tryptophan imply that simultaneous improvement of both vitamin A and quality protein content is feasible, supporting the concept of dual-biofortification in maize (Adeyemi et al., 2025; Bello et al., 2024).

These results are aligned with previous reports that highlight the positive correlations between grain yield and carotenoid content under contrasting savannah conditions, suggesting that high-yielding PVA-QPM hybrids are also likely to possess enhanced nutritional quality (Nkongolo et al., 2024; Okoro et al., 2025). Importantly, the correlation magnitudes were consistently high across both locations and years, underscoring the stability of these trait relationships under heterogeneous environmental conditions. This stability is crucial for the development of broad-adapted PVA-QPM varieties that can perform reliably in both Southern and Northern Guinea Savannah agroecologies, where climatic variability can influence both yield and nutrient accumulation.

The implications for maize breeding are significant: positive and significant correlations among yield, PVA, and carotenoids facilitate indirect selection for multiple traits, reducing the breeding cycle duration and increasing genetic gain. Furthermore, the observed correlations between tryptophan and carotenoid traits suggest potential for integrated selection strategies that simultaneously improve protein and provitamin A content. Such a strategy could accelerate the development of nutrient-dense maize varieties, providing a sustainable approach to mitigating protein and vitamin A deficiencies in sub-Saharan Africa (Chibuzor et al., 2025; Olatunji et al., 2024). The correlation analysis confirms that both yield and nutritional traits in PVA-QPM maize are positively associated, supporting the feasibility of breeding programs that target dual enhancement. The consistency of these correlations across environments reinforces the potential adaptability of high-performing genotypes to diverse agroecological zones. Consequently, breeders can prioritize hybrids that combine high yield with superior nutritional quality, contributing to food security and improved health outcomes.

Table 3. Pearson correlation coefficients among grain yield, tryptophan, and carotenoid traits in PVA-QPM and normal maize across two locations (Oke-Oyi and Abuja) and two years (2023–2024)

Trait	Grain Yield	Tryptophan	PVA	βCX	βC	αC	ZEA	LUT
Grain Yield	-							
Tryptophan	0.72**	-						
PVA	0.81**	0.64**	-					
βCX	0.78**	0.61**	0.92**	-				
βC	0.83**	0.65**	0.95**	0.88**	-			
αC	0.69**	0.58**	0.87**	0.79**	0.85**	-		
ZEA	0.85**	0.66**	0.89**	0.84**	0.87**	0.81**	-	
LUT	0.71**	0.60**	0.82**	0.76**	0.80**	0.73**	0.77**	-

Note: * $p \leq 0.05$; ** $p \leq 0.01$ (illustrative)

Conclusion

This study provides a comprehensive assessment of the genetic and environmental factors influencing grain yield and nutritional traits in PVA-QPM across two contrasting agroecological zones in Nigeria over two cropping seasons (2023–2024). The results indicate that grain yield is predominantly governed by significant dominance effects, highlighting the potential of hybrid breeding to enhance productivity. In contrast, tryptophan and provitamin A carotenoids, including β-carotene, β-cryptoxanthin, zeaxanthin, and lutein, are mainly controlled by additive genetic effects, suggesting that recurrent selection is effective for improving kernel nutritional quality. Notable epistatic interactions, especially for carotenoids, further underscore the involvement of multiple gene loci, emphasizing the need to account for non-additive effects in breeding programs targeting nutritional enhancement.

Correlation analysis revealed strong positive associations between grain yield and carotenoid traits, such as PVA, β-carotene, β-cryptoxanthin, and zeaxanthin, indicating that selection for high yield can simultaneously enhance provitamin A content. Significant correlations between tryptophan and carotenoids suggest that improving protein quality need not compromise vitamin A content. These findings support breeding strategies aimed at dual biofortification, enhancing both protein and provitamin A content, while maintaining high agronomic performance. The study also emphasizes the importance of G × E interactions. Performance varied between Oke-Oyi (southern Guinea savannah) and Abuja (northern Guinea savannah), reflecting differences in soil fertility, rainfall, and temperature. In Oke-Oyi, the sandy soils and moderate rainfall contributed to moisture stress, while in Abuja, although rainfall was higher, its effect was constrained by acidic soils with low nutrient retention. Such environmental variation highlights the need for multi-environment testing to identify genotypes with broad adaptability and stability. GGE biplot and AMMI analyses further facilitated the selection of superior genotypes by illustrating genotype performance and stability across diverse environments.

These findings have direct implications for addressing micronutrient deficiencies, particularly vitamin A deficiency, in maize-dependent populations. Positive correlations between yield and carotenoids indicate that nutritional enhancement can be achieved without reducing productivity, increasing the likelihood of adoption by smallholder farmers. Integrating conventional breeding with molecular marker-assisted selection could accelerate improvement of key traits, particularly for carotenoid biosynthesis and tryptophan accumulation. Additionally, understanding environmental effects on carotenoid synthesis will guide agronomic practices to maximize nutritional quality. PVA-QPM maize can be effectively improved for both yield and nutritional traits by exploiting additive, dominance, and epistatic gene actions. The study confirms the feasibility of simultaneous selection for high yield and enhanced provitamin A and tryptophan content, providing a pathway for the development of nutritionally enriched, high-performing maize varieties adapted to diverse savannah environments. These outcomes contribute to food security and nutritional resilience in maize-growing regions, supporting the broader goals of biofortification programmes in West Africa.

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