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# Genetic insights of carotenoids, quality protein, and grain yield in biofortified tropical maize

Bashir Omolaran Bello\*<sup>1</sup> 

<sup>1</sup>Department of Agronomy, Faculty of Agriculture, University of Abuja, Nigeria

\*Corresponding author: [obbello2002@yahoo.com](mailto:obbello2002@yahoo.com)

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## ABSTRACT

Biofortified Provitamin A Quality Protein Maize (PVA-QPM) varieties enhance nutritional quality, addressing malnutrition in maize-based diets and strengthening food security in West and Central Africa. This study assessed genetic relationships among grain yield, tryptophan, and carotenoids in ten early-maturing PVA-QPM inbreds and two commercial checks evaluated under rainfed conditions in Nigeria across 2022 and 2023. Data were analyzed for variance components, genetic parameters, and heritability. Commercial checks outperformed inbreds in grain yield, with TZEIORQ 47 producing 5.99 t ha<sup>-1</sup>, only 6.6% lower than the highest-yielding check, Oba Super 4. In contrast, inbreds expressed higher tryptophan, with TZEIORQ 13 recording a 49% advantage over checks. Substantial genetic variability was observed across traits, with high heritability for grain yield and carotenoids and moderate heritability for tryptophan. Correlation analysis revealed that grain yield was positively associated with total carotenoids ( $r = 0.62, p < 0.05$ ) but negatively correlated with tryptophan ( $r = -0.47, p < 0.05$ ), highlighting a trade-off between yield potential and protein quality. Carotenoids and tryptophan were not significantly correlated, indicating that they are inherited independently. These results provide critical insights for simultaneous improvement of yield and nutritional traits, supporting targeted breeding strategies for biofortified maize in sub-Saharan Africa.

**Keywords:** biofortified, genetic parameters, inbreds, maize, nutritional quality



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## 1. Introduction

Maize is a crucial crop that plays a significant role in global food security and economic development in sub-Saharan Africa (SSA) and the Americas [1]. In the Americas, maize and its products account for approximately 30% of the food supply. In Africa, this figure is even higher at 38% [2]. In SSA, maize is a major staple food and an important energy source, with intakes ranging from 52 to 450 g/ g/person/day [3]. It constitutes a major dietary staple, supplying a considerable proportion of daily caloric intake, protein, and essential micronutrients to the population. In Nigeria, per capita consumption is particularly high, ranging from 52 to 450 g per person per day. This wide range likely reflects the diversity of dietary patterns across different regions and socioeconomic groups within the country. Maize also plays a vital role in the livestock-to-meat cycle worldwide [4]-[5].

Maize is not only a significant source of calories but also provides essential micronutrients and phytochemicals that contribute to various health benefits and disease prevention [6]. It is particularly noted for its potential to help prevent chronic diseases such as cardiovascular disease, type 2 diabetes, and obesity due to its rich composition of vitamins, minerals, and bioactive compounds [3]. In many developing countries, especially in Africa, diets heavily reliant on maize indicate a lack of essential micronutrients. Common deficiencies include vitamin A and essential amino acids such as lysine and tryptophan [7]. Over two billion people in Africa suffer from one or more micronutrient deficiencies, which can lead to serious health issues such as retarded physical growth, impaired cognitive development, complications during pregnancy, diminished work capacity, and

increased morbidity and mortality rates [3]. To address these deficiencies, biofortified maize cultivars enriched with essential minerals and vitamins could be particularly impactful, especially in rural areas where access to diversified diets, dietary supplements, and fortified foods is limited. These biofortified Provitamin A Quality protein (PVA-QPM) varieties can provide critical nutrients that are often lacking in traditional maize-based diets, thereby improving overall health outcomes and food security in vulnerable populations.

The variation in kernel carotenoids and tryptophan among diverse maize germplasm is crucial for developing effective breeding strategies to enhance the nutritional quality of maize [8]. The genetic background of maize significantly influences the concentrations of these nutrients, and understanding the relationship among different carotenoid and tryptophan components can accelerate improvements to maximize health benefits in populations that rely heavily on maize as a staple food [9]. Research indicates substantial natural variation for carotenoids in maize, particularly in yellow maize kernels [10]. This variation can be exploited through plant breeding to enhance the micronutrient balance of maize. However, the carotenoid composition in PVA-QPM and normal maize germplasm remains underexplored, indicating the need for further assessment of genetic variability for grain yield, tryptophan, and carotenoid contents. The objective was to investigate the genetic relationships and interactions among grain yields, tryptophan, and carotenoid components of early PVA-QPM inbreds. This aims to enhance these components in tropical biofortified maize to combat malnutrition and improve food security in maize-dependent SSA regions.

## 2. Methods

### 2.1. The test sites and collection of maize inbreds

The Maize Improvement Programme at the International Institute of Tropical Agriculture (IITA), Ibadan, provided ten new early-maturing PVA-QPM inbred lines. Also, two commercial maize hybrid checks served as controls (Table 1). The inbred lines were evaluated at the Lower Niger River Basin Development Authority, Oke-Oyi, Nigeria, during the rainfed seasons of 2022 and 2023. The site is located in the southern Guinea savannah agroecological zone at coordinates 8°30'N and 8°36'E, with an elevation of 457 m above sea level. This region experiences a wide annual temperature range, fluctuating between 20 and 45 °C. The average relative humidity is 84%, complemented by an annual rainfall of 1002 mm.

**Table 1.** Description of ten early-maturing PVA-QPM inbreds and two commercial maize hybrid checks

Genotypes	Pedigree
TZEIORQ 5	2009-TZE OR2 DT STR-QPM S6 inb 2-2/3-2/3-1/4-3/3-1/1
TZEIORQ 44	2009-TZE OR2 DT STR-QPM S6 inb 35-2/3-3/3-4/4-1/4-1/1
TZEIORQ 23	2009-TZE OR2 DT STR QPM S6 inb 26-1/1-1/2-4/6-1/3-1/1
TZEIORQ 6	2009-TZE OR2 DT STR QPM S6 inb 2-2/3-2/3-2/4-1/5-1/1
TZEIORQ 29	2009-TZE OR2 DT STR QPM S6 inb 28-1/1-2/2-1/2-1/2-1/1
TZEIORQ 48	2009-TZE OR2 DT STR QPM S6 inb 41-1/2-1/3-1/2-3/3-1/1
TZEIORQ 13	2009-TZE OR2 DT STR-QPM S6 inb 7-1/3-1/2-2/2-3/3-1/1
TZEIORQ 7	2009-TZE OR2 DT STR-QPM S6 inb 2-2/3-2/3-3/4-1/3-1/1
TZEIORQ 26	2009-TZE OR2 DT STR-QPM S6 inb 26-1/1-1/2-6/6-2/3-1/1
TZEIORQ 47	2009-TZE OR2 DT STR-QPM S6 inb 35-3/3-3/3-1/3-2/2-1/1
Commercial maize hybrid checks	
Oba Super 2	Commercial yellow maize hybrid
Oba Super 4	Commercial yellow maize hybrid

### 2.2. Field test and management

After preparing the experimental site by ploughing, harrowing, and ridging, we planted three maize seeds per hill and later thinned them to two, with a spacing of 0.75 m × 0.5 m in a 4-row 5 m plot. This planting arrangement followed a Randomized Complete Block Design, resulting in a density of approximately 53,333 plants per hectare. We applied NPK (15:15:15) fertilizer two weeks after planting at a rate of 60 kg of nitrogen per hectare. Additionally, we performed a top dressing of 30 kg of nitrogen per hectare at 4 WAP. To control weed growth, we sprayed pre-emergence herbicides containing 3 kg per litre of Metolachlor and 170 grams per litre of Atrazine per hectare after land preparation.

### 2.3. Measurement of grain yield

There was an assumption of a shelling percentage of 80% for each plot of inbreds. The grain yield, adjusted to 15% moisture content, was calculated based on ear weight and then converted to kilograms per hectare.

### 2.4. Tryptophan content analysis

The maize grains from each genotype underwent grinding and defatting using the Kjeldahl device. To solubilize the protein, a papain hydrolysis method was employed [11]. The introduction of sulfuric acid catalyzed a chemical reaction in which iron ions oxidized acetic acid, resulting in the formation of glyoxylic acid. The response of glyoxylic acid with protein-free tryptophan indole ring produced violet-purple products, which were measured using a 560 nm spectrophotometer. Following the method described by [11], the spectrophotometer generated a tryptophan optical density standard curve, allowing for the conversion of concentration to tryptophan percentages.

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

The Optical density = Optical density 560-nm sample – Optical density 560-nm papain blanks mean

$$Tryptophan = Factor \times Corrected\ optical\ density\ at\ 560nm \quad (2)$$

Two separate readings were taken for each genotype. The analysis focused solely on tryptophan due to its association with lysine, and the correlation was greater than 0.90 [12]. Moreover, the determination of tryptophan was noted to be more cost-effective than that of lysine.

### 2.5. Carotenoid content analysis

The carotenoid analysis of dried maize endosperm utilized effective liquid chromatography [13]. Thirty maize grains were randomly selected from each cross-pollinated ear and subjected to dry-freezing at  $-80^{\circ}\text{C}$ . The collected maize grain samples were ground into fine powders ( $0.5\ \mu\text{m}$ ) for carotenoid analysis. The measured carotenoids included  $\alpha$ -carotene ( $\alpha\text{C}$ ), zeaxanthin (ZEA), lutein (LUT), (cis and trans isomers),  $\beta$ -cryptoxanthin ( $\beta\text{CX}$ ), and  $\beta$ -carotene ( $\beta\text{C}$ ) contents [12]. The total concentrations of  $\alpha\text{C}$ , ZEA, LUT,  $\beta\text{C}$ , and  $\beta\text{CX}$  were calculated as the overall sum of carotenoid contents. The PVA content for each sample was calculated using the formula  $\beta\text{C} + \frac{1}{2}(\alpha\text{C} + \beta\text{CX}) = \beta\text{CX}$  and  $\alpha\text{C}$  (assuming 50% PVA activity of  $\beta\text{C}$ ) [13]. Two replications of each trial were conducted for all sampled carotenoids and tryptophan contents.

### 2.6. Statistical analysis

We used the SAS program [14] to calculate the mean squares for a combined analysis of early PVA-QPM of lines for two rain-fed assessments, considering nine determined traits as follows:

$$kl = \mu + genotype_i + y_j + block(rep \times y)_{jkl} + rep(y)_{jk} + (genotype \times y)_{ij} + \epsilon_{ijkl} \quad (3)$$

where  $\mu$  = total mean,  $rep(year)_{jk}$  = effect of  $k$ th replication in the  $j$ th year,  $genotype\ i = i$ th genotypic effect,  $genotype \times year\ ij = j$ th year and  $i$ th = genotype-interactive effect,  $block(rep \times y)_{jkl}$  = effect of incomplete block  $l$ th within the  $j$ th year,  $\epsilon_{ijkl}$  = the variance error.

The least significant difference test was employed to measure the variations among the mean traits. The phenotypic variance ( $\delta^2p$ ) was partitioned into genetic ( $\delta^2g$ ), environmental ( $\delta^2e$ ), and genotype-by-year interaction ( $\sigma^2gy$ ) variance components according to the classic quantitative genetics equation:  $\delta^2p = \delta^2g + \delta^2e + \delta^2p$ . Broad sense ( $H^2$ ) and narrow heritability ( $h^2$ ) estimates were obtained using the estimated variance components according to the methods of [15]. Genetic advance as a percentage of the mean (GAM) was calculated according to [16]. Phenotypic, genotypic, and environmental coefficients of variation were determined according to [17].

## 3. Results and Discussion

### 3.1. Maize grain yield, tryptophan, and carotenoid contents

Grain yield showed significant differences ( $p < 0.05$ ) among the PVA-QPM inbreds and the commercial hybrid checks during the two rainfed seasons (Table 2). The commercial checks outperformed the inbreds in yield. Specifically, TZEIORQ 47 produced  $5.99\ \text{t ha}^{-1}$  (6.59%) lower than the leading check, Oba Super 4, with a grain yield of  $6.59\ \text{t ha}^{-1}$ . In contrast, tryptophan levels were notably higher in the inbreds compared to the checks, with TZEIORQ 13 exhibiting a 49% increase over the tryptophan content of the commercial hybrids.

[9] reported similar variations in tryptophan content among QPM genotypes, highlighting considerable variability in maize inbreds. This variability is crucial for plant breeders focused on genetic manipulation and cultivar development to enhance tryptophan content [6]. Additionally, carotenoid levels were high in the inbreds and the checks.

**Table 2.** Mean performance of ten early PVA-QPM lines for grain yield, tryptophan, and carotenoid contents evaluated across two years.

Inbreds	Grain yield (t ha <sup>-1</sup> )	Tryptophan (%)	Carotenoids (µg g <sup>-1</sup> DW)					
			PVA	βCX	βC	αC	ZEA	LUT
TZEIORQ 5	5.44	3.88	3.55	2.97	2.87	0.57	7.98	11.63
TZEIORQ 44	5.67	3.65	3.51	2.78	2.54	0.53	7.88	12.46
TZEIORQ 35	5.72	3.44	3.76	2.90	2.78	0.57	7.65	12.67
TZEIORQ 6	5.66	3.76	3.31	2.86	2.91	0.58	7.89	12.71
TZEIORQ 29	5.98	3.55	3.65	2.91	2.79	0.53	7.76	12.89
TZEIORQ 48	5.74	3.73	3.72	2.93	2.62	0.65	7.88	11.66
TZEIORQ 13	5.58	3.99	3.91	2.88	2.77	0.62	7.90	12.72
TZEIORQ 7	5.93	3.56	3.57	2.87	2.56	0.61	7.94	12.88
TZEIORQ 26	5.97	3.55	3.73	2.90	2.98	0.56	7.90	12.69
TZEIORQ 47	5.99	3.21	3.34	2.84	2.83	0.73	6.93	12.54
<b>Hybrid Checks</b>								
Oba Super 2	6.56	2.03	2.13	2.95	2.71	0.72	7.12	12.81
Oba Super 4	6.59	2.07	2.22	2.89	2.83	0.64	7.01	12.79
SE	0.061	0.041	0.051	0.063	0.056	0.065	0.066	0.072
CV (%)	9.43	12.88	7.63	12.43	8.88	10.45	12.45	7.88
LSD (0.05)	0.69	0.23	0.67	0.79	0.98	0.55	0.63	0.97

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

3.2. Combined analysis of variance for provitamin A quality protein maize

Table 3 presents the combined analysis of variance for provitamin A quality protein maize (PVA-QPM) inbreds concerning grain yield, tryptophan, and carotenoid contents, evaluated over two years. The mean squares for the inbreds were highly significant across all nine parameters examined, indicating a broad diversity among the inbreds used in this study [18]. This heritable variation is crucial for selection processes in breeding programs. Additionally, comparable mean squares were observed for both the year and the inbreds-by-year interaction across all studied parameters, suggesting that the performance of the inbreds remained consistent throughout the two growing seasons [19].

**Table 3.** Combined ANOVA mean squares for grain yield, tryptophan, and carotenoid contents of early PVA-QPM inbreds evaluated across two years.

Source of variation	Df	Grain yield (t ha <sup>-1</sup> )	Tryptophan (%)	PVA	βCX	βC	αC	ZEA	LUT
Year	1	10.33	5.55	24.76	34.12	23.43	7.55	23.77	9.11
Rep (Year)	6	11.09	23.76	12.45	14.14	12.00	12.00	10.23	11.23
Inbreds	9	88.72**	107.72**	93.11**	97.29**	97.45**	88.84**	98.54**	99.22**
Inbreds × Year	9	12.55	9.34	11.89	9.66	12.34	9.67	13.65	12.74
Pool error	64	11.23	9.34	2.67	7.11	9.45	9.87	15.23	7.55

Note: \*\* and \* significant at 0.01 and probability levels, respectively

3.3. Variance components, heritability, and genetic advance

The partitioning of variance components provided critical insights into the relative contributions of genetic, environmental, and genotype × environment interaction effects across traits (Table 4). Each variance component plays a distinct role in shaping phenotypic expression. Specifically, genotypic variance reflects the heritable fraction of variability available for exploitation through selection, whereas environmental variance captures the extent of uncontrolled external influences. Furthermore, the genotype × environment interaction

variance represents the sensitivity or stability of traits across environments, while phenotypic variance integrates all sources of variability to reflect the total observable variation. The genotypic coefficient of variation and the phenotypic coefficient of variation are indispensable indicators of trait variability. In particular, the genotypic coefficient of variation quantifies the portion of variation attributable to genetic factors, while the phenotypic coefficient of variation captures overall variability [10]. A narrow phenotypic coefficient of variation–genotypic coefficient of variation gap indicates that phenotypic variation is predominantly genetic, thereby improving the reliability of selection [20]. Conversely, a wide gap suggests a strong environmental influence that reduces selection efficiency. Additionally, the environmental coefficient of variation directly measures environmental noise in trait expression and provides a useful benchmark for determining the stability of traits across diverse environments [21]–[22].

Equally important, broad-sense heritability quantifies the proportion of phenotypic variance attributable to genetic factors, thereby serving as a predictor of selection response. Traits with high heritability values are more amenable to direct selection, while those with moderate or low heritability require multi-environment testing or indirect selection approaches [10]. In addition, genetic advance and genetic advance as a percentage of the mean complement heritability indicate the magnitude of expected progress under selection. Traits with high heritability, genetic advance, and genetic advance as a percentage of the mean point to additive gene action and strong prospects for genetic gain, whereas low genetic advance and genetic advance as a percentage of the mean combined with low to moderate heritability imply non-additive gene action or substantial environmental masking [10]. Collectively, these parameters are essential for identifying priority traits, refining selection strategies, and accelerating genetic improvement.

For grain yield, the relatively high genotypic variance ( $\sigma^2g = 67.32$ ) compared with phenotypic variance ( $\sigma^2p = 79.41$ ) reflects a strong genetic basis. However, the large genotype  $\times$  environment interaction variance ( $\sigma^2gy = 61.55$ ) and substantial environmental variance ( $\sigma^2e = 77.41$ ) underscore the pronounced sensitivity of yield to environmental fluctuations. Consequently, heritability was moderate ( $H^2 = 0.50$ ), with a modest genetic advance (0.82) and genetic advance as a percentage of the mean (13.73), suggesting limited efficiency of direct selection. These findings are aligned with [7], [10], who emphasized that maize yield is often constrained by genotype  $\times$  environment interaction variance, which diminishes selection efficiency despite considerable genetic variability. For tryptophan, phenotypic variance ( $\sigma^2p = 7.21$ ) was largely explained by environmental variance ( $\sigma^2e = 24.93$ ) and genotype  $\times$  environment interaction variance ( $\sigma^2gy = 12.89$ ), with only a minor contribution from genotypic variance ( $\sigma^2g = 3.34$ ). This was reflected in a wide gap between phenotypic coefficient of variation (12.31%) and genotypic coefficient of variation (5.62%), coupled with moderate heritability ( $H^2 = 0.50$ ). These results indicate that environmental factors outweigh genetic control, complicating selection. This trend is similar to [6], who reported low genetic regulation of tryptophan accumulation under variable conditions, and corroborated with [10], who highlighted the necessity of multi-environment evaluation when breeding for nutritional traits.

In contrast, carotenoid components exhibited stronger genetic determination. For provitamin A, genotypic variance ( $\sigma^2g = 4.22$ ) accounted for much of the total phenotypic variance ( $\sigma^2p = 7.88$ ), resulting in high heritability ( $H^2 = 0.80$ ) and appreciable genetic advance (GA = 1.65; GAM% = 23.54%). This finding is corroborated by [13], [22], who emphasized the predominance of additive gene action in carotenoid accumulation. Similarly,  $\beta$ -carotene displayed high heritability ( $H^2 = 0.80$ ) despite relatively low genotypic variance, indicating its stability across environments. This is aligned with [11] who reported that  $\beta$ -carotene can be effectively improved even under limited testing conditions. Lutein and zeaxanthin also exhibited moderate to high heritability ( $H^2 = 0.60$ – $0.70$ ), consistent with [10], who highlighted their potential as auxiliary indices for enhancing nutritional quality.

The comparative assessment of coefficients of variation reinforced these trends. For grain yield and provitamin A, the narrow gap between phenotypic coefficient of variation and genotypic coefficient of variation indicated that most of the phenotypic variability was genetically controlled, suggesting favourable prospects for selection. In contrast, the wide disparity between phenotypic coefficient of variation and genotypic coefficient of variation for tryptophan underscored the predominance of environmental factors in trait expression. These interpretations are aligned with [1], [10], who emphasized the value of integrating variance components, heritability, and genetic advance when designing efficient selection strategies. These results demonstrate that while genetic improvement of grain yield in early PVA-QPM inbreds is constrained by strong genotype  $\times$  environment interaction variance effects, carotenoid traits, particularly provitamin A and  $\beta$ -carotene, are under

stronger genetic control and can be effectively enhanced through selection. This conclusion is corroborated by [23], [24], who highlighted biofortified maize as a sustainable pathway to improve food and nutrition security in SSA.

**Table 4.** Estimates of variance components, heritability, and genetic advance for grain yield, tryptophan, and carotenoid contents of early PVA-QPM inbreds evaluated across two years.

	$\delta^2_p$	$\delta^2_g$	$\sigma^2_{gy}$	$\delta^2_e$	PCV	GCV	ECV	H <sup>2</sup>	GA	GAM%
Grain yield	79.41	67.32	61.55	77.41	24.11	31.54	67.33	0.50	0.82	13.73
Tryptophan	7.21	3.34	12.89	24.93	12.31	5.62	6.55	0.50	0.01	17.17
PVA	7.88	4.22	2.72	6.82	26.41	0.72	33.56	0.8	1.65	23.54
$\beta$ CX	6.32	1.45	5.89	0.98	16.16	7.82	18.83	0.7	1.44	28.84
$\beta$ C	9.22	0.83	6.03	6.56	36.11	8.74	5.11	0.8	1.65	23.54
$\alpha$ C	7.09	9.77	8.62	2.76	18.33	8.08	6.43	0.7	0.72	24.03
ZEA	5.09	8.12	5.45	6.22	18.67	7.64	9.78	0.7	4.33	28.84
LUT	9.34	8.11	5.21	0.98	12.33	9.78	7.22	0.6	2.47	24.72

Note: phenotypic variance = ( $\sigma^2_p$ ), genotypic variance = ( $\sigma^2_g$ ), genotype by year variance = ( $\sigma^2_{gy}$ ), error variance = ( $\sigma^2_e$ ), phenotypic coefficient of variability = (PCV), genotypic coefficient of variability = (GCV), environmental coefficient of variability = (ECV), broad sense heritability = (H<sup>2</sup>), narrow sense heritability = (h<sup>2</sup>), expected genetic advances = (GA) and genetic advance as per cent of mean = (GAM)

### 3.4. Correlation analysis of grain yield, tryptophan, and carotenoid components

The correlation analysis among grain yield, tryptophan, and carotenoid components in early PVA-QPM inbreds provides critical insights into the genetic relationships that influence nutritional and agronomic performance (Table 5). Understanding these interdependencies is essential for breeding strategies aimed at simultaneously enhancing yield and nutritional quality in biofortified maize varieties. A strong negative correlation between grain yield and tryptophan content ( $r = -0.773$ ) suggests that selecting for higher tryptophan may reduce yield potential, a finding corroborated by [22], who reported that improving protein quality traits in maize often incurs agronomic penalties due to underlying physiological trade-offs. Nevertheless, breaking this unfavourable association is feasible through marker-assisted and genomic selection, which have been shown to decouple yield from quality attributes in maize breeding pipelines [25].

Carotenoid components exhibited diverse associations with grain yield and tryptophan, while  $\beta$ -carotene ( $\beta$ C) showed a weak positive correlation with grain yield ( $r = 0.017$ ), provitamin A (PVA) and  $\beta$ -cryptoxanthin displayed weak negative associations ( $r = -0.119$  and  $r = -0.224$ , respectively). These results indicate that increasing carotenoid content is unlikely to significantly compromise yield, reinforcing the feasibility of biofortification without yield penalties. This aligns with [21], who emphasized that strategic carotenoid enrichment in biofortified maize can be achieved while sustaining productivity. The positive correlation between  $\beta$ -cryptoxanthin and  $\beta$ -carotene ( $r = 0.385$ ) further suggests that selection for higher  $\beta$ -cryptoxanthin levels could indirectly enhance  $\beta$ C accumulation, thereby increasing PVA content. Notably, the strong positive correlation between tryptophan and zeaxanthin ( $r = 0.773$ ) implies that nutritional enhancement in protein quality could inadvertently increase zeaxanthin concentrations. Conversely, the negative correlation between zeaxanthin and  $\alpha$ -carotene ( $r = -0.688$ ) highlights the need for careful balancing of carotenoid profiles to optimize PVA levels without reducing other beneficial compounds.

Interestingly, lutein showed a moderate positive correlation with grain yield ( $r = 0.544$ ), indicating that its accumulation does not compromise productivity. This corroborates the findings of [19], who demonstrated that non-provitamin A carotenoids, such as lutein, can accumulate without adverse agronomic trade-offs. However, lutein displayed a moderate negative correlation with  $\beta$ -cryptoxanthin ( $r = -0.494$ ), implying that increasing  $\beta$ -cryptoxanthin may reduce lutein content. This is noteworthy since lutein plays an important role in eye health and protection against age-related macular degeneration. Collectively, these correlations highlight the complex genetic architecture underlying yield and nutritional traits in early PVA-QPM inbreds. The results emphasize that while increasing tryptophan content poses challenges for yield improvement, targeted breeding approaches and advanced molecular tools can mitigate these trade-offs. Furthermore, the feasibility of enhancing carotenoid content without compromising yield provides a promising pathway for developing nutritionally enhanced maize varieties capable of addressing vitamin A deficiency in SSA.

**Table 5.** Correlation of grain yield, tryptophan, and carotenoid traits of early PVA-QPM inbreds evaluated across two years.

Traits	Grain Yield	Tryptophan	PVA	$\beta$ CX	$\beta$ C	$\alpha$ C	ZEA	LUT
Grain Yield	-							
Tryptophan	-0.773**	-						
PVA	-0.119	0.365	-					
$\beta$ CX	-0.224	0.312	0.388	-				
$\beta$ C	0.017	-0.024	-0.088	0.385	-			
$\alpha$ C	0.217	-0.327	-0.209	-0.049	-0.023	-		
ZEA	-0.467	0.773**	0.398	0.295	-0.155	-0.688*	-	
LUT	0.544	-0.342	0.005	-0.494	0.079	-0.177	-0.151	-

Note: PVA, total provitamin A;  $\beta$ CX,  $\beta$ -cryptoxanthin; ZEA, zeaxanthin; LUT, lutein;  $\beta$ C =  $\beta$ -carotene;  $\alpha$ C,  $\alpha$ -carotene.

#### 4. Conclusion

This study revealed significant genetic variability among early-maturing PVA-QPM inbred lines, underscoring the potential for simultaneous improvement of grain yield and nutritional quality. High heritability and genetic advance for grain yield and carotenoid concentrations indicate that additive genetic effects play a dominant role in their inheritance, making selection highly effective. Tryptophan exhibited moderate heritability, suggesting its enhancement is attainable but influenced by environmental factors. The observed negative correlation between grain yield and tryptophan content highlights the complexity of improving yield and nutritional traits concurrently. Nonetheless, the substantial genetic control and variability in carotenoid content offer strong prospects for developing biofortified maize that combats vitamin A deficiency in SSA. To maximize genetic gain, integrated breeding approaches combining conventional selection with genomic-assisted tools are recommended to accelerate improvement while maintaining yield stability and kernel quality. However, the study was limited to a single environment, lacked assessment of gene interactions and bioavailability, and did not fully explore the effects of environmental and agronomic factors on nutrient stability. Therefore, future studies should include multi-environment evaluations, genomic dissection of quantitative loci, and post-harvest nutritional assessments. Developing high-yielding, nutrient-dense hybrids resilient to environmental variability will be essential for enhancing food and nutritional security in maize-dependent regions. Ultimately, integrating genomic selection, recurrent selection, and nutritional bioavailability analyses will ensure that PVA-QPM varieties deliver sustainable agronomic performance and human health benefits across diverse agroecological zones.

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