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Unravelling economic heterosis for nutritional profiles and grain yield in extra-early provitamin a quality protein maize

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Abstract

Heterosis is a crucial genetic mechanism for enhancing grain yield and nutritional quality in extra-early provitamin A (PVA) Quality Protein Maize (QPM). This study investigated the extent of heterosis in grain yield, tryptophan content, and carotenoid composition among 10 PVA-QPM hybrids. A partial diallel mating design, excluding reciprocals, generated 45 F_1 hybrids from 10 extra-early PVA-QPM inbred lines. These hybrids, along with their 10 parental lines and two commercial hybrid checks, were evaluated under rainfed conditions at the Lower Niger River Basin Authority, Oke-Oyi, Nigeria, over two consecutive years. Significant economic heterotic effects were observed for grain yield, with values ranging from -4.29% in TZEIORQ 26 × TZEQI 82 to 19.38% in TZEIORQ 11 × TZEIORQ 24, demonstrating the genetic advantage of certain hybrids over commercial varieties. The Tryptophan content exhibited notable positive heterosis, surpassing 50% in the derived hybrids, further validating the effectiveness of QPM breeding initiatives. Provitamin A and different carotenoids showed significant hybrid vigour, especially marked by substantial gains in α-carotene (295.65%), β-carotene (46.80%), and β-cryptoxanthin (40.52%). The low standard error of difference values (0.0044-0.0124) and highly significant critical differences at 5% (0.0087-0.0247) and 1% (0.0114-0.0319) levels confirmed the statistical reliability of the findings. These results underscore the potential of hybrid breeding for biofortification, reinforcing its role in addressing vitamin A deficiency. Further studies are needed to evaluate genotype-by-environment interactions, ensuring the stability and adaptability of these hybrids in various agroecological regions.

***Keywords**: Heterosis, quality protein maize, provitamin A, maize hybrid, grain yield, biofortification*

Introduction

Maize (*Zea mays* L.) is one of the most widely cultivated cereal crops, serving as a staple food for millions, particularly in sub-Saharan Africa, Latin America, and Asia (Dhliwayo *et al.*, 2024). Its adaptability to diverse agroecological conditions, high yield potential, and wide-ranging usage in food, feed, and industry make it a key component of global agriculture (Bello, 2017). However, Conventional maize varieties are deficient in key micronutrients, notably provitamin A, and possess inadequate levels of quality protein, thereby contributing to the persistence of malnutrition in populations that depend predominantly on maize as a primary food source (Bello *et al*., 2013). To overcome these nutritional shortcomings, maize varieties have been biofortified with traits that boost provitamin A carotenoid

levels and essential amino acids such as lysine and tryptophan (Bello *et al.,* 2013; Babu and Prasanna, 2020). Provitamin A Quality Protein Maize (PVA-QPM) is a significant breakthrough, offering a dual advantage of high provitamin A content and improved protein quality (Suwarno *et al.,* 2022). This innovation is particularly relevant in combating vitamin A deficiency (VAD) and protein malnutrition, which remain prevalent public health issues in developing regions. Malnutrition due to micronutrient deficiencies poses serious health challenges, particularly in vulnerable populations. VAD is a leading cause of preventable blindness and weakened immunity, especially in children and pregnant women (Bello *et al.,* 2019). Since conventional maize varieties contain low levels of provitamin A carotenoids, they fail to meet the nutritional needs of populations dependent on maize-based diets (Ige *et al.,* 2023). Commercial maize varieties lack adequate levels of lysine and tryptophan, making protein deficiency another significant concern (Bello *et al.,* 2024). PVA-QPM hybrids present a comprehensive solution by integrating provitamin A and QPM traits, thereby addressing micronutrient and protein deficiencies. Effective improvement of these hybrids requires an understanding of heterosis, which is fundamental to enhancing yield performance and nutritional quality traits (Muthusamy & Hossain, 2023).Heterosis, also known as hybrid vigour, refers to the superior performance of hybrid offspring compared to their inbred parents, contributing to increased yield, stress resilience, and improved nutritional traits (Chen *et al.,* 2021). Economic heterosis refers to the superiority of a hybrid over the best commercially available cultivar, emphasizing improvements that translate into greater financial returns for producers. This phenomenon has been used in maize breeding programmes for productivity and adaptation to diverse environmental conditions (Tang *et al.,* 2022). Although widely studied in conventional maize breeding, the impact of heterosis on extra-early PVA-QPM hybrids is underexplored. Given the unique genetic composition of these hybrids, further research Given the unique genetic composition of these hybrids, further research to determine the extent to which economic heterosis contributes to agronomic performance and enhanced nutritional profiles is essential (Wang and Li, 2021). (Wang and Li, 2021).

Extra-early maturing maize varieties, which reach physiological maturity in under 90 days, are crucial for regions with short growing seasons and unpredictable climatic conditions. In sub-Saharan Africa, where unpredictable rainfall patterns and extended drought periods pose significant challenges to food security, extra-early maturing maize varieties offer a strategic advantage by allowing cultivation within shortened growing seasons, thereby minimizing the risk of crop failure (Dhliwayo *et al*., 2024). However, breeding extra-early PVA-QPM hybrids presents a challenge, maintaining high grain yield and nutritional quality while preserving the early maturity advantage. Exploiting economic heterosis in these hybrids offers a potential solution to enhance their productivity without compromising their short-duration growth cycle (Babu and Prasanna, 2020).

The genetic basis of heterosis in maize is complex, influenced by genetic distance between parental lines, combining ability, and environmental interactions (Tang *et al.,* 2022).

In PVA-QPM hybrids, heterosis expression for provitamin A carotenoid

content and quality protein traits is regulated by numerous genetic loci, with additive and non-additive gene effects contributing to their inheritance patterns (Muthusamy & Hossain, 2023). (Muthusamy and Hossain, 2023). The protein-enhancing trait associated with the opaque-2 (o2) gene demonstrates heterotic advantages in hybrid crosses. Understanding how these genetic interactions influence economic heterosis in extra-early PVA-QPM hybrids is crucial for breeding programs (Chen *et al.*, 2021).

Although progress has been made in biofortifying maize and developing hybrid varieties, several critical areas remain insufficiently explored. First, there is limited empirical evidence on economic heterosis in extra-early PVA-QPM hybrids, particularly concerning yield, provitamin A, and protein traits. Second, there has been limited evaluation of how integrating early maturity traits with biofortified characteristics influences yield and nutritional performance. Third, the contribution of parental inbred lines to heterosis expression in provitamin A carotenoid content remains poorly understood, leaving a critical gap in the strategic selection of parental lines for hybrid improvement.

The research objectives are to:

i. Quantify economic heterosis for grain yield, provitamin A content, and protein quality traits in extra-early maturing PVA-QPM maize hybrids.
ii. Evaluate the impact of parental genetic backgrounds on the manifestation of heterosis in nutritional quality and agronomic performance traits. Identify superior hybrid combinations that combine high economic heterosis with early maturity, improved grain yield, and enhanced nutritional quality.

By addressing these objectives, the study aims to generate actionable insights that will support the development of high-performing, nutritionally enhanced maize hybrids adapted to the constraints of low-input, short-season environments. The findings aim to improve breeding strategies for nutritionally enriched maize, thereby contributing significantly to efforts aimed at alleviating food insecurity and micronutrient deficiencies in vulnerable populations.

Materials and Methods

The trial location, inbreds and hybrids

We used ten novel PVA-QPM inbred lines, selected for drought and nitrogen tolerance, from the International Institute of Tropical Agriculture (IITA), Ibadan Maize Improvement Programme (MIP), as shown in Table 1. A partial diallel mating design was employed to generate a total of 45 hybrid combinations from the selected parental lines. A total of 47 genotypes, along with two commercial hybrids, Oba 98 and Oba Super 6, were evaluated at the Lower Niger River Basin Development Authority, Oke-Oyi, Nigeria, during the 2023 and 2024 rainy seasons.

Table 1. Ten extra-early maturing PVA-QPM inbreds and two commercial hybrids as checks used in the diallel cross

Genotypes	Pedigree
TZEEIORQ 28	2009 TZEE-OR2 STR QPM S6 21-2/6-3/3-2/3-1/3-1/2 5
TZEEIORQ 30	2009 TZEE-OR2 STR QPM S6 21-2/6-3/3-3/3-1/3-1/2 5
TZEEIORQ 32	2009 TZEE-OR2 STR QPM S6 21-5/6-1/2-1/2-3/3-1/1 5
TZEEIORQ 42	2009 TZEE-OR2 STR QPM S6 22-3/3-2/3-3/3-1/3-1/2 6
TZEEIORQ 54	2009 TZEE-OR2 STR QPM S6 27-1/5-3/3-1/3-2/2-1/1 6
TZEEIORQ 57	2009 TZEE-OR2 STR QPM S6 27-1/5-3/3-3/3-1/1-1/1 2
TZEEIORQ 61	2009 TZEE-OR2 STR QPM S6 27-5/5-1/2-1/3-2/3-1/1 4
TZEEIORQ 62	2009 TZEE-OR2 STR QPM S6 27-5/5-1/2-3/3-2/3-1/1 4
TZEEIORQ 64	2009 TZEE-OR2 STR QPM S6 27-5/5-2/2-1/2-2/2-2/2 6
TZEEIORQ 75	2009 TZEE-OR2 STR QPM S6 82-2/2-2/2-1/4-1/3-1/2 2
Varietal checks	
OBA 98	Commercial quality protein maize hybrid
OBA Super 6	Commercial Provitamin A maize hybrid

The field management of the trials

At the research location, land preparation included ploughing, harrowing, and ridging. Maize was planted by placing three seeds in each hill and later reduced to two seedlings per hill to establish a planting distance of 0.75 meters between rows and 0.5 meters between plants within the row. The trial was laid out in plots consisting of four rows, each 5 meters long, using a Randomised Complete Block Design (RCBD) to reduce variability and ensure accurate treatment comparisons. The experiment was established with a target planting density of approximately 53,333 plants per hectare to ensure optimal plant population for evaluation. Fertilizer application included NPK (15:15:15) at a rate of 60 kg N ha^{-1} applied two weeks after planting, followed by an additional 30 kg N ha^{-1} as top dressing at four weeks. To manage weeds, a combination of Metolachlor (3 kg L^{-1}) and atrazine (170 g L^{-1}) was applied as a pre-emergence treatment before planting, aiming to prevent initial weed establishment.

Cross-pollination approach

Bello *et al*. (2019) outlined a systematic procedure for controlled cross-pollination in maize. Daily monitoring of pollen shed and ear shoot emergence enabled accurate synchronization and timely execution of controlled pollination. To avoid accidental cross-pollination, emerging ear shoots were shielded before silk emergence, and tassels were enclosed in bags to ensure the collection of pure pollen. Crosses were conducted by manually applying the collected pollen to the receptive silks. After pollination, workers wrapped the ears of corn to keep their genetic traits pure until harvest. The hybrid seeds produced were then isolated and preserved for later analysis and performance assessment.

Grain yield evaluation

Upon maize ear maturity, ears in each plot were harvested and weighed separately. The grain yield in kilograms per hectare (kg ha^{1}) was determined using the formula provided by Bello *et al.* (2013). This process involved adjusting yield data for factors such as harvested plot size, grain

weight, and moisture content, thereby ensuring accurate and reliable comparisons across experimental plots.

Analysis of carotenoids, lysine, and tryptophan contents

Carotenoids were extracted and quantified through HPLC at IITA, using the method described by Howe and Tanumihardjo (2006). Provitamin A (PVA) content involved summing β-carotene with 50% of the amounts of β-cryptoxanthin and α-carotene, as outlined by the US Institute of Medicine (2001). Duplicate tests ensured accuracy. Tryptophan levels in maize flour were evaluated via a colourimetric method (Hernández and Bates 1969), calibrated against a standard curve. Tryptophan content was exploited as a protein quality marker, multiplied by four to estimate lysine levels (Sentayehu, 2008), indicating its relevance in assessing maize nutritional quality.

Analysis of crude zein, zein in dry matter, crude protein, quality index and carbohydrate contents

The amino acid composition was determined using the analytical procedure described by Sentayehu (2008). The ammonium content was quantified through distillation and titration (Aykroyd *et al.*, 1964). The crude zein was quantified using the method described by Drochioiu *et al.* (2002). Crude protein content was determined using the standard micro-Kjeldahl method (AOAC, 2006). Other nutritional components, including moisture content, crude fat (via solvent extraction), crude fibre, and ash, were assessed using standard procedures (AOAC, 2006). Carbohydrate content was calculated by deducting the percentages of moisture, fat, crude protein, ash, and crude fibre from 100%, representing the nitrogen-free extract (NFE). The quality index was evaluated by determining the ratio of tryptophan to total protein content.

Statistical analyses

Data collected from the 2023 and 2024 evaluations were analyzed using the General Linear Model (PROC GLM) procedure in SAS software, version 9.10 (SAS Institute, 2021). In this analysis, entries were treated as fixed effects, while replications were considered random effects and specified using the RANDOM statement in the model. Economic heterosis was calculated as a percentage relative to best commercial hybrids for traits with significant differences, following Falconer and Mackay (1996):

$$Economic\ Heterosis\ (\%) = \frac{F_1 - Best\ Commercial\ Variety}{Best\ Commercial\ Variety} \times 100$$

Where F_1 is the mean hybrid value.

Differences in heterosis were determined using Panse and Sukhatme's (1961) method. A t-test was applied for significance among crosses and checks, with standard error (SE) and critical difference (CD) calculated as follows:

$$SE(d) = \sqrt{\frac{2Me}{r}}$$

$$CD = SE(d)t$$

Where t is the tabulated t-value at the error degree of freedom. The significance of the results was evaluated using probability thresholds of 5% and 1% to determine meaningful differences among treatments.

Results and Discussion

Analysis of variance (ANOVA) for grain yield, tryptophan, and carotenoid content in extra-early PVA-QPM hybrids

The ANOVA results presented in Table 2 demonstrate the influence of various

sources of variation on grain yield, tryptophan concentration, and carotenoid content among extra-early PVA-QPM hybrids evaluated over two years under rainfed conditions. The analysis revealed significant contributions of genetic and environmental factors to trait expression, consistent with previous findings in maize breeding research (Badu-Apraku *et al*., 2020). The year effect was highly significant for grain yield, tryptophan, provitamin A (PVA), β-carotene, and α-carotene, while β-cryptoxanthin showed moderate significance. Conversely, zeaxanthin and lutein remained unaffected by annual fluctuations, indicating that these traits maintain relative stability across different growing seasons (Prasanna & Mazumdar, 2020). Similar patterns corroborated with the results of Menkir *et al*. (2018), where specific carotenoids remained stable across environments, underscoring their potential utility in developing stable nutritional profiles in maize.

The results highlight how environmental factors such as rainfall patterns, soil nutrient levels, and temperature fluctuations, on the expression of particular traits in maize hybrids. Conversely, certain traits exhibited stable performance across different seasons, indicating regulation predominantly by inherent genetic factors rather than environmental influences (Dhliwayo *et al*., 2024). The non-significant replication (Rep[Year]) effects across all traits indicate minimal experimental error and high precision, consistent with findings by Yan and Holland (2021), who reported that such consistency is crucial for reliable trait evaluation in breeding trials.

The hybrid effect was highly significant for all traits, highlighting substantial genetic variability among the evaluated hybrids. This is consistent with the findings of Pixley *et al*. (2023), who reported substantial genetic variation among PVA-QPM hybrids in terms of yield performance and micronutrient composition. The detected genetic diversity provides a solid foundation for progressing genetic enhancement efforts via targeted hybrid selection strategies. Similar outcomes of Zunjare and Hossain (2023) demonstrated that genetic variability in agronomic and nutritional traits is essential in hybrid breeding approaches.

Notably, the hybrid × year interaction was highly significant ($P < 0.01$) for all evaluated traits, indicating substantial genotype × environment (G × E) interactions. The result emphasizes the critical role of testing across diverse environments to pinpoint hybrids that combine high performance with environmental adaptability (Wang & Li, 2021). These findings are consistent with the study by Badu-Apraku and Akinwale (2011), who highlighted the critical role of genotype × environment interactions in selecting maize hybrids with broad adaptability across sub-Saharan African environments.

The general combining ability (GCA) effects were significant ($P < 0.05$) for most traits, suggesting that additive genetic variance predominantly governs the inheritance of grain yield, tryptophan, and carotenoid content. This finding is consistent with Bernardo (2020), who noted that GCA-driven inheritance is often more predictable and heritable than specific combining ability (SCA) controlled by non-additive effects. The lack of significant SCA effects suggests a minimal contribution of dominance and epistatic interactions, underscoring the predominance of additive gene action in governing these traits.

Moreover, the consistently high Baker ratios (GCA/SCA > 0.5) across all traits affirm the dominance of additive gene effects. This observation aligns with the

conclusions of Menkir *et al*. (2024), who suggested that breeding strategies emphasizing GCA can enhance provitamin A content and grain yield in maize. Consequently, parental selection based on strong GCA effects becomes a practical strategy for achieving genetic gain in PVA-QPM maize, particularly for traits governed by additive effects.

The absence of significant Year × GCA and Year × SCA interactions confirms the stability of additive and non-additive genetic contributions across different environments. It supports the reliability of parental performance over time, similar to observations made by Prasanna and Mazumdar (2020) in their evaluation of vitamin-enriched maize hybrids.

Pooled error variances were low, and coefficients of variation (CV %) for traits such as grain yield (1.07%), PVA (1.36%), β-cryptoxanthin (1.32%), and β-carotene (1.56%) were minimal, suggesting high experimental accuracy and reliability. These CV % values fall within acceptable thresholds for field trials (Yan & Holland, 2021), while the slightly higher CV % for α-carotene (5.20%) suggests comparatively higher variability, possibly due to environmental responsiveness or lower heritability of this specific carotenoid. The results emphasize the significant interplay between genetic makeup and environmental influences in shaping the agronomic traits and nutritional attributes of extra-early PVA-QPM hybrids. The pronounced hybrid and GCA effects validate the efficacy of selection-driven breeding approaches in improving key agronomic and nutritional traits.

The high Baker ratios and consistent performance across years provide evidence for the utility of additive genetic variance in improving grain yield and micronutrient content (Muthusamy & Hossain, 2023). These insights contribute valuable knowledge to improvement programmes in maize cultivars, particularly in breeding nutrient-dense, high-yielding cultivars adapted to rainfed and resource-limited environments.

Table 2. Combined ANOVA mean squares for grain yield, tryptophan and carotenoid contents of extra-early PVA-QPM hybrids across two of years rainfed seasons

Source of variation	Df	Grain yield (t ha^{-1})	Tryptophan (%)	Carotenoids (µg g^{-1} DW)					
				PVA	βCX	βC	αC	ZEA	LUT
Year	1	0.698**	0.046**	0.075**	0.014*	0.035**	0.016*	0.001	0.020
Rep (Year)	6	0.019	0.0013	0.00096	0.00004	0.00002	0.00011	0.00017	0.000034
Hybrids	44	0.067**	0.019**	0.037**	0.014**	0.012**	0.013**	0.013**	0.077**
Hybrids × Year	44	0.035**	0.018**	0.035**	0.014**	0.011**	0.013**	0.013**	0.076**
GCA	9	0.036	0.010*	0.020*	0.008*	0.006*	0.007*	0.007*	0.042**
SCA	35	0.0000036	0.0000036	0.0000069	0.0000026	0.0000022	0.0000025	0.0000024	0.0000143
Year × GCA	9	0.0020	0.00058	0.0011	0.00042	0.00036	0.00041	0.00039	0.0023
Year × SCA	35	0.00087	0.00025	0.00048	0.00018	0.00015	0.00018	0.00017	0.00010
Pool error	86	0.00023	0.000058	0.000093	0.000035	0.000029	0.000041	0.000035	0.000116
GCA/SCA (Baker ratio) %		88.81	76.84	93.66	92.82	90.87	88.33	91.41	94.79
CV%		1.07	1.90	1.36	1.32	1.56	5.20	0.74	0.58

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

Mean performance of extra-early PVA-QPM hybrids

The average values for grain yield, tryptophan levels, and carotenoid content in extra-early PVA-QPM hybrids demonstrate their high capacity to enhance maize yield and nutritional value in rainfed environments across two growing seasons (Table 3). The hybrids generally outperformed commercial checks such as OBA SUPER 6 and OBA 98, indicating significant genetic gains in yield and nutritional attributes (Badu-Apraku *et al*., 2020; Muthusamy & Hossain, 2023). An average grain yield of 6.57 t ha^{-1}, ranging from 5.58 to 6.96 t ha^{-1}, reflects a significant improvement over earlier findings, reinforcing the effectiveness of hybrid breeding in boosting maize productivity under low-input conditions (Chen *et al*., 2021). Notably, the hybrid TZEIORQ 11 × TZEIORQ 24 achieved a 17.1% higher grain yield than the commercial checks, highlighting its strong potential for deployment in rainfed maize production systems where enhancing yield is a key priority.

Tryptophan content, a critical measure of protein quality in quality protein maize, ranged from 2.34% to 3.97%, with all hybrids exhibiting significantly higher values than the commercial checks, as reported by Muthusamy and Hossain (2023). These elevated tryptophan levels align with findings by Suwarno *et al*. (2022), revealing the potential of these hybrids to contribute to alleviating protein-energy malnutrition in maize-dependent populations. Such improvements confirm the effectiveness of the QPM breeding approach in enhancing essential amino acid profiles alongside yield gains.

Carotenoid analysis revealed substantial variation among hybrids, reinforcing their potential role in biofortification strategies in combating vitamin A deficiency (Prasanna & Mazumdar, 2020; Tang *et al*., 2022). The highest provitamin A content recorded was 4.92 µg g^{-1} in hybrid TZEIORQ 20 × TZEIORQ 24. The β-cryptoxanthin, β-carotene, α-carotene, zeaxanthin, and lutein concentrations varied across hybrids, indicating diverse carotenoid profiles that can collectively contribute to improved vitamin A intake. These results align with Tang *et al*. (2022), who highlighted the nutritional advantages of carotenoid-enriched maize hybrids and reported that advancing biofortification strategies could sustainably improve provitamin A.

The low coefficients of variation for grain yield (1.07%), PVA (1.36%), β-cryptoxanthin (1.32%), and β-carotene (1.56%) indicate high experimental precision and reliability (Dhliwayo *et al*., 2024), and while the somewhat higher variability observed in α-carotene (5.20%) suggests trait-specific sensitivity to environmental or genetic factors. The significant differences revealed by the least significant difference (LSD) tests confirm the presence of meaningful genetic diversity among the hybrids, validating selection efforts (Zunjare & Hossain, 2023). These results demonstrate the feasibility of breeding extra-early PVA-QPM hybrids that combine superior agronomic performance with enhanced nutritional qualities.

Importantly, these findings build upon previous research highlighting the dual challenges of increasing maize yield and improving nutritional content, particularly in sub-Saharan Africa's rainfed agro-ecosystems (Babu & Prasanna, 2020). The observed enhancements underscore the vital importance of combining biofortification with hybrid breeding as a sustainable strategy to address food insecurity and

combat micronutrient deficiencies. Future multi-location trials across diverse agroecologies are essential to validate the stability and adaptability of these hybrids, ensuring their broad utility and impact in smallholder farming systems.

Table 3: Mean performance of selected extra-early PVA-QPM hybrids for grain yield, tryptophan and carotenoid contents across two years of rainfed cropping seasons

Genotypes	Grain yield (t ha^{-1})	Tryptophan (%)	Carotenoids (µg g^{-1} DW)					
			PVA	βCX	βC	αC	ZEA	LUT
TZEIORQ 2 × ZEIORQ 11	6.78	3.88	4.35	3.69	2.94	0.71	7.54	12.98
TZEIORQ 2 × TZEIORQ 20	6.82	3.91	4.11	3.58	2.89	0.66	7.92	12.78
TZEIORQ 2 × TZEIORQ 24	6.84	3.97	4.63	3.47	2.71	0.82	7.45	12.89
TZEIORQ 2 × TZEIORQ 42	6.80	3.89	4.78	3.66	2.98	0.61	7.91	12.92
TZEIORQ 2 × TZEQI 82	6.54	3.54	4.83	3.34	2.94	0.45	7.56	12.77
TZEIORQ 2 × TZEIORQ 70	6.11	3.61	4.91	3.56	2.87	0.71	7.82	12.65
TZEIORQ 11 × TZEIORQ 20	6.94	3.45	4.64	3.37	2.71	0.49	7.74	12.79
TZEIORQ 11 × TZEIORQ 24	6.96	3.55	4.53	3.44	2.88	0.54	7.90	12.51
TZEIORQ 20 × TZEIORQ 24	6.93	3.71	4.92	3.57	2.91	0.63	7.74	12.58
TZEIORQ 22 × TZEIORQ 42	6.92	3.65	4.67	3.54	2.65	0.76	7.89	12.71
TZEIORQ 22 × TZEQI 82	6.77	3.55	4.22	3.61	2.78	0.89	7.67	12.88
TZEIORQ 24 × TZEIORQ 26	6.80	3.67	4.63	3.78	2.53	0.67	7.92	11.56
TZEIORQ 24 × TZEIORQ 42	6.93	3.88	4.39	3.22	2.62	0.44	7.90	12.73
TZEIORQ 24 × TZEIORQ 59	6.80	3.29	4.34	3.36	2.89	0.79	7.77	12.90
TZEIORQ 24 × TZEIORQ 69	6.73	3.76	4.69	3.59	2.72	0.88	7.97	12.97
TZEIORQ 26 × TZEIORQ 42	6.12	3.53	4.57	3.37	2.75	0.91	7.84	12.94
TZEIORQ 26 × TZEIORQ 59	6.39	3.67	4.11	3.51	2.98	0.54	7.60	12.91
TZEIORQ 26 × TZEIORQ 69	6.33	3.58	4.84	3.59	2.765	0.72	7.88	12.88
TZEIORQ 26 × TZEIORQ 70	6.43	3.66	4.35	3.33	2.60	0.59	7.79	12.74
TZEIORQ 26 × TZEQI 82	5.58	3.52	4.23	3.57	2.77	0.61	7.67	11.89
TZEIORQ 42 × TZEIORQ 59	6.84	3.90	4.67	3.41	2.89	0.79	7.80	12.87
TZEIORQ 42 × TZEIORQ 69	6.85	3.68	4.35	3.56	2.54	0.63	7.79	12.91
TZEIORQ 42 × TZEIORQ 70	6.83	3.52	4.53	3.76	2.61	0.85	7.90	12.78
TZEIORQ 42 × TZEQI 82	6.79	3.67	4.61	3.56	2.79	0.90	7.89	12.93
TZEIORQ 59 × TZEIORQ 69	6.54	3.44	4.67	3.44	2.88	0.67	7.61	12.85
TZEIORQ 59 × TZEIORQ 70	6.22	3.34	4.23	3.69	2.70	0.34	7.93	12.90
TZEIORQ 69 × TZEIORQ 70	6.34	3.69	4.11	3.56	2.53	0.69	7.72	12.93
TZEIORQ 69 ×TZEQI 82	6.53	3.45	4.54	3.88	2.79	0.71	7.54	11.89
Commercial hybrid checks								
OBA SUPER 6	5.71	2.34	3.67	2.76	2.01	0.21	6.61	11.83
OBA 98	5.83	2.36	3.54	2.69	2.03	0.23	6.73	11.90
SD	0.386	0.370	0.331	0.252	0.233	0.184	0.313	0.404
SE (d)	0.0704	0.0675	0.0605	0.0461	0.0425	0.0337	0.0572	0.0737
CV (%)	1.07	1.90	1.36	1.32	1.56	5.20	0.74	0.58
LSD (0.05)	0.54	0.71	0.88	0.41	0.64	0.87	0.52	0.83
Mean	6.57	3.56	4.46	3.48	2.72	0.65	7.70	12.66
Maximum	6.96	3.97	4.92	3.88	2.98	0.91	7.97	12.98
Minimum	5.58	2.34	3.54	2.69	2.01	0.21	6.61	11.56

SD = Standard Deviation SE(d) = standard error difference, PVA, total provitamin A; βCX, β-cryptoxanthin; ZEA, zeaxanthin; LUT, lutein; βC = β-carotene; αC, α-carotene.

Economic heterosis for grain yield, tryptophan, and carotenoid contents in extra-early PVA-QPM hybrids

The analysis of economic heterosis for grain yield, tryptophan, and carotenoid contents in extra-early PVA-QPM hybrids revealed considerable variability, underscoring the genetic potential for simultaneous yield improvement and nutritional quality (Table 4). The grain yield heterosis ranged from -4.29% to 19.38%, with the highest heterosis observed in TZEIORQ 11 × TZEIORQ 24 (19.38%), corroborating findings by Muthusamy and

Hossain (2023) who reported similar heterotic gains in elite PVA-QPM hybrids. Such positive heterosis suggests that dominant alleles and favourable gene interactions contribute substantially to enhanced grain production, revealing the importance of hybrid vigour in maize breeding. The observed negative heterosis in the TZEIORQ 26 × TZEQI 82 hybrid (-4.29%) indicates that not all parental crosses result in hybrid vigour, indicating the necessity for careful and deliberate selection of parental lines to achieve optimal genetic improvement, as noted by Dhliwayo *et al*. (2024).

Tryptophan content exhibited exceptionally high and significant heterosis across all hybrids, ranging from 46.19% to 68.22%, with TZEIORQ 2 × TZEIORQ 24 attaining the highest value. These results align closely with Badu-Apraku *et al*. (2020), who demonstrated marked heterotic improvements in tryptophan levels within QPM hybrids, thereby validating the efficacy of biofortification breeding programs to enhance protein quality. The strong heterotic expression for tryptophan highlights its promise in combating protein-energy malnutrition among poor-resource communities by providing genotypes with improved nutritional quality.

Table 4. Estimates of economic heterosis for grain yield, tryptophan and carotenoid contents of selected extra-early PVA-QPM hybrids across two years of rainfed cropping seasons

Genotypes	Grain yield (t ha^{-1})	Tryptophan (%)	Carotenoids (µg g^{-1} DW)					
			PVA	βCX	βC	αC	ZEA	LUT
TZEIORQ 2 × ZEIORQ 11	16.30*	64.41**	22.88*	37.17**	44.83**	208.70**	12.04*	9.08
TZEIORQ 2 × TZEIORQ 20	16.98*	65.68**	16.10*	33.09**	42.36**	186.96**	17.68*	7.39
TZEIORQ 2 × TZEIORQ 24	17.32*	68.22**	30.79**	29.00*	33.50**	256.52**	10.70*	8.32
TZEIORQ 2 × TZEIORQ 42	16.64*	64.83**	35.03**	36.06**	46.80**	165.22**	17.53*	8.57
TZEIORQ 2 × TZEQI 82	12.18*	50.00**	36.44**	24.16*	44.83**	95.65**	12.33*	7.31
TZEIORQ 2 × TZEIORQ 70	4.80	52.97**	38.70**	32.34**	41.38**	208.70**	16.20*	6.30
TZEIORQ 11 × TZEIORQ 20	19.04*	46.19**	31.07**	25.28*	33.50**	113.04**	15.01*	7.48
TZEIORQ 11 × TZEIORQ 24	19.38*	50.42**	27.97*	27.88*	41.87**	134.78**	17.38*	5.13
TZEIORQ 20 × TZEIORQ 24	18.87*	57.20**	38.98**	32.71**	43.35**	173.91**	15.01*	5.71
TZEIORQ 22 × TZEIORQ 42	18.87*	64.41**	24.01*	19.70*	29.06*	91.30**	17.38*	6.97
TZEIORQ 22 × TZEQI 82	16.12**	50.42**	19.21*	34.20*	36.95**	286.96**	13.97	8.24
TZEIORQ 24 × TZEIORQ 26	16.64**	55.51**	30.79**	40.52**	24.63*	191.30**	17.68*	-2.86
TZEIORQ 24 × TZEIORQ 42	18.91**	64.41**	24.01**	19.70*	29.06*	91.30**	17.36*	6.98
TZEIORQ 24 × TZEIORQ 59	16.64**	39.41**	22.60**	24.91*	42.36**	243.48**	15.47*	8.40
TZEIORQ 24 × TZEIORQ 69	15.45**	59.32**	32.49**	33.46**	34.00**	282.61**	18.44*	+9.00
TZEIORQ 26 × TZEIORQ 42	4.97*	49.58**	29.10**	25.28**	35.47**	295.65**	16.52**	8.74
TZEIORQ 26 × TZEIORQ 59	9.61	55.51**	16.10*	30.48**	46.80**	134.78**	12.93*	8.49
TZEIORQ 26 × TZEIORQ 69	8.58*	51.69**	36.72**	33.46**	36.21**	213.04**	17.07**	8.24
TZEIORQ 26 × TZEIORQ 70	10.29*	55.08**	22.88*	23.79*	28.08*	156.52**	15.75*	7.06
TZEIORQ 26 × TZEQI 82	-4.29	49.15**	19.49*	32.71**	36.45**	165.22**	13.97*	-0.08
TZEIORQ 42 × TZEIORQ 59	17.32*	65.25**	31.92**	26.77*	42.36**	243.48**	15.90*	8.15
TZEIORQ 42 × TZEIORQ 69	17.50*	55.93**	22.88*	32.34**	25.12*	173.91**	15.75*	8.49
TZEIORQ 42 × TZEIORQ 70	17.15*	49.15**	27.97*	39.78**	28.57*	269.57**	17.38*	7.39
TZEIORQ 42 × TZEQI 82	16.47*	55.51**	30.23**	32.34**	37.44**	291.30**	17.24*	.66
TZEIORQ 59 × TZEIORQ 69	12.18*	45.76**	31.92**	27.88*	41.87**	191.30**	13.08*	7.98
TZEIORQ 59 × TZEIORQ 70	6.69*	41.53**	19.49**	37.17**	33.00**	47.83**	17.84**	8.40
TZEIORQ 69 × TZEIORQ 70	8.73*	56.36**	16.10**	32.34**	24.63**	200.00**	14.71**	8.66
TZEIORQ 69 ×TZEQI 82	12.02*	46.19**	28.25**	44.24**	37.44**	208.70**	12.04**	0.08
SE(d)	0.0124	0.0062	0.0079	0.0048	0.0044	0.0052	0.0048	0.0088
CD5%	0.0247	0.0123	0.0157	0.0095	0.0087	0.0103	0.0095	0.0175
CD1%	0.0319	0.0159	0.0204	0.0124	0.0114	0.0135	0.0124	0.0229

Similarly, provitamin A (PVA) and associated carotenoid compounds showed substantial heterotic responses, confirming genetic variability for carotenoid biosynthesis in these hybrids. TZEIORQ 20 × TZEIORQ 24 recorded the highest PVA heterosis (38.98%), consistent with Menkir *et al*. (2024), who reported comparable increases in PVA through targeted biofortification. Elevated heterosis for β-cryptoxanthin and β-carotene, notably in hybrids such as TZEIORQ 2 × TZEIORQ 70 and TZEIORQ 2 × TZEIORQ 42, further corroborates the genetic potential for enhancing these key provitamin A carotenoids (Prasanna & Mazumdar, 2022). The observed heterotic gains in these carotenoids are particularly significant, given their direct role in combating vitamin A deficiency, a major public health challenge in many poor-resource regions.

Remarkably, α-carotene and zeaxanthin exhibited exceptionally high heterosis values, peaking at 295.65% in TZEIORQ 26 × TZEIORQ 42. This exceptional heterosis aligns with prior reports by Zunjare *et al*. (2023), which identified these carotenoids as highly heritable and responsive to hybrid breeding strategies. Such pronounced heterotic responses for α-carotene and zeaxanthin suggest untapped genetic reservoirs for further nutritional enhancement in maize breeding programs.

The consistently low standard error and critical difference values across traits reinforce the robustness and reliability of these heterosis estimates, validating the precision of the experimental design (Wang & Li, 2021). The significant variation in heterosis among hybrids confirms that hybrid breeding is a potent approach for improving grain yield and enhancing key nutritional traits such as tryptophan and carotenoid content. These findings have profound implications for maize biofortification efforts, indicating that carefully designed breeding schemes can deliver nutrient-dense, high-yielding maize varieties tailored for rainfed and resource-limited environments. Future breeding programs should strategically exploit additive and non-additive genetic variation to enhance food and nutritional security in at-risk populations.

Conclusion

This study highlights the significant role of heterosis in enhancing grain yield and nutritional quality in extra-early PVA-QPM hybrids. The findings demonstrate that breeding effectively improves agronomic performance, with several hybrids exhibiting substantial heterotic gains over commercial checks. Notably, grain yield heterosis ranged from -4.29% to 19.38%, underscoring the genetic advantage of selected hybrids. Tryptophan content also showed high positive heterosis, exceeding 50% in several hybrids, reinforcing the effectiveness of QPM breeding in enhancing protein quality. Heterotic effects for carotenoid composition varied significantly, with β-carotene (46.80%), β-cryptoxanthin (40.52%), and α-carotene (295.65%) displaying the highest improvements. These findings confirm the potential of hybrid breeding as a biofortification strategy to address vitamin A deficiency in poor-resource populations. The statistical reliability of the results, as indicated by low standard error values and significant critical differences, further validates the genetic gains achieved. The results stressed the importance of selecting superior parental lines and breeding strategies to enhance yield and nutritional profiles in maize. Future research should focus on evaluating genotype-by-

environment interactions to ensure the stability of these heterotic effects across diverse agroecological regions. By leveraging heterosis, maize breeding programs can develop nutritionally superior, high-yielding hybrids that contribute to food security and improve public health outcomes, particularly in areas where maize is a staple food.

Recommendations

1. Selecting superior parental lines exhibiting strong combining ability for grain yield and nutritional characteristics is essential to enhance hybrid performance within breeding programs.
2. Conducting multi-location trials across varied agroecological zones is essential for evaluating the stability and adaptability of genotypes. Incorporating hybrid breeding into biofortification initiatives will further strengthen strategies aimed at reducing vitamin A deficiency and malnutrition.
3. Efforts should also focus on farmer adoption and seed production through awareness campaigns, subsidies, and improved seed distribution systems.
4. Sustained monitoring of hybrid performance in terms of yield, nutritional benefits, and farmer adoption is crucial for guiding future breeding efforts. This approach will support the development of resilient, nutrient-rich maize hybrids that contribute to long-term food security in resource-constrained areas.

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