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CANONICAL DISCRIMINANT ANALYSIS OF AGRONOMIC, CAROTENOID, AND PROTEIN QUALITY PARAMETERS IN BIOFORTIFIED MAIZE UNDER FALL ARMYWORM STRESS

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ABSTRACT: Fall armyworm (FAW) infestation poses a critical constraint to maize production across sub-Saharan Africa, with disproportionate impacts on extra-early maturing genotypes that are vital for ensuring food security in climate-sensitive and resource-constrained environments. This study provides the first systematic evaluation of extra-early Provitamin A Quality Protein Maize inbred lines for grain yield, biofortification traits, and FAW resistance under natural infestation. It employs multivariate analysis to integrate earliness, nutritional quality, and pest resilience for climate-smart maize breeding. Field evaluations were conducted during the 2023 and 2024 rainy seasons at Oke-Oyi, Nigeria, using a randomised complete block design. Multivariate analysis of variance revealed highly significant differences among inbred lines for key agronomic and nutritional traits. Notably, top-performing genotypes such as TZEEIORQ 14 exhibited grain yield advantages of 25.9% and 41.7% over standard checks. Multiple inbred lines surpassed the biofortification threshold of 12 µg/g for β-carotene and consistently demonstrated elevated tryptophan concentrations relative to the standard checks. Canonical discriminant analysis grouped the inbreds into distinct clusters, with grain yield, β-carotene, and tryptophan emerging as the primary discriminating traits. Conversely, leaf damage score and anthesis–silking interval were the most negative contributors to genotype separation. Inbreds TZEEIORQ 5, 10, 11, 14, and 18 emerged as promising candidates for breeding programmes aimed at enhancing FAW tolerance, nutritional quality, and grain yield. These findings demonstrate the effectiveness of multivariate tools in deciphering trait interrelationships and facilitating the selection of elite genotypes for sustainable maize improvement.

Keywords: Multivariate analysis, grain yield, *Spodoptera frugiperda* resistance, β-carotene, leaf damage score

INTRODUCTION

Maize (*Zea mays* L.) remains a staple crop of global significance, particularly in sub-Saharan Africa, where it underpins food and nutritional security. Yet, conventional maize varieties often lack essential micronutrients, notably provitamin A, and key amino acids such as lysine and tryptophan, deficiencies that exacerbate vitamin A deficiency and protein-energy malnutrition in maize-dependent populations (Naik *et al*., 2024). In response, biofortification through plant breeding has emerged as a sustainable strategy to enhance the nutritional profile of maize. The development of Provitamin A Quality Protein Maize (PVA-QPM), which incorporates elevated levels of β-carotene, lysine, and tryptophan, marks a significant milestone in this endeavour (Oyekale *et al*., 2021).

In regions with erratic rainfall and abbreviated growing seasons, extra-early maturing PVA-QPM hybrids offer advantages by supporting rapid crop turnover and yield stability (Bello *et al*., 2024). Nevertheless, the productivity and large-scale

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adoption of these genotypes are increasingly threatened by fall armyworm (FAW) (*Spodoptera frugiperda*), it is an invasive and highly destructive lepidopteran pest that has rapidly spread across Africa since its emergence in 2016, inflicting substantial damage on maize production systems. FAW-induced yield losses in maize have been reported to range from 8% to over 70%, corresponding to economic damages estimated between USD 72.4 and 675.3 per hectare across affected regions (Togola *et al*., 2025). The pest's prolific reproductive capacity, broad host range, and increasing resistance to insecticides have undermined the effectiveness of conventional chemical control. This renders the conventional method unsustainable, especially for resource-limited smallholder farmers (Asare-Nuamah, 2020). Consequently, breeding for host-plant resistance within biofortified genetic backgrounds presents a cost-effective and ecologically sound alternative.

Morphological and biochemical traits such as leaf toughness, trichome density, and secondary metabolite production contribute to reduced FAW damage in maize (Woolfolk *et al*., 2025). However, resistance to FAW is a quantitatively inherited trait governed by complex genetic architecture and significant genotype-by-environment interactions. Achieving simultaneous improvement in resistance, grain yield, and nutritional quality necessitates the application of advanced statistical tools capable of dissecting multi-trait relationships and guiding effective selection. Canonical discriminant analysis is a multivariate classification tool that constructs linear combinations of traits (canonical variables) to maximize separation among predefined groups. Its application in genotype discrimination has proven effective in several crops, including maize, for agronomic, physiological, and stress-response traits (Öz, 2012). The Canonical discriminant analysis offers the added advantage of identifying the attributes that contribute most significantly to group differentiation, thereby supporting targeted selection. Notably, this study represents the first comprehensive effort to evaluate extra-early maturing PVA-QPM inbred lines for combined grain yield, nutritional quality (β-carotene and tryptophan), and FAW resistance under natural infestation using multivariate analytical tools. As a pioneer in integrating these three critical components (earliness, biofortification, and FAW tolerance), this work provides a novel framework for accelerating the development of climate-smart, nutrition-sensitive maize hybrids. Agronomic parameters such as grain yield, plant height, days to silking, and the anthesis-silking interval (ASI) are key indicators of genotype performance under stress. The ASI, in particular, reflects reproductive resilience, while plant height influences canopy structure and pest susceptibility (Zia *et al*., 2021). On the nutritional front, β-carotene and tryptophan are primary targets in QPM biofortification efforts (Naik *et al*., 2024). The FAW resistance is commonly evaluated through phenotypic traits such as leaf damage scores and stay-green ability, which reflect tolerance or compensatory growth (Kasoma *et al*., 2020). However, these traits are often assessed in isolation, limiting a comprehensive understanding of genotype performance. To bridge this gap, this study applied Canonical and discriminant analysis to a diverse panel of extra-early maturing PVA-QPM inbred lines evaluated under natural FAW infestation. The objectives were as follows:

i. identify key traits that discriminate among genotypes;
ii. classify genotypes into distinct performance groups; and
iii. inform breeding strategies aimed at developing high-yielding, nutritionally enhanced, and pest-resilient maize cultivars adapted to Sub-Saharan Africa's production systems.

MATERIALS AND METHODS

Plant materials

Eighteen extra-early maturing PVA-QPM inbred lines and two varietal checks were evaluated (Table 1). These inbred lines, developed by the International Institute of Tropical Agriculture (IITA), were selected based on their contrasting performance in agronomic traits, nutritional composition, and resistance to FAW. The varietal checks, TZEE-Y Pop STR QPM and Obatanpa, are widely cultivated maize varieties with well-documented nutritional profiles and established responses to FAW infestation. Their inclusion facilitated the benchmarking of the inbred lines for grain yield, β-carotene, and Tryptophan contents, as well as resistance to FAW under natural field conditions.

Trial location and environmental description

Field trials took place during the 2023 and 2024 main growing seasons at the experimental station of the Lower Niger River Basin Development Authority in Oke-Oyi, Kwara State, Nigeria. The site lies within the southern Guinea savannah agroecological zone. It is positioned between latitudes 8°30′N and 8°50′N and longitudes 4°20′E and 4°35′E, at an elevation of approximately 378 meters above sea level. The region is characterized by a tropical savannah climate (Köppen classification Aw), with distinct rainy (April–October) and dry (November–March) seasons (Climate-Data.org, 2024). The annual rainfall averages 852 mm, with peak precipitation (≈211 mm) typically recorded in September (Climate.top, 2024). Average minimum and maximum temperatures are 21.8°C and 32.6°C, respectively. The soils at the experimental site are predominantly alluvial, with moderate fertility and good drainage characteristics, making them well-suited for maize cultivation (Tutiempo Network, 2024).

Table 1. Description of the 18 extra-early PVA-QPM inbred lines used in the study

Inbred code	Pedigree/Line name	Grain color	Provitamin A level	FAW resistance
TZEEIORQ 1	TZE-W DT STR QPM x ACR 91 SU1	Orange	High	Moderate
TZEEIORQ 2	TZE-W DT QPM x KU1409	Orange	Medium	High
TZEEIORQ 3	TZE-W Pop DT QPM	Orange	High	Low
TZEEIORQ 4	ACR 91 SU1 x KU1409	Orange	Medium	High
TZEEIORQ 5	KU1414 x TZE-W QPM	Orange	High	High
TZEEIORQ 6	TZE-Y QPM DT STR x ACR97 SU1	Yellow-Orange	Medium	Moderate
TZEEIORQ 7	ACR 97 SU1 x ACR 91 SU1	Orange	High	High
TZEEIORQ 8	KU1409 x KU1414	Orange	Low	Moderate
TZEEIORQ 9	KU1414 x TZE-W QPM	Orange	High	Moderate
TZEEIORQ 10	TZE-W QPM x DTSTR SYN	Yellow	Medium	High
TZEEIORQ 11	DTSTR QPM x ACR97 SU1	Orange	High	High
TZEEIORQ 12	ACR91 SU1 x KU1409	Orange	Medium	Low
TZEEIORQ 13	TZE-W Pop DT QPM	Yellow	High	Moderate
TZEEIORQ 14	KU1414 x DTSTR QPM	Orange	High	High
TZEEIORQ 15	TZE-Y QPM x ACR97 SU1	Orange	Medium	High
TZEEIORQ 16	KU1409 x TZE-W DT QPM	Orange	High	High
TZEEIORQ 17	ACR97 SU1 x KU1409	Orange	Medium	Moderate
TZEEIORQ 18	DTSTR QPM x KU1414	Orange	High	High
Varietal checks				
TZEE-Y Pop STR QPM	Open-Pollinated Variety	Yellow	Medium	Moderate
Obatanpa	Local Improved White Maize	White	Low	Low

Experimental design and field management

The trials followed a randomised complete block design (RCBD) with three replications. Each genotype was sown in a 5-meter-long single row, with inter-row and intra-row spacing of 75 cm and 50 cm, respectively. It accommodated two plants per hill, resulting in a planting density of approximately 53,333 plants per hectare. Land preparation involved ploughing followed by harrowing to achieve a well-tilled seedbed. Weed control at planting was achieved through the application of a pre-emergence herbicide formulation (Primextra Gold®, comprising Atrazine and Metolachlor) at a rate of 4.0 L/ha using 200 L/ha of water immediately after sowing. Post-emergence weed control was conducted four weeks after emergence (WAE) with Glyphosate (Force-Up®) at 2.5 L/ha as a directed spray. Nutrient application was carried out at the rate of 60 kg N, 30 kg P, and 30 kg K per hectare using a combination of NPK 15–15–15 and urea fertilizers. Fifty per cent of the nitrogen was applied at planting, while the remaining half was used to top-dress four weeks after emergence (WAE). No insecticides were applied during the trial to allow for the natural infestation of FAW resistance under ambient pest pressure."

Trait measurement: agronomic, resistance, and physiological traits

Grain yield (t/ha) was estimated by harvesting ears from the central plot rows and adjusting the weights to a standard moisture content of 12.5%. Plant height (cm) was measured from the base of the plant at the soil surface to the tip of the tassel at physiological maturity. Days to 50% silking was recorded as the number of days from planting until 50% of the plants in a plot exhibited visible silk emergence. The anthesis–silking interval (ASI), expressed in days, was calculated at the plot level as the difference between the dates when 50% of plants in a plot had shed pollen (anthesis) and when 50% had extruded silks (silking). We assessed resistance to FAW using two phenotypic indicators. Leaf damage was visually scored three weeks after emergence on a 1-9 scale, where a score of 1 denoted no visible injury and 9 indicated severe foliar damage caused by FAW feeding. Stay-green ability, reflecting the plant's capacity to maintain green foliage under FAW pressure, was evaluated at physiological maturity on a 1–9 scale, with 1, denoting complete senescence and 9 indicating full foliage retention.

Quantification of β-Carotene and Tryptophan

Grain β-carotene content was quantified using high-performance liquid chromatography (HPLC), following the protocol described by Biswas *et al.* (2011). Each sample was analyzed in duplicate to ensure reproducibility. Tryptophan content was measured using a modified colourimetric method (Hosokawa *et al.*, 2023), calibrated using standard solutions, and based on whole-grain flour. Final concentrations were reported as a percentage of the sample's dry weight (Drochioiu *et al.*, 2024).

Data analysis

To evaluate variation among the genotypes for the set of traits, a multivariate analysis of variance (MANOVA) was conducted using SAS® Viya® 4.0 (SAS Institute Inc., 2024). Mean comparisons were performed using Tukey's Honestly Significant Difference (HSD) procedure at a significance level of $p \leq 0.05$. Experimental precision and data reliability were assessed using descriptive statistics, including the coefficient of variation (CV), standard error (SE), and standard error of the difference (SED). Canonical discriminant analysis was applied to categorize genotypes according to their trait profiles and to identify the variables that most effectively contributed to group differentiation. Before analysis, trait values were transformed into standardized z-scores to eliminate scale disparities and facilitate unbiased comparisons.

RESULTS

The evaluation of 18 extra-early PVA-QPM inbred lines and two varietal checks under natural FAW infestation revealed substantial genetic variability across agronomic, phenological, resistance, and nutritional traits. Grain yield ranged from 2.1 t/ha in TZEEIORQ 8 to 3.4 t/ha in TZEEIORQ 14, with TZEEIORQ 14 outperforming TZEE-Y Pop STR QPM (2.7 t/ha) and Obatanpa (2.5 t/ha) by 25.9% and 36.0%, respectively. Notably, eight inbreds surpassed TZEE-Y Pop STR QPM in terms of yield, and 14 outperformed Obatanpa, reflecting substantial genetic gains under FAW pressure. Plant height ranged from 130 to 160 cm, with most inbreds exhibiting moderate stature that supported yield potential and lodging resistance. Days to 50% silking spanned 54–57 days, while 10 inbreds recorded a short anthesis–silking interval (ASI) of 1 day, indicating enhanced stress adaptability. FAW resistance traits varied widely among the inbreds. Leaf damage scores ranged from 2.6 (TZEEIORQ 11) to 6.5 (TZEEIORQ 3), with TZEEIORQ 11, 14, and 5 showing the lowest scores (≤2.8), translating to up to 40% lower injury compared to TZEE-Y Pop STR QPM and 37% relative to Obatanpa. These lines also demonstrated superior stay-green ratings (2.6–2.8) compared to the varietal checks (3.5 and 3.8), reflecting better physiological resilience. Nutritionally, 13 inbreds exceeded the β-carotene biofortification threshold of 12 µg/g, with TZEEIORQ 16 and 18 achieving >75% higher concentrations than TZEE-Y Pop STR QPM. Tryptophan content ranged from 0.068% to 0.078%, with TZEEIORQ 14 leading at 0.078%, representing an 11.4% improvement over the checks. Overall, TZEEIORQ 14 stood out as the most promising genotype, effectively combining elevated grain yield, strong FAW resistance, and superior nutritional quality. The experimental data were robust, with low coefficients of variation and statistically significant genotype differences confirmed by Tukey's HSD test, underscoring the precision and reliability of the results.

Canonical discriminant analysis among maize inbred groups

Canonical discriminant analysis effectively identified the key traits contributing to the differentiation of extra-early PVA-QPM inbred groups (Figure 1). Grain yield was the strongest discriminant, exhibiting the highest structure (0.81) and standardized (0.69) coefficients, confirming its central role in genotype separation. Nutritional traits, particularly β-carotene (structure: 0.72; standardized: 0.58) and tryptophan (structure: 0.64; standardized: 0.52), also contributed significantly, underscoring their importance in selecting nutritionally enhanced maize lines. Plant height (0.65) and ear height (0.52) showed moderate positive effects, reflecting their relevance in optimizing plant architecture for standability and resource use efficiency.

Table 2. Mean performance of 18 extra-early PVA-QPM inbred lines and two varietal checks across evaluated traits

Inbreds code	Grain yield (t/ha)	Plant height (cm)	Days to silking (days)	Anthesis-silking interval (days)	Stay-green score	Leaf damage score	β-carotene (µg/g)	Tryptophan (%)
TZEEIORQ 1	2.6	145	56	2	4.0	4.5	14.2	0.074
TZEEIORQ 2	3.0	135	55	1	3.0	3.2	12.5	0.070
TZEEIORQ 3	2.2	160	57	3	5.0	6.5	14.8	0.069
TZEEIORQ 4	2.9	140	55	1	3.2	3.8	12.0	0.072
TZEEIORQ 5	3.1	138	54	1	2.8	3.0	14.5	0.075
TZEEIORQ 6	2.5	142	56	2	4.1	4.7	11.9	0.071
TZEEIORQ 7	3.2	136	55	1	2.9	2.9	14.7	0.076
TZEEIORQ 8	2.1	134	57	3	4.5	5.5	10.5	0.068
TZEEIORQ 9	2.7	139	56	2	4.0	4.2	13.8	0.072
TZEEIORQ 10	3.0	130	54	1	3.0	3.1	11.5	0.073
TZEEIORQ 11	3.3	137	54	1	2.6	2.7	14.9	0.076
TZEEIORQ 12	2.4	144	57	3	5.1	5.9	12.3	0.070
TZEEIORQ 13	2.8	141	56	2	4.0	4.0	13.5	0.074
TZEEIORQ 14	3.4	138	54	1	2.7	2.8	14.6	0.078
TZEEIORQ 15	2.9	135	55	1	3.0	3.4	12.4	0.071
TZEEIORQ 16	3.1	143	55	1	2.9	2.9	15.1	0.077
TZEEIORQ 17	2.6	140	56	2	4.0	4.5	11.7	0.069
TZEEIORQ 18	3.2	139	54	1	2.8	3.0	15.0	0.076
Varietal checks								
TZEE-Y Pop STR QPM	2.7	136	55	1	3.5	4.0	8.5	0.070
Obatanpa	2.5	142	56	2	3.8	4.3	7.2	0.069
SE	0.088	1.485	0.256	0.183	0.191	0.271	0.347	0.001
SED	0.125	2.101	0.362	0.259	0.270	0.383	0.491	0.001
CV (%)	13.21	4.51	1.96	48.26	22.91	29.29	11.05	4.19
HSD (0.05)	0.853	14.371	2.474	1.773	1.846	2.620	3.358	0.007

Conversely, FAW damage score (−0.79) and ASI (−0.63) had strong negative loadings, indicating that genotypes with reduced foliar injury and shorter reproductive intervals are favoured under FAW pressure and environmental stress. The alignment between the standardized coefficients and the structure matrix highlights the robustness of the discriminant model. Overall, grain yield, β-carotene, and tryptophan emerged as the primary positive discriminators while FAW damage and ASI remained key negative indicators, providing clear targets for multi-trait selection in biofortified, pest-resilient maize breeding.

Multivariate analysis of variance for genotypic effects of extra-early PVA-QPM inbred lines

The MANOVA showed highly significant differences among the 18 extra-early PVA-QPM inbred lines and two varietal checks across eight evaluated traits (Table 3). All four multivariate test statistics—Pillai's Trace, Wilks' Lambda, Hotelling's Trace, and Roy's Largest Root—were significant at $p < 0.0001$, confirming strong overall genotypic effects. Pillai's Trace value of 1.982 with an F-value of 2.745 (df = 136, 172) indicated a significant multivariate response to genotypic differences. Wilks' Lambda was notably low (0.021), with an associated F-value of 3.012 (df = 136, 152), supporting the existence of meaningful trait variation across genotypes. Similarly, Hotelling's Trace (4.671) and Roy's Largest Root (2.752; F = 5.912) confirmed strong multivariate genotype discrimination. These results highlight that the inbred lines differed significantly when traits were considered jointly, justifying the application of canonical discriminant analysis. The significant multivariate tests validate the presence of substantial variation in agronomic, resistance, and nutritional traits, supporting the identification of superior genotypes for use in hybrid development and stress-resilient, nutritionally enhanced maize breeding programmes.

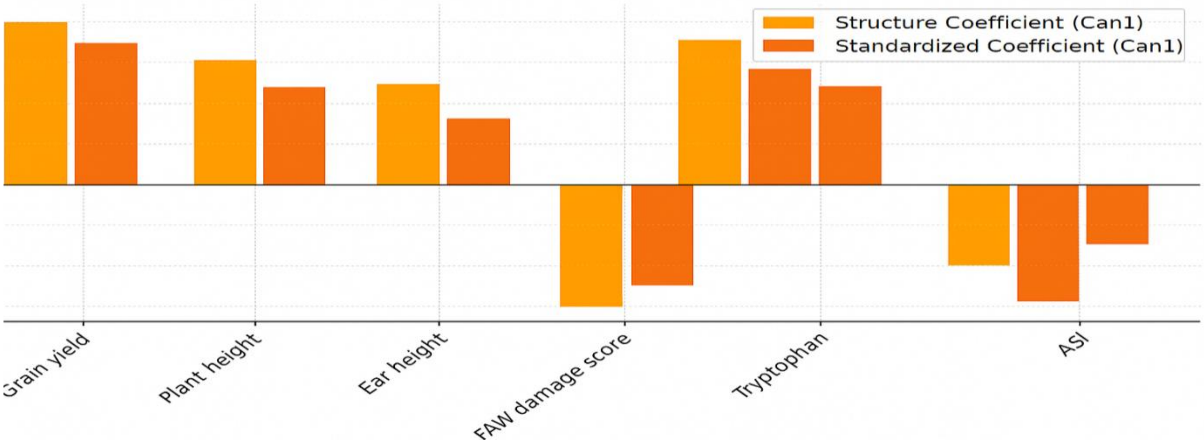

Figure 1. Canonical structure matrix and standardized canonical coefficients for discriminating among inbred groups

Table 3. Multivariate analysis of variance for genotypic effects across eight traits in extra-early PVA-QPM inbred lines

Multivariate Test	Value	F-value	Hypothesis df	Error df	p-value	Decision
Pillai's Trace	1.982	2.745	136	172	<0.0001	Significant
Wilks' Lambda	0.021	3.012	136	152	<0.0001	Significant
Hotelling's Trace	4.671	2.583	136	160	<0.0001	Significant
Roy's Largest Root	2.752	5.912	17	21	<0.0001	Significant

Canonical classification and breeding implications

The canonical discriminant analysis effectively classified the inbred lines into three distinct groups: Low, Medium, and High, based on their discriminant functions. The first two canonical variates accounted for the major portion of the between-group variability, facilitating clear group separation. Inbred lines classified as "High" (represented by red squares) clustered predominantly on the negative side of Canonical Variate 1, indicating distinct discriminant characteristics from the other groups. The "Medium" group (orange X markers) occupied intermediate canonical scores, while the "Low" group (yellow circles) clustered on the positive side of Canonical Variate 1. The clear separation of these groups highlights the efficiency of the discriminant functions in distinguishing among the inbred lines based on the measured traits.

DISCUSSION

This investigation is the earliest to systematically assess extra-early maturing PVA-QPM inbred lines for the simultaneous improvement of grain yield, nutritional traits, and resistance to FAW under natural infestation, employing multivariate statistical approaches. By combining earliness, biofortification, and insect tolerance in a single analysis, it establishes a new paradigm for developing climate-resilient and nutritionally oriented maize hybrids. The evaluation of 18 extra-early PVA-QPM maize inbred lines, alongside two varietal checks, under natural FAW infestation revealed substantial genetic variability across key agronomic, nutritional, and pest resistance traits. The observed significant variation highlights the robustness of the evaluated genetic materials. It emphasizes their promising potential for incorporation into breeding programmes aimed at improving food and nutritional security across sub-Saharan Africa. Among the inbreds, TZEEIORQ 14 emerged as a standout genotype, achieving the highest grain yield (3.4 t/ha) while also exhibiting elevated β-carotene content (14.6 µg/g) and tryptophan level (0.078%). These findings suggest that this genotype is suitable for dual-purpose breeding goals, combining agronomic productivity with enhanced nutritional quality. It is particularly relevant considering the current breeding emphasis on stress-resilient and nutrient-dense maize genotypes to improve yield and dietary quality (Sebayang *et al*., 2022; Gedil *et al*., 2024).

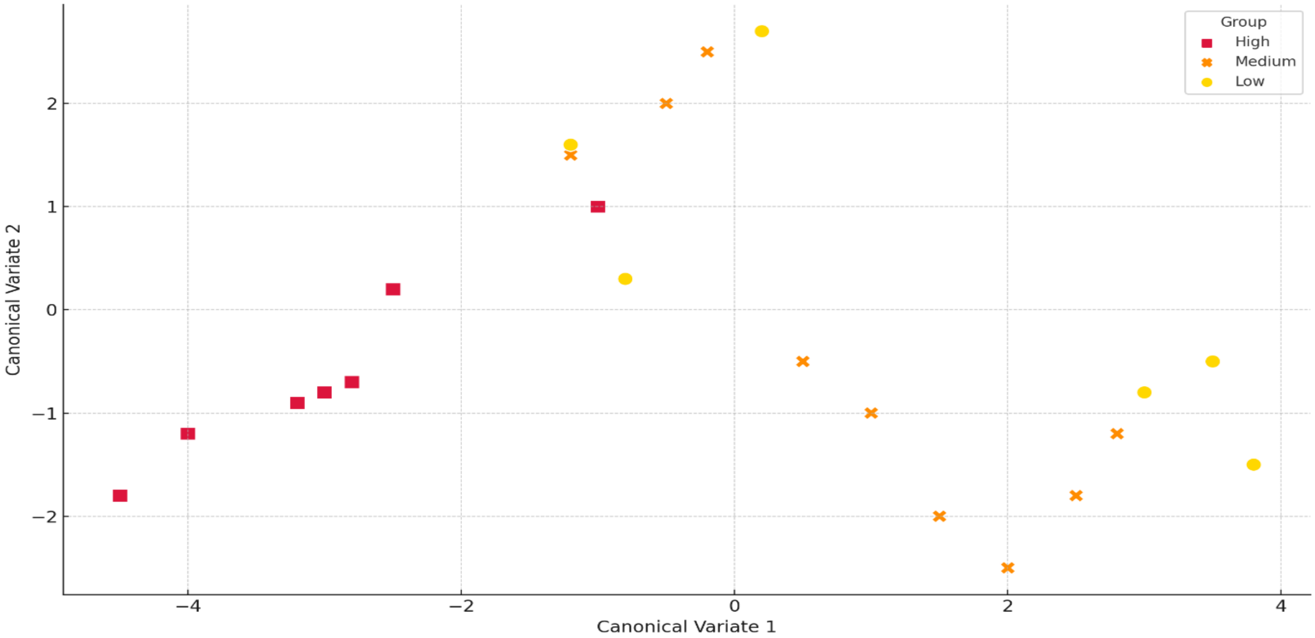

Figure 2. Canonical classification plot

Phenological attributes, ASI, and silking time further reinforce the adaptability of certain inbreds. Short ASI observed in TZEEIORQ 5, 10, 11, 14, and 18 suggests enhanced synchronization of male and female flowering, which is critical under FAW stress conditions. A short ASI has been identified as a key trait associated with reproductive resilience and efficient pollination under abiotic stress (Oyekale *et al*., 2021). Its expression under FAW pressure in the current study highlights the underlying genetic robustness of these lines. Plant height showed moderate variability among the evaluated inbreds, with TZEEIORQ 3 attaining a maximum height of 160 cm, suggesting its potential for good biomass production and contribution to improved lodging resistance under field conditions. Although tall plants may have an advantage in terms of light interception, excessively tall maize can be prone to lodging. Hence, intermediate plant height (~138 cm) observed in superior inbreds TZEEIORQ 11 and 14 offers an ideal compromise, supporting both photosynthetic capacity and standability. These results corroborate earlier research that associated favourable plant architecture traits with enhanced yield stability across diverse and fluctuating environmental conditions, thereby highlighting their potential as reliable selection criteria in breeding programmes (Johnstone *et al*., 2017).

Another important agronomic attribute observed was the stay-green trait, particularly in TZEEIORQ 11, 14, and 5. Stay-green phenotypes delay leaf senescence, allowing prolonged photosynthesis and extended grain filling under stress conditions, including pest infestation. This physiological advantage has been previously associated with higher yield and stress tolerance in cereals (Anderegg *et al*., 2021) and likely contributed to the superior yield performance observed in these inbreds. Pest resistance, particularly to FAW, was a distinguishing factor among the inbreds. Genotypes TZEEIORQ 11 and TZEEIORQ 14 recorded significantly lower FAW damage scores (2.7 and 2.8, respectively) relative to the varietal checks, indicating superior tolerance to *Spodoptera frugiperda* and highlighting their potential as valuable parents for breeding resistant hybrids. These findings reveal intrinsic resistance traits within the host plants that are advantageous in developing long-term pest management solutions. Job *et al*. (2022) emphasized the strategic importance of breeding for host resistance to minimize reliance on synthetic pesticides and reduce environmental impacts.

From a nutritional standpoint, the enhanced β-carotene content observed in 13 inbreds, each exceeding the HarvestPlus threshold of 12 µg/g, is a significant milestone. For instance, TZEEIORQ 16 (15.1 µg/g) and TZEEIORQ 18 (15.0 µg/g) surpassed the levels recorded in orange maize checks by more than 75%, reinforcing their potential in biofortification efforts. It aligns with ongoing initiatives aimed at mitigating vitamin A deficiency through PVA-enriched maize (Gedil *et al*., 2024). Concurrently, the enhanced tryptophan levels observed in multiple inbred lines affirm the preservation of QPM traits, which are vital for improving the protein quality of maize-based diets. The experiment demonstrated strong statistical reliability, as evidenced by low values of standard error and standard error of difference. The observed trait differences were substantiated by Tukey's HSD test, confirming the robustness and reliability of the findings. Although moderate-to-high coefficients of variation for ASI and FAW damage reflect the inherent variability associated with genotype × environment interactions and non-uniform pest pressure in field evaluations, the identification of stable, high-performing genotypes such as TZEEIORQ 14, 11, 5, and 16 provides promising material for breeding pipelines.

The Canonical discriminant analysis further elucidated the multivariate trait structure, providing critical insights into genotype classification. Grain yield contributed the most to genotype separation, reaffirming its central role in maize improvement (Amegbor, 2022). Nutritional quality traits, β-carotene and tryptophan, also loaded strongly on the first canonical variate, underscoring their growing importance in modern breeding objectives that integrate agronomic performance with health-focused outcomes (Chandrasekharan *et al*., 2022). Although plant height exhibited a relatively modest discriminant weight in the canonical analysis, it remains an important secondary trait influencing environmental adaptation, crop architecture, and standability in breeding programmes. Traits linked to stress resilience, FAW damage score, and ASI contributed negatively to the discriminant function, reinforcing their inverse association with genotype performance. Their inclusion improves the discrimination of lines with superior pest resistance and stress tolerance, as confirmed in multivariate studies focused on host plant resistance (Wambi *et al*., 2025). MANOVA results supported the robustness of trait differentiation among genotypes. The significance of all four multivariate test statistics (Pillai's Trace, Wilks' Lambda, Hotelling's Trace, and Roy's Largest Root) validates the presence of meaningful genotypic variation. Particularly, the low Wilks' Lambda value and high Roy's Largest Root statistic suggest strong linear trait combinations that effectively distinguish among genotypes (Jadhav & Dolas, 2023). These findings corroborate the conclusions of Mubushar *et al*. (2022), highlighting the effectiveness of multivariate approaches in selecting genotypes amid complex trait interactions and challenging stress conditions.

Lastly, the clear groupings derived from the canonical classification plot support the application of multivariate tools for pre-breeding and parental selection. The elite cluster, comprising TZEEIORQ 7, 11, 14, and 18, integrates high yield, nutritional value, and FAW resistance, making these lines ideal candidates for hybrid development. In contrast, genotypes with lower multivariate scores may still possess specific traits that warrant targeted introgression. As emphasized by Amegbor (2022), integrating multivariate selection approaches streamlines the identification of complementary parental lines and accelerates breeding progress. This study highlights the critical role of multivariate statistical approaches, including Canonical discriminant analysis and MANOVA, in elucidating intricate genotype-by-trait interactions. Such tools facilitate precise and informed selection decisions in breeding programmes focused on developing stress-resilient, nutrient-enriched, extra-early maize cultivars tailored to the demanding agroecological conditions of sub-Saharan Africa.

CONCLUSION

The evaluation of 18 extra-early PVA-QPM inbred lines under natural FAW infestation revealed significant genetic variability for agronomic, phenological, nutritional, and resistance traits. Several inbreds, notably TZEEIORQ 14, showed superior multi-trait performance. Grain yield advantages were 25.9% and 41.7% over TZEE-Y Pop STR QPM and Obatanpa, respectively. This underscores their potential to enhance productivity in FAW-prone, low-input production systems. The narrow silking window and reduced ASI in TZEEIORQ 5, 10, 11, 14, and 18 suggest improved reproductive synchrony and stress adaptability. Additionally, several inbreds recorded FAW damage scores up to 37% lower than the checks. This confirms the possibility of combining heritable insect tolerance with desirable agronomic and nutritional traits. Importantly, these FAW-tolerant genotypes exceeded the β-carotene biofortification threshold (12 µg/g) and maintained elevated tryptophan levels, reinforcing their dual potential for improving both food security and nutritional quality. Multivariate analyses validated the distinctiveness of these lines, with grain yield, β-carotene, and tryptophan emerging as the most important discriminators. The integration of MANOVA and canonical discriminant analysis provided a powerful multivariate framework for selecting elite parental lines and accelerating hybrid development. Collectively, these findings demonstrate the feasibility of breeding extra-early PVA-QPM hybrids with high yield, enhanced nutritional value, and durable FAW resilience, a critical step toward building climate-smart. These nutrition-sensitive maize breeding pipelines address the challenges faced by smallholder farmers in sub-Saharan Africa.

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