RESEARCH | Egyptian Journal of Agricultural Research | Agronomy

Differentiation of agronomic, β-carotene, and tryptophan traits in provitamin A quality protein maize under fall armyworm infestation

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Received: 3-6-2025; **Accepted**: 22-10-2025; **Published:** 30-10-2025 **DOI:** 10.21608/EJAR.2025.391760.1676

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 randomized 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, anthesis–silking interval, *Spodoptera frugiperda resistance*

INTRODUCTION

Maize (*Zea mays* L.) is a critical staple crop of global importance, particularly in sub-Saharan Africa (SSA), where it plays a central role in food and nutritional security. However, conventional maize varieties are often deficient in key micronutrients, particularly provitamin A, as well as essential amino acids such as lysine and tryptophan. This deficiency contributes significantly to vitamin A deficiency and protein-energy malnutrition in populations that rely heavily on maize-based diets (Naik *et al.,* 2024). In response, biofortification through plant breeding has emerged as a sustainable approach to improving the nutritional quality of maize. The development of provitamin A Quality Protein Maize (PVA-QPM), which combines enhanced β-carotene concentration with elevated levels of lysine and tryptophan, represents a significant advancement in this direction (Oyekale *et al.,* 2021).

In regions with erratic rainfall and short growing seasons, extra-early maturing PVA-QPM hybrids provide vital benefits by enabling rapid crop turnover and more stable yields (Bello *et al.,* 2024). However, the productivity and widespread adoption of these nutritionally improved genotypes face growing challenges due to the Fall Armyworm (*Spodoptera frugiperda*) (FAW), an invasive and highly destructive lepidopteran pest. Since its introduction to Africa in 2016, FAW has rapidly spread across the continent, causing yield losses ranging from 8% to over 70% and resulting in estimated economic damages between USD 72.4 and 675.3 per hectare (Togola *et al.,* 2025). FAW's polyphagous nature, high fecundity, and resistance to commonly used insecticides have rendered conventional chemical control methods unsustainable and inaccessible for smallholder farmers (Asare-Nuamah, 2020). In this context, breeding maize for intrinsic resistance to FAW, particularly within biofortified backgrounds such as PVA-QPM, offers an ecologically sound and cost-effective solution. Several resistance-related traits, including leaf toughness, trichome density, and secondary metabolite production, have been associated with reduced FAW damage (Woolfolk *et al.,* 2025). However, resistance to FAW is typically quantitative, complex, and influenced by environmental interactions. The challenge of concurrently improving resistance, grain yield, nutritional composition, and agronomic performance necessitates robust statistical tools to dissect multi-trait interactions.

Canonical Discriminant Analysis (CDA) is a multivariate technique that constructs linear combinations of traits (canonical variables) to maximize the separation among predefined groups, making it well suited for genotype classification in breeding programmes. CDA has been successfully applied in maize and other crops to differentiate genotypes based on physiological, agronomic, and stress-response traits (Öz, 2012). Its utility lies in its ability to identify the traits contributing most strongly to group differentiation, providing breeders with informed targets for selection. However, a few studies have employed CDA to evaluate PVA-QPM genotypes under natural FAW infestation, integrating agronomic, nutritional, and resistance traits simultaneously. Notably, this study represents the first comprehensive effort to assess 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 traits, including grain yield, plant height, days to silking, and anthesis-silking interval (ASI), serve as critical indicators of crop performance, especially under stress conditions. ASI, for example, is a reliable marker of reproductive resilience under biotic and abiotic stress, while plant height influences canopy architecture, light capture, and pest vulnerability (Zia *et al.,* 2021). Nutritional traits such as β-carotene and tryptophan content are equally essential, as they contribute directly to the biofortification goals of QPM breeding (Naik *et al.,* 2024).

Resistance to FAW is typically assessed through phenotypic traits such as leaf damage scores and stay-green ability, which reflect a plant's tolerance or capacity to recover from pest attacks (Kasoma *et al.,* 2020). However, these traits are often analyzed in isolation rather than within a comprehensive framework. To address this gap, the present study employs CDA on a diverse set of extra-early PVA-QPM inbred lines evaluated under natural FAW infestation, enabling integrated assessment of resistance, agronomic performance, and nutritional quality. This study aims to identify traits that best discriminate among genotypes, classify genotypes into distinct performance categories, and inform breeding strategies for developing high-yielding, nutritionally enhanced, and pest-resilient maize cultivars suitable for SSA.

MATERIALS AND METHODS

1. Plant materials:

We used eighteen extra-early maturing PVA-QPM inbred lines and two variety checks to serve as controls in this study (Table 1). These lines were developed and provided by the International Institute of Tropical Agriculture (IITA) and selected for their variability in agronomic performance, nutritional quality, and resistance to FAW. These checks represent widely adopted and high-performing varieties with established stable performance and resistance to FAW. Their inclusion allows for benchmarking the performance of the new inbreds in terms of agronomic, nutritional, and FAW damage-related traits. Additionally, comparing PVA-QPM lines with the checks helps validate their FAW resistance and nutritional superiority, ensuring their significant improvements in provitamin A and protein quality without sacrificing yield. The checks also assess the agronomic competitiveness of the new inbreds by determining whether they can match or outperform existing checks under similar conditions.

2. Trial site and georeferencing:

Field trials were carried out at the Lower Niger River Basin Development Authority's experimental station in Oke-Oyi, Kwara State, Nigeria, during the main growing seasons of 2023 and 2024, commencing on July 2, 2023, and July 5, 2024, respectively. The experimental site lies between latitudes 8°30′N and 8°50′N and longitudes 4°20′E and 4°35′E, at an average altitude of 378 meters above sea level. This location falls within the southern Guinea savannah agroecological zone, characterized by a tropical savannah climate (Köppen climate classification Aw), marked by distinct wet and dry seasons (Climate-Data.org, 2024). The rainy season typically extends from April to October, with peak rainfall occurring between July and September. September has the highest average rainfall of approximately 211.1 mm (Climate.top, 2024). The dry season spans November to March, with a monthly rainfall average of about 4.6 mm and negligible precipitation in December. The location experiences an average annual rainfall of approximately 852 mm, with atmospheric moisture levels peaking at around 1500 mm (Tutiempo Network, 2024). Average minimum and maximum temperatures are approximately 21.8°C and 32.6°C, respectively. The soil type is alluvial, with moderate fertility and adequate drainage, suitable for maize production.

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

3. Experimental design and field evaluation:

The field experiments followed a randomized complete block design (RCBD) with three replications. Each maize inbred line was sown on a single 5-m row, spaced 75 cm between rows and 50 cm between plants, with two plants maintained per hill. This arrangement resulted in a planting density of approximately 53,333 plants ha^{-1}. Each treatment was represented by one row per replicate, providing uniform experimental units and reducing error variation. Before planting, the land was tilted using ploughing and harrowing to create a smooth and well-aerated seedbed. A pre-emergence herbicide mixture of Atrazine and Metolachlor (Primextra Gold®) was applied at 4.0 L ha^{-1} using 200 L ha^{-1} of water immediately after planting. Post-emergence weed control was conducted four weeks after emergence (WAE) using Glyphosate (Force-Up®) applied at 2.5 L ha^{-1} as a directed spray. Fertilizer was applied at a total rate of 60 kg N, 30 kg P, and 30 kg K per hectare using NPK 15–15–15 and urea. Fifty per cent of the nitrogen fertilizer was applied at planting, with the remainder top-dressed four weeks after emergence (WAE). Insecticides were completely withheld during the study to enable a precise evaluation of natural resistance to FAW.

4. Trait measurements for agronomic, resistance, and physiological attributes:

Grain yield (GY, t ha^{-1}) was determined by harvesting ears from the central two rows of each plot and adjusting the weight to a standard moisture content of 12.5%. Plant height (PH, cm) was measured from the soil surface to the tassel tip at physiological maturity. The number of days from planting to when half of the plants in a plot showed silk emergence was recorded as days to 50% silking. Anthesis-silking interval (ASI, days) was calculated by subtracting the silking date from the anthesis date. Leaf damage caused by fall armyworm was visually rated three weeks after emergence using a 1–9 scale. A score of 1 indicated no visible damage with intact leaves. Score 2 showed a few pinholes or minor feeding marks on one leaf; 3 signified scattered pinholes or short lesions on several leaves with light overall damage. Score 4 reflected noticeable feeding with elongated lesions and window-paning on several leaves, affecting up to 25% of the foliage. A score of 5 indicated moderate damage, characterized by ragged edges and window-paning on multiple leaves, covering approximately 25–50% of the leaf area. Plants rated 6 exhibited heavy feeding on central whorl leaves and larger lesions, resulting in roughly 50% of the foliage being damaged. A score of 7 indicated severe damage, with most leaves showing ragged holes, shredding, and whorl injury. Score 8 described very severe defoliation, where nearly all leaves were damaged, leaving only midribs and minimal tissue. The maximum score of 9 indicated destruction of the whorl and leaves, resulting in plant death or near-death due to extensive FAW feeding.

Stay-green was evaluated at physiological maturity using a 1–9 scale. A score of 1 indicated complete senescence, with all leaves brown and desiccated, whereas a score of 2 represented nearly complete senescence, with only a few patches of green remaining. A score of 3 indicated extensive senescence, with the majority of leaves brown and less than 25% of the foliage remaining green. A score of 4 reflected moderate to high senescence, with approximately 25–35% of the foliage still green, while a score of 5 indicated an intermediate condition with about 40–50% of leaf area green. A score of 6 represented moderate stay-green expression, with 55–65% of the foliage green, and a score of 7 described high stay-green expression, with 70–80% of the leaves retained as green. A score of 8 corresponded to very high stay-green, where most leaves remained green with only minimal senescence, and the maximum score of 9 signified sustained stay-green, with nearly all foliage green and functional despite pest pressure.

In addition to foliar damage ratings, the percentage of ears infected by FAW and the percentage of ear damage were recorded at physiological maturity. Ear infection (%) was calculated as the proportion of harvested ears showing larval entry, frass deposition, or tunnelling relative to the total number of ears sampled per plot. Ear damage was visually rated using the 1–9 CIMMYT/IBCR scale, where 1 = no visible ear damage; 2 = only slight feeding marks on a few kernels (<5%); 3 = light damage, with 6–10% of kernels affected; 4 = moderate feeding, 11–20% of kernels damaged; 5 = intermediate damage, 21–30% of kernels affected; 6 = moderately severe damage, with 31–40% of kernels destroyed; 7 = severe ear damage, with 41–60% of kernels affected; 8 = very severe ear damage, with 61–80% of kernels destroyed; and 9 = complete ear destruction, with >80% kernel loss. For analysis, these scores were also expressed as percentages of kernels damaged per ear. Genotypes with <10% kernel damage (scores 1–3) were considered tolerant, while those with >30% damage (scores ≥5) were classified as susceptible. These measures, combined with leaf damage ratings and stay-green assessments, provided a comprehensive evaluation of host plant resistance to FAW.

5. β-Carotene and tryptophan quantification:

Grain samples were analyzed for β-carotene content using high-performance liquid chromatography (HPLC), following the procedure described by (Biswas *et al.,* 2011). Each analysis was performed in duplicate to ensure reproducibility and accuracy. Tryptophan content was determined using a colourimetric method adapted from (Hosokawa *et al.,* 2023), based on the analysis of whole-grain maize flour. A standard calibration curve derived from known tryptophan concentrations was used to estimate the tryptophan levels in the samples. Final values were expressed as a percentage of the sample's dry weight (Drochioiu *et al.,* 2024).

6. Statistical analysis:

Multivariate analysis of variance (MANOVA) was performed using SAS® Viya® 4.0 (SAS Institute Inc., 2024) to evaluate the statistical significance of trait variation among the different inbred lines. Treatment means were compared using Tukey's honestly significant difference (HSD) test at a 5% significance level. Additional statistical parameters, including the coefficient of variation (CV% %), standard error of the mean (SE), and standard error of the difference (SED), were computed to assess data reliability and experimental precision. CDA was applied to identify the primary traits responsible for differentiating genotypes and group them based on their overall trait profiles. Before analysis, all variables were standardized to eliminate differences in measurement scales, allowing for meaningful comparisons across diverse traits.

RESULTS

1. Performance of extra-early PVA-QPM inbred lines and two varietal checks:

Evaluation of 18 extra-early PVA-QPM inbred lines under natural FAW infestation revealed significant variation across agronomic, phenological, pest resistance, and nutritional traits (Table 2). Grain yield ranged from 2.1 t/ha (TZEEIORQ 8) to 3.4 t/ha (TZEEIORQ 14), with eight inbreds yielding more than the varietal checks TZEE-Y Pop STR QPM (2.7 t/ha) and Obatanpa (2.5 t/ha). Notably, TZEEIORQ 14 recorded the highest yield, outperforming TZEE-Y Pop STR QPM by 25.9% and Obatanpa by 41.7%. Plant height varied from 130 cm (TZEEIORQ 10) to 160 cm (TZEEIORQ 3), with most lines maintaining a moderate height that is advantageous for light interception without increasing lodging risk. Phenological differences were relatively narrow, with days to 50% silking spanning 54 to 57 days. ASI, an indicator of stress tolerance, was shortest (1 day) in eight inbred lines: TZEEIORQ 2, 4, 5, 7, 10, 11, 14, and 18, while there was a maximum ASI of 3 days in TZEEIORQ 3, 8, and 12.

Stay-green scores, which reflect delayed leaf senescence, ranged from 2.6 to 5.1, with TZEEIORQ 11 (2.6), 14 (2.7), and 5 (2.8) showing superior foliage retention under FAW pressure compared to the checks. Leaf damage scores due to FAW varied significantly, ranging from 2.7 (TZEEIORQ 11) to 6.5 (TZEEIORQ 3). In terms of nutritional traits, β-carotene concentration ranged from 10.5 µg/g (TZEEIORQ 8) to 15.1 µg/g (TZEEIORQ 16), with 13 inbreds exceeding the biofortification target of 12 µg/g. Tryptophan content varied between 0.068% (TZEEIORQ 8) and 0.078% (TZEEIORQ 14), with ten inbreds outperforming the varietal checks, particularly TZEEIORQ 14, 11, and 16, which had the highest tryptophan levels.

Highly significant differences were detected among the inbred lines and varietal checks for ear infection and ear damage attributable to fall armyworm (FAW) (Table 2). The proportion of infected ears among the inbreds varied widely, ranging from 10% in TZEEIORQ 14 to 60% in TZEEIORQ 3, while the varietal checks recorded

28% in TZEE-Y Pop STR QPM and 36% in Obatanpa. Genotypes such as TZEEIORQ 14 (10%), TZEEIORQ 11 (12%), and TZEEIORQ 16 (14%) exhibited the lowest levels of ear infection, reflecting a high degree of resistance. In contrast, TZEEIORQ 3 (60%), TZEEIORQ 8 (55%), and TZEEIORQ 12 (50%) showed the highest infection levels and were classified as highly susceptible. Patterns of ear damage closely mirrored those of ear infection. Damage scores ranged from 5% in TZEEIORQ 14 to 35% in TZEEIORQ 3. Severe kernel destruction was observed in TZEEIORQ 3 (35%), TZEEIORQ 8 (28%), and TZEEIORQ 12 (25%), whereas minimal kernel loss occurred in TZEEIORQ 14 (5%), TZEEIORQ 11 (6%), and TZEEIORQ 16 (7%).

Among the checks, Obatanpa (18%) sustained slightly greater ear damage than TZEE-Y Pop STR QPM (14%), suggesting moderate susceptibility in both. These results highlight TZEEIORQ 11, TZEEIORQ 14, and TZEEIORQ 16 as stable sources of FAW resistance, combining low ear infection and damage with favourable agronomic performance. Conversely, TZEEIORQ 3, TZEEIORQ 8, and TZEEIORQ 12 were consistently vulnerable across both infection and damage traits, underscoring their limited utility in resistance breeding. Statistical parameters confirmed the precision and reliability of the experiment. Standard errors (SE) and standard errors of the difference (SED) were low across traits, enhancing confidence in the means. Coefficients of variation (CV) were below 15% for most traits, except for ASI (48.26%) and leaf damage (29.29%), which reflected environmental influences and pest pressure (Table 2). Tukey's HSD test at a 5% significance level indicated significant differences among genotypes, validating the observed variability and supporting the selection of genotypes for hybrid breeding.

Table 2. Mean performance of the 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	Ear infection (%)	Ear damage (%)	β-carotene (µg/g)	Tryptophan (%)
TZEEIORQ 1	2.6	145	56	2	4.0	4.5	35	18	14.2	0.074
TZEEIORQ 2	3.0	135	55	1	3.0	3.2	20	10	12.5	0.070
TZEEIORQ 3	2.2	160	57	3	5.0	6.5	60	35	14.8	0.069
TZEEIORQ 4	2.9	140	55	1	3.2	3.8	25	12	12.0	0.072
TZEEIORQ 5	3.1	138	54	1	2.8	3.0	18	9	14.5	0.075
TZEEIORQ 6	2.5	142	56	2	4.1	4.7	38	20	11.9	0.071
TZEEIORQ 7	3.2	136	55	1	2.9	2.9	15	8	14.7	0.076
TZEEIORQ 8	2.1	134	57	3	4.5	5.5	55	28	10.5	0.068
TZEEIORQ 9	2.7	139	56	2	4.0	4.2	30	15	13.8	0.072
TZEEIORQ 10	3.0	130	54	1	3.0	3.1	20	10	11.5	0.073
TZEEIORQ 11	3.3	137	54	1	2.6	2.7	12	6	14.9	0.076
TZEEIORQ 12	2.4	144	57	3	5.1	5.9	50	25	12.3	0.070
TZEEIORQ 13	2.8	141	56	2	4.0	4.0	32	16	13.5	0.074
TZEEIORQ 14	3.4	138	54	1	2.7	2.8	10	5	14.6	0.078
TZEEIORQ 15	2.9	135	55	1	3.0	3.4	22	11	12.4	0.071
TZEEIORQ 16	3.1	143	55	1	2.9	2.9	14	7	15.1	0.077
TZEEIORQ 17	2.6	140	56	2	4.0	4.5	34	17	11.7	0.069
TZEEIORQ 18	3.2	139	54	1	2.8	3.0	18	9	15.0	0.076
TZEE-Y Pop STR QPM	2.7	136	55	1	3.5	4.0	28	14	8.5	0.070
Obatanpa	2.5	142	56	2	3.8	4.3	36	18	7.2	0.069
SE	0.088	1.485	0.256	0.183	0.191	0.271	1.6	0.9	0.347	0.001
SED	0.125	2.101	0.362	0.259	0.270	0.383	2.2	1.3	0.491	0.001
CV (%)	13.21	4.51	1.96	48.26	22.91	29.29	11.8	15.6	11.05	4.19
HSD (0.05)	0.853	14.371	2.474	1.773	1.846	2.620	9.1	5.2	3.358	0.007

2. Canonical discriminant analysis among maize inbred groups:

The analysis generated a canonical structure matrix and standardized canonical coefficients for the first canonical variate (Can1), which accounted for the majority of between-group variability (Fig. 1). Grain yield emerged as the most influential trait in separating the inbred groups, with the highest structure coefficient (0.812) and a substantial standardized coefficient (0.678). β-carotene content (structure coefficient = 0.711; standardized = 0.590) and tryptophan content (structure coefficient = 0.634; standardized = 0.508). It indicates the significant role of nutritional traits in group differentiation. Plant height showed moderate positive contributions, with structure coefficients of 0.640 and 0.523 and standardized coefficients of 0.422 and 0.309, respectively. It shows some influence on group separation. Conversely, traits associated with stress tolerance displayed negative loadings. Leaf damage score due to FAW infestation had a strong negative structure coefficient (–0.785) and standardized coefficient (–0.641). The ASI exhibited a negative contribution to the first canonical variate, with a structure coefficient of –0.553 and a standardized canonical coefficient of –0.412, indicating its inverse association with the trait complexes underlying genotype differentiation. These results suggest that inbreds with higher resistance to FAW and more synchronized flowering were positioned opposite to those with superior yield and nutritional traits along the canonical axis.

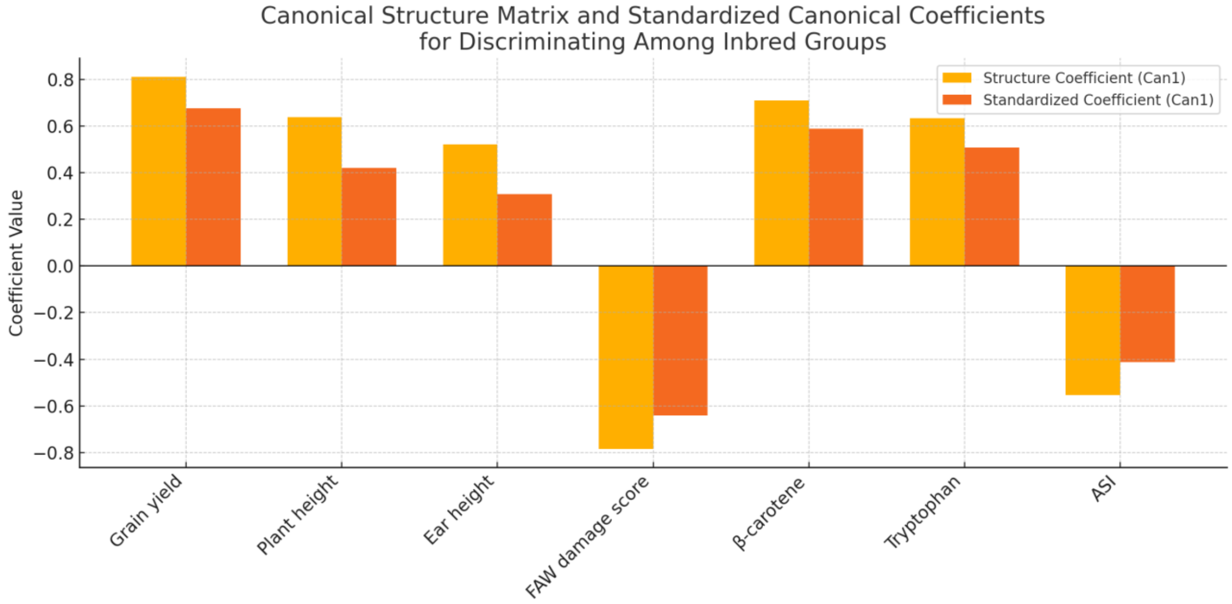

Fig. 1. Canonical structure matrix and standardized canonical coefficients for discriminating among inbred groups

3. Multivariate analysis of variance of extra-early PVA-QPM inbred lines:

As MANOVA presented in (Table 3), all four multivariate test statistics, Pillai's Trace, Wilks' Lambda, Hotelling's Trace, and Roy's Largest Root, produced significant results ($p < 0.0001$), indicating substantial genotypic variability in the trait set. Pillai's Trace yielded a value of 1.982 with an F-value of 2.745 (df = 136, 172), suggesting that a considerable proportion of multivariate variance was attributable to genotypic differences. Wilks' Lambda was exceptionally low (0.021), with a corresponding F-value of 3.012, indicating that the variation observed across traits was high due to genotype rather than residual error. Hotelling's Trace (4.671, F = 2.583) and Roy's Largest Root (2.752, F = 5.912) also pointed to strong multivariate effects, with Roy's statistic especially highlighting one or more trait combinations with dominant discriminatory influence.

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**

4. Group classification and breeding implications:

The canonical classification plot (Fig. 2) displayed the clustering of inbred lines based on multivariate trait performance. Distinct genotype groupings emerged, illustrating the effectiveness of CDA in classifying genetically diverse inbred lines using integrated phenotypic traits. In particular, four inbred lines, TZEEIORQ 7, 11, 14, and 18, formed a compact, high-performing cluster characterized by superior grain yield, elevated levels of β-carotene and tryptophan, low FAW damage scores, and short ASI. These lines consistently combined agronomic performance with enhanced nutritional quality and pest resistance. Conversely, genotypes such as TZEEIORQ 3, 8, and 12 formed a separate cluster exhibiting relatively lower values across these critical traits, indicating limited suitability for direct use in hybrid development.

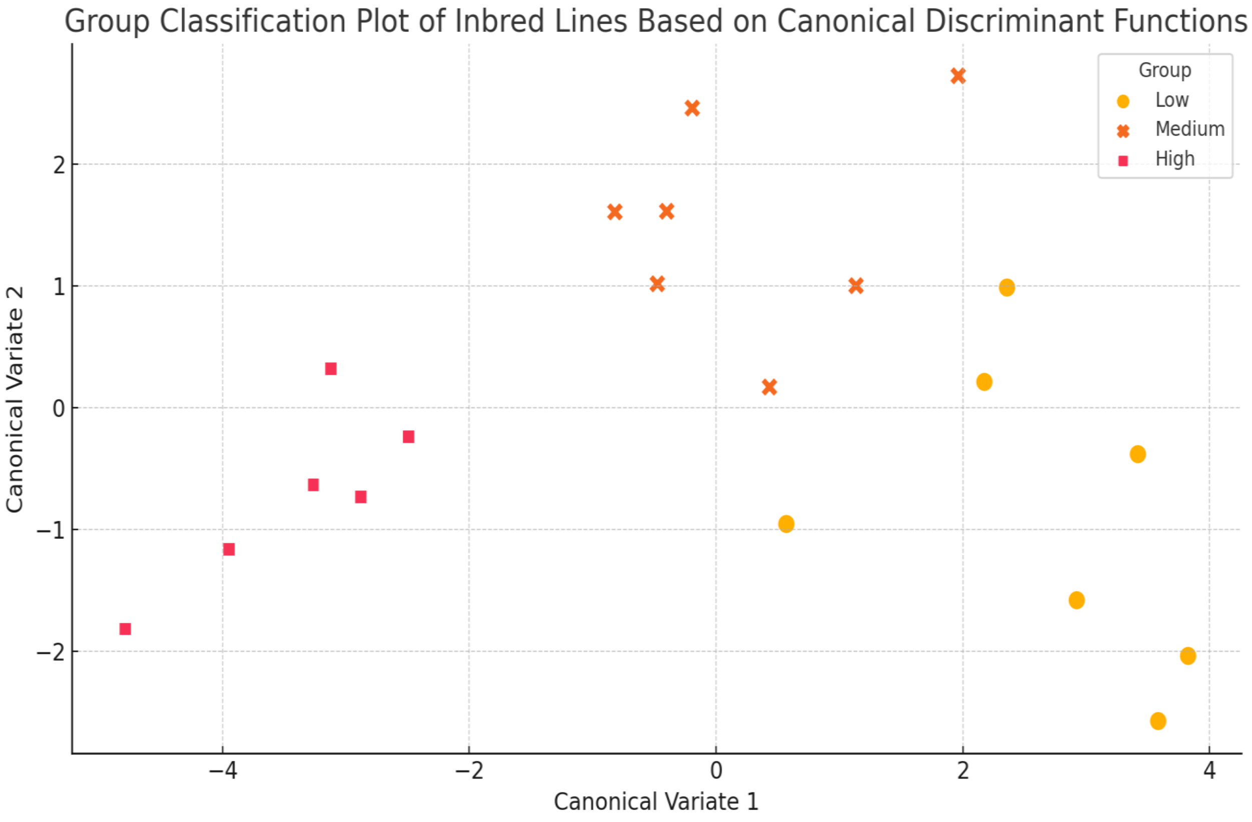

Fig. 2. Canonical classification plot

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 programs 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 both yield and dietary quality (Sebayang *et al.,* 2022; Gedil *et al.,* 2024).

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 exhibited moderate variability, with genotypes like TZEEIORQ 3 reaching 160 cm. 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 stand ability. These results corroborate earlier research that associates favourable plant architecture traits with enhanced yield stability across diverse and fluctuating environmental conditions (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 such as TZEEIORQ 11 and 14 exhibited significantly lower FAW damage scores (2.7 and 2.8, respectively, compared to the varietal checks. These findings reveal intrinsic resistance traits within the host plants 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 Harvest Plus 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 significant genotypic variation observed for ear infection and damage highlights contrasting levels of FAW resistance among the evaluated maize inbreds. Resistant lines, TZEEIORQ 11, TZEEIORQ 14, and TZEEIORQ 16 exhibited consistently low infection (<15%) and kernel damage (<7%), suggesting the presence of heritable resistance factors that could be exploited in breeding (Amegbor *et al.,* 2022). In contrast, highly susceptible lines (TZEEIORQ 3, TZEEIORQ 8, and TZEEIORQ 12) sustained infection above 50% and kernel destruction exceeding 25%, similar to previous reports of strong genotype-dependent FAW responses in tropical maize (Kasoma *et al.,* 2020; Sebayang *et al.,* 2022). The moderate susceptibility of the varietal checks (Obatanpa and TZEE-Y Pop STR QPM) reinforces the importance of incorporating resistance into widely grown germplasm to safeguard productivity. Given FAW's continued threat to African maize systems, breeding programmes must prioritize integrating genetic resistance with farmer-adapted varieties to ensure durable protection and resilience (Togola *et al.,* 2025). CDA 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 *et al.,* 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 with relatively modest discriminant weight remains relevant as a secondary trait for environmental adaptation and crop architecture.

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 (Jadhavand 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 CDA 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 maize inbred lines revealed substantial genetic variability for key traits. Several inbreds, notably TZEEIORQ 14, significantly outperformed standard checks with yield advantages of 25.9% and 41.7%, demonstrating high potential for FAW-prone areas. Superior lines also exhibited strong FAW resistance, with up to 37% less leaf damage and low ear infection/kernel damage (<15% and <7%). These pest-resistant genotypes also surpassed nutritional targets, with β-carotene over 12 μg/g and high tryptophan, enhancing both food security and nutrition. The study confirms the feasibility of combining high yield, pest resilience, and improved nutrition in a single variety. Statistical analyses validated the findings and identified key traits for selection, providing elite candidates for breeding climate-adaptive, nutrition-focused maize.

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التمييز بين الصفات الزراعية والبيتا كاروتين والتريبتوفان في الذرة الغنية بالبروتين عالي الجودة بروفيتامين أ تحت الإصابة بدودة الحشد الخريفية

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الملخص

تُعد إصابة دودة الجيش الخريفية (*Spodoptera frugiperda*)من أبرز القيود التي تواجه إنتاج الذرة في منطقة إفريقيا جنوب الصحراء، لما تسببه من خسائر كبيرة، خاصة في الأنماط الوراثية المبكرة جدًا في النضج، التي تُعد أساسية لضمان الأمن الغذائي في البيئات الحساسة مناخيًا والمحدودة الموارد. تُقدّم هذه الدراسة أول تقييم منهجي لخطوط الذرة الغنية بفيتامين)أ (والبروتين عالي الجودة والمبكرة جدًا، من حيث إنتاج الحبوب، وصفات الإغناء الغذائي، ومقاومة دودة الجيش الخريفية تحت ظروف الإصابة الطبيعية. استخدمت الدراسة التحليل المتعدد المتغيرات لدمج صفات التبكير في النضج، والجودة الغذائية، والقدرة على مقاومة الآفات، من أجل تعزيز برامج التربية الذكية مناخيًا للذرة .أُجريت التجارب الحقلية خلال موسمي الأمطار لعامي 2023و2024 في محطة أوكي-أوي، نيجيريا، باستخدام تصميم القطاعات العشوائية الكاملة. أظهر تحليل التباين المتعدد المتغيرات وجود فروق معنوية عالية بين الخطوط الوراثية المدروسة في الصفات الزراعية والتغذوية الرئيسة .وقد تفوّقت بعض الأنماط الوراثية مثل TZEEIORQ 14 في إنتاج الحبوب بنسبة 25.9٪ و41.7٪ مقارنة بالأصناف القياسية .كما تجاوزت عدة خطوط وراثية العتبة المستهدفة لمحتوى بيتا-كاروتين 12)ميكروغرام/غرام(، وأظهرت مستويات مرتفعة من التريبتوفان مقارنة بالضوابط. أظهر التحليل التمييزي الكانوني تجميع الخطوط الوراثية في مجموعات مميزة، حيث مثّلت صفات إنتاج الحبوب، وبيتا-كاروتين، والتريبتوفان أهم الصفات المميزة للفصل بين المجموعات، بينما كانت درجة تلف الأوراق والفترة بين التزهير الذكري والأنثوي أكثر الصفات السلبية في تحديد الفروق بين الأنماط الوراثية. وقد برزت الأنماط الوراثية TZEEIORQ 5، 10، 11، 14، و18 كأفضل المرشحين لبرامج التربية الرامية إلى تحسين مقاومة دودة الجيش الخريفية، ورفع الجودة الغذائية، وتعزيز إنتاج الحبوب .تُبرز هذه النتائج فاعلية التحليل المتعدد المتغيرات في توضيح العلاقات بين الصفات، ودعم عملية اختيار السلالات المتفوقة لتحقيق تحسين مستدام لإنتاج الذرة في البيئات الاستوائية.

الكلمات المفتاحية: التحليل المتعدد المتغيرات، إنتاج الحبوب، الفاصل بين التزهيرين، مقاومة *Spodoptera frugiperda*