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Development of Extra-Early Provitamin A Quality Protein Maize Inbreds with Resistance/Tolerance to *Striga hermonthica* and Soil Nitrogen Stress

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Abstract: A hemiparasitic plant, *Striga hermonthica* (Del.) Benth and soil nitrogen stress are the key constraints to maize (*Zea mays* L.) productivity in sub-Saharan Africa, where commonly cultivated maize is the normal endosperm type that is deficient in provitamin A, tryptophan and lysine (PVATL). Seventy-six extra-early maize inbreds with provitamin A, tryptophan, and lysine qualities (TZEEIORQ) were developed to address these constraints, and four checks were assessed under *Striga*, low and high nitrogen conditions at three locations in Nigeria. The inbreds were further genotyped with two beta-carotene hydroxylase 1 (crtRB1) markers, and their seeds were quantified for provitamin A content. Significant ($p < 0.01$) genetic variations were observed for grain yield and other agronomic attributes of the inbreds under varying environmental conditions. Levels of PVATL for the inbreds ranged from 2.21–10.95 µg g^{-1}, 0.04–0.08%, and 0.19–0.39%, respectively. Beta-carotene marker, crtRB1-3'TE, was polymorphic and grouped the inbreds into two. The marker was effective in identifying inbreds with moderate provitamin A content. Inbreds TZEEIORQ 5, TZEEIORQ 52, and TZEEIORQ 55 exhibited resistance to *Striga*, tolerance to nitrogen stress with moderate levels of PVATL and could be invaluable sources of favorable alleles for breeding nutritionally improved maize varieties with resistance/tolerance to *Striga* and soil nitrogen stress.

Keywords: *Striga hermonthica*; low soil nitrogen; high soil nitrogen; extra-early; provitamin A quality protein maize; beta-carotene markers

1. Introduction

Maize (*Zea mays* L.) is one of the major cereal crops in sub-Saharan Africa (SSA). The contribution of maize to human calorific intake is 50%, 30% and 15% for southern, eastern, and West and Central Africa (WCA), respectively [1]. Normal endosperm maize, known to be lacking in provitamin A [2], tryptophan and lysine (PVATL) [3,4], is a major component of the food fed to babies (from two to three-month-old) and children of preschool age; this is usually done with no supplements in many developing countries [5]. In SSA, vitamin A deficiency (VAD) has been reported in about 40% and 15% of children and pregnant women, respectively [6,7]. VAD causes night blindness, increased childhood mortality, delayed growth, and a depressed immune system [8,9]. On the other hand, tryptophan and lysine are important building blocks of protein required by humans and monogastric [10]. Tryptophan deficiency causes a reduction in food intake, reduction in growth rate, impairment of skeletal development, increased pain sensitivity, increased

aggression and anxiety [11], while lysine deficiency results in fatigue and reduction in growth rate, among others.

Varieties of maize (in the late, intermediate, early and extra-early maturity groups) that can mitigate the effects of VAD [12–14] and protein malnutrition [15,16] have been developed. However, no maize hybrid or variety with adequate provitamin A, tryptophan, and lysine contents with extra-early characteristic (matures around 80–85 days), *Striga* resistance and tolerance to nitrogen stress is yet to be developed and commercialized in WCA. With a population growth rate greater than 2% for many SSA countries, the need for maize has been predicted to triple by 2050 [17]. The current maize grain yield in farmers' fields in the subregion is abysmally low to meet the projected demand. The mean maize grain yield in Nigeria is 1.8 t ha^{-1}, while 2.4 t ha^{-1} has been reported for SSA; these values are lower than the 5.6 t ha^{-1} mean grain yield of the crop all over the world [18]. The lower yield of maize in the subregion is attributed to a combination of factors, namely: *Striga hermonthica* parasitism [19], stem borer attacks [20], low and declining soil nitrogen [21], recurrent drought [22], and more recently armyworm invasion [23]. Reduction in grain yield of maize due to nitrogen stress can be as high as 52% [24], while drought can result in 34% yield loss [25]. *Striga hermonthica* alone can cause about 53.7% [26] to 100% [27] yield losses, armyworms about 47% [28] and stem borers about 21% [29]. The widespread cultivation of maize with normal endosperm features and low grain yield of the crop is expected to exacerbate the nutritional problems in SSA.

Striga hermonthica is widespread in the savannas of WCA, where environmental conditions are considered excellent for maize production. Maize yield losses due to *Striga* stress varied from 30–90%, and the parasite can cause total crop failure where the infestation is severe, compelling farmers to abandon their fields [19]. Adetimirin and colleagues [26] identified ears per plant as the primary component of yield most severely affected under *Striga* infestation. Effects of *Striga* are most severe in the soils with low nutrients, particularly nitrogen, [30] which is a key constraint in major maize-producing areas of WCA [31,32]. Oikeh and colleagues [33] reported that most farmers in WCA grow maize under low nitrogen stress. This is because the soils of the area are inherently low in nitrogen, and many farmers cannot afford inorganic fertilizers to augment the low soil nitrogen [34]. About 50% reduction in maize yield has been reported due to nitrogen deficiency [35]. Therefore, developing improved maize with *Striga* resistance, tolerance to nitrogen stress, extra-earliness, provitamin A, and quality protein maize traits offers a sustainable and economic strategy to combat *Striga* and soil nitrogen stresses while improving human nutrition and health in WCA.

Seventy-six maize-inbred lines have been developed by the International Institute of Tropical Agriculture (IITA) with a view to breeding extra-early maize hybrids/varieties that combine improved levels of PVATL and tolerance/resistance to multiple stresses in SSA. However, the reactions of these newly developed inbreds have not been thoroughly investigated under *Striga* and nitrogen stress. Although the inbreds with appropriate modification of endosperm for tryptophan and lysine were repeatedly selected for using lightbox [16] and kernels with relatively deep orange color were assumed to have increased provitamin A levels, information on the per se PVATL concentration of each of the inbreds is lacking. The information will facilitate selecting suitable inbreds as parents in hybrid breeding programs in the subregion.

The deep orange kernel color in maize, though presumed to correlate with provitamin A levels, has been reported not to be sufficiently indicative of the levels of beta-carotene [2,36]. Safawo and colleagues [2] reported that using high-performance liquid chromatography (HPLC) is very costly in breeding maize with increased provitamin A and proposed that marker-assisted selection could be more efficient than using only maize kernel color for beta-carotene content—A major provitamin A carotenoid. Beta-carotene hydroxylase 1 (crtRB1-3′TE and crtRB1-5′TE) is one of the three major genetic markers, which play an important role during the accumulation of beta-carotene in the endosperm of maize [37]. The crtRB1-3′TE marker is a favorable DNA marker in maize for effecting

an increase in the level of beta-carotene from 2 to about 10-fold in the kernels [38,39]. This study, therefore, aimed at developing and identifying Tropical *Zea* extra-early provitamin A quality protein maize inbred (TZEEIORQ) lines that possess tolerance/resistance to *Striga* and tolerance to nitrogen stress and determine the usefulness of beta-carotene hydroxylase 1 (crtRB1-3′TE and crtRB1-5′TE) in identifying inbreds with high kernel provitamin A content.

2. Materials and Methods

2.1. Germplasm

In 2007, IITA initiated a breeding program to develop varieties of maize that combine drought tolerance, tolerance to low nitrogen stress, *Striga* resistance and high PVALT for WCA. A variety of extra-early maize possessing *Striga* resistance and quality protein traits (with both yellow and orange endosperm color) was crossed to Syn-KU1409/DES/1409 (OR2), a donor of beta-carotene alleles. The resulting cross was backcrossed to multiple stress-resistant varieties, TZEE-Y STR QPM. Following the backcrossing that was aimed at introgressing genes for increased provitamin A content of maize, a total of 76 inbred lines were developed after seven cycles of selfing and selection for agronomically desirable traits.

2.2. Research Locations, Experimental Design, and Field Management

Evaluation of the 76 inbreds and the four extra-early normal endosperm checks (Supplementary Materials Table S1) was carried out under artificial *Striga* environment at Abuja (9°15′ N, 7°20′ E, 1700 mm annual precipitation, 300 m altitude) in 2016 and Mokwa (9°18′ N, 5°4′ E, 1100 mm annual precipitation, 457 m altitude) in 2017; the two locations are found in the southern Guinea savanna agroecological zone of Nigeria. Ethylene gas was injected into the soil following land preparation—To rid the soil of *Striga* seeds native to it. The gas was applied 12 cm deep into the soil. This activity was repeated at intervals of 100 cm to ensure good coverage of the field with the gas. Seeds of *Striga* obtained from the fields previously planted to sorghum were used for artificial infestation, following the procedure described by [31]. Well-sieved sand and the *Striga* seeds were mixed carefully by weight in the ratio 99:1. Before planting maize seeds per hill, each hill was infested with *Striga* seed-sand mixture (8.5 g containing about 5000 germinable seeds of *Striga hermonthica*). At four weeks after planting, NPK fertilizer (NPK 15–15–15) was applied at the rate of 30 kg ha^{-1} of K_2O, P_2O_5 and N to the established plants. All other unwanted plants, apart from *Striga*, were controlled by hand-pulling.

In addition, the 76 inbreds and the four checks were evaluated in adjacent blocks in high and low soil nitrogen environments both at Ile-Ife (7°28′ N, 4°33′ E, 1350 mm annual precipitation, 244 m altitude) in the rainforest agroecological zone in 2016 and Mokwa in 2017. The soils at Mokwa and Ile-Ife are Luvisol and Alfisol, respectively [40]. Depletion of N from the low-N fields at Mokwa and Ile-Ife was achieved through regular planting of maize for many years and removing the stover following every harvest. At both locations, soil samples obtained (with a soil auger) before land preparation from zero to fifteen-centimeter depth were subjected to analysis. The total potassium (K), phosphorus (P) and nitrogen (N) contents of the soils were determined by Kjeldahl digestion and colorimetric method [41]. The soil analyses at Ile-Ife and Mokwa used in this study are the same as reported by [42]. Following the soil test, the total N available in the high- and low-N plots were augmented with urea to 90 and 30 kg N ha^{-1}, respectively. Nitrogen fertilizer was applied, in two equal splits, under nitrogen experiments at 2 and 4 weeks after planting (WAP). In addition, 60 kg K ha^{-1} as muriate of potash (K_2O) and 60 kg P ha^{-1} as single superphosphate (P_2O_5) were applied to the two N treatments at 2 WAP.

A 10 × 8 alpha lattice design, replicated two times, was used for all evaluations in the *Striga*, high- and low-N trials. Single row plots, 3 m long, were used under the *Striga* experiment. Within-row spacing was 0.40 m, while between-row spacing was 0.75 m. However, in 2016, single -ow plots 4 m long were used during evaluations in high- and low nitrogen fields. Three maize kernels were sown per hill, and the seedlings thinned

to two per hill at 2 WAP. In low- and high-N fields, weeds were controlled by applying atrazine and gramoxone, supplemented with hand weeding. Fall armyworms (*Spodoptera frugiperda*) were controlled by using ampligo at 300 mL ha^{-1}. Ampligo contained 100 g per liter of chlorantraniliprole and 50 g per liter of lambda-cyhalothrin.

2.3. Collection of Data on Various Characters

Data collection was carried out on plants per plot. The traits measured include days to 50% anthesis (DA) and 50% silking (DS), respectively determined as the total number of days from sowing to the time 50% of the plants had shed pollen and showed silk extrusion. Anthesis-silking interval (ASI) was calculated by subtracting DA from DS. Plant height (PLHT) represented the distance from the first tassel branch to the base of the plant, while ear height (EHT) was recorded as the distance from the node bearing the topmost ear (in prolific lines) to the base of the maize ear. In addition, stalk lodging (SLPER) was recorded as the proportion of plants with the broken stalk at (or below) the node bearing the uppermost ear. Ear aspect (EASP) was determined on a scale of 1–9, where 1 = uniform, large, clean and well-filled ears, and 9 = variable, small, rough and poorly filled ears [43]. Husk cover was assessed on a scale of 1–9, where 1 = tightly arranged husks extending beyond the tip of the ear and 9 = husks loosely arranged with ear tip exposed. The number of ears per plant (EPP) was calculated as the ratio of the total number of harvested ears for each plot to the number of harvested plants in the plot. For the high nitrogen and *Striga* environments, grain yield was calculated from ear weight, on the assumption of 80% shelling percentage, adjusted to 15% moisture content.

Plant aspect (PASP), assessed only under low- and high-nitrogen conditions, was scored on a scale of 1–9 using plant type and overall appeal, where 1 = excellent plant type and 9 = poor plant type. Stay-green characteristic (STGR) was determined under low soil nitrogen alone on a scale of 1–9, where 1 = nearly all foliage were lush green, and 9 = practically all foliage were dead. Determination of grain yield under low-N involved shelling of harvested ears per plot, weighing of the kernels and measuring of grain moisture content. Afterward, grain yield was calculated, per plot, using grain weight adjusted to 15% moisture content.

Additional data collected under *Striga* infestation were: host plant damage (by *Striga*) syndrome rating at 8 and 10 WAP, an indication of *Striga* tolerance [27,40] and the number of emerged *Striga* plants, which is indicative of resistance to *Striga*. Host plant damage was scored per plot on a scale of 1–9, where 1 = no visible damage, suggesting plant with normal growth and high tolerance to *Striga*, and 9 = severe damage or total collapse of the plant, indicating high susceptibility to *Striga* [19,44].

2.4. Identification of Beta-Carotene Rich Inbreds Using Allele-Specific Beta-Carotene Markers-crtRB1-3′TE and crtRB1-5′TE.

Leaf samples for each of the inbred genotypes were accumulated from seven to eight plants at 3 WAP. Thereafter, the samples were lyophilized using Free Zone 18 liter console dry system (Labconco Inc., Missouri, USA). Genomic DNA was extracted from the lyophilized samples using a DNA extraction protocol, modified cetyl trimethyl ammonium bromide (CTAB), described by [45]. The following markers, according to [37], were used to identify crtRB1-3′TE: (i) the forward primer (F) (5′ACACCACATGGACAAGTTCG 3′), (ii) the first reverse primer (R1) (5′ACACTCTGGCCCATGAACAC 3′) and (iii) the second reverse primer (R2) (5′ACAAGCAATACAGGGGACCAG3′). In addition, crtRB1-5′TE was identified with: (i) the forward primer (F) (5′TTAGAGCCTCGACCCTCTGTG 3′) and (ii) the reverse primer (R) (5′AATCCCTTTCCATGTTACGA 3′). A polymerase chain reaction (PCR) was conducted in 25 μL volume for each of the functional markers. The quantity of genomic DNA, beta-carotene DNA markers and other reaction mixtures used are, as shown in Table 1. The thermocycler standard cycling conditions provided by [37] were used for the PCR. Resolution of amplicons was carried out on 2% agarose gel. DNA bands were viewed on a UV Transilluminator.

Table 1. Cocktail and reaction mix used for polymerase chain reaction. [a] Primer 3 R was not used under crtRB1-5′TE.

Reagent	Volume per Reaction (in µL)	
	crtRB1-3′TE	crtRB1-5′TE
Nuclease-free H_2O	15.75	15.65
$10 \times$ PCR buffer	2.50	2.50
50 mM $MgCl_2$	1.00	1.50
25 mM dNTP	0.20	0.20
Primer1 R	0.80	1.00
Primer2 F	0.80	1.00
Primer3 R	0.80	- [a]
DMSO	1.00	1.00
Taq	0.15	0.15
Template DNA	2.00	2.00
Total volume	25.00	25.00

Photographs of the bands were taken and then scored for absence or presence of the favorable allele of crtRB1-3′TE gene (allele 1) and favorable allele of crtRB1-5′TE gene (allele 2) [39].

2.5. Generation and Processing of Seed Samples of Inbreds for Carotenoid, Tryptophan, and Lysine Analyses

The first two and last two plants in each plot, under high nitrogen conditions at Mokwa and Ile-Ife in 2016, were self-pollinated to develop seed samples of S_8 lines that were used for carotenoids [46] tryptophan and lysine analyses. The ears were harvested at each location, dried, processed and stored at 4 °C [12]. Thereafter, two samples, each containing 60 kernels, were prepared for analyses; the first sample was used for carotenoids analysis, while the second was used for lysine and tryptophan analyses. Analyses for carotenoids, lysine and tryptophan were carried out at CIMMYT, Mexico. Samples of 20–30 maize kernels of each inbred were frozen at −80 °C until when required for analysis, at which time they were ground to (0.5 µm) powder. Carotenoids analysis was carried out using ultra-high-performance liquid chromatography (UPLC) (Waters, Milford, MA, USA) Apex Track. It involved extraction, separation, and quantification by UPLC using protocols described by [47]. Beta-carotene (13-*cis*, all-trans and 9-*cis* isomers), beta-cryptoxanthin, zeaxanthin and lutein were measured. Overall, provitamin A content of each inbred line was calculated thus: beta-carotene (13-*cis* + all-trans + 9-*cis*) + 0.5 (beta-cryptoxanthin) [46]. The amount of lysine and tryptophan in whole grains of the inbred lines were determined as reported by [47]; briefly, Kjeldahl apparatus was used to grind and de-fat whole grain sample for each inbred line, followed by the addition of papain to hydrolyze the protein. A purple coloration was induced with the addition of a combination of glacial acetic acid and H_2SO_4. The deepness/concentration of the induced color was quantified using a spectrophotometer at 560 nm. The percent tryptophan content of each inbred line was then obtained from the reading of the spectrophotometer converted to percent tryptophan. Two measurements were taken for each inbred line.

2.6. Statistical Analysis

Prior to statistical analyses, log transformation was carried out to achieve homogeneity of variances for data collected on *Striga* (hostplant) damage rating and the number of emerged *Striga* plants. Year–location–treatment combination was considered a test environment [48]. Analysis of variance (ANOVA) was first carried out for each research condition (*Striga*, low and high soil nitrogen). Thereafter, a combined ANOVA was carried out across the six environments for yield and other characters. The performance of the inbreds was determined under *Striga*, low nitrogen and across all research environments using the following base indices:

(i)　　*Striga* base index = (2*YLI* + *EPP* − *SDR1* − *SDR2* − 0.5*ESP1* − 0.5*ESP2*). where *YLI* = yield of maize in *Striga*-infested plots (kg ha^{-1}), *EPP* = number of ears per plant, *SDR1* and *SDR2* = *Striga* damage ratings at 8 and 10 WAP, *ESP1* and *ESP2* = number of emerged *Striga* plants at 8 and 10 WAP [49];

(ii)　　Low nitrogen base index = (2*YLDL* + *EPP* − (*PASP* + ASI + *EASP* + *STGREN*)). where *YLDL* = yield of low nitrogen plots (kg ha^{-1}), *EPP* = ears per plant, *PASP* = plant aspect, *ASI* = anthesis-silking interval, *EASP* = ear aspect, *STGREN* = stay-green characteristic [50].

(iii)　　Multiple-character—base-index = (2*YLD* + *EPP* − 0.5*ESP1* − 0.5*ESP2* − *SD1* − *SD2* − (*PASP* + *EASP* + *STGREN*)) [5] where *YLD* = yield across six research environments, *EPP* = number of ears per plant across research environments, *ESP1* = number of emerged *Striga* plants at 8 WAP under *Striga*-infested environment, *ESP2* = number of emerged *Striga* plants at 10 WAP under *Striga*-infested environment, *SD1* = *Striga* damage rating at 8 WAP under *Striga*-infested environment, *SD2* = *Striga* damage rating at 10 WAP under *Striga*-infested environment, *PASP* = plant aspect across high- and low nitrogen environments, *EASP* = ear aspect across six research environments, *STGREN* = stay-green characteristic under low nitrogen environment [5].

Adjusted means for each character of each genotype were standardized to minimize the effects of the different scales used to measure them. In each case, a positive base index value suggested that the inbred was tolerant to that stress, whereas negative values were indicative of the susceptibility of the inbreds to the stress [5]. Chi-squared analysis was carried out to determine if the groups formed from the results of the molecular screening were associated with the amount of provitamin A in the inbreds determined by HPLC. In addition, stepwise regression of total provitamin A on provitamin A carotenoids was carried out.

3. Results

3.1. Mean Squares of Inbreds Evaluated in Striga-Infested, Low- and High Nitrogen Conditions

Results of ANOVA across six research conditions (two environments each of *Striga* infestation, low and high nitrogen) showed significant ($p < 0.01$) environment, inbred and inbred × environment variance for all traits except inbred × environment interaction mean square for EPP (Table 2). ANOVA under *Striga*-infested environments indicated significant ($p < 0.01$) environment, inbred and inbred × environment interaction variance for grain yield and other traits (Table 3). Similarly, the ANOVA under low nitrogen revealed significant ($p < 0.01$) environment, inbred and inbred × environment interaction variance for all characters, except environment variance for STGR (Table 3). ANOVA under high nitrogen environments indicated significant ($p < 0.01$) environment, inbred, and inbred × environment interaction mean squares for all traits, except environment mean squares for EPP and PASP, and inbred × environment interaction variance for PLHT, EHT and EPP (Table 3).

Table 2. Mean squares for grain yield and other agronomic traits of extra-early provitamin A quality protein maize inbreds evaluated across six (low-N, *Striga*-infested and high-N) environments at Ile-Ife, Mokwa and Abuja in Nigeria in 2016 and 2017.

SOURCE	DF	YIELD	DS	DA	EASP	EPP	PLHT	EHT	HUSK	SLPER	DF	† PASP
		(kg/ha)	(Days)	(Days)	(1–9)		(cm)	(cm)	(1–5)	(%)		(1–9)
Env	5	69,659,566.00 ***	1062.74 ***	574.96 ***	46.70 ***	1.87 ***	91,895.65 ***	17,831.99 ***	388.06 ***	3995.50 ***	3	9.86 ***
Rep (Env)	6	4,190,018.80 ***	20.33 ***	10.89 ***	4.44 ***	0.26 **	1837.52 ***	394.56 ***	5.33 ***	291.70 ***	4	0.98 *
Blk (Env × Rep)	84	5,66,701.80 ***	7.84 ***	5.90 ***	2.15 ***	0.12 **	241.79 **	148.87 ***	0.81 **	111.00 **	56	0.78 ***
Inbred	79	1,519,243.80 ***	53.64 ***	54.35 ***	6.41 ***	0.20 ***	1112.78 ***	242.04 ***	1.72 ***	154.54 ***	79	3.19 ***
Inbred × Env	382	526,858.80 ***	7.50 ***	5.98 ***	1.87 ***	0.10 ns	367.82 ***	118.31 ***	1.02 ***	118.04 ***	230	0.69 ***
Error	368	263,908.7	3.85	2.37	0.89	0.08	147.29	77.26	0.5	73.66	253	0.35

*, **, *** = significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively; ns = not significant; DF = degrees of freedom; YIELD = grain yield; DS = days to 50% silking; DA = days to 50% anthesis; EASP = ear aspect (rated on a scale of 1–9); EPP = number of ears per plant; PLHT = plant height; EHT = ear height; HUSK = husk cover (rated on a scale of 1–5); SLPER = percent stalk lodging; † PASP = plant aspect evaluated across four (two low-N and two high-N) environments (on a scale of 1–9). DF of sources were adjusted for missing plots.

Table 3. Mean squares for grain yield and other agronomic traits of extra-early provitamin A quality protein maize inbreds evaluated under *Striga*, low-N and high-N environments at Abuja, Mokwa and Ile-Ife in Nigeria in 2016 and 2017.

SOURCE	DF	YIELD	DA	DS	PLHT	EASP	EHT	HUSK	EPP	SLPER	SDR1	SDR2	ESP1	ESP2
		(kg/ha)	(Days)	(Days)	(cm)	(1–9)	(cm)	(1–5)	(1–9)	(%)	(1–9)	(1–9)		
Under *Striga*														
Env	1	10,156,972.90 ***	878.01 ***	1218.51 ***	32,041.36 ***	32.92 ***	281.01 **	55.82 ***	1.12 ***	958.53 **	54.01 ***	19.48 ***	2612.84 ***	8870.10 ***
Rep (Env)	2	338,039.56 ns	4.27 ns	19.79 ns	31.02 ns	1.50 ns	29.64 ns	13.37 ***	0.04 ns	105.15 ns	2.32 *	10.13 ***	184.77 ***	334.88 ***
Blk (Env × Rep)	28	741,044.44 ***	11.12 ***	16.49 **	173.04 ns	2.46 **	86.09 ***	1.09 ns	0.10 *	189.54 ns	0.75 ns	1.20 ns	16.75 *	78.23 ***
Inbred	79	515,964.45 ***	27.79 ***	30.28 ***	244.47 ***	2.92 ***	63.14 **	1.18 **	0.08 *	238.71 ***	0.79 *	1.38 **	16.83 **	39.36 *
Inbred × Env	79	424,495.41 **	8.73 ***	13.38 **	262.15 ***	2.40 ***	85.60 ***	1.42 ***	0.08 *	205.80 **	0.97 **	1.58 **	16.54 *	37.57 *
Error	126	233,057.4	3.32	7.44	114.1	1.14	37.33	0.72	0.06	125.68	0.52	0.86	10.64	26.13
Under Low N											**PASP (1–9)**	**STGR (1–9)**		
Env	1	89,123,724.10 ***	91.58 ***	58.54 ***	7271.70 ***	6.25 **	790.48 **	631.34 **	4.41 ***	346.42 **	4.89 ***	0.75 ns	-	-
Rep (Env)	2	1,130,174.13 **	17.56 ***	23.88 ***	4418.27 ***	1.29 ns	334.82 **	0.12 ns	0.19 **	445.44 ***	0.05 ns	0.85 ns	-	-
Blk (Env × Rep)	28	546,090.83 ***	3.80 **	3.04 *	302.02 ***	2.58 **	196.97 **	0.70 **	0.07 *	68.10 *	1.04 ***	1.06 ***	-	-
Inbred	79	671,288.80 ***	20.72 ***	19.50 ***	564.46 ***	3.89 ***	149.64 **	1.00 ***	0.080 **	69.13 ***	1.72 ***	1.63 ***	-	-
Inbred × Env	79	429,192.31 ***	6.23 ***	6.24 ***	508.70 ***	2.48 ***	130.68 **	1.07 ***	0.05 **	52.03 *	0.75 ***	0.93 ***	-	-
Error	124	227,697.2	1.86	1.87	101.62	0.78	70.01	0.37	0.03	37.25	0.32	0.34	-	-
Under high-N														
Env	1	57,477,304.10 ***	141.26 ***	50.11 ***	85,728.41 ***	55.53 ***	38,050.66 **	546.69 ***	0.15 ns	346.42 **	0.03 ns	-	-	-
Rep (Env)	2	11,386,215.00 ***	10.83 **	17.31 ***	1063.26 **	10.55 ***	819.21 **	2.51 **	0.40 **	445.44 ***	1.91**	-	-	-
Blk (Env × Rep)	28	421,403.70 ns	2.77 ns	3.98 *	250.29 ns	1.42 **	163.55 ns	0.65 *	0.06 ns	68.10 *	0.52 ns	-	-	-
Inbred	79	1,338,936.80 ***	15.11 ***	16.14 ***	1059.55 ***	2.78 ***	290.17 ***	1.07 ***	0.10 **	69.13 ***	2.04 ***	-	-	-
Inbred × Env	74	772,242.00 ***	5.24 ***	5.74 ***	291.96 ns	1.39 **	112.02ns	1.11 ***	0.05 ns	52.03 *	0.81 ***	-	-	-
Error	119	332,792.10	1.92	2.24	226.33	0.74	125.13	0.41	0.06	37.25	0.38	-	-	-

*, **, *** = Significant at *p* < 0.05, *p* < 0.01 and *p* < 0.001, respectively; ns = Not significant; DF = degrees of freedom; YIELD = grain yield; DA = days to 50% anthesis; DS = days to 50% silking; PLHT = plant height; EASP = ear aspect (rated on a scale of 1–9); EHT = ear height; HUSK = husk cover (rated on a scale of 1–5); EPP = number of ears per plant; SLPER = percent stalk lodging; SDR1 = *Striga* damage rating at 8 WAP (rated on a scale of 1–9); SDR2 = *Striga* damage rating at 10 WAP (rated on a scale of 1–9); ESP1 = number of emerged *Striga* plant at 8 WAP; ESP2 = number of emerged *Striga* plant at 10 WAP. DF of sources adjusted for missing plots; PASP = plant aspect (scored on a scale of 1–9); STGR = stay green characteristic (scored on a scale of 1–9).

3.2. Performance of Inbreds for Grain Yield and Other Agronomic Traits and Identification of Striga-Resistant/Tolerant and Nitrogen Stress-Tolerant Inbreds

Out of the 80 inbreds evaluated under *Striga*, 34 were resistant/tolerant to *Striga* based on their positive *Striga* base index values (Table 4). Eighteen of the 34 lines had higher *Striga* base index values than the best check-TZEEI 73. The index values for the top ranking 15 lines ranged from 3.97 to 11.34 compared to 3.38 obtained for TZEEI 73 (Table 4). A total of 41 lines were identified as low nitrogen tolerant based on their low nitrogen base index values (Table 5). Of these lines, 12 had higher low nitrogen base index values (4.93–9.28) than TZEEI 73 (4.87)—The best *Striga* and low nitrogen tolerant checks were: TZEEIORQ 57, TZEEIORQ 21, TZEEIORQ 64, TZEEIORQ 53, TZEEIORQ 43, TZEEIORQ 55, TZEEIORQ 63, TZEEIORQ 20, TZEEIORQ 51, TZEEIORQ 42, TZEEIORQ 52, and TZEEIORQ 14 (Table 5).

Table 4. Grain yield and other agronomic traits of 76 extra-early provitamin A quality protein maize inbreds with four checks evaluated under *Striga*-infested environments at Abuja and Mokwa in Nigeria in 2016 and 2017.

LINE	YIELD	EPP	DS	PLHT	EASP	HUSK	SDR1	SDR2	ESP1	ESP2	STR-BI
	(kg/ha)		(Days)	(cm)	(1–9)	(1–5)	(1–9)	(1–9)			
TZEEIORQ 62	1938	0.9	57	115	2.7	3.7	2.7	4.0	1	1	11.34
TZEEIORQ 46	1547	1.1	63	92	4.4	4.1	3.6	4.2	1	2	8.24
TZEEIORQ 63	2032	0.8	57	114	3.1	4.3	3.3	4.6	6	5	7.20
TZEEIORQ 72	1621	0.9	59	100	3.9	4.1	3.0	4.5	4	7	6.67
TZEEIORQ 69	1181	0.8	59	109	4.8	3.9	2.9	4.0	2	1	6.38
TZEEIORQ 42	1275	0.8	64	111	4.1	4.0	3.2	4.5	0	4	5.56
TZEEIORQ 41	1173	0.8	59	110	4.5	3.9	3.3	4.2	0	1	5.54
TZEEIORQ 61	1836	0.8	58	112	3.5	4.6	3.4	4.9	4	8	5.49
TZEEIORQ 68	1477	0.7	62	110	3.9	4.4	3.2	4.7	1	2	5.41
TZEEIORQ 62B	1743	0.7	62	107	6.3	4.3	3.6	4.9	1	3	5.35
TZEEIORQ 8	1469	0.9	59	114	4.0	4.3	3.4	4.8	2	9	4.58
TZEEIORQ 17	1235	0.9	58	99	4.2	4.7	3.3	5.0	0	4	4.55
TZEEIORQ 53	1592	0.7	61	128	4.0	4.7	3.5	4.7	2	6	4.52
TZEEIORQ 5	1540	1.0	58	107	3.3	4.5	3.5	4.8	7	11	4.28
TZEEIORQ 55	1381	0.7	59	128	4.9	4.3	3.2	4.8	3	3	3.97
TZEEIORQ 15	1403	0.7	62	104	5.7	4.1	3.5	4.6	2	5	3.93
TZEEIORQ 22	1346	0.8	62	103	5.0	4.3	3.8	4.9	2	4	3.77
TZEEIORQ 18	1057	0.7	59	110	4.0	4.1	3.3	4.4	0	4	3.65
TZEEIORQ 43	1128	0.8	62	101	5.1	4.3	3.8	4.5	2	2	3.38
TZEEI 73	1423	0.7	57	99	5.0	4.3	3.2	4.7	4	9	3.38
TZEEIORQ 64	1792	0.7	60	114	4.1	4.4	4.2	4.8	6	7	3.24
TZEEIORQ 52	1678	0.7	58	128	3.9	4.4	3.8	5.3	3	6	3.19
TZEEIORQ 57	1633	0.7	59	118	4.1	5.0	3.5	5.3	7	6	3.17
TZEEIORQ 25	1099	0.8	64	116	4.6	4.5	3.5	4.6	2	4	3.00
TZEEIORQ 58	1405	0.7	59	106	4.4	4.5	3.9	4.9	1	5	2.82
TZEEIORQ 33	998	0.6	59	97	5.5	4.5	3.0	4.8	2	3	2.66
TZEEIORQ 73	1154	0.9	60	119	4.6	4.9	3.5	5.4	3	5	2.37
TZEEIORQ 7	964	0.8	60	101	4.2	4.2	3.1	4.5	5	9	2.00
TZEEIORQ 29	1045	0.9	62	111	4.5	4.6	3.6	4.8	3	10	1.85
TZEEIORQ 45	788	0.7	64	104	5.0	4.5	3.2	4.9	2	4	1.02
TZEEIORQ 3	1053	0.5	60	112	4.9	4.1	3.0	5.4	2	6	0.88
TZEEIORQ 75	1082	0.8	62	104	4.2	4.3	3.6	4.6	6	10	0.77
TZEEIORQ 77	1060	0.7	65	75	5.2	4.8	3.9	5.2	1	3	0.73
TZEEIORQ 4	1018	0.7	60	106	5.3	4.8	3.4	5.1	4	9	0.12
TZEEIORQ 56	1208	0.6	61	121	5.1	4.8	4.1	5.3	3	3	−0.12
TZEEIORQ 66	902	0.6	60	109	5.2	4.2	3.8	5.0	1	5	−0.23
TZEEIORQ 1	984	0.7	57	110	5.0	4.8	3.5	5.3	4	11	−0.48
TZEEIORQ 49	878	0.6	62	101	5.5	4.4	3.8	5.2	2	1	−0.48
TZEEIORQ 19	992	0.8	59	103	4.2	5.5	4.0	5.7	3	6	−0.55

Table 4. *Cont.*

LINE	YIELD	EPP	DS	PLHT	EASP	HUSK	SDR1	SDR2	ESP1	ESP2	STR-BI
	(kg/ha)		(Days)	(cm)	(1–9)	(1–5)	(1–9)	(1–9)			
TZEEI 76	1341	0.7	56	93	4.7	5.2	4.1	5.4	7	10	−0.58
TZEEI 94	798	0.7	57	97	5.2	4.5	3.7	5.2	2	8	−0.77
TZEEIORQ 60	1300	0.7	57	104	4.9	5.5	4.3	5.8	3	6	−0.86
TZEEIORQ 2	934	0.7	59	94	5.7	5.5	3.8	6.0	2	3	−0.88
TZEEIORQ 26	1015	0.7	62	111	5.4	5.3	3.4	5.5	7	10	−0.89
TZEEIORQ 65	859	0.6	59	108	5.0	4.8	3.8	4.9	4	4	−0.92
TZEEI 75	765	0.7	55	93	4.9	4.5	3.8	5.1	3	5	−1.05
TZEEIORQ 70	1137	0.6	64	107	5.0	5.1	3.8	5.7	3	7	−1.05
TZEEIORQ 10	1130	0.8	57	106	4.0	5.4	4.2	5.6	7	5	−1.18
TZEEIORQ 9	1117	0.8	57	96	4.2	5.1	3.9	5.4	7	15	−1.55
TZEEIORQ 74	844	0.7	59	111	4.9	5.0	3.6	5.2	5	11	−1.56
TZEEIORQ 28	942	0.6	63	98	5.7	5.1	3.6	5.5	4	9	−1.62
TZEEIORQ 76	854	0.6	64	102	5.1	5.0	4.5	5.3	2	0	−1.81
TZEEIORQ 34	768	0.5	64	110	5.5	5.1	4.2	5.1	1	3	−1.93
TZEEIORQ 14	1061	0.8	60	111	4.6	4.7	3.9	5.7	9	11	−2.05
TZEEIORQ 32	886	0.7	59	102	5.1	5.7	4.1	6.0	3	5	−2.14
TZEEIORQ 11	685	0.6	57	100	5.2	4.9	3.8	5.2	4	5	−2.18
TZEEIORQ 48	723	0.5	64	114	6.9	4.8	3.3	5.2	3	9	−2.41
TZEEIORQ 50	1035	0.7	60	107	4.9	5.2	4.5	5.7	4	8	−2.51
TZEEIORQ 51	1166	0.5	63	109	5.3	5.5	4.2	5.8	5	6	−2.84
TZEEIORQ 16	706	0.4	64	101	6.3	4.8	3.7	5.8	0	4	−3.12
TZEEIORQ 47	630	0.4	65	100	5.8	4.1	3.8	4.9	3	6	−3.22
TZEEIORQ 27	898	0.6	65	106	5.0	4.9	4.2	6.1	3	9	−3.53
TZEEIORQ 24	724	0.5	65	115	5.6	5.8	3.7	6.1	3	3	−3.57
TZEEIORQ 71	828	0.6	62	100	5.7	5.2	3.9	5.4	4	16	−3.68
TZEEIORQ 40	669	0.4	68	100	6.5	4.9	4.0	5.4	3	5	−4.00
TZEEIORQ 6	732	0.6	59	102	5.9	5.3	3.5	5.7	8	10	−4.12
TZEEIORQ 44	666	0.4	67	94	6.4	4.8	4.2	5.6	2	5	−4.91
TZEEIORQ 13	431	0.4	60	103	6.9	4.9	4.1	5.4	3	7	−5.66
TZEEIORQ 39	483	0.5	66	106	5.4	4.9	4.6	6.0	1	3	−5.69
TZEEIORQ 59	606	0.7	61	106	6.4	5.8	4.6	6.1	6	7	−6.28
TZEEIORQ 21	898	0.6	60	103	5.1	5.7	4.9	6.4	5	11	−6.48
TZEEIORQ 23	250	0.3	64	98	6.9	5.0	3.9	5.6	2	6	−7.03
TZEEIORQ 30	535	0.2	.	117	7.7	5.1	4.5	6.2	3	3	−8.03
TZEEIORQ 54	529	0.4	61	113	6.0	5.9	4.6	6.4	6	7	−8.53
TZEEIORQ 50B	491	0.3	66	98	7.0	6.0	4.9	7.0	6	7	−11.17
TZEEIORQ 12	333	0.4	66	101	6.3	6.9	5.1	7.3	9	12	−13.37
Mean	1064	0.7	61	106	5.0	4.8	3.8	5.2	3	6	
Max	2032	1.1	68	128	7.7	6.9	5.1	7.3	9	16	
Min	250	0.2	55	75	2.7	3.7	2.7	4.0	0	0	
LSD (0.05)	966	0.5	5	21	2.1	1.7	1.4	1.9	7	10	

YIELD = grain yield; EPP = number of ears per plant; DS = days to 50% silking; PLHT = plant height; EASP = ear aspect (rated on a scale of 1–9); HUSK = husk cover (rated on a scale of 1–5); SDR1 = *Striga* damage rating at 8 WAP (rated on a scale of 1–9); SDR2 = *Striga* damage rating at 10 WAP (rated on a scale of 1–9); ESP1 = number of emerged *Striga* plant at 8 WAP; ESP2 = number of emerged *Striga* plant at 10 WAP; STR-BI = *Striga* base index.

Table 5. Grain yield and other agronomic traits of 76 extra-early provitamin A/QPM inbreds with four checks evaluated across low-N environments at Ile-Ife and Mokwa between 2016 and 2017 in Nigeria.

LINE	YIELD	PASP	EASP	STGR	HUSK	EPP	DS	DA	PLHT	EHT	LN-BI
	(kg/ha)	(1–9)	(1–9)	(1–9)	(1–5)		(Days)	(Days)	(cm)	(cm)	
TZEEIORQ 57	2017	3.5	3.1	1.7	2.9	0.8	56	57	164	64	9.28
TZEEIORQ 21	2282	3.9	2.5	3.5	2.6	0.9	55	55	140	64	8.46
TZEEIORQ 64	1749	3.5	3.0	2.1	1.9	0.8	56	57	152	60	7.88
TZEEIORQ 53	1685	3.4	3.1	2.3	2.5	0.7	58	58	175	70	6.55
TZEEIORQ 43	1534	3.5	4.2	2.2	2.5	0.8	57	57	138	61	5.96
TZEEIORQ 55	1729	3.6	3.8	2.2	3.1	0.8	58	58	166	61	5.76
TZEEIORQ 63	1708	3.7	4.0	1.9	2.2	0.7	55	56	161	60	5.69
TZEEIORQ 20	2227	4.2	3.8	3.7	2.4	0.8	57	56	147	58	5.61
TZEEIORQ 51	1792	4.2	3.2	3.0	3.0	0.8	56	56	146	64	5.58
TZEEIORQ 42	1520	4.0	4.5	2.4	2.5	1.0	60	60	151	66	5.57
TZEEIORQ 52	1749	4.1	3.7	2.5	3.1	0.8	54	54	154	58	5.25
TZEEIORQ 14	1850	4.7	4.2	3.0	3.2	1.0	55	54	154	69	4.93
TZEEI 73	1977	4.1	4.5	2.7	2.9	0.7	54	53	147	60	4.87
TZEEIORQ 75	1375	3.5	3.2	2.5	2.2	0.7	60	60	135	52	4.83
TZEEIORQ 76	1697	3.8	3.5	2.9	2.7	0.7	59	59	149	53	4.49
TZEEIORQ 1	1713	4.3	3.9	2.8	2.5	0.8	54	54	137	56	4.15
TZEEIORQ 7	1453	4.5	4.1	3.0	3.3	1.0	56	56	131	55	4.12
TZEEIORQ 5	1515	4.5	3.2	3.4	2.8	0.9	56	56	153	64	4.06
TZEEIORQ 50	1572	4.8	3.8	2.4	3.1	0.8	56	56	138	62	4.01
TZEEIORQ 74	1650	4.2	4.1	3.0	3.3	0.8	55	56	151	48	3.99
TZEEIORQ 41	1359	3.9	3.8	2.9	2.6	0.8	57	58	159	64	3.77
TZEEI 76	1623	4.9	3.4	3.3	2.9	0.8	56	56	145	43	3.35
TZEEIORQ 26	1631	4.5	4.1	2.5	3.1	0.6	59	59	143	55	3.26
TZEEIORQ 69	1830	4.9	4.1	3.4	3.1	0.8	57	56	153	64	2.93
TZEEIORQ 61	1658	4.6	3.7	3.0	3.0	0.8	55	54	155	54	2.67
TZEEIORQ 34B	762	5.2	4.9	2.3	3.3	0.5	-	63	143	60	2.54
TZEEIORQ 48	1332	3.7	5.2	2.8	2.2	0.8	60	60	149	66	2.53
TZEEIORQ 59	1745	4.7	4.4	2.8	2.9	0.6	55	56	155	58	2.39
TZEEIORQ 77	755	4.8	5.5	3.3	3.4	0.7	63	63	151	60	2.14
TZEEIORQ 25	1693	4.3	4.9	3.0	2.6	0.6	60	59	151	70	1.92
TZEEIORQ 9	1294	5.0	4.4	2.6	3.9	0.8	54	55	131	51	1.74
TZEEIORQ 54	1306	4.5	3.9	2.3	2.9	0.6	57	57	151	65	1.61
TZEEIORQ 27	1330	4.5	4.6	3.4	3.0	0.8	60	60	146	61	1.41
TZEEIORQ 29	1524	4.4	4.2	2.6	3.0	0.6	59	58	145	58	1.25
TZEEIORQ 60	1072	4.4	4.3	2.7	3.4	0.7	55	55	150	53	0.88
TZEEIORQ 10	1391	4.9	3.8	3.6	3.3	0.7	55	55	144	59	0.53
TZEEIORQ 3	1268	4.9	4.7	2.8	5.0	0.7	58	58	138	61	0.48
TZEEIORQ 56	985	3.6	4.7	2.7	3.5	0.6	58	58	174	66	0.35
TZEEIORQ 44	1099	4.7	4.0	2.7	2.6	0.6	61	61	139	62	0.15
TZEEIORQ 47	1430	4.8	5.8	2.8	3.2	0.7	60	60	154	68	0.10
TZEEI 75	1182	5.3	4.3	2.3	3.3	0.7	56	56	138	53	0.06
TZEEIORQ 33	729	4.7	4.7	2.3	2.8	0.7	57	57	139	64	−0.14
TZEEIORQ 28	1010	4.7	4.9	1.8	3.3	0.6	59	58	153	66	−0.35
TZEEIORQ 17	1519	5.1	4.5	4.1	3.3	0.7	55	56	141	65	−0.46
TZEEIORQ 24	1010	4.9	4.7	2.8	2.9	0.8	62	61	141	62	−0.54
TZEEIORQ 2	1781	4.9	5.7	4.0	3.5	0.7	57	56	159	70	−0.58
TZEEIORQ 18	1462	5.4	4.3	3.7	3.7	0.7	56	56	145	64	−1.23
TZEEIORQ 49	1030	4.8	5.3	2.4	3.4	0.6	61	62	141	51	−1.59
TZEEIORQ 50B	971	5.0	4.8	2.3	3.2	0.6	57	57	141	56	−1.63
TZEEIORQ 46	1053	4.5	5.3	3.3	2.6	0.6	61	60	132	60	−1.94
TZEEIORQ 22	611	5.1	5.3	2.1	2.9	0.7	59	59	146	66	−2.04
TZEEIORQ 71	1174	5.8	5.0	3.0	3.7	0.6	58	57	142	60	−2.36

Table 5. *Cont.*

LINE	YIELD	PASP	EASP	STGR	HUSK	EPP	DS	DA	PLHT	EHT	LN-BI
	(kg/ha)	(1–9)	(1–9)	(1–9)	(1–5)		(Days)	(Days)	(cm)	(cm)	
TZEEIORQ 45	1063	4.8	5.1	3.2	3.3	0.6	61	60	150	58	−2.49
TZEEIORQ 31	1324	5.3	6.3	2.7	3.5	0.6	60	60	150	60	−2.57
TZEEIORQ 62	1188	5.5	4.1	4.3	3.3	0.7	56	55	146	52	−2.87
TZEEIORQ 40	891	4.2	5.8	2.9	2.6	0.5	61	61	153	68	−2.88
TZEEIORQ 12	829	5.6	4.2	3.4	3.4	0.7	58	57	138	59	−2.91
TZEEIORQ 70	928	5.0	5.4	2.7	3.2	0.5	57	56	149	80	−3.51
TZEEIORQ 58	736	5.1	5.5	3.0	3.6	0.6	59	59	155	67	−3.54
TZEEI 94	1056	5.1	4.6	3.6	3.4	0.5	54	54	135	54	−3.61
TZEEIORQ 62B	1093	4.2	5.7	4.1	3.8	0.5	62	62	158	76	−3.65
TZEEIORQ 68	934	5.5	5.3	2.6	2.8	0.6	61	60	133	53	−3.65
TZEEIORQ 19	1221	5.5	5.6	4.1	3.9	0.6	55	55	134	56	−4.13
TZEEIORQ 65	939	5.5	5.5	3.2	4.1	0.6	59	59	160	54	−4.33
TZEEIORQ 34	861	5.3	5.4	3.1	3.2	0.6	60	60	146	67	−4.53
TZEEIORQ 15	522	5.1	5.9	2.8	3.8	0.7	62	62	131	55	−4.67
TZEEIORQ 39	637	4.6	5.7	3.0	3.0	0.5	61	60	137	61	−5.07
TZEEIORQ 6	614	5.8	4.7	2.8	4.2	0.6	56	55	125	48	−5.14
TZEEIORQ 23	819	4.9	6.8	2.6	3.4	0.4	60	59	156	65	−6.22
TZEEIORQ 67	881	5.8	6.4	3.4	3.4	0.5	62	61	79	48	−7.03
TZEEIORQ 66	873	5.3	7.1	3.6	3.2	0.4	59	58	165	56	−8.23
TZEEIORQ 11	494	5.7	6.8	2.9	3.7	0.4	55	54	133	55	−8.77
TZEEIORQ 73	494	6.0	5.2	4.7	3.8	0.5	54	54	140	57	−9.28
TZEEIORQ 32	711	6.2	5.3	5.3	3.8	0.5	56	56	135	54	−10.26
TZEEIORQ 13	451	5.8	6.9	4.0	3.5	0.4	56	55	127	54	−11.43
TZEEIORQ 16	595	6.3	7.1	4.9	3.9	0.3	60	60	137	60	−13.48
Mean	1257	4.7	4.7	3.0	3.2	0.7	58	58	145	60	
Max	2282	6.3	7.1	5.3	5.0	1.0	63	63	175	80	
Min	451	3.4	2.5	1.7	1.9	0.3	54	53	79	43	
LSD (0.05)	954	1.1	1.8	1.2	1.2	0.4	3	3	20	17	

YIELD = grain yield; DA = days to 50% anthesis; DS = days to 50% silking; PLHT = plant height; EHT = ear height; EASP = ear aspect (rated on a scale of 1–9); STGR = stay-green characteristic (rated on a scale of 1–9); PASP = plant aspect (rated on a scale of 1–9); HUSK = husk cover (rated on a scale of 1–5); EPP = number of ears per plant; LN-BI = low-N base index.

Averaged over inbred lines, grain yield was 1064 kg ha^{-1} under *Striga*-infested environment, 1257 kg ha^{-1} under low nitrogen and 2120 kg ha^{-1} under high nitrogen; thus, compared to high nitrogen plots, grain yield reduction due to *Striga* and low nitrogen averaged 49.8% and 40.7%, respectively (Table 6). The number of ears per plant for these environments was 0.7, 0.7, and 0.8, respectively. Grain yield across the environments ranged from 658 kg ha^{-1} for TZEEIORQ 16 to 2337 kg ha^{-1} for TZEEIORQ 63 with an average of 1492 kg ha^{-1}, while ears per plant ranged from 0.3 for TZEEIORQ 16 to 1.3 for TZEEIORQ 57 with a mean of 0.7. Ear aspect was lowest for TZEEIORQ 62 and TZEEIORQ 64 (3.2) and highest for TZEEIORQ16 (6.9) (Table 7). There were no significant differences in grain yield among the top five ranking inbreds (TZEEIORQ 57, TZEEIORQ 63, TZEEIORQ 42, TZEEIORQ 55, and TZEEIORQ 64), identified as resistant/tolerant to both *Striga* and nitrogen stresses based on the multiple character base index, and the best check TZEEI 73 (Table 6). Inbred TZEEIORQ 57 had the highest mean value (1.3) for ears per plant across research environments, but it had the lowest mean values for stay-green characteristic (1.7) under low nitrogen stress and *Striga* (host–plant) damage rating at 8 WAP (2.7) under *Striga* condition. The expression of these desirable attributes by the inbred contributed to its excellent performance across research environments.

Table 6. Grain yield and other agronomic traits of 20 best and 5 worst extra-early maturing provitamin A quality protein maize inbreds evaluated under *Striga*-infested, low-N and high-N environments.

Line	Grain Yield				Ears per Plant				Ear Aspect				‡ Plant Aspect			§ STGR	SDR1(1–9) [þ]	SDR2(1–9)	ESP1 [d]	ESP2	MI [Ħ]
	Striga	(kg/ha) LN	HN	Acrs	*Striga*	LN	HN	Acrs	*Striga*	LN	† (1–9) HN	Acrs	LN	(1–9) HN	Acrs	(1–9) LN					
Best																					
TZEEIORQ 57	1632	2017	3074	2241	0.7	0.8	0.8	1.3	4.1	3.1	2.9	3.3	3.5	3.3	3.4	1.7	2.7	4.0	1	1	13.10
TZEEIORQ 63	2032	1708	3270	2337	0.8	0.7	0.9	0.8	3.1	4.0	2.5	3.2	3.7	3.2	3.5	3.5	3.6	4.2	1	2	11.50
TZEEIORQ 42	1275	1520	3241	2012	0.8	1.0	1.1	1.0	4.1	4.5	3.6	4.1	4.0	3.1	3.6	2.1	3.3	4.6	6	5	11.20
TZEEIORQ 55	1381	1729	3056	2055	0.7	0.7	0.8	0.8	4.9	3.8	3.6	4.1	3.6	3.9	3.7	2.3	3.0	4.5	4	7	8.97
TZEEIORQ 64	1792	1749	3148	2230	0.7	0.8	0.8	0.8	4.1	3.0	2.4	3.2	3.5	2.9	3.2	2.2	2.9	4.0	2	1	8.70
TZEEI 73	1423	494	3141	2184	0.7	0.5	1.0	0.8	5.0	5.2	3.5	4.3	6.0	3.5	3.8	3.7	3.2	4.5	0	4	7.85
TZEEIORQ 53	1592	1684	2323	1863	0.7	0.7	0.8	0.7	3.9	3.1	3.9	3.7	3.4	4.1	3.8	2.2	3.3	4.2	0	1	6.80
TZEEIORQ 52	1678	1749	2724	2046	0.7	0.8	1.0	0.8	3.9	3.7	3.0	3.5	4.1	3.8	4.0	1.9	3.4	4.9	4	8	6.58
TZEEIORQ 43	1128	1534	2447	1699	0.8	0.8	0.8	0.8	5.0	4.2	4.5	4.6	3.5	3.6	3.5	2.5	3.2	4.7	1	2	6.18
TZEEIORQ 62	1938	1188	1672	1599	0.9	0.7	0.9	0.8	2.7	4.1	3.8	3.5	5.5	4.9	5.2	2.4	3.6	4.9	1	3	5.48
TZEEIORQ 25	1099	1693	2794	1862	0.8	0.6	0.9	0.7	4.6	4.9	3.5	4.3	4.3	4.0	4.2	2.7	3.4	4.8	2	9	5.44
TZEEIORQ 61	1836	1658	2019	1841	0.8	0.8	0.7	0.8	3.5	3.7	3.6	3.6	4.6	3.7	4.2	3.0	3.3	5.0	0	4	5.31
TZEEIORQ 5	1540	1515	2506	1854	1.0	0.9	1.1	0.9	3.3	3.2	3.9	3.5	4.5	3.8	4.1	2.9	3.5	4.7	2	6	4.98
TZEEIORQ 41	1173	1359	1491	1341	0.8	0.8	0.6	0.7	4.5	3.8	5.0	4.4	3.9	4.1	4.0	3.0	3.5	4.8	7	11	4.75
TZEEIORQ 7	964	1453	2426	1614	0.8	1.0	0.8	0.9	4.2	4.1	3.7	4.0	4.5	4.5	4.5	2.5	3.2	4.8	3	3	4.53
TZEEIORQ 46	1547	1053	2045	1549	1.1	0.6	0.9	0.9	4.4	5.3	4.4	4.7	4.5	3.9	4.2	2.8	3.5	4.6	2	5	4.44
TZEEIORQ 69	1181	1830	1537	1512	0.8	0.8	0.6	0.8	4.8	4.0	4.6	4.5	4.9	5.1	5.0	3.0	3.8	4.9	2	4	4.03
TZEEIORQ 18	1057	1462	2636	1718	0.7	0.7	0.8	0.8	4.0	4.3	4.0	4.1	5.4	4.8	5.1	2.4	3.3	4.4	0	4	3.50
TZEEIORQ 74	844	1650	2934	1809	0.7	0.8	1.1	0.9	4.9	4.1	3.7	4.2	4.2	3.8	4.0	3.4	3.8	4.5	2	2	3.32
TZEEIORQ 76	854	1697	2724	1759	0.6	0.7	0.9	0.8	5.1	3.5	3.1	3.9	3.8	4.1	3.9	3.0	3.2	4.7	4	9	3.14
Worst																					
TZEEIORQ 23	250	819	1038	702	0.3	0.4	0.5	0.4	6.9	6.8	5.5	6.4	4.9	4.7	4.8	2.9	3.9	5.6	2	6	−9.53
TZEEIORQ 32	886	711	1615	1070	0.7	0.5	0.8	0.7	5.1	5.3	4.1	4.8	6.2	5.0	5.6	4.7	4.5	6.2	3	3	−9.70
TZEEIORQ 13	431	451	1297	730	0.4	0.4	0.6	0.4	6.9	6.9	6.0	6.6	5.8	5.6	5.7	5.3	4.6	6.4	6	7	−13.30
TZEEIORQ 12	333	829	1109	757	0.4	0.7	0.7	0.6	6.3	4.2	4.9	5.2	5.6	4.8	5.2	4.0	4.9	7.0	6	7	−15.10
TZEEIORQ 16	706	595	672	658	0.4	0.3	0.2	0.3	6.3	7.1	7.2	6.9	6.3	6.5	6.4	4.9	5.1	7.3	9	12	−15.90
Grand mean	1064	1257	2120	1492	0.7	0.7	0.8	0.7	5.0	4.7	4.1	4.6	4.7	4.3	4.5	3.0	3.8	5.2	3	6	
LSD (0.05)	966	954	1154	1027	0.5	0.4	0.5	0.6	2.1	1.8	1.7	1.9	1.1	1.2	1.2	1.2	1.4	1.9	7	10	

*, ** and *** = significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively; *Striga* = *Striga*-infested environment; LN = low-N environment; HN = high-N environment; Acrs = across research environments; § STGR = stay-green characteristic; ‡ Plant aspect = estimated across low-N and high-N environments þ SDR1 and SDR2 = *Striga* damage rating at 8 and 10 WAP, respectively; d ESP1 and ESP2 = number of emerged *Striga* plant at 8 and 10 WAP, respectively; † (1–9) = rated on a scale of 1–9; Ħ MI = multiple-trait base index; LSD (0.05) = least significant difference at 5% probability level.

Table 7. Tropical *Zea* extra-early maturing provitamin A quality protein maize inbreds with and without favorable allele for provitamin A marker crtRB1-3′TE and corresponding levels of beta carotene and total provitamin A as determined by high-performance liquid chromatography.

S/N	Line	Presence/Absence of crtRB1-3′TE Allele	β-Carotene Level (µg/g)	Total Provitamin A (µg/g)
1	TZEEIORQ 49	1	4.06	7.25
2	TZEEIORQ 50	1	4.70	7.75
3	TZEEIORQ 51	1	5.50	8.25
4	TZEEIORQ 52	1	6.40	10.01
5	TZEEIORQ 53	1	4.33	7.36
6	TZEEIORQ 54	1	6.43	10.95
7	TZEEIORQ 55	1	5.97	10.61
8	TZEEIORQ 57	1	5.75	9.99
9	TZEEIORQ 58	1	6.05	10.68
10	TZEEIORQ 60	1	5.19	7.95
11	TZEEIORQ 62	1	5.77	9.22
12	TZEEIORQ 63	1	4.13	6.68
13	TZEEIORQ 64	1	3.41	5.83
14	TZEEIORQ 50B	1	-	-
15	TZEEIORQ 73	1	2.83	4.35
16	TZEEIORQ 34B	1	-	-
17	TZEEIORQ 1	0	3.56	6.89
18	TZEEIORQ 2	0	1.85	2.74
19	TZEEIORQ 3	0	2.73	6.37
20	TZEEIORQ 4	0	2.92	4.65
21	TZEEIORQ 5	0	5.75	10.25
22	TZEEIORQ 6	0	3.78	7.48
23	TZEEIORQ 7	0	3.36	7.46
24	TZEEIORQ 8	0	3.86	7.92
25	TZEEIORQ 9	0	3.40	6.89
26	TZEEIORQ 10	0	3.78	6.34
27	TZEEIORQ 11	0	2.40	4.44
28	TZEEIORQ 12	0	3.56	4.82
29	TZEEIORQ 13	0	2.44	3.72
30	TZEEIORQ 14	0	2.91	3.95
31	TZEEIORQ 15	0	2.60	4.96
32	TZEEIORQ 16	0	3.74	6.47
33	TZEEIORQ 17	0	2.50	3.47
34	TZEEIORQ 18	0	2.35	3.04
35	TZEEIORQ 19	0	2.54	3.30
36	TZEEIORQ 20	0	2.43	3.21
37	TZEEIORQ 21	0	2.24	3.16
38	TZEEIORQ 22	0	3.45	5.58
39	TZEEIORQ 23	0	2.51	3.77
40	TZEEIORQ 24	0	2.86	3.96
41	TZEEIORQ 25	0	2.28	3.68
42	TZEEIORQ 26	0	2.15	3.90
43	TZEEIORQ 27	0	1.48	2.21
44	TZEEIORQ 28	0	1.88	3.93
45	TZEEIORQ 29	0	2.09	3.78
46	TZEEIORQ 30	0	2.47	4.62
47	TZEEIORQ 31	0	2.38	4.51
48	TZEEIORQ 32	0	2.38	3.94
49	TZEEIORQ 33	0	2.93	6.40
50	TZEEIORQ 34	0	2.80	7.24
51	TZEEIORQ 35	0	2.79	6.50
52	TZEEIORQ 82	0	4.82	7.55
53	TZEEIORQ 37	0	2.91	6.49

Table 7. *Cont.*

S/N	Line	Presence/Absence of crtRB1-3′TE Allele	β-Carotene Level (µg/g)	Total Provitamin A (µg/g)
54	TZEEIORQ 38	0	2.75	6.00
55	TZEEIORQ 39	0	3.05	5.29
56	TZEEIORQ 40	0	2.50	6.58
57	TZEEIORQ 41	0	2.36	5.63
58	TZEEIORQ 42	0	3.75	7.65
59	TZEEIORQ 43	0	3.82	7.44
60	TZEEIORQ 44	0	4.07	8.40
61	TZEEIORQ 45	0	3.04	6.20
62	TZEEIORQ 46	0	3.97	7.46
63	TZEEIORQ 47	0	3.76	7.35
64	TZEEIORQ 48	0	3.43	6.11
65	TZEEIORQ 56	0	4.07	6.63
66	TZEEIORQ 61	0	3.31	5.90
67	TZEEIORQ 65	0	3.94	5.37
68	TZEEIORQ 67	0	4.11	6.39
69	TZEEIORQ 45	0	3.63	5.12
70	TZEEIORQ 69	0	2.95	5.12
71	TZEEIORQ 70	0	2.14	4.51
72	TZEEIORQ 35	0	3.88	5.29
73	TZEEIORQ 72	0	5.57	8.89
74	TZEEIORQ 75	0	3.59	5.75
75	TZEEIORQ 76	0	4.36	6.86
76	TZEEIORQ 59	-	5.86	8.82
	Mean		3.53	6.18

1 = presence of crtRB1-3′TE; 0 = absence of crtRB1-3′TE; - = missing sample during molecular analysis.

3.3. Molecular Screening and Biochemical Analyses of Inbreds Derived from Tropical Zea Extra-Early Striga-Resistant Maize Population Improved for Provitamin A and Quality Protein Maize Properties

Of the two allele-specific provitamin A markers used, only crtRB1-3′TE was polymorphic among the inbred lines (Figures 1 and 2). The polymorphic marker differentiated the 76 biofortified lines into two groups (Table 7). The first group comprised 16 inbred lines with the favorable allele of crtRB1-3′TE, while the second group of 60 inbred lines was without the favorable provitamin A allele. HPLC did not detect beta carotene and provitamin A in two of the 16 samples possessing the favorable allele of crtRB1-3′TE (Table 7). Levels of provitamin A in all the inbred lines analyzed ranged from 2.21 µg g^{-1} for TZEEIORQ 27 to 10.95 µg g^{-1} for TZEEIORQ 54 with an average of 6.18 µg g^{-1}. A total of 12 of the 16 inbreds with the favorable allele of crtRB1-3′TE marker had provitamin A levels greater than the mean provitamin A value (Table 7). Two inbred lines (TZEIORQ 64 and TZEEIORQ 73) with the favorable provitamin A allele had lower levels of provitamin A than the average provitamin A value for the inbred lines analyzed (Table 7). Chi-squared analysis showed a significant association ($p < 0.01$) between the desirable provitamin A marker allele and the provitamin A content of the inbred lines (Table 8). Stepwise regression of provitamin A carotenoids (beta-carotene and beta-cryptoxanthin) on the overall provitamin A in the inbreds showed significant contributions, of 81.9% and 18.3% for beta-carotene and beta-cryptoxanthin, respectively, to the levels of total provitamin A in the inbreds studied (Table 9). Tryptophan content varied from 0.04% in TZEEIORQ 53 to 0.08% in TZEEIORQ 72 with an average of 0.05%, while lysine content ranged from 0.19% in TZEEIORQ 50 to 0.39% in TZEEIORQ 74 with a mean of 0.27% (Table 10). In all, 39 inbred lines had provitamin A levels above 6.18 µg g^{-1}; these inbred lines showed different combinations of *Striga* tolerance/resistance, low nitrogen stress tolerance, provitamin A, tryptophan and lysine contents (Tables 7 and 10). Three lines viz. TZEEIORQ 55, TZEEIORQ 5 and TZEEIORQ 52 combined high provitamin A content (>10.0 µg g^{-1}), tolerance/resistance to

Striga and low nitrogen stress with improved tryptophan and lysine contents—Not lower than the average values of 0.05 and 0.27%, respectively (Tables 7 and 10). An inbred line with a positive base index value was identified as resistant/tolerant to the stress, while a negative base index value indicated susceptibility to the stress.
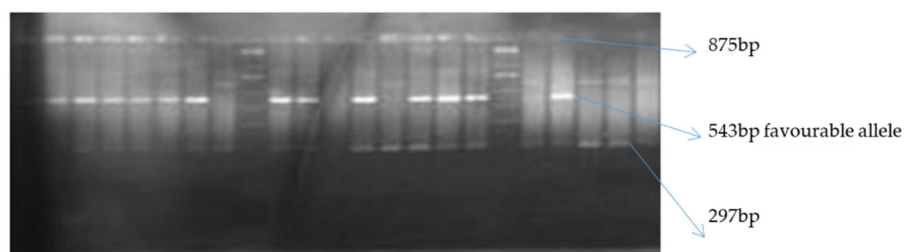

Figure 1. Gel image of crtRB1-3′TE PCR product resolved on a 2% agarose gel indicating polymorphism of the marker for beta carotene allele.
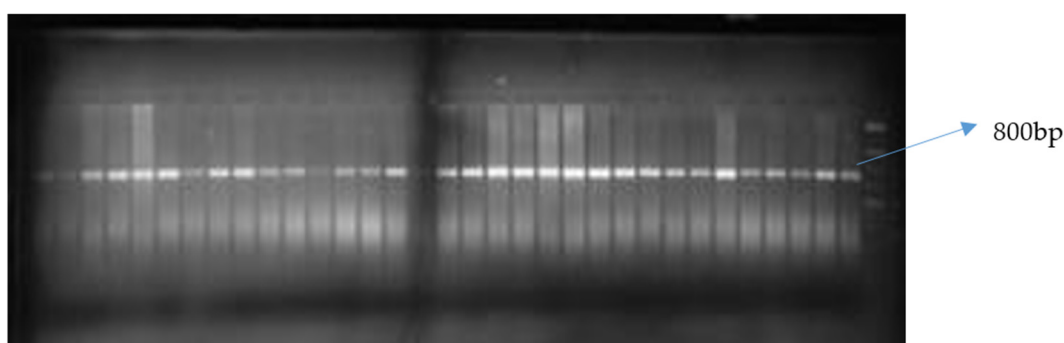

Figure 2. Gel image of *crtRB1*-5′TE PCR product resolved on a 2% agarose gel indicating monomorphism of the marker for beta carotene allele.

Table 8. Presence or absence of desirable allele of beta-carotene hydroxylase gene (crtRB1-3′TE) in the 76 extra-early provitamin A quality protein maize inbreds and provitamin A content of the inbred lines determined by high-performance liquid chromatography.

	Provitamin A Content (μg g^{-1})			
Desirable Marker Allele	**Low (<7.49)**	**Moderate (7.50–8.49)**	**High (>8.49)**	**Chi-Squared Value**
Present	5.00	3.00	6.00	19.10 **
Absent	52.00	5.00	3.00	

**, significant at $p < 0.01$.

Table 9. Regression of provitamin A carotenoids on total provitamin A content of extra-early provitamin A quality protein maize inbreds analyzed using high-performance liquid chromatography.

Provitamin A Carotenoids	**Partial R-Squared**
Beta cryptoxanthin	0.1834 ***
All-trans beta carotene	0.8192 ***
9-*cis*-beta carotene	0.0069 ***
13-*cis*-beta carotene	0.0005 ***

***, significant at $p < 0.001$.

Table 10. Levels of tryptophan and lysine in the extra-early provitamin A quality protein maize inbreds with the most stable check and their responses to *Striga* and low-N, determined by *Striga* and low-N base index values.

Line	Tryptophan (%)	lysine (%)	Reaction to *Striga*	Reaction to Low N
TZEEIORQ 54	0.06 ± 0.005	0.31 ± 0.008	Susceptible	Tolerant
TZEEIORQ 58	0.06 ± 0.001	0.29 ± 0.013	Tolerant	Susceptible
TZEEIORQ 55	0.07 ± 0.003	0.36 ± 0.018	Tolerant	Tolerant
TZEEIORQ 5	0.05 ± 0.001	0.30 ± 0.012	Tolerant	Tolerant
TZEEIORQ 52	0.05 ± 0.001	0.27 ± 0.003	Tolerant	Tolerant
TZEEIORQ 57	0.04 ± 0.002	0.24 ± 0.011	Tolerant	Tolerant
TZEEIORQ 62	0.05 ± 0.001	0.28 ± 0.004	Tolerant	Susceptible
TZEEIORQ 72	0.08 ± 0.006	0.35 ± 0.010	Tolerant	Susceptible
TZEEIORQ 59	0.04 ± 0.002	0.22 ± 0.011	Susceptible	Tolerant
TZEEIORQ 44	0.05 ± 0.004	0.27 ± 0.003	Susceptible	Tolerant
TZEEIORQ 51	0.04 ± 0.005	0.25 ± 0.029	Susceptible	Tolerant
TZEEIORQ 60	0.05 ± 0.003	0.28 ± 0.032	Susceptible	Tolerant
TZEEIORQ 8	0.05 ± 0.002	0.25 ± 0.005	Tolerant	Susceptible
TZEEIORQ 50	0.04 ± 0.004	0.23 ± 0.009	Susceptible	Tolerant
TZEEIORQ 74	0.08 ± 0.001	0.39 ± 0.028	Susceptible	Tolerant
TZEEIORQ 42	0.05 ± 0.001	0.32 ± 0.007	Tolerant	Tolerant
TZEEIORQ 50B	0.04 ± 0.001	0.19 ± 0.008	Susceptible	Susceptible
TZEEIORQ 6	0.05 ± 0.002	0.24 ± 0.005	Susceptible	Susceptible
TZEEIORQ 7	0.05 ± 0.000	0.25 ± 0.006	Tolerant	Tolerant
TZEEIORQ 46	0.05 ± 0.005	0.30 ± 0.013	Tolerant	Susceptible
TZEEIORQ 43	0.05 ± 0.004	0.27 ± 0.005	Tolerant	Tolerant
TZEEIORQ 53	0.04 ± 0.001	0.26 ± 0.015	Tolerant	Tolerant
TZEEIORQ 47	0.05 ± 0.004	0.30 ± 0.007	Susceptible	Tolerant
TZEEIORQ 49	0.06 ± 0.002	0.33 ± 0.015	Susceptible	Susceptible
TZEEIORQ 34B	0.06 ± 0.001	0.26 ± 0.001	Susceptible	Susceptible
TZEEIORQ 1	0.05 ± 0.001	0.28 ± 0.007	Susceptible	Tolerant
TZEEIORQ 9	0.05 ± 0.006	0.26 ± 0.003	Susceptible	Tolerant
TZEEI 76	0.05 ± 0.002	0.23 ± 0.015	Susceptible	Tolerant
TZEEIORQ 76	0.04 ± 0.001	0.24 ± 0.005	Susceptible	Tolerant
TZEEIORQ 63	0.06 ± 0.001	0.28 ± 0.004	Tolerant	Tolerant
TZEEIORQ 56	0.05 ± 0.006	0.25 ± 0.001	Susceptible	Tolerant
TZEEIORQ 66	0.04 ± 0.002	0.28 ± 0.010	Susceptible	Susceptible
TZEEIORQ 40	0.06 ± 0.001	0.31 ± 0.010	Susceptible	Susceptible
TZEEIORQ 35	0.06 ± 0.000	0.27 ± 0.013	Susceptible	Tolerant
TZEEIORQ 62B	0.06 ± 0.001	0.26 ± 0.001	Tolerant	Susceptible
TZEEIORQ 16	0.06 ± 0.000	0.30 ± 0.005	Susceptible	Susceptible
TZEEIORQ 33	0.06 ± 0.000	0.26 ± 0.014	Tolerant	Susceptible
TZEEIORQ 67	0.05 ± 0.001	0.26 ± 0.004	Susceptible	Susceptible
TZEEIORQ 3	0.04 ± 0.001	0.23 ± 0.002	Tolerant	Tolerant
TZEEIORQ 10	0.05 ± 0.002	0.29 ± 0.001	Susceptible	Tolerant
TZEEIORQ 45	0.05 ± 0.002	0.29 ± 0.013	Tolerant	Susceptible
TZEEIORQ 48	0.05 ± 0.003	0.27 ± 0.022	Susceptible	Tolerant
TZEEIORQ 38	0.05 ± 0.002	0.29 ± 0.021	Susceptible	Susceptible
TZEEIORQ 61	0.05 ± 0.003	0.27 ± 0.023	Tolerant	Tolerant
TZEEIORQ 64	0.05 ± 0.000	0.29 ± 0.004	Tolerant	Tolerant
TZEEIORQ 75	0.04 ± 0.003	0.23 ± 0.006	Tolerant	Tolerant
TZEEIORQ 41	0.06 ± 0.002	0.30 ± 0.005	Tolerant	Tolerant
TZEEIORQ 22	0.07 ± 0.005	0.36 ± 0.006	Tolerant	Susceptible
TZEEIORQ 65	0.04 ± 0.001	0.24 ± 0.002	Susceptible	Susceptible
TZEEIORQ 71	0.06 ± 0.002	0.27 ± 0.003	Susceptible	Susceptible
TZEEIORQ 39	0.06 ± 0.006	0.30 ± 0.001	Susceptible	Susceptible

Table 10. *Cont.*

Line	Tryptophan (%)	lysine (%)	Reaction to *Striga*	Reaction to Low N
TZEEIORQ 69	0.06 ± 0.004	0.30 ± 0.003	Tolerant	Tolerant
TZEEIORQ 68	0.05 ± 0.007	0.22 ± 0.004	Tolerant	Susceptible
TZEEIORQ 15	0.05 ± 0.003	0.23 ± 0.003	Tolerant	Susceptible
TZEEIORQ 12	0.04 ± 0.002	0.22 ± 0.002	Susceptible	Susceptible
TZEEIORQ 4	0.04 ± 0.003	0.22 ± 0.003	Tolerant	Susceptible
TZEEIORQ 30	0.06 ± 0.004	0.30 ± 0.008	Susceptible	Susceptible
TZEEIORQ 31	0.06 ± 0.002	0.28 ± 0.001	Susceptible	Susceptible
TZEEIORQ 70	0.06 ± 0.001	0.25 ± 0.008	Susceptible	Susceptible
TZEEIORQ 11	0.06 ± 0.006	0.26 ± 0.003	Susceptible	Susceptible
TZEEIORQ 73	0.06 ± 0.002	0.28 ± 0.004	Tolerant	Susceptible
TZEEIORQ 24	0.06 ± 0.001	0.27 ± 0.001	Susceptible	Susceptible
TZEEIORQ 14	0.05 ± 0.002	0.20 ± 0.001	Susceptible	Tolerant
TZEEIORQ 32	0.05 ± 0.002	0.28 ± 0.001	Susceptible	Susceptible
TZEEIORQ 28	0.06 ± 0.000	0.30 ± 0.002	Susceptible	Susceptible
TZEEIORQ 26	0.06 ± 0.001	0.28 ± 0.005	Susceptible	Tolerant
TZEEIORQ 29	0.05 ± 0.001	0.30 ± 0.003	Tolerant	Tolerant
TZEEIORQ 23	0.05 ± 0.002	0.27 ± 0.001	Susceptible	Susceptible
TZEEIORQ 13	0.06 ± 0.001	0.22 ± 0.001	Susceptible	Susceptible
TZEEIORQ 25	0.05 ± 0.005	0.24 ± 0.002	Tolerant	Tolerant
TZEEIORQ 17	0.05 ± 0.002	0.28 ± 0.001	Tolerant	Susceptible
TZEEIORQ 19	0.05 ± 0.001	0.30 ± 0.000	Susceptible	Susceptible
TZEEIORQ 20	0.05 ± 0.001	0.26 ± 0.010	Susceptible	Tolerant
TZEEIORQ 21	0.07 ± 0.002	0.33 ± 0.007	Susceptible	Tolerant
TZEEIORQ 18	0.05 ± 0.004	0.31 ± 0.002	Tolerant	Susceptible
TZEEIORQ 2	0.05 ± 0.003	0.24 ± 0.010	Susceptible	Susceptible
TZEEIORQ 27	0.06 ± 0.003	0.29 ± 0.004	Susceptible	Tolerant
MEAN	0.05	0.27		
CV (%)	15.18	13.38		
SE ±	0.001	0.004		

4. Discussion

In the moist savanna of WCA, where maize is considered to have great potential, *Striga hermonthica* [19], soil nitrogen stress [21], and recurrent drought [22] limit maize production and productivity. In the present study, *Striga hermonthica* reduced grain yield by 49.8%, while the average yield loss due to nitrogen stress was 40.7%. These stresses present unique, challenging conditions as evidenced by the significant variations obtained for grain yield and the majority of the research environments' characteristics. Abiotic and biotic stresses in the WCA savanna often occur simultaneously in farmers' fields [31], resulting in geometric losses in yield. In an earlier study, [26] reported a 53.7% reduction in maize grain yield due to *Striga* stress alone and 85% yield loss when in combination with drought stress. Although the grain yield reduction obtained under *Striga* infestation in this study is lower than the 68% yield reduction reported by [51], it is higher than the 39% reported by [49]. In addition, the 40.7% yield reduction under low nitrogen stress in this study is higher than the 35% reduction in yield reported by [32]. The differences in yield reduction observed in this study and those earlier reported could be attributed to the dissimilarities in the genotypes evaluated, severity and uniformity of the stresses and other management practices.

Of the nutrient elements in tropical soils, nitrogen is the most restraining [21,32]. Low soil nitrogen is widespread in WCA, where average fertilizer application is 6.11 kg ha^{-1}, an amount lower than the 8.89 kg ha^{-1} average fertilizer consumption in SSA [52], a consequence of non-availability and/or non-affordability by the predominant resource-limited farmers in the area [34]. The cultivation of extra-early maize varieties is one of the strategies for mitigating end-of-season drought. Consequently, developing extra-early maturing maize germplasm, which combines tolerance/resistance to *Striga* and nitrogen

stress, is an important approach for improving maize productivity under the biotic and abiotic stresses of low nitrogen, *Striga* and drought in WCA. The incidence of *Striga* in farmlands is erratic and often influenced by environmental factors. Maize cultivars developed for the savanna of WCA, therefore—in addition to showing resistance/tolerance to these stresses—must also demonstrate capability for high-performance in nonstress environments.

In the current study, the inbreds TZEEIORQ 55, TZEEIORQ 52 and TZEEIORQ 5 were among the thirteen most promising extra-early provitamin A quality protein maize inbreds identified across the research environments (evidenced by their relatively high and positive multiple-character base index values). Coupled with their performance under each of the stresses, these inbreds showed tolerance/resistance to *Striga*, tolerance to low nitrogen, and better performance in stress-free environments. The inbreds also had moderate to relatively high levels of tryptophan and lysine. The consistently higher base index values of TZEEIORQ 55 than those of TZEEI 73, in each and across environments, indicated the outstanding performance of the inbred across the research conditions. The different base indices used in this study integrated several important traits under the respective stresses. For example, under *Striga*, high yield, reduced host–plant damage (tolerance index), and reduced number of emerged *Striga* plants (resistance index) were important for sustainability. While tolerance ensured high yield and low host damage, resistance reduced the number of emerged parasites and buildup of the seeds of *Striga* in the soil [44,52].

The significant genotype × environment interaction obtained in the current study indicated that the genotypes varied in their response patterns to each of the stresses and nonstress conditions. In effect, performance under the conditions in any of the research environments cannot be used to extrapolate performance in other environments. These results justified our approach of developing a considerable number of lines from the source population and screening the lines for their responses under the different stresses and in stress-free environments, thus allowing identifying lines that possessed alleles for both tolerance/resistance to the stresses and good performance under nonstress conditions. Ifie [53] reported significant genotype × environment interaction for yield and other traits of 100 early-maturing maize inbreds studied under *Striga* and low nitrogen environments. Similarly, Akaogu and colleagues [49] observed significant genotype × environment interaction for many characters of 90 extra-early yellow maize inbreds in *Striga*-free and *Striga*-infested environments. The similarity in the results of this study and those of the previous authors suggests that the environments where the maize genotypes were evaluated might be similar.

While breeding for improved levels of provitamin A in maize, several workers have identified and used different molecular markers linked to provitamin A carotenoids [37,39]. Of the two provitamins A markers used in the present study, only crtRB1-3′TE was polymorphic among the inbreds. In a previous study, [12] reported polymorphism for both markers. The variation in the results of this study and that of [12] might be attributed to differences in the genetic materials used for the studies. The allele 1 of crtRB1-3′TE has been reported to bring about a 2 to 10-fold favorable increase in kernel beta-carotene in maize [38,39]. The range of provitamin A levels (2.21–10.95 μg g^{-1}) and the average beta-cryptoxanthin (5.25 μg g^{-1}) observed in the present study are comparable to values (provitamin A levels = 3.01–11.90 μg g^{-1}; average beta-cryptoxanthin = 4.23 μg g^{-1}) obtained by [54]. The similarity in our results is suggestive of the fact that the inbreds used in this study may be genetically related, concerning provitamin A, to those studied by [54]. The significant chi-squared value obtained between the results of the molecular markers and provitamin A content of the inbred lines indicates that the marker was associated with the levels of provitamin A in the inbreds. This suggests that the marker was effective in identifying inbreds with increased levels of provitamin A.

All the inbreds used in the current study, with or without the favorable marker alleles, had lower provitamin A content than the HarvestPlus target of 15 μg g^{-1} [55]. The lines with relatively high content of provitamin A in this study (7.50–10.25 μg g^{-1}) can,

therefore, be regarded as being moderate in provitamin A. Of the 60 inbred lines without the favorable marker allele, seven had provitamin A content in the range 7.50–10.25 µg g^{-1}. This suggests the involvement and effectiveness, in some of the inbred lines, of other provitamin A carotenoid(s) apart from the one linked to the favorable allele of the crtRB1-3′TE. The results of the stepwise regression analyses of the provitamin A carotenoids on the total provitamin A levels in the inbreds revealed that β-cryptoxanthin (with half the vitamin A activity of β-carotene) also contributed significantly to the increased levels of total provitamin A in the inbred lines with or without the favorable allele of crtRB1-3′TE. Similar to our result is the finding of [54], who observed a strong positive relationship between β-cryptoxanthin and provitamin A concentration in maize.

Inbreds TZEEIORQ 55, TZEEIORQ 72 and TZEEIORQ 74 had relatively high levels of tryptophan and lysine, indicating that they possess quality protein properties. Kostadinovic [56] reported a similar range of 0.06–0.08% tryptophan for 13 maize lines. In general, inbreds TZEEIORQ 58, TZEEIORQ 55, TZEEIORQ 5, TZEEIORQ 52, TZEEIORQ 57, TZEEIORQ 62, TZEEIORQ 72, TZEEIORQ 59 and TZEEIORQ 54 had the highest levels of PVATL.

The lines developed in the present study, which are the first set of extra-early maize lines with combined resistance/tolerance to *Striga* and tolerance to nitrogen stress and moderate levels of PVATL, showed exploitable genetic variation for these traits. In addition to their use in developing open-pollinated maize varieties/hybrids for increasing maize productivity in WCA, the lines offer promise for addressing the prevalent problems of VAD and protein deficiency in the subregion. Opportunities exist to further improve the levels of these nutrients in the inbreds through selection.

5. Conclusions

Exploitable genetic variability exists for grain yield and other agronomic characters of the TZEEIORQ lines studied under *Striga*, low- and high-nitrogen soil conditions. The beta-carotene marker, crtRB1-3′TE, was polymorphic and grouped the inbreds into two. The marker was effective in identifying inbreds with moderate provitamin A content. Inbred lines TZEEIORQ 55, TZEEIORQ 52 and TZEEIORQ 5 combined resistance/tolerance to *Striga* and nitrogen stress with improved performance under high nitrogen conditions. These inbreds are invaluable pools of favorable alleles in breeding for extra-earliness, *Striga* resistance, nitrogen stress tolerance, and PVATL.

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