Performance of provitamin A- quality protein maize inbred lines and derived hybrids in contrasting environments

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Abstract

Background Early maturing provitamin A (PVA) quality protein maize (QPM) inbred lines with tolerance to drought and low-N are needed to develop superior hybrids in West and Central Africa (WCA). This study aimed to (i) identify inbred lines that combined drought and low-N tolerance with increased levels of PVA and tryptophan and (ii) assess the relationship among PVA carotenoids, tryptophan and grain yield. Sixty-four inbred lines plus six inbred checks were evaluated under induced drought, low-N and optimal conditions in 2016 and 2017 in Nigeria. The inbred lines were assayed for PVA and tryptophan contents. Results Thirty-three of the lines were found to be tolerant to drought and low-N. Ninety percent of the inbred lines had tryptophan contents > 0.075 % per sample in whole grain substantiating the presence of the QPM trait in the inbred lines. Inbred lines TZEIORQ 55 and TZEIORQ 29 combined high PVA contents (15.38 and 12.10µg g-1, respectively) with low-N tolerance while nine inbred lines combined moderate PVA levels (5.06 – 8.34µg g-1) with drought and low-N tolerance. Conclusions These maize inbred lines could be utilized to develop superior drought and low-N tolerant hybrids and synthetics with elevated levels of PVA and tryptophan for WCA. The correlations observed among grain yield, PVA and tryptophan of the lines were not significant suggesting that these traits could be improved independently.

Background

Maize (*Zea mays* L.) production in West and Central Africa (WCA) is limited by major abiotic constraints including drought and poor soil fertility (particularly low-N) which are the two most frequent yield reduction factors in the sub-region [1]. The occurrence of drought within the two weeks before and after flowering can lead to drastic grain yield losses of 40-90% [2]. Also, annual yield reduction in maize from the impact of low-N has been estimated to vary between 10 and 50% [3]. The frequent occurrence of drought and low-N on maize production fields in WCA contributes to low grain yield annually hovering around 1.8 t/ha [4]. The synergistic effect of the two stresses could even lead to total yield loss of maize [1]. However, studies have revealed that genetic improvement in maize under stress conditions could result in genetic gains under drought [1,5,6] and under low-N [7,8,9]. Assessing the efficiency of induced stress screening of advanced hybrid maize under drought and low-N for grain yield in favourable environments in Southern Africa, [10] showed that, for early maturing genotypes, indirect selection under low-N and optimal conditions was more efficient than direct selection under random abiotic stress. A similar report was found for indirect selection under induced drought. However, direct selection was most efficient for predicting performance under low-N conditions.

Vitamin A, an essential micronutrient, is needed by human beings for improved vision, skin health and enhanced immune system, especially, in infants. However, vitamin A cannot be synthesised by human beings and its requirements must be met through external sources. [11] revealed that inadequate supply of vitamin-A causes weak immune system and increases the risk of child mortality resulting from communicable diseases and disorders. Unfortunately, the conventional yellow maize can only provide 0.5 to 1.5 µg g^{-1} PVA [12] which is extremely inadequate compared to the breeding target of 15 µg g^{-1} dry weight established by the HarvestPlus Challenge program [13] to prevent vitamin A deficiency (VAD) in diets dominated by maize. The PVA maize could provide more than 15 µgg^{-1} dry weight of PVA to combat VAD and its health-related problems including night blindness and depressed immune response [14]. Maize β-carotene was more effective when PVA maize was consumed as a staple food and could avoid the potential of hypervitaminosis A that could occur with the utilization of preformed vitamin A supplementation [15].

The conventional maize grain has a major shortfall as food or feed because the protein lacks adequate levels of tryptophan and lysine which are the two amino acids responsible for the quality differences between QPM and conventional maize. As a result, heavy reliance on conventional maize by infants with less protein supplement could predispose them to childhood disorders such as “kwashiorkor” (a fatal condition emanating from initial growth failure). Conversely, quality protein maize (QPM) has the potential to provide more than double the contents of tryptophan and lysine to prevent protein energy malnutrition. The QPM is capable of supplying about 73% of the protein required by humans as compared with about 46% obtainable in conventional maize [16]. [17] found that the protein of QPM contains about 80% biological value (a measure of protein absorption in human beings) relative to 40-57% of conventional maize. Consumption of QPM resulted in a 12% increase in the rate of growth in weight and a 9% increase in the rate of growth in height of a mild to moderately malnourished infant population [18].

In view of the greater potential of maize for drought and low-N tolerance as well as accumulation of elevated levels of PVA, tryptophan and lysine in its endosperm, there is the need to develop maize hybrids that combine tolerance to drought and low-N with increased contents of PVA, tryptophan and lysine to ensure food and nutrient security in WCA. In response to this need, the IITA-maize improvement program has between 2007 and 2015, developed inbred lines with varying reactions to drought and low-N stresses and also biofortified for PVA and tryptophan. This was necessary because successful performance of maize hybrids both under stress and favourable growing conditions largely depends on the genetic potentials of their parental lines especially when the inbred lines combine superior performance for important traits that are highly heritable [19]. However, in the course of the inbreeding program, the inbred lines were screened based on visual observation to select for kernels with deep orange colour for the PVA trait, and the endosperms with opaqueness ranging from 25 – 50% for the QPM trait. Also, stay-green characteristic (STGR), anthesis-silking interval (ASI), plant and ear aspects (PASP and EASP) and number of ears per plant (EPP) have strong associations with grain yield (GY) especially, under stressful environments. Therefore, these traits have been successfully employed in a multiple-trait base index (MI) to select superior maize genotypes [1, 20]. It was, therefore, important to evaluate the recently developed early maturing PVA-QPM inbred lines for superior performance under stressful (drought and low-N) and non-stressful environments and also investigate the levels of PVA and tryptophan using chemical analysis to establish the basis for developing drought and low-N tolerant PVA-QPM hybrids and synthetics. This study aimed at (i) identifying inbred lines that combined drought and low-N tolerance with increased contents of PVA and tryptophan, (ii) assess the relationship among PVA, tryptophan and grain yield of the inbred lines under favourable growing conditions, (iii) determine the inbred-hybrid relationships under the contrasting environments and (iv) examine grain yield performance of hybrids derived from selected outstanding inbred lines in hybrid combinations.

Results

The ANOVA under two managed drought conditions revealed significant (P< 0.01) variation among environments (E) and inbreds (G) except ASI, PASP, EASP and EPP for environments. However, inbred × environment interactions (GEI) were significant (P< 0.05) for only GY and ASI (Table 1). Repeatability (R) estimates based on plot means obtained for GY was 58% under drought. Across the three low-N environments, the combined ANOVA showed significant (P < 0.01) variations among E, G and GEI mean squares for the traits measured except GEI mean squares for ear height (EHT) (Table 1). Estimates of R ranged from 18% for PASP to 63% for DA (days to 50% anthesis) with GY recording 42%. Under optimal environments, the ANOVA showed significant (P< 0.05) differences among E, G and GEI mean squares for all traits measured and the few exceptions were GEI mean squares for DA, plant height (PLHT) and EASP (Table 2). Estimates of Rranged from 52% for EPP to 87% for DA. High Restimate of 58% was recorded for GY. Across the eight test environments, the observed differences among E, G, research condition and GEI mean squares for the traits measured were statistically significant (P < 0.01) except GEI mean squares for DA (Table 2). Although the interactions between mean squares of G and research condition was not significant for the major response variable (GY), significant variations were detected for other important traits, notably, ASI, EASP and STGR. Across environments, ASI recorded the lowest R estimate (18%) while the highest (83%) was observed for PLHT. A relatively high R^2 estimate of 56% was obtained for GY.

Grain yield of the inbreds under drought conditions varied from 100 kg ha^{-1} for TZEIORQ 41 to 895 kg ha^{-1} for TZEIORQ 8 with a mean of 321 kg ha^{-1} (Table 3). Comparison of the GY under drought to that under non-stress conditions revealed high variation in yield reductions of 25% for TZEIORQ 8 to 94% for TZEIORQ 11 with a mean of 76%. Averages of ASI and STGR under drought were 4.27 and 4.51 respectively. Under low-N environments, GY of the inbreds varied from 299 kg ha^{-1} for TZEQI 85 to 1652 kg ha^{-1} for TZEIORQ 12 with a mean of 980 kg ha^{-1} (Table 3). Grain yield reduction due to the effects of the low-N ranged from 8% for TZEIORQ 48 to 82% for TZEQI 85 with a mean of 35%. The inbreds recorded greater yield reductions under drought than under low-N conditions. Averages of ASI and STGR under low-N were 1.07 and 3.49 respectively. The combined data across test environments showed a wide variation in GY from 394 kg ha^{-1} for TZEQI 74 to 1307 kg ha^{-1} for TZEIORQ 27 with an average of 942 kg ha^{-1}. Grain yield reduction across drought and low-N environments ranged from 18% for TZEIORQ 8 to 87% for TZEQI 85 with a mean of 56%. Generally, from the performance across stress environments, the inbred lines that recorded increased ASI, higher STGR and high percentage GY reduction also had lower GY and negative selection indices (Table 3).

Under drought, 46% (32 out of 70) of the inbred lines recorded positive base indices indicating drought tolerance, 53% (37) of the inbreds assessed under low-N conditions had positive base indices, indicating low-N tolerance, 16 inbreds showed tolerance to drought and low-N simultaneously while 33 inbreds showed tolerance across stress environments using the multiple trait base index with performance under optimal conditions serving as a check (Additional file 1).

Most of the measured traits exhibited significant (P < 0.05) genetic correlations with GY (Additional file 2). Grain yield recorded highly significant (P < 0.01) negative correlations with PASP and EASP under each and across environments. However, GY had highly significant (P< 0.01) and negative correlation coefficients with ASI, and STGR under drought but not under low-N conditions. Under drought, ASI exhibited highly significant positive genetic correlations with PASP (0.98), EASP (0.98) and STGR (0.86) but displayed highly significant negative correlations with ears per plant (-0.98) as well as plant height (-0.98). Ear aspect consistently displayed significant (P< 0.05) positive correlations with the flowering traits including DA, days to 50% silking (DS) and ASI under each and across environments.

The ANOVA for carotenoids and tryptophan revealed significant (P < 0.01) variation among the early PVA-QPM inbreds, except for α-carotene (Additional file 3). However, no significant differences were detected among the lines for kernel colour. The effects of replications were not significant for most carotenoids except for β-cryptoxanthin and β-carotene. Estimated PVA contents of inbred lines varied from 3.47 μg g^{-1} for TZEIORQ 48 to 15.38 μg g^{-1} for TZEIORQ 55 with a mean of 6.47μg g^{-1}(Table 4). The levels of the three PVA carotenoids ranged from 1.15 to 6.61 μg g^{-1} with an average of 2.88μg g^{-1} for β-cryptoxanthin, from 0.45 to 2.10μg g^{-1} with an average of 0.99μg g^{-1} for α-carotene, and from 2.18 to 11.23 μg g^{-1} with an average of 4.52μg g^{-1} for β-carotene. Relative to the other carotenoid contents, the α-carotene levels of the selected inbred lines were very low. Kernel colour scores of the inbred lines varied from 7.0 (moderately deep yellow) to 10.0 (dark orange) with a mean of 7.84. Ninety-five percent of the inbred lines had > 0.075% tryptophan per sample in whole grain. “Obatanpa”, the standard QPM check, recorded the highest level of tryptophan and was 37% higher than the second best inbred, TZEIORQ 42. Ten out of the 19 selected inbred lines assayed were tolerant to drought, while 11 including TZEIORQ 29 and TZEIORQ 55 which recorded PVA contents of 12.10 and 15.38μg g^{-1} were tolerant to low-N environments. Also, ten inbred lines displayed combined drought and low-N tolerance out of the 19 inbred lines across drought, low-N and optimal environments (Table 4).

Highly significant (P < 0.01) positive phenotypic correlations were observed among PVA and its component carotenoids including β-cryptoxanthin (r = 0.75), α-carotene (r = 0.67) and β-carotene (r = 0.93) (Table 5). Significant (P < 0.05) and positive correlations were also observed between PVA and total carotenoids (r = 0.73) as well as kernel colour (r = 0.52), but the correlations between PVA and the non-PVA carotenoids, lutein and zeaxanthin, were not significant. The correlations among grain yield and the nutritional traits were not significant for the selected inbred lines except for β-cryptoxanthin and zeaxanthin. The highest significant (P < 0.01) correlation coefficient was found between PVA and β-carotene (r = 0.93), followed by the correlation between β-cryptoxanthin and α-carotene (r = 0.77).

The 100 hybrids including four commercial hybrid checks evaluated showed varying degrees of drought and low-N tolerance. The 20 best performing hybrids including two commercial hybrid checks (Table 6) were identified using the multiple trait base index (MI). Although the best commercial hybrid check, TZEI 124 x TZEI 25 was outstanding under optimal conditions it suffered significant grain yield penalties under drought and low-N conditions. The best hybrid check was therefore ranked 11th according to the MI across the two stress environments. On the other hand, as many as seven of the top performing hybrids including TZEIORQ 40 x TZEIORQ 26, TZEIORQ 26 x TZEIORQ 47, TZEIORQ 42 x TZEIORQ 20, TZEIORQ 20 x TZEIORQ 45, TZEIORQ 29 x TZEIORQ 43, TZEIORQ 48 x TZEIORQ 43 and TZEIORQ 29 x TZEIORQ 24 significantly outperformed the best commercial check, TZEI 124 x TZEI 25 across drought and low-N conditions. Moreover, the yields of these top performing hybrids were statistically equal to the best check and also recorded relatively lower percentage grain yield reductions compared to the best check.

The correlation between mid-parent values of inbred lines and the means of measured traits of the hybrids were significant for grain yield, plant and ear heights, plant and ear aspects, ears per plant and stay green characteristic under drought (Table 7). Under low-N and across environments, the inbred-hybrid relationship was significant for grain yield, days to 50% anthesis and silking, plant and ear heights, plant and ear aspects and stay green characteristic. Also, under optimal environments, mid-parent values of inbred lines had significant correlations with means of measured traits of the hybrids for grain yield, days to 50% anthesis and silking, plant height, and plant aspect. Furthermore, grain yield recorded significant positive average heterosis of 513, 237, 235 and 315% under drought, low-N, optimal and across environments respectively. Similarly, plant and ear heights had highly significant positive heterosis under each and across research environments. Significant negative heterosis was observed for days to 50% anthesis and silking under drought, low-N, optimal and across environments, while anthesis-silking interval recorded significant negative heterosis under drought. Highly significant positive heterotic values were obtained for ear aspect and ears per plant under drought environments.

Discussion

The significant variation (P< 0.01) observed among G, E, and GEI for grain yield and most of the traits measured under each and across test environments implied the existence of genetic variability in the early maturing PVA-QPM inbred lines. The significance of E and GEI observed for GY and several other traits under each and across environments suggested the inconsistent rankings of the traits measured in varying environments and that inbred evaluations in more environments was necessary to identify outstanding genotypes as reported in other studies under drought [1,6,16] and under low-N [21].

The high R estimates recorded for PASP, EASP, EPP and STGR compared with the relatively moderate R estimate for GY under drought indicated that R estimates of GY under drought could be low and that it is important to use the yield related traits (with high R) to complement GY in the identification of drought tolerant inbred lines [1,22]. This result was supported by the highly significant negative genetic correlation coefficients observed between GY and PASP, EASP and STGR, and the highly significant and positive association recorded between GY and EPP. However, the very low R estimate (< 0.30) obtained for ASI among the inbred lines under drought contradicted the findings of earlier workers [22,23] who identified ASI as a reliable secondary trait for selecting drought tolerant maize cultivars. Nonetheless, a highly significant and negative genetic correlation was found between ASI and GY under the contrasting environments suggesting that R of GY and other adaptive traits and their genetic correlations were important components to guide breeding strategies in the improvement of the early PVA-QPM inbred lines.

The high repeatability estimates recorded for GY, DA, DS, PLHT and EHT under low-N indicated that early generation testing of the inbred lines using these traits under low-N conditions would be successful as reported by [7,8]. However, among these traits, DA was the only trait which recorded significant genetic correlation with GY, indicating that it was the most reliable trait that complemented GY under low-N conditions. Similarly, the strong genetic correlations of PASP and EASP with GY under low-N suggested the high probability of these yield related traits being reliable under low-N. This result agreed with the report of [24], who identified PASP and EASP as reliable traits for improving GY under low-N using a set of extra-early maize genotypes. The unreliability of EPP to complement GY under low-N corroborated the results of [25] who detected EPP as an unpredictable trait in selecting for improved GY in low soil N conditions. This result also revealed that STGR is more reliable than ASI for selection for GY in limited soil N conditions. The high R values observed for the six agronomic traits (except ASI) employed in the combined drought and low-N multiple-trait index and their strong genetic associations with GY across test environments confirmed the reliability of those traits in selecting inbred lines tolerant to limited water and nitrogen availability.

Under optimal environments, the very high repeatability estimates recorded for DA, DS, PLHT and EASP compared to that of GY underscored the need to select for these secondary traits in early generations to improve GY. The 32 out of the 70 inbred lines identified as drought tolerant based on the drought base index would serve as an important source of drought tolerant genes for developing superior drought tolerant hybrids [19], synthetics and for the improvement of the early PVA-QPM inbred lines to tolerate drought. Similarly, the 37 low-N tolerant inbred lines identified in this study would be invaluable for the exploitation of low-N tolerant genes to develop superior hybrids and synthetic varieties under limited nitrogen environments [9, 21]. Moreover, the 33 inbred lines (47% of 70) identified as drought and low-N tolerant based on the multiple-trait base index underscored the earlier finding that similar adaptive mechanisms were involved in the tolerance to the two stresses and that selection under drought could also indirectly result in improved low-N tolerance as reported by several authors [7, 21,24].

The PVA contents of the selected inbred lines assayed which ranged from 3.47 to 15.38μg g^{-1} with an average of 6.47μg g^{-1} indicated the existence of significant variability for the PVA carotenoids in the inbred lines used [26,27]. This range of PVA values exceeded the 5.00 to 7.80μg g^{-1} reported by [28] for 15 tropically adapted yellow maize inbred lines, but was comparable to the 0.06 to 17.25 μg g^{-1} with an average of 5.87μg g^{-1} reported by [29] for 130 intermediate/late maturing PVA inbred lines. However, only TZEIORQ 55 recorded a PVA value comparable to the 15μg g^{-1} which is the current breeding target set by HarvestPlus Challenge Program [13,30,31]. Although these results have signalled the potential of meeting the set target using this inbred set, there is the need to introgress the best favourable alleles of PVA from other germplasm sources into the tropically adapted inbred lines to speed-up the development of high PVA hybrids adapted to limited moisture and nitrogen conditions. The highest estimated mean of total carotenoids was 60.22μg g^{-1} which was higher than the 42.71μg g^{-1} obtained by [29] but was far below the 100μg g^{-1} reported by [32]. The significance of high total carotenoids is that inbred lines harbouring higher contents of total carotenoids could serve as invaluable sources of the PVA carotenoids especially if the influx of assimilates to the carotenoid biosynthetic pathway favours the build-up of the PVA in the endosperm. The results of the present study revealed relatively higher levels of lutein and zeaxanthin (synthesized from the PVA carotenoids) at the expense of the PVA carotenoids for most of the inbred lines. This result corroborated the findings of [33] who identified lutein and zeaxanthin as the most predominant carotenoids in the maize endosperm. These results however, contradicted the findings of [34] who found many genotypes having high PVA contents (ranging from 15 to 20 μg g^{-1}) comparable to the non-PVA carotenoids when improved PVA inbred lines and populations were assayed.

Ninety percent of the inbred lines had tryptophan contents > 0.075 % per sample in whole grain and thus, the lines met the quality standards of a QPM genotype as reported by [35, 36]. However, new sources of PVA genes would be necessary to improve the existing early maturing PVA-QPM inbred lines to speed-up the process of developing high PVA maize hybrids for commercialization in SSA to combat "hidden hunger" due to VAD. The two inbred lines, TZEIORQ 55 and TZEIORQ 29 identified as possessing high levels of PVA (15.38 and 12.10µg g^{-1}, respectively) and low-N tolerance could be invaluable sources of PVA genes for the improvement of the early PVA-QPM source population and for the development of PVA-QPM inbred lines, hybrids and synthetics with tolerance to the two stresses. Furthermore, multiple stress tolerant extra-early maize inbred lines with PVA levels higher than the target of 15 ug/g established by the HarvestPlus Challenge Program have been identified in IITA [37]. The extra-early (80-85 days to physiological maturity) inbred lines, TZEEIOR 202 and TZEEIOR 205 have 22.58 ug g^{-1} and 23.98 ug g^{-1}, respectively. These normal endosperm PVA inbred lines could be invaluable sources of high PVA genes for developing high PVA-QPM early maturing inbred lines through introgression of the favourable PVA alleles into tropical early maturing PVA-QPM source populations and extracted inbred lines. The lack of significant correlations among GY, PVA and tryptophan suggested that the individual traits could be readily improved simultaneously. A different result was earlier reported by [14] who found higher levels of tryptophan to be associated with PVA levels in the PVA-QPM varieties evaluated. Moreover, the non-significant correlations observed between PVA and lutein as well as zeaxanthin indicated that the contents of the PVA carotenoids could be improved without significant negative association with the synthesis of lutein and zeaxanthin which are by-products in the PVA biosynthetic pathway. Furthermore, the weak positive and significant correlations observed between kernel colour and PVA, and β-carotene suggested that to some extent, the intensity of the orange colour of kernels could be a quick (but not the most reliable) approach to identifying inbred lines with high PVA levels. In contrast, [29] reported non-significant correlations between kernel colour and PVA as well as β-carotene contents in the set of intermediate and late inbred lines studied. The differences in the results of the two studies may be attributed to the differences in the germplasm used in the two different studies.

An important objective of the present study was to compare the grain yield performance of the two most outstanding hybrid checks, TZEI 124 x TZEI 25 and TZEIOR 127 x TZEIOR 57 with the performance of the PVA-QPM hybrids derived from the outstanding drought and low N tolerant inbred lines identified in the present study. This was important for the assessment of the level of heterosis among the early maturing PVA-QPM inbred lines since they were all extracted from the same QPM-PVA variety. It was striking that seven of the top performing hybrids, TZEIORQ 40 x TZEIORQ 26, TZEIORQ 26 x TZEIORQ 47, TZEIORQ 42 x TZEIORQ 20, TZEIORQ 20 x TZEIORQ 45, TZEIORQ 29 x TZEIORQ 43, TZEIORQ 48 x TZEIORQ 43 and TZEIORQ 29 x TZEIORQ 24 significantly out-yielded the most outstanding commercial check, TZEI 124 x TZEI 25 across drought and low-N conditions. It was worth noting that the six inbred lines TZEIORQ 40, TZEIORQ 26, TZEIORQ 20, TZEIORQ 29, TZEIORQ 48 and TZEIORQ 24 identified among the best performing inbred lines were involved in the best hybrid combinations. This observation demonstrated that the outstanding performance of the hybrids under varying environments was attributable largely to the superior performance of the PVA-QPM inbred lines for important agronomic traits [19]. The most outstanding commercial hybrid check, TZEI 124 x TZEI 25, was released and commercialized in Mali in 2014 as Tamalaka, in Nigeria in 2014 as Sammaz 41 and in Ghana in 2015 as CSIR- Denbea [16]. This result implied that, the seven best hybrids would be the hybrids of choice under drought prone and nitrogen deficient environments in West and Central Africa.

Assessment of the relationship between hybrids and their parental lines for grain yield and other important agronomic traits under drought, low-N and optimal environments was necessary to ascertain the possible improvement in those traits of the hybrids under each and across environments based on the performance of parents. The significant correlations recorded between mid-parent values and the means of the corresponding hybrids for grain yield, plant height, ear aspect and stay-green characteristic under drought, low-N, optimal and across environments suggested that initial screening and selection of drought and low-N tolerant inbred lines for the development of superior hybrids would be effective. The significant but weak correlation observed between mid-parent values and means of hybrids for grain yield under low-N and optimal environments indicated the need for using secondary traits to support grain yield in predicting hybrid performance based on their parents under low-N and optimal conditions. This result corroborated the findings of [21]. However, the result is not entirely consistent with the report by [19] who observed significant and strong correlation between mid-parent and hybrid means under low-N but a weak correlation under optimal conditions. On the other hand, the non-significant correlations between parental lines and their hybrids for ears per plant under low-N and optimal conditions suggested that predicting hybrid performance based on prolificacy may not be effective under low-N and optimal environments. Similar result was reported by [38] under optimal environments. The inconsistencies in the results of the different studies could be attributed to the different parental inbred lines used as well as the levels of inbreeding [21]. The highly significant positive heterosis observed for grain yield, plant height and ear height under drought, low-N, optimal and across test environments indicated that there was high potential for the exploitation of superior hybrid performance to increase grain yield under each and across test environments. This result, coupled with the significant negative heterosis observed for days to 50% anthesis and silking under each and across environments implied that the hybrids matured faster than their parents, produced taller and more vigorous plants which translated into high grain yield. Similar results were reported by [39] under optimal conditions and [21] under low-N conditions.

Conclusions

Thirty-three of the inbred lines assessed were found to be tolerant to drought and low-N. Ninety percent of the inbred lines had tryptophan contents > 0.075 % per sample in whole grain indicating that most of the lines possessed the quality protein trait. However, low to moderate levels of PVA were recorded for most of the inbred lines including those tolerant to the two stresses. This result suggested the need to introgress favourable PVA alleles from the recently identified extra-early inbred lines, TZEEIOR 202 and TZEEIOR 205 (with PVA levels of 22.58 and 23.98 ug g^{-1}, respectively) into the early PVA-QPM inbred lines. These two inbred lines are also multiple stress tolerant (combined tolerance to drought and low N and resistant to *Striga*) and therefore their exploitation could accelerate progress in developing high PVA-QPM hybrids adapted to the stressful drought and low-N conditions in WCA. Furthermore, inbred lines TZEIORQ 55 and TZEIORQ 29 combined high PVA contents (15.38 and 12.10µg g^{-1} respectively) with low-N tolerance while nine inbred lines combined moderate PVA levels (5.06 – 8.34µg g^{-1}) with drought and low-N tolerance. These inbred lines could also be important sources of PVA beneficial alleles for improvement of the early PVA-QPM inbred lines from which drought and low-N tolerant PVA-QPM hybrids and synthetics could be developed. The lack of significant correlations observed among grain yield, PVA and tryptophan suggested that these traits could be readily improved independently without significant trade-offs. The superior performance of the identified hybrids, the significant correlation between mid-parent values and their hybrids, as well as the significant average

heterosis observed for grain yield and other important agronomic traits under each and across stress environments suggested the existence of adequate genetic variability within the early maturing PVA-QPM inbred lines. Thus, the inbred lines could be exploited for the development of superior early maturing PVA-QPM drought and low-N tolerant hybrids and synthetics.

Methods

Genetic materials

The inbred lines were extracted from the early maturing PVA-QPM variety, 2009 TZE - OR2 DT STR QPM. The background of this variety is highly diverse with its development commencing in 2007 by crossing a drought and *Striga* resistant early QPM variety, TZE-Y-Pop-DT-STR-QPM with an intermediate maturing (105-110 days to maturity) high PVA maize [Syn-KU1409/DES/1409-(OR2)] from the IITA-MIP to introgress genes for high β-carotene into the QPM variety. This was followed by one cycle of backcrossing to the recurrent parent for recovery of earliness. In 2008, the BC_1F_1 lines with deep orange colour (for PVA) and/or appropriate endosperm modifications were selected and advanced to the F_2 and the F_3 generations. In 2009, the F_3 lines were selected based on their reactions to *Striga*, drought and low-N, and recombined to constitute the early PVA-QPM variety, 2009 TZE-OR2-DT-STR-QPM. From 2011 to 2014, another program was initiated to extract the first generation of early maturing inbred lines from the PVA-QPM variety and the S_1 lines were advanced using the pedigree method to S_6 generation. In 2015, a set of 73 early maturing PVA-QPM inbreds were selected based on the orange colour and opaqueness of the kernels [16]. Sixty-four of these inbred lines plus six inbred checks were evaluated in this study. Four out of the six checks, TZEQI 85, TZEQI 91, TZEQI 74 and TZEQI 82 are early maturing, yellow, QPM inbred lines, while the remaining two, TZEI 129 and TZEI 24 are early maturing, normal endosperm yellow inbred lines. These checks were used because early maturing PVA QPM inbred checks were not available. Furthermore, 24 outstanding inbred lines identified on the basis of their reactions to drought and low-N stresses (after one-year inbred evaluations) were grouped into six sets with each set containing four inbreds and used to generate 96 early maturing PVA-QPM single- cross hybrids at IITA, Ibadan, Nigeria. Each inbred line served as a female parent in one set and a male parent in another according to the North Carolina Design 2 mating scheme [40]. The PVA-QPM single-cross hybrids plus 4 commercial hybrid checks were evaluated across drought, low-N and optimal environments in Nigeria for two years.

Field trials

Evaluations of the 70 inbred-lines were carried out under managed drought, low-N and optimal conditions in 2016 and 2017 while the hybrid evaluations were conducted in 2017 and 2018 in Nigeria. The managed drought experiments were conducted at Ikenne (6^o 50'N, 30^o 45'E, 62 m altitude, 1200 mm mean rainfall annually) in the 2016/2017 and 2017/2018 dry seasons. Drought stress was achieved by supplying 17 mm of sprinkler irrigation water in a week up to 25 days after planting (DAP) after which the irrigation was terminated and the maize plants depended on the available soil moisture to reach physiological maturity. The managed drought trials received NPK fertilizer at the rate of 60 kg ha^{-1} each of N, P and K (15-15-15) during planting. Additionally, top dressing was done with 60 kg ha^{-1} of N (supplied as urea) at 3 weeks-after-planting (WAP).

Evaluations of the inbred-lines under low-N (30-kg ha^{-1}) conditions were carried out at Ile-Ife-(7 30' N, 5 31' E, and 240 m altitude, 1250 mm mean rainfall annually) and Mokwa-(10^{o}20'N, 5^o 6'E, 459 m above sea level, 1050 mm mean rainfall annually) in the 2016 and 2017 major growing seasons. The hybrids were also evaluated in an adjacent field in 2017 and 2018 major seasons. According to [41], the Mokwa topsoil is a luvisol while that of Ile-Ife is Alfisol. Low soil N conditions at both locations were accomplished by depleting the fields of N through continuous cultivation of densely populated maize without fertilizer application for 3 cropping seasons and complete removal of all crop residues at the end of every harvest. Prior to field preparation, topsoil samples were collected at the depth of 0 – 15 cm for analysis of the contents of nitrogen (N), phosphorus (P) and potassium (K) using the Kjeldahl digestion and colorimetric procedure [42] at the IITA analytical services laboratory, Ibadan, Nigeria. The low-N experimental field at Mokwa had 0.085 % (N), 6.32 ppm (P) and 0.20 cmol kg^{-1} (K), whereas that of Ile-Ife contained 0.084 % (N,) 2.05 ppm (P) and 0.358 cmol kg^{-1} (K). Based on the soil tests, NPK fertilizer was formulated using urea (N source), triple superphosphate (P_2O_5 source) and muriate of potash (K_2O source), respectively, and it was applied immediately after thinning (2 WAP). The urea provided a basal available N of 15 kg ha^{-1}, P_2O_5 and K_2O fertilizers supplied 60 kg ha^{-1} each of P and K. Additionally, top dressing of 15 kg ha^{-1} of N (supplied as urea) was done at 4 WAP to bring the total available N received on the low-N fields to 30 kg ha^{-1}.

The inbred trials under favourable growing conditions were planted at Mokwa, Ikenne and Ile-Ife in the 2016 and 2017 rainy seasons, while the hybrids were planted in the 2017 and 2018 major seasons. The optimal trials received NPK (15:15:15) fertilizer at 60 kg ha^{-1} each for N, P and K at 2 WAP immediately after thinning. Top dressing was done at 4 WAP with additional 30 kg N ha^{-1}. In all the experiments, 7 × 10 and 10 × 10 randomized incomplete-block designs were used for the inbreds and the hybrids respectively, with two replications in each case. The experimental units consisted of 3m long one-row plots with a spacing of 0.75 x 0.40m. Three seeds were sown per hill and later, seedlings were thinned to two at 2 WAP to give a population density of about 66,667 plants ha^{-1}. Weeds were managed in all trials by the application of pre- and post- emergence herbicides, atrazine (primextra) and gramozone (paraquat) respectively, each at the rate of 5 litres ha^{-1}.

Data collection

Based on individual plots for inbred lines and hybrids, data were taken for DA and DS as well as PLHT and EHT. PASP and EASP were rated on a scale of 1-9 (1= excellent plants or ears and 9= extremely poor plants or ears). The difference between DA and DS was calculated as ASI. EPP was obtained as the ratio of the number of harvested ears in a plot to the total number of plants in that plot. At 70 DAP, visual ratings for STGR were carried out for the trials under managed drought and low-N using a scale of 1-9 (1= less than 10 % dead leaf area and 9= more than 80 % dead leaf area). The harvested ears from each plot under the two stress conditions were shelled and grain weight was measured. Grain moisture content was determined using Kett moisture tester PM-450Grain

weight was adjusted to 15% moisture content, and GY in kg ha^{-1} was computed on plot basis. For the optimal trials on the other hand, an assumption of 80% shelling percentage was considered per plot to compute GY from ear weight adjusted to 15% moisture content.

Production of inbred kernel samples for carotenoid and tryptophan analyses

Nineteen inbred lines with good agronomic performance were selected and planted under well-watered growing conditions in January 2018 at IITA Ibadan, to produce fresh kernel samples for carotenoid and tryptophan analyses. The inbred lines were planted in 1m long single row plots with a spacing of 0.75 x 0.20 m. Two seeds were sown per hill and subsequently thinned to one to provide at least 5 plants per inbred line. Kernel samples for analyses of maize carotenoids were produced by selfing all plants in a plot. Harvested ears of the self-pollinated inbred lines in each plot were well-dried at room temperature (12% moisture content) and shelled separately (individual ears from an inbred line). Equal number of kernels were taken from each ear to form a bulk representative sample per inbred line. One hundred seeds were drawn for carotenoid and tryptophan analyses in the Food and Nutritional Laboratory of IITA, Ibadan. In addition, kernel colour was scored following the standard colour scale for determining total carotenoid content [43], and this was converted to a scale of 1 to 12 representing shades of colours from pale yellow to darkest orange as observed within the early maturing PVA-QPM inbred line set evaluated.

Quantification of PVA carotenoids and tryptophan

Carotenoids were extracted and quantified by High Performance Liquid Chromatography at the Food and Nutritional Laboratory of IITA, Ibadan, Nigeria. The protocol for extraction and the method of carotenoid analysis employed were based on the procedure described by [44]. Total carotenoids were computed as the total of α-carotene, β-carotene, β-cryptoxanthin, zeaxanthin and lutein concentrations. The PVA was derived by adding the value of β-carotene, and 50% each of the concentrations of β-cryptoxanthin and α-carotene, since β-cryptoxanthin and α-carotene contribute half each of the amount of β-carotene as PVA [45]. Values of PVA and other carotenoid levels of each sample were obtained from two independent measurements to allow for statistical analysis.

The selected early maturing PVA-QPM inbred lines plus an intermediate maturing open-pollinated QPM variety standard check "Obatanpa" were analysed for only tryptophan content but not lysine or both in endosperm flour. This is because lysine content of the maize endosperm is highly correlated with that of tryptophan (greater than 0.9) [46,47]. Moreover, analysis for tryptophan is far cheaper than lysine and so it is economically prudent for the breeder to use tryptophan content to determine the nutritional potential of QPM genotypes at early breeding stages. Estimated levels of tryptophan were obtained by the colorimetric technique as described by [48]. Levels of tryptophan were quantified with the aid of a standard curve of a well-known check. Tryptophan content for each sample was obtained from two independent measurements to allow for data analysis.

Data analysis

Agronomic data recorded for the inbreds and hybrids under each environment were subjected to analysis of variance (ANOVA) separately with PROC GLM (the general linear model procedure) in SAS including a random statement and a test option [49]. A combined ANOVA was later performed across all environments. Location by year combination was considered as an environment whereas the managed drought, low-N and favourable growing environments represented research conditions. Environments, replications within environments and also incomplete blocks within replications × environment interactions were treated as random factors and the inbreds and hybrids were regarded as a fixed factor. The model used to reflect the layout of the experiments was as follows:

$$y_{klmi} = \mu_i + E_{ki} + R(E)_{klji} + G_{mi} + GE_{kmi} + \epsilon_{kljmi}$$

Where Y_{klmi} is the measurement of trait i with mean effect μi, E_{ki} is the environmental effect k on trait i, $RB(E)_{klji}$ represents the effect of replicates l and block j within environment k on trait i, G_{mi} is the genotypic effect m on trait i, GE_{kmi} is the genotype m × environment k interaction effect on trait i, and ϵ_{kljmi} represents the effect of experimental error due to genotype m, replicates l and block j within environment k on trait i. Significant differences among means were assessed using standard error of a difference. Genetic and phenotypic variance components of the inbred lines were estimated under each and across research conditions with the restricted maximum likelihood (REML) method using PROC Varcomp in SAS to compute the repeatability (R) for each of the traits measured using the formula;

$$R = \sigma g 2 \sigma g 2 + \sigma g e 2 e + \sigma 2 r e$$

where $\sigma g 2$ is the additive genetic variance (since the genetic materials are inbred lines) [50], $\sigma g e 2$ is the variance of genotype × environment interaction, $\sigma 2$ is the experimental error variance, e represents the environments while r is the replications per environment. Furthermore, the relationship between grain yield and the other agronomic traits measured under each and across environments were examined by estimating the genetic correlation coefficients for each pair of traits using the meta menus program in SAS [49]. Drought and low-N tolerant inbred lines and hybrids were identified using the MI proposed by [1]. Grain yield (GY), number of ears per plant (EPP), ASI, plant aspect (PASP), ear aspect (EASP) and stay-green characteristic (STGR) were employed to compute the MI as follows;

$$M.I = [2 x GY + EPP - ASI - PASP - EASP - STGR$$

To reduce the effects of differences in scales/units of measurements, each of the parameters was standardized using a standard deviation of 1 and a mean of zero. Positive MI values indicated drought or low N tolerance while negative values implied susceptibility. Also, the MI was applied to data across drought and low-N environments to assess the combined reactions of the inbred lines to the two stresses. In addition, data for PVA carotenoids and tryptophan of the inbred lines were subjected to ANOVA in SAS and means were separated using standard error (SE). Phenotypic correlations between PVA and other carotenoids, kernel colour, tryptophan, and grain yield were estimated to assess the relationships among the traits. Phenotypic correlation coefficients were computed using the Spearman rank correlation method in SAS.

Furthermore, for the agronomic traits, mid-parent values for a cross was computed as the mean of the two parental lines averaged for each research conditions, and across all research conditions. The relationship between per se performance of the parental lines and the grain yield of their respective hybrids under each and across research conditions was estimated using Spearman's rank correlation analysis implemented in SAS. Thus, the correlation coefficient of traits of parental lines with grain yield of their hybrids were calculated using mean grain yields of hybrids and the corresponding mid-parent values. Also, the percent increase or decrease of F_1 hybrids over mid parent values was calculated to estimate possible heterotic effects for the measured traits [51]. Values for mid-parent heterosis (MPH) for a cross were computed for each trait as follows:

$$M P H = F 1 - M P M P \times 100$$

Where;

F_1 = Mean of the hybrid,

MP = the mean of the parents that constituted the hybrids.

Mid-parent heterosis were averaged separately under drought, low-N, optimal and across all the twelve test environments. T-test was performed to determine whether F_1 hybrid means were statistically different from mid parent means as follows [52].

$$t i j = F 1 i j - M P 3 8 E M S$$

Where;

F_1ij = The mean of the ij^{th} F_1 cross

MPij = The mid parent for the ij^{th} cross

EMS = Error mean square

List Of Abbreviations

ANOVA, analysis of variance; ASI, anthesis-silking interval; DA, days to 50% anthesis; DAP, days after planting; DS, days to 50% silking; E, environment; EASP, ear aspect; EPP, number of ears per plant; G, inbred; GEI, inbred x environment interaction; GY, grain yield; IITA, International Institute of Tropical Agriculture; MI, multiple-trait base index; PASP, plant aspect; PVA, Provitamin A; QPM, quality protein maize; SE, standard error; VAD, vitamin A deficiency; WAP, Weeks after planting; WCA, West and Central Africa.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Authors' contributions

EO and BB conceived, designed and executed the experiment as well as drafted the manuscript; EO, AT and NT analysed the data, executed the experiment and drafted the manuscript; EI, AD, EB and MD critically reviewed the manuscript. All contributing authors agreed to the final version of the manuscript.

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Tables

Table 1. Mean squares of early- maturing PVA-QPM inbreds under contrasting environments at Ikenne, Ile-Ife and Mokwa, 2016 - 2018.

Source	DF	GY	DA	DS	ASI	PLHT	EHT	PASP	EASP	EPP	STGR
Induced drought condition											
Env	1	480952.72**	278.01**	284.01**	0.03	19881.61**	9165.73**	2.41	0.01	0.07	38.63**
Rep(Env)	2	11038.47	2.8	3.21	11.51*	294.78*	66.58	0.46	2.35	0.03	5.16**
Block(Env*Rep)	36	65311.25**	17.35**	21.68**	3.93	330.43**	109.03**	1.92**	3.60**	0.06**	1.71**
Inbred	69	131623.07**	14.85**	21.2**	5.92**	475.15**	165.85**	1.86**	4.29**	0.08**	2.33**
Env*Inbred	69	55852.2*	3.9	7.15	4.96*	91.73	59.43	0.64	1.11	0.02	0.92
Error	102	34510.18	6.81	10.62	3.45	93	54.13	0.62	1.07	0.03	0.64
Repeatability(R)	**-**	**0.58**	**0.74**	**0.66**	**0.16**	**0.81**	**0.64**	**0.66**	**0.74**	**0.75**	**0.61**
Low-N condition											
Env	2	33597047.68**	41.40**	318.44**	115.78**	6370.30**	474.82**	56.12**	24.99**	4.35**	170.53**
Rep(Env)	3	673976.31**	4.03	3.47	0.05	1170.16**	946.18**	3.13**	3.25	0.06	0.37
Block(Env*Rep)	54	302824.7**	2.15	2.54	0.96	277.44**	108.01*	1.61**	2.60**	0.04*	0.72*
Inbred	69	526260.25**	9.38**	13.03**	4.75**	749.38**	186.99**	1.28**	3.18**	0.11**	1.57**
Env*Inbred	138	307000.74**	3.49**	5.88**	3.01**	295.84**	80.45	1.05**	3.14**	0.08**	0.94**
Error	153	143160.7	1.85	2.45	0.83	110.53	70.74	0.62	1.36	0.03	0.50
Repeatability(R)	**-**	**0.42**	**0.63**	**0.55**	**0.37**	**0.61**	**0.57**	**0.18**	**0.20**	**0.27**	**0.40**

*, ** = Significant at 0.05 and 0.01 probability levels, respectively; Env = environment; Rep=replication; GY = Grain yield (kg ha-1); DA= days to 50% anthesis; DS = days to 50% silking; ASI = anthesis-silking interval; PLHT = plant height (cm); EHT = ear height (cm); PASP = plant aspect (1-9); EASP = ear aspect (1-9); EPP =ears per plant; STGR= stay-green characteristic (1-9).

Table 2. Mean squares of early- maturing PVA-QPM inbreds under optimal and across research conditions, 2016-2017.

Source	DF	GY	DA	DS	ASI	PLHT	EHT	PASP	EASP	EPP	DF
Optimal conditions											
Env	2	134313818.6**	101.29**	362.52**	81.52**	45577.87**	20840.08**	118.98**	173.25**	7.55**	-
Rep(Env)	3	21773.4	0.54	1.57	0.93	694.99**	420.69**	10.25**	9.62**	0.13**	-
Block(Env*Rep)	54	103579.7	5.55**	7.96**	1.00**	311.75**	118.27**	1.33*	1.31	0.05*	-
Inbred	69	768236.7**	14.95**	18.25**	1.43**	633.41**	220.14**	1.68**	2.53**	0.06**	-
Env*Inbred	138	490480.8**	2.18	3.05*	1.02**	141.65	85.31*	1.12*	1.22	0.04*	-
Error	153	73194.1	1.86	2.26	0.58	111.5689	60.91	0.83	1.03	0.03	-
Repeatability(R)	**-**	**0.58**	**0.85**	**0.83**	**0.29**	**0.63**	**0.61**	**0.33**	**0.52**	**0.33**	**-**
Across research conditions											
Env	7	82217326.1**	191.49**	1051.91**	404.54**	64747.46**	10398.83**	98.33**	221.51**	9.13**	4
Rcond	2	119609298.8**	405.11**	2800.80**	1228.83**	164727.14**	10498.15**	167.84**	577.05**	20.05**	1
Rep(Env)	8	263665.7**	2.16	2.96	3.26*	773.13**	529.22**	5.13**	5.41**	0.08**	5
Block(Env*Rep)	144	168729.5**	7.24**	9.33**	1.7	303.55**	112.11**	1.58**	2.37**	0.05**	90
Inbred	69	661182.5**	31.09**	37.33**	5.02**	1477.70**	432.19**	1.95**	4.30**	0.08**	69
Rcond*Inbred	138	399198.2	4.94	7.5	3.64**	209.15	72.7	1.46	2.85*	0.08	69
Env*Inbred	483	346564.9**	3.54	5.66**	2.96**	195.14**	75.56*	1.12**	2.24**	0.06**	276
Error	408	89760.6	3.17	4.37	1.45	106.54	62.9	0.7	1.16	0.03	255
Repeatability(R)	**-**	**0.48**	**0.89**	**0.85**	**0.41**	**0.87**	**0.83**	**0.43**	**0.48**	**0.25**	**-**

*, ** = Significant at 0.05 and 0.01 probability levels, respectively; Env = environment; Rep=replication; GY = Grain yield (kg ha-1); DA= days to 50% anthesis; DS = days to 50% silking; ASI = anthesis-silking interval; PLHT = plant height (cm);EHT = ear height (cm); PASP = plant aspect (1-9); EASP = ear aspect (1-9); EPP =ears per plant; STGR= stay-green characteristic (1-9).

Table 3. Performance of early PVA-QPM inbreds (best 15 and worst 10) including checks under contrasting environments.

	Grain Yield (kg ha-1)				Yield Reduction (%)			ASI			STGR		M.I Across
Inbred	Drought	Low-N	Optimal	Across Env	Drought	Low-N	Across Stress	Drought	Low-N	Optimal	Drought	Low-N	Stress
TZEIORQ 12	307.29	1651.55	1882	1280.34	83.67	12.25	39.99	3.71	0.02	0.59	4.44	3.48	9.90
TZEIORQ 48	321.02	1617.67	1773	1237.36	81.90	8.78	36.88	3.64	0.29	0.56	4.69	3.94	8.26
TZEIORQ 6	701.74	994.21	1180	958.51	40.51	15.71	28.11	3.75	-0.01	0.31	3.68	4.16	8.26
TZEIORQ 8	895.12	1051.06	1194	1046.57	25.00	11.94	18.47	2.65	0.96	1.03	3.25	4.93	8.01
TZEIORQ 37	462.79	1329.34	1803	1198.38	74.33	26.27	50.30	3.97	0.73	0.73	3.36	3.32	7.97
TZEIORQ 27	701.48	1320.87	1900	1307.35	63.07	30.47	46.77	3.91	0.27	0.40	4.89	3.08	7.50
TZEIORQ 5	518.62	1023.99	1194	912.10	56.55	14.22	35.38	1.69	0.17	0.39	3.56	4.73	7.03
TZEIORQ 9	523.25	979.67	1765	1089.33	70.36	44.50	57.43	3.22	1.19	0.64	4.10	4.26	6.20
TZEIORQ 28	567.56	1297.60	2075	1313.43	72.65	37.47	55.06	3.57	0.92	0.12	4.22	4.41	5.70
C6-TZEI 24	349.98	1437.46	1920	1235.92	81.77	25.14	53.46	6.60	0.55	0.33	4.88	2.86	5.17
TZEIORQ 17	270.38	1175.9	1351	932.35	79.98	12.95	46.46	3.30	0.52	0.24	4.61	3.34	4.79
TZEIORQ 40	227.28	1292.32	1457	992.10	84.40	11.28	47.84	3.23	0.66	0.7	4.70	3.78	4.69
TZEIORQ 26	342.42	1096.03	1310	916.11	73.86	16.33	45.09	3.09	0.06	0.37	3.70	4.75	4.65
C5-TZEI 129	336.09	1273.23	1843	1150.85	81.77	30.92	56.34	4.47	0.42	0.63	4.59	3.22	4.57
TZEIORQ 60	283.41	917.45	1149	783.23	75.33	20.14	47.74	4.19	1.01	1.23	2.94	2.82	3.47
TZEIORQ 65	418.76	1160.89	2034	1204.48	79.41	42.92	61.16	3.36	0.48	0.96	4.21	3.79	1.87
TZEIORQ 24	399.10	401.64	519	439.75	23.03	22.54	22.78	3.57	-0.26	0.15	3.87	4.77	1.55
TZEIORQ 29	297.27	857.81	1139	764.66	73.90	24.68	49.29	5.78	0.90	1.23	4.27	3.34	-0.20
TZEIORQ 59	147.54	769.54	2086	1001.16	92.93	63.12	78.02	4.25	0.55	1.50	4.56	2.90	-0.67
TZEIORQ 11	122.10	844.8	2189	1051.84	94.42	61.40	77.91	4.57	1.50	0.10	5.62	3.96	-1.74
TZEIORQ 20	364.47	571.58	1055	663.78	65.46	45.84	55.65	3.85	0.28	1.16	4.14	4.63	-3.59
TZEIORQ 13	191.53	1138.79	2171	1167.03	91.18	47.54	69.36	4.67	2.43	0.07	6.25	4.03	-3.85
TZEIORQ 55	272.98	1227.95	1427	976.11	80.88	13.97	47.42	5.42	0.01	1.46	4.78	4.23	-4.45
C4-TZEQI 82	109.31	853.60	1342	768.34	91.86	36.40	64.13	6.15	2.26	1.94	3.54	3.86	-5.21
TZEIORQ 2	112.67	606.95	1462	699.25	92.29	58.49	75.39	3.27	1.24	1.02	4.54	3.83	-5.52
TZEIORQ 52	119.43	599.08	1548	755.48	92.28	61.30	76.79	4.29	0.69	0.56	4.97	4.46	-7.26
C2 - TZEQI 91	144.89	986.91	1685	938.78	91.40	41.41	66.41	5.60	2.22	0.05	5.45	4.05	-7.49

TZEIORQ 46	106.55	458.35	1396	653.74	92.37	67.17	79.77	4.72	2.03	0.54	6.65	5.13	-10.89
TZEIORQ 41	100.00	842.94	1191	711.14	91.60	29.19	60.40	6.55	2.56	0.79	6.07	4.31	-11.82
C1-TZEQI 85	104.62	297.96	1610	670.77	93.50	81.49	87.50	6.14	3.34	1.56	5.15	3.83	-13.22
C3-TZEQI 74	123.98	298.79	760	394.18	83.68	60.67	72.18	4.05	5.21	1.21	4.26	4.00	-13.26
Mean	**321**	**980**	**1529**	**942**	**76.62**	**34.73**	**55.68**	**4.27**	**1.07**	**0.73**	**4.51**	**3.94**	
Sed	**105.21**	**175.23**	**125.**	**84.88**	**-**	**-**	**-**	**1.05**	**0.42**	**0.35**	**0.45**	**0.33**	

C1 to C6 = Checks 1 to 6 respectively; Env= environment; ASI=anthesis silking interval; STGR= stay green characteristics; M. I= multiple trait base index

Table 4. Carotenoid and tryptophan contents, and reactions of selected PVA-QPM inbreds to drought and low-N environments.

	Carotenoids§(µg g-1dry weight)								Reaction to drought and low-N			
Inbred lines	Lut	Zeax	β-cryp	α-caro	β-caro	PVA	Tcaro	Tryp (%)	Kern-col	Drought BI	Low-NBI	MI
TZEIORQ 55	19.19	21.49	6.61	1.71	11.23	15.38	60.22	0.096	8.0	-1.00	-7.61	-6.49
TZEIORQ 29	27.07	6.31	6.11	2.10	7.69	12.10	49.28	0.119	9.0	-2.02	1.57	-0.2
TZEIORQ 20	15.04	21.70	3.84	1.31	5.78	8.36	47.67	0.087	8.0	1.93	-5.52	-3.59
TZEIORQ 42	10.63	23.19	4.99	1.27	5.21	8.34	45.30	0.124	8.0	-2.06	4.50	2.20
TZEIORQ 13	3.68	10.11	1.40	0.54	6.72	7.70	22.46	0.120	7.0	-5.25	1.18	-3.85
TZEIORQ 24	7.89	20.86	3.71	1.16	4.17	6.60	37.79	0.104	10.0	8.44	-2.85	1.55
TZEIORQ 59	25.79	15.17	2.11	1.00	4.64	6.19	48.71	0.121	8.0	-1.31	-0.04	-0.67
TZEIORQ 40	13.30	16.97	1.53	0.44	4.62	5.61	36.86	0.065	9.0	4.05	2.78	4.69
TZEIORQ 7	10.43	19.33	3.36	1.11	3.37	5.61	37.61	0.101	8.0	11.25	2.35	7.79
TZEIORQ 6	10.30	19.07	3.18	0.94	3.44	5.50	36.93	0.118	7.0	12.46	2.47	8.26
TZEIORQ 26	9.62	18.04	2.88	0.89	3.40	5.28	34.82	0.102	7.0	5.51	1.90	4.65
TZEIORQ 5	9.78	18.06	2.76	0.89	3.35	5.18	34.85	0.110	8.0	12.01	0.59	7.03
TZEIORQ 43	16.20	18.01	1.79	0.45	3.99	5.11	40.44	0.113	8.0	-3.66	0.99	-1.60
TZEIORQ 45	18.77	16.27	1.46	0.99	3.88	5.10	41.36	0.106	8.0	-4.52	5.62	0.25
TZEIORQ 23	9.52	18.58	2.82	0.91	3.20	5.06	35.03	0.091	7.0	6.97	-3.02	1.52
TZEIORQ 44	14.34	16.53	1.68	0.50	3.80	4.89	36.85	0.089	8.0	-2.35	-1.80	-2.81
TZEIORQ 2	4.89	16.58	1.83	0.50	2.65	3.82	26.44	0.102	7.0	-2.93	-5.79	-5.52
TZEIORQ 47	17.75	13.65	1.15	1.00	2.48	3.55	36.03	0.056	7.0	0.34	-2.33	-2.14
TZEIORQ 48	8.84	17.23	1.56	1.00	2.18	3.47	30.83	0.115	7.0	1.70	9.03	8.26
†OBATANPA	-	-	-	-	-	-	-	0.198	-	-	-	-
Sed	2.19	2.44	0.54	0.43	0.27	0.44	3.29	0.008	1.12	-	-	-
Min.	3.68	6.31	1.15	0.45	2.18	3.47	22.46	0.056	7.0	-5.25	-7.61	-6.49
Max.	27.07	23.19	6.61	2.10	11.23	15.38	60.22	0.198	10.0	12.46	9.03	8.26
Mean	13.32	17.22	2.88	0.99	4.52	6.47	38.92	0.110	7.84	-	-	-

§Carotenoids are abbreviated as Lut= iutein; Zeax= zeaxanthin; β-cryp= β-cryptoxanthin; α-caro= alpha-carotene; β-caor= β-carotene; PVA= provitamin A; Tcaro= total carotenoids; Kern-col= kernel colour (scored on a scale of 1 to 12, 1= pale yellow, 12= darkest orange); Tryp= tryptophan; B.I= base index; MI= multiple trait base index, with positive value= Tolerance, and negative value= Susceptibility; †= QPM standard check.

Table 5. Phenotypic correlation co-efficients among traits of selected provitamin A quality protein maize inbred lines

Trait	PVA	β-cryp	α-caro	β -caro	Tcaro	Kern-col	Lut	Zeax	Tryp
β-cryp	0.75***								
α-caro	0.67**	0.77***							
β-caro	0.93***	0.58*	0.54*						
Tcar	0.73**	0.69**	0.74**	0.75***					
Kern-col	0.52*	0.37	0.32	0.55*	0.63**				
Lut	0.19	0.07	0.24	0.32	0.62**	0.43*			
Zeax	0.23	0.55*	0.28	0.05	0.25	0.11	-0.24		
Tryp	0.20	0.27	0.20	0.14	0.04	-0.02	-0.04	-0.07	
GY	-0.39	-0.73**	-0.41	-0.16	-0.32	-0.34	0.05	-0.64**	0.08

***, ** ,*= Significant at P< 0.001, 0.01 and 0.05 respectively; Carotenoids are abbreviated as PVA= provitamin A; β-cryp= β-cryptoxanthin; α-caro= alpha-carotene; β-caor= β-carotene; Tcaro= Total carotenoid; Kern-col= Kernel colour (scored on a scale of 1 to 12, 1= pale yellow, 12= darkest orange) Lut= Lutein; Zeax= Zeaxanthin; Tryp= Tryptophan; GY= grain yield across drought, low-N and optimal environments.

Table 6. Grain yield performance of early PVA-QPM hybrids under contrasting environments for two years in Nigeria.

Hybrid	Drought conditions	Low-N conditions	Across stress conditions	Optimal conditions	%YRD across stress	MI across stress
TZEIORQ 40 x TZEIORQ 26	4853	4155	4388	5641	22.21	8.09
TZEIORQ 26 x TZEIORQ 47	5085	4160	4468	5834	23.41	6.71
TZEIORQ 42 x TZEIORQ 20	3908	4584	4359	5286	17.55	6.67
TZEIORQ 24 x TZEIORQ 41	4499	3950	4134	5776	28.42	6.66
TZEIORQ 20 x TZEIORQ 45	5012	3961	4312	5396	20.09	6.57
TZEIORQ 29 x TZEIORQ 43	2866	5055	4474	5278	15.23	6.29
TZEIORQ 48 x TZEIORQ 43	3519	4694	4302	5322	19.17	6.05
TZEIORQ 43 x TZEIORQ 5	3886	4359	4201	5862	28.32	5.87
TZEIORQ 6 x TZEIORQ 29	4728	3322	3790	5042	24.82	5.45
TZEIORQ 29 x TZEIORQ 24	5500	3792	4362	5413	19.42	5.29
Check 2 - TZEI 124 x TZEI 25	3607	3690	3663	5895	37.87	5.19
TZEIORQ 26 x TZEIORQ 13	4160	4384	4309	5795	25.65	5.02
TZEIORQ 44 x TZEIORQ 29	3214	3920	3684	5691	35.25	4.72
TZEIORQ 43 x TZEIORQ 26	4557	4030	4205	5379	21.82	4.54
TZEIORQ 26 x TZEQI 82	4030	3935	3967	6013	34.03	4.45
TZEIORQ 45 x TZEIORQ 24	4826	4017	4287	4929	13.03	4.14
Check 1 - TZEIOR 127 x TZEIOR 57	2749	3805	3453	5083	32.07	4.13
TZEIORQ 6 x TZEIORQ 45	4773	3615	4001	5372	25.53	4.12
TZEIORQ 29 x TZEIORQ 40	3680	3784	3749	5252	28.61	3.97
TZEIORQ 5 x TZEIORQ 13	4311	3749	3925	5525	28.97	3.91
Sed	522	356	295	344	-	-
Min.	2749	3322	3453	5042	13.03	3.91
Max.	5500	5055	4474	6013	37.87	8.09
Mean	4188	4048	4102	5489	25.07	5.39

MI= multiple trait base index

Table 7. Correlation coefficients of selected early PVA-QPM parents and hybrids under contrasting environments in Nigeria, 2017-2018

	Correlation coefficient (r)				Average heterosis (%)			
Trait	Drought	Low-N	Optimal	Across Env	Drought	Low N	Optimal	Across Env
Grain yield (kgha-1)	0.30**	0.23*	0.21*	0.26*	512.89***	236.61**	234.58**	314.63**
Days to 50% anthesis (days)	0.06	0.27**	0.29**	0.29**	-2.63*	-5.20**	-5.49**	-5.47**
Days to 50%silking (days)	0.04	0.30**	0.29**	0.29**	-5.23**	-5.53**	-5.40**	-6.25**
Anthesis silking interval	0.02	0.14	0.04	0.11	-38.90**	-102.07	1.76	-35.62
Plant height (cm)	0.45**	0.51**	0.46**	0.50**	114.05**	28.37**	35.02**	44.60**
Ear height (cm)	0.34**	0.23*	0.16	0.22*	137.19**	52.54**	58.98**	71.26**
Root lodging	0.08	0.07	0.13	0.11	22.02	187.42	160.88	28.63
Stalk lodging	0.07	0.04	0.01	0.03	0.424	10.38	34.95	16.1
Husk cover	0.12	0.09	0.17	0.01	11.88	30.45*	31.59	10.87
Plant aspect (1-9)a	0.24*	0.29**	0.14	0.31**	10.07	11.91	28.65**	11
Ear aspect (1-9)b	0.45**	0.33**	0.24*	0.32**	24.78***	-0.45	15.38	-4.44
Number of ears per plant (No.)	0.26*	0.06	0.07	0.11	214.51***	18.41	12.27	31.28*
Stay green characteristic (1-9)c	0.23*	0.34**	-	0.21*	-9.81	-6.11	-	-9.45

*, ** Significant at 0.05 and 0.01 probability levels, respectively, Env= environment.

a Plant aspect (on a scale of 1-9), where 1= excellent overall phenotypic appeal and 9= poor overall phenotypic appeal.

b Ear aspect (on a scale of 1-9), where 1= clean, uniform, large and well-filled ears and 9= rotten, variable, small and partially filled ears.

c Stay green characteristics (on a scale of 1-9), where 1= less than 10% overall dead leaf area and 9= more than 80% overall dead leaf area.

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