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GENOTYPIC VARIATION IN ENDOSPERM PROTEIN, LYSINE AND TRYPTOPHAN CONTENTS OF NORMAL EXTRA-EARLY MAIZE CULTIVARS AND THEIR QUALITY PROTEIN HYBRIDS UNDER NITROGEN STRESS AND NON-STRESS ENVIRONMENTS

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Abstract
Drought and nitrogen (N) tolerance quality protein maize (QPM) could serve as a succor for malnourishment in the Sub-Saharan Africa, and adoption should be intensified where resource poor farmers cannot afford N fertilizer and drought on maize at grain filling stage is frequent. This study compares the quality index, protein, tryptophan and lysine contents of normal extra-early drought-tolerant and their quality protein maize hybrids under sub-optimal and optimal soil N conditions. Four normal drought-tolerant and their respective QPM hybrids were planted under no (0 kg N ha^{-1}), low (30 kg N ha^{-1}) and optimal (90 kg N ha^{-1}) fertilizations at Oke Oyi, Ilorin in the southern Guinea savanna of Nigeria in 2012 and 2013 cropping seasons. The trials were set up in a split plot arrangement with the N rates as main plot and the eight

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cultivars as sub-plots. Each plot within N levels was four-row, laid out in a randomized complete block design with four replications. The normal cultivars outyielded the QPM versions across N rates with no significance difference. There is a linear increase in all protein qualities with increase in N rates among QPM, while normal maize counterparts had a linear decline with increase in N fertilization. The QPM cultivars also maintained their endosperm protein qualities across N rates. Across N environments, the grain quality characters, such as crude protein, tryptophan, and lysine contents in grain, showed a significant negative relationship with grain yields, which were relatively much stronger under low-N stress. 99TY, TYEEC4, 99TYQ and 20SYNEEWQ have viable traits that could be explored for the development of maize varieties with good grain yield and better protein qualities to improve protein availability in maize based diets and feed for large population of man and livestock.

Keywords: Lysine, N rates, protein, quality index, tryptophan.

INTRODUCTION

Food security and health of the people are very important for the development of societies, but malnutrition still remains pervasive problem in the developing countries with low per capita income [Kassahun and Prasanna 2004]. According to United Nation Development Programme (UNDP), out of one billion people undernourished for protein in the developing countries, 200 million are children under five years of age, leading to several health problems including stunt growth, weaken immune system and impaired intellectual development [WHO 2006]. Maize grain is a major staple food accounting for about 15% of the total calorific intake of people in the rural communities of West and Central Africa (WCA), where animal protein is scarce and expensive and consequently, unavailable to a vast sector of the population [Badu-Apraku and Lum 2010, Wegarya *et al.* 2011]. It is cultivated in highly heterogeneous environments and consumed by people with varying food preferences and socio-economic backgrounds [Badu-Apraku *et al.* 2006]. Normal maize proteins have poor nutritional value for monogastric animals including humans, because of reduced content of essential amino acids such as lysine and tryptophan. Normal maize's average protein content is about 2% lysine that is less than half of the concentration recommended by Food and Agricultural Organization of the United Nations [FAO 2008]. Therefore, maize consumed by human must contain alternate sources of lysine and tryptophan. Increased lysine and tryptophan content was observed in the protein composition of the endosperm of the *opaque-2* (*o2*) mutant gene of maize [Mertz 1964]. The homozygous recessive *o2* gene restricts synthesis of zein that contains little lysine and tryptophan and a pleiotropic increase of the non-zein; proteins that contain high lysine and tryptophan levels in endosperm [Habben *et al.* 1993]. Through backcrossing and recurrent selection however, breeders were able to combine a fairly complex system of genetic modifiers with the opaque-2 (*o2*) gene to convert normal maize populations into quality protein maize (QPM). This leads to the development of cultivars with modified endosperm kernel, and increased concentrations of lysine and tryptophan [Vasal 2001, Prasanna *et al*. 2001, Wegarya *et al.* 2011]. The genetic system of *o2* is qualitative and increases lysine and tryptophan contents in endosperm by suppressing or reducing the synthesis of zein storage proteins and increasing that of glutelin storage proteins [Ngaboyisonga *et al.* 2012]. Parties of the modified endosperm are vitreous and hard instead of being opaque and soft [Villegas *et al.* 1992]. The *o2*-modified endosperm cultivars also possessed agronomic characteristics comparable with those of normal maize. The biological value of the protein that measures how well the body can absorb and use a protein is 80% for QPM compared to 40 to 57% for normal maize and 86% for eggs [Bressani 1992]. Besides increased biological value, QPM has additional nutritional advantages, such as higher concentration of niacin (vitamin B3), improved absorption of potassium [Graham *et al*. 1980] and carotene [De Bosque *et al.* 1988]. The protein of QPM has 90% of the relative value of milk compared to 40% for normal maize [Olakojo *et al.* 2007, Upadhyay *et al.* 2009, Mohammed *et al.* 2012]. Furthermore, QPM hybrid yields are 10 percent higher than those of other hybrids [Prasanna *et al.* 2001, Vasal, 2001]. The QPM cultivars can therefore be effective means to reach malnourished populations in the WCA [Bello *et al.* 2013, 2014a]. At a time when UNICEF reports that 1,000,000 infants and small children are starving each month, the inclusion of QPM in daily rations improves health and saves their lives [Vasal 2001]. Krivanek *et al.* [2007] and Sofi *et al.* [2009] earlier studies in Ghana showed that QPM fed children (0-15 months) were healthier, suffered fewer fatalities and had better growth rates than children fed with normal maize. The authors also reported similar results in India, Guatemala and Brazil, Mexico and Ethiopia. However, unlike other West African countries such as Ghana, the adoption and cultivation of QPM is still low in Nigeria [Jaliya *et al.* 2008].

In WCA including Nigeria, there is a high risk of crop failure especially in the drought-prone environments, due to high price ratios between fertilizer and grain, limited availability of fertilizer and low purchasing power of farmers [Worku *et al.* 2008, Badu-Apraku *et al.* 2011, Bello *et al.* 2011, Abe *et al.* 2013]. Research efforts of International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria yielded appreciable results since 2002. Adapted normal early and extra-early maize maturity groups were converted to QPM with increased levels of lysine and tryptophan, competitive performance in yield, and improved resistance to drought, *Striga*, and diseases [Olakojo *et al.* 2001, 2003, 2005, 2007, Badu-Apraku and Lum 2010, Olawuyi *et al.* 2011, 2013]. Cultivation of early and extra-early maize cultivars in this drought-susceptible agro-ecology is a strategy for breeding adaptable maize cultivars that are drought escaping, to withstand the effect of short rainy season, and prevent drought stress that occurs during the grain-filling stage, especially in the late season [Bello *et al.* 2014b]. These cultivars are endowed with favorable genes for high yield (ranging from 20-50% yield increase than other maize varieties) potential and stability across a broad range of water availability [Olaoye *et al.* 2009]. Bänziger *et al.* [1999] showed that improvement for drought tolerance also resulted in specific adaptation and improved performance under low nitrogen (N) conditions, suggesting that tolerance to either stress involves a common adaptive mechanism. CIMMYT [2003] also reported tryptophan levels of QPM under both low N and optimum conditions that were higher than those of normal maize under optimum conditions, while the quality index (percent of tryptophan in protein) remained unchanged. Cognizant of the foregoing, together with the information on skepticism and myths about QPM, this study was therefore conducted to assess the quality index, protein, tryptophan and lysine contents of extra-early QPM hybrids measure up to their normal counterparts under sub-optimal and optimal soil N conditions for the benefit of both QPM breeders and potential consumers.

MATERIALS AND METHODS

GERMPLASM AND THE SOIL SITE

Field studies were conducted during the 2012 and 2013 cropping seasons at the Lower Niger River Basin Development Authority station, Oke-Oyi, Ilorin (8°30'N, 8°36'E and 945 m above sea level.) in the southern Guinea savannah of Nigeria. The soil in the region was inherently low in both natural fertility and moisture retention capacity. The predominant soil is sandy loam (classified as Typic paleustalf according to the US Soil Taxonomy) by texture within the 0–30 cm soil depth, and moderately well drained. The soil is slightly acidic (pH of 6.3) with organic carbon of 8.3 g kg^{-1}, adequate to enhance maize productivity. Available phosphorus was mild (6.1 g kg^{-1}), though below the critical level of 10 mg kg^{-1}. Base saturation (16.3 g kg^{-1}) as well as exchangeable bases of calcium (1.4 cmol kg^{-1}), potassium (0.3 cmol kg^{-1}) and magnesium (1.4 cmol kg^{-1}) were within the acceptable limit for optimum plant growth, while the nitrogen level (0.6 g kg^{-1}) was insufficient for maize growth and development at the experimental site. Generally, the average rainfall was 1201.2 and 944.3 mm in the year 2012 and 2013 respectively.

The method adopted for the development of the maize cultivars has been described in details by Badu-Apraku and Lum [2010]. The sources of the QPM traits used in the conversion program at IITA, Ibadan, Nigeria for the normal endosperm, white early maturing populations, cultivars, and inbred lines were Pool 15 SR QPM and DMR-ESR-W QPM (both white grained) and Pool 18 SR QPM (yellow grained) for the yellow materials

(Table 1). Four normal white extra-early (TZEE-W Pop C3, 2000 Syn EE-W STR TZEE-Y Pop STR C3, and 99 TZEE-Y STR) cultivars that were *Striga* resistant and/or drought tolerant were crossed in 2002 to QPM donors (EV 99 QPM) for partial conversion to QPM. Characteristics of extra-early normal endosperm cultivars and their quality protein maize (QPM) HYBRIDs are given in Table 1.

Table 1: Characteristics of extra-early normal endosperm cultivars and their quality protein maize (QPM) traits.

Genotypes	Code	Parentage	Grain type	Reaction to biotic, abiotic stresses
99 TZEF-Y STR	99TY	Various	Yellow-Flint	Drought escaping and *Striga* tolerant
99 TZEF-Y STR QPM C_0	99TYQPM	99 TZEF-Y STR × QPM source	Yellow-Flint	Drought escaping and *Striga* tolerant
TZEE-W Pop STR C_4	TWEEC_4	Local and introduced extra-early germplasm	White-Dent/ Flint	Drought escaping and *Striga* resistant
TZEE-W Pop STR QPM C_0	TWEEQPM	Local and introduced extra early germplasm × QPM source	White-Dent/ Flint	Drought escaping and *Striga* tolerant
2000 Syn EE-W	20SynEEW	TZEE-W Pop STR S4 F2	White-Dent/ Flint	Drought escaping and *Striga* tolerant
2000 Syn EE-W QPM C_0	20SynEEWQ	TZEE-W Pop STR S4 F2 × QPM Source	White-Dent	Drought escaping and *Striga* tolerant
TZEE-Y Pop STR C_4	TYEEC_4	Local and introduced extra-early germplasm source	Yellow-Flint	Drought escaping and *Striga* tolerant
TZEE-Y Pop STR QPM C_0	TYEEQPM	Local and introduced extra-early germplasm × QPM source	Yellow-Flint	Drought escaping and *Striga* tolerant

PLANTING AND CULTURAL PRACTICES

A split-plot factorial in a randomized complete block design with four replications was used in each year. The main plot treatments consisted of three N fertilization rates (0, 30 and 90 kg N ha^{-1}), while the subplot treatments were the eight (four normal extra early and their four QPM hybrid hybrids) maize cultivars. Each subplot experimental unit consisted of four rows 5 m in length spaced 0.75 m apart with 0.25 m between plants within each row.

The experimental field was disc-harrowed and ridged before planting. Planting was carried out on 18th July, 2012 and 25th July, 2013. Two seeds were planted per hill and later thinned to one to obtain a plant population density of 53,333 plants per hectare. At planting, the experiments in the two-year period received a basal application of P in form of single super phosphate and K as muriate of potash at the rate of 60 kg P_2O_5 ha^{-1} and 30 kg K_2O ha^{-1} respectively. N fertilizer in form of urea was applied in two equal split doses in which the first half was 2 weeks after planting (WAP) while the second dose was at 4 WAP. The trials were kept weed-free with the application of Atrazine and

Gramoxone as pre- and post-emergence herbicides at 5 L ha^{-1} each of Primextra and Paraquat, and subsequently, by hand weeding.

At crop maturity ears were harvested and weighed for each plot separately and grain yield ha^{-1} at 12.5% grain moisture content was determined with the following formula:

$$\text{Grain yield (kg ha}^{-1}\text{)} = \frac{\text{Fresh wt.} \times (100 - \text{MC}) \times 0.8 \times 10{,}000\ \text{m}^2}{(100 - 12.5) \times 7.5\ \text{m}^2}$$

Where Fresh weight = weight of ears $plot^{-1}$ at the time of harvest,
0.8 = shelling percentage,
10,000 m^2 = area per hectare,
12.5% = standard grain moisture for grain storage,
7.5 m^2 = area of plot harvested (5m x 0.75m x 2),

Grain yield was obtained from ear weight per plot (assuming 80% shelling percentage) and converted to tonnes per hectare after adjusting to 12.5% moisture content.

SIB-MATING AND PROXIMATE ANALYSIS OF THE MAIZE ENDOSPERM CONTENTS

Eight plants were sib-mated plant-to-plant for the QPM and normal cultivars in each plot to avoid crosses between QPM and normal cultivars in the grain of these plants. At harvest the S_2 grain was shelled from the middle of five ears for all sibbed plots and 20 seeds were then taken at random and analyzed in the laboratory. The grains obtained were grinded to form a fine powder and each sample was oven dried to a constant weight at 72°C for 48 hours to obtain grain moisture content percentage. Three replicates were analyzed for each genotype and the mean recorded.

Crude protein determination was estimated using standard micro-Kjeldahl procedure [AOAC 2006]. Moisture content, crude protein (Kjeldahl method), crude fat (solvent extraction), crude fibre and ash were determined using standard methods [AOAC 2005]. The carbohydrate content was determined by difference, i.e. addition of moisture, fat crude protein, ash and crude fibre, was subtracted from 100%. This gave the amount of nitrogen free extract otherwise known as carbohydrate, as follows:

Carbohydrate% = 100% - (Moisture% + Fat % + Ash% + Crude fibre %+ Crude protein%).

The amino acid was determined according to the procedure described by Sentayehu [2008]. Maize flour samples of 0.5 gm were weighed in tarred scoop and transferred to boiling tubes. A catalyst tablet, selenium was dropped into each tube and about 25ml of concentrated sulfuric acid was added. The tubes were then placed in an automatic controlled heater and set at 200 °C. The mixtures were heated until the color changed to light blue. For samples which had a digest color of light brown or yellow, the digestions were repeated twice or more times. Thirty ml of distilled water was added into the digestion tube. During these events, the organic matter of maize flour was oxidized, and the nitrogen in the protein was converted to ammonium by sulfuric acid as described by Aykroyd *et al.* [1964] and Purseglove *et al.* [1968].

Ammonium in the digestion mixture was determined by distillation and titration [Aykroyd *et al.* 1964]. The digestion tube was placed on to the Tecator steam distillation apparatus. The distiller was set, the digestion tube inserted in the system and 150 ml ammonia was collected in the receiver flask containing 50 ml of boric acid solution at 4%. Then ammonia was titrated against a standard acid (0.1 N 10%HCl). Since 1 ml of 0.1 N HCl is equivalent to 1.401 mg N, the nitrogen content of the sample was calculated as follows:

$$\%N = \frac{\text{ml HCL ml black) x normality x 14.007 x 14.007 x 100}}{\text{mg sample}} \qquad (1)$$

% Crude protein = %N x 6.25

Due to the simplicity of the estimation of tryptophan, its content has been used as a criterion for screening materials with superior protein quality. For estimation of tryptophan of *opaque-2* maize materials, the papain hydrolysis method was used [Hornandez *et al.* 1969].

A single step papain hydrolysis was utilized for protein solubilization. Iron ions oxidized acetic acid to glyoxylic acid in the presence of sulphuric acid. The indole ring of free tryptophan that bound in soluble proteins reacted with glyoxylic acid and a violet-purple compound was produced. The intensity of the violet-purple color was measured at 545 nm with a spectrophotometer. By drawing a standard curve of optical density vs. tryptophan concentration, percent tryptophan in sample was recorded as follows:

$$\%\text{ tryptophan in protein} = \frac{\%\text{ tryptophan in sample}}{\%\text{ protein in sample}} \qquad (2)$$

The relationship between tryptophan and lysine in the maize endosperm protein had been observed by various researchers [Mertz *et al.* 1964, Hornandez *et al.* 1969, Doll *et al.* 1975], thus the tryptophan was used as parameter for protein quality evaluation, and the value was increased by 4 times to obtain the value of lysine [Sentayehu 2008]. The zein crude was determined using the procedure as described by Drochioiu *et al.* [2002], while zein dry matter percentage were estimated by the formula the authors suggested given as:

% Zein in dry matter = % crude protein x 0.386-2.22. (3)

Quality index (QI), defined as tryptophan to protein ratio in the sample, was calculated according to Villegas et al. (1984) as:

$$QI = 100 \times \frac{\text{tryptophan content in the sample}}{\text{protein content in the sample}} \quad (4)$$

STATISTICAL ANALYSIS

Statistical analysis was performed on all measured traits using the SAS for Windows Release 9.2 [SAS Institute 2009]. The SAS GLM procedure used for the ANOVA was mixed model. Replication was treated as a random effect, while nitrogen rate and hybrids were fixed effects. Differences between two treatment means were compared using least significant difference (LSD). Pearson's correlation coefficient was used to test for an association between grain yield and other variables at each N level using PROC CORR of SAS.

Table 2: Interactions of Genotype x Nitrogen x Year on grain yield and protein qualities response of extra-early normal endosperm and their QPM versions to varying N fertilizations.

Source	Grain yield	Crude protein	Carbo-hydrate	Lysine	Tryptophan	Zein crude	Zein dry matter	Quality index
Replication	12.1	21.4	13.9	12.6	15.5	17.6	10.6	8.0
Year	22.5	11.6	9.1	31.4	9.9	12.3	11.4	6.9
SE±	13.6	10.2	11.3	9.4	4.6	12.6	9.9	11.3
Nitrogen	134.2*	355.5**	114.5*	322.9**	113.1*	376.9**	111.8*	378.5**
Year x Nitrogen	11.1	10.4	10.2	9.4	13.7	11.9	9.5	15.1
SE±	11.1	10.5	17.5	11.8	23.7	19.4	14.6	17.6
Genotype	14.7	31.9	131.7*	321.5**	122.8*	314.5**	362.1**	346.9**
Genotype x Year	11.4	19.3	22.7	31.6	14.6	19.1	21.4	7.6
Genotype x Nitrogen	131.3*	367.7**	362.1**	346.9**	134.2*	167.7*	112.7*	109.6*
Genotype x Nitrogen x Year	33.9	24.8	19.2	62.2	44.5	62.7	78.9	13.7
SE±	2.3	7.5	13.7	8.8	16.6	21.5	8.4	10.3
%CV	11.5	10.0	8.6	9.1	16.2	11.7	12.2	15.3

*, ** Significant at the 0.05 and 0.01 probability level respectively.

RESULTS

Combined ANOVAs of the extra-early maturing maize cultivars showed significant mean squares for nitrogen (N) and genotype x nitrogen interaction for grain yield and all the analyzed qualities in grain at the three N fertilizer applications (Table 2). The differences among the extra-early cultivars were also significant for all the parameters studied except grain yield and crude protein under the varying N regimes. Differences between the year, genotype x year and genotype x year x nitrogen interactions were not significant. The results also showed that genotype x nitrogen mean squares were of most significance, accounting for 64.6%

of the total sum of squares for grain yield and the qualities of the cultivars under optimal-N (90 kg N ha^{-1}) and for 73.4% and 63.9% under no-N (0 kg N ha^{-1}) and low-N (30 kg N ha^{-1}) respectively (data not presented). On the other hand, the year x genotype x nitrogen (11.7, 14.8 and 17.3% under no-, low- and optimal-N respectively) and genotype x year (7.2% under optimal-N, 6.4% and 6.4% for low-N and No-N) differences constituted only a small proportion of the total sums of squares of the cultivars under the varying N rates.

Grain yield of QPM hybrids was significantly affected by different N levels (Table 3). The normal cultivars outyielded the QPM hybrids across N rates with no significance difference. The highest average grain yield (4.71 t ha^{-1}) for $TWEEC_4$ under optimal-N proved significantly superior over the rest of N treatments. The lowest grain yield of 0.77 t ha^{-1} was recorded for 99TYQPM under no-N. On the average, the magnitude of increase of grain yield was 75.52% with the optimal-N application over no-N among the cultivars. The highest yielding extra-early cultivar, $TWEEC_4$, outyielded the lowest yield cultivar 99TYQPM by 39% under low-N. Under low-N and optimal-N conditions across years, cultivar $TWEEC_4$ and its QPM version TWEEQPM were closest to the ideal yield of the region. Under optimal-N conditions, there was no significant difference in the mean grain yield of the normal endosperm extra-early cultivars $TWEEC_4$ and 20SynEEW as well as their QPM hybrids, TWEEQ and 20SynEEWQPM, and they were the most superior cultivars under low-N. In contrast, the normal endosperm extra-early cultivars 99TY, and $TYEEC_4$ significantly outyielded their QPM counterparts, 99TYQPM and TWEEQPM. However, the grain yields of 99TY, 99TYQPM and TYEEQPM were below the mean grain yield of the cultivars at low-N, Nitrogen efficiency (above average grain yield under low-N stress) varied among QPM cultivars in almost the same magnitude as among normal cultivars. On the other hand, carbohydrate content of the cultivars increased linearly with increase in N rates among normal cultivars, while a linear decrease was recorded in QPM counterparts. The carbohydrate content of the cultivars across N rates ranged from 61.15% in TYEEQPM to 79.62% in 20SynEEW under optimal-N. $TYEEC_4$ and 20SynEEW gave the highest mean carbohydrate content. The best QPM cultivar 20SynEEW was among the high yielding cultivars under low-N stress condition, outyielding the N-inefficient QPM cultivar TYEEQPM by 16% under low-N stress. However, it also yielded less than the best QPM cultivars in each N fertility environment. Among the cultivars, zein crude and zein dry matter concentrations also followed the same trend with carbohydrate contents by increasing as N fertilizer application increased in normal cultivars and they were superior compared to the QPM versions. 99TY was the most outstanding cultivar for zein crude contents in each N rate with 10.96% yield advantage over N-unresponsive QPM, TYEEQPM at low-N.

Table 3: Grain yield, carbohydrate, zein crude and zein dry matter contents of normal endosperm extra-early cultivars and their QPM versions under low and optimum nitrogen environments at Ilorin, Nigeria in 2012 and 2013.

	Grain yield ($t\ ha^{-1}$)			Carbohydrate (%)			Zein crude ($ml\ \mu g^{-1}$)			Zein dry matter (%)		
Nitrogen ($kg\ N\ ha^{-1}$)	0	30	90	0	30	90	0	30	90	0	30	90
Year												
2012	0.94	2.86	3.87	69.20	70.94	72.82	184.81	185.15	185.36	0.81	0.81	0.69
2013	0.95	3.00	3.88	69.30	71.00	72.98	185.00	187.24	188.55	0.78	0.82	0.70
SE±	8.43	1.83	12.83	9.12	3.63	7.22	13.36	1.84	11.68	1.12	3.34	9.21
Cultivars												
99TY	0.81	2.22	3.56	70.89	72.78	75.56	192.93	195.67	198.73	0.78	0.81	0.87
99TYQPM	0.77	2.28	3.45	68.34	67.74	64.32	194.45	190.89	188.67	0.82	0.78	0.75
$TWEEC_4$	1.26	3.44	4.71	72.69	73.82	76.93	190.71	193.23	195.86	0.78	0.80	0.84
TWEEQPM	1.11	3.31	4.62	69.73	67.41	65.13	186.55	185.63	184.22	0.78	0.74	0.72
20SynEEW	1.03	3.13	3.87	76.01	77.69	79.62	176.97	178.73	181.27	0.90	0.92	0.95
20SynEEWQ	1.00	3.10	3.82	67.56	66.32	64.95	176.91	174.62	171.68	0.86	0.80	0.78
$TYEEC_4$	0.75	3.02	3.58	75.81	76.88	78.63	187.94	188.56	191.44	0.86	0.90	0.93
TYEEQPM	0.89	2.55	3.40	66.86	65.11	61.15	180.82	174.23	171.83	0.87	0.83	0.80
SE±	2.63	0.95	0.99	11.4	0.87	0.83	1.67	13.69	11.22	7.83	3.75	0.63
$LSD_{(0.05)}$	0.35	0.63	0.83	10.56	10.42	12.02	13.62	13.11	12.54	0.11	0.10	0.13
Mean	0.95	2.88	3.88	70.99	70.97	65.41	185.91	185.20	185.46	0.83	0.82	0.83

Table 4: Crude protein, lysine and tryptophan and quality index contents of normal endosperm extra-early cultivars and their QPM versions under low and optimum nitrogen environments at Ilorin, Nigeria in 2012 and 2013.

	Crude protein (%)			Lysine (%)			Tryptophan (%)			Quality index (%)		
Nitrogen ($kg\ N\ ha^{-1}$)	0	30	90	0	30	90	0	30	90	0	30	90
Year												
2012	9.17	9.65	10.52	2.68	2.76	2.91	0.74	0.76	0.83	0.69	0.70	0.71
2013	9.16	9.70	10.52	2.69	2.71	2.90	0.74	0.79	0.83	0.65	0.69	0.71
SE±	12.22	9.63	0.35	13.53	0.97	0.69	11.72	10.11	13.63	17.62	3.31	10.55
Cultivars												
99TY	9.84	9.45	8.11	2.18	1.86	1.79	0.62	0.60	0.58	0.55	0.51	0.48
99TYQPM	10.82	10.88	10.90	3.56	3.57	3.59	0.80	0.81	0.82	0.80	0.80	0.81
$TWEEC_4$	9.72	9.06	8.02	2.10	1.76	1.71	0.61	0.60	0.60	0.60	0.58	0.55
TWEEQPM	10.79	10.88	10.90	3.58	3.60	3.61	0.86	0.88	0.88	0.79	0.80	0.82
20SynEEW	9.41	7.85	6.73	2.24	1.98	1.77	0.54	0.51	0.50	0.69	0.67	0.65
20SynEEWQ	10.37	10.39	10.43	3.60	3.62	3.66	0.80	0.81	0.82	0.78	0.79	0.80
$TYEEC_4$	9.62	8.43	8.01	2.38	2.02	1.98	0.60	0.58	0.56	0.67	0.65	0.54
TYEEQPM	10.48	10.50	10.57	3.50	3.51	3.54	0.82	0.84	0.84	0.77	0.79	0.81
SE±	12.11	1.93	6.56	12.4	2.66	8.83	7.93	12.34	6.82	11.82	13.23	10.73
$LSD_{(0.05)}$	1.12	1.11	1.34	0.76	0.83	1.23	0.24	0.27	0.31	0.11	0.15	0.17
Mean	10.13	9.68	9.21	2.89	2.74	2.71	0.71	0.78	0.70	0.71	0.70	0.69

There is a linear increase in all protein qualities with increase in N rates among QPM, while normal cultivars had a linear decline with increase in N fertilization of the study (Table 4). The QPM cultivars also maintained their endosperm protein qualities across N rates. Significant differences were observed among QPM and normal hybrids for endosperm protein quality index, QI (the ratio of tryptophan content to total protein content in the endosperm, expressed as a percentage) at varying N rates. The profile of QI was higher for QPM than normal cultivars in all N environments. The mean QI of the cultivars across N rates ranged from 0.50% in non-QPM, 20SynEEW to 0.88% in QPM, TWEEQPM with 43.18% yield advantage at both low-N and optimal-N. Furthermore, QI was above the QPM threshold of 0.80 in all the four hybrids studied at all N regimes compared with normal cultivars.There was no differential response among either QPM or normal cultivars for crude protein at varying N rates , but N stress significantly reduced the crude protein in normal compared to QPM cultivars. The best QPM, TWEEQPM for crude protein outyielded the N-inefficient normal 99TY with 41.46% advantage across N rates. The most outstanding QPM cultivar 20SynEEWQ for lysine and tryptophan across N had advantage of 53% for tryptophan over respective normal hybrid 20SynEEW.

Table 5: Genotypic correlation coefficients of protein quality parameters with grain yield in stress and non-stress N fertilizers

	Grain yield	Crude protein	Carbo-hydrate	Lysine	Tryptophan	Zein crude	Zein dry matter	Quality index
Grain yield								
Crude protein	-0.63**							
Carbohydrate	0.31	-0.86**						
Lysine	-0.57*	0.68*	-0.78**					
Tryptophan	-0.49*	0.71**	-0.81**	0.91**				
Zein crude	0.33	-0.76**	0.34	-0.74**	-0.81**			
Zein dry matter	0.26	-0.63*	0.29	-0.79**	-0.79**	0.72**		
Quality index	-0.51*	0.65**	-0.73**	0.73**	0.67**	-0.76**	-0.82**	

*, ** Significant at the 0.05 and 0.01 probability level respectively.

Interrelationship of grain yield and grain qualities (Table 5) showed that grain yield not only highly associated negatively with all the studied protein qualities (QI, crude protein, lysine and tryptophan), but also correlated positively with carbohydrate and zein contents with no significant difference. There was significant negative correlation (r=-0.86**) between carbohydrate and crude protein contents, and crude protein content decreased with increase in carbohydrate content of the maize grain. Similarly, QI, lysine and tryptophan contents correlated negatively $(p \leq 0.01)$ with carbohydrate and zein concentrations. Endosperm amino acid contents (lysine and tryptophan) were highly correlated positively (*r* = 0.91**) across N rates. While the amino acid contents was positively related $(p \leq 0.01)$ with QI, zein was highly associated negatively $(p \leq 0.01)$ with the amino acid contents.

DISCUSSION

The main problem with QPM hybrids is to achieve high levels of essential amino acids and high grain yield at the same time. The eight cultivars (four normal extra early and their QPM cultivars) studied differed in their response pattern to N in both years. This result corroborates with Agrama *et al.* [1999] and Mosisa *et al.* [2007] in tropical maize as well as O'Neill *et* *al.* [2004] in temperate maize. It was also observed that increase in N fertilizer rate increased grain yield, but was lowered in QPM compared with non-QPM counterparts with no significant difference as reported by earlier workers [Bhatnagar *et al.* 2004, Krivanek *et al.* 2007, Gissa *et al.* 2008]. The comparable magnitude in grain yield between QPM and normal cultivars at the same level of N fertilizer application for N-uptake and utilization could be attributed to the same gene pools in which both cultivars were developed. Earlier workers [Jaliya *et al.* 2008, Badu-Apraku *et al.* 2014] attributed N fertilizer being part of the essential nutrients required for the promotion of the meristematic and physiological activities such as plant leaf spread, root development and plant dry matter production, leading to an efficient absorption and translocation of water and nutrients as well as interception of solar radiation and assimilation of carbon dioxide.

Protein qualities of QPM were also consistently stabled at all N regimes in accordance with those reported by Pixley and Bjarnason [2002] and Vivek *et al.* [2008]. The relatively stable performance of these cultivars is highly desirable because under low-N in farmers fields, erratic, unpredictable rainfall and soil infertility (especially N level) are predominant. Low-income rural and urban communities are increasing their dependence on maize in WCA. Therefore, QPM of high grain yield with high nutritional qualities demand promotion for adoption by the farmers, as it becomes a powerful means of helping the millions of people living in poverty to improve their food security and reduce malnourishments. Some varieties of maize have been reported to combine high grain yield and protein quality parameters under low N and optimal N. For example, TWEEQPM and 20SynEEWQ that expressed a high capacity for N absorption and storage as well as maintaining the protein qualities are promising cultivars for improvement in the region. According to Badu *et al.* [2010], the outstanding performance of the QPM cultivars may be attributed to the large population sizes sampled during QPM conversion which might have ensured that the favourable drought tolerant alleles were maintained in the respective populations during selection for high grain yield, desirable agronomic characteristics, and appropriate endosperm modification. This also indicates the effectiveness of the backcross, inbreeding, and hybridization methods adopted in the conversion programme. Bänziger *et al.* [1999] showed that improvement for drought tolerance also resulted in specific adaptation and improved performance under low nitrogen (N) conditions, suggesting that tolerance to either stress involves a common adaptive mechanism.

According to Balconi *et al.* [1997] carbohydrate and crude protein are the major components of maize endosperm, primarily sinks for carbon and N compounds. In this group of cultivars, grain yield, carbohydrate and zein contents in the grain tended to increase linearly in normal cultivars as N level in the soil increased, and declined in QPM as N increased, with a resultant increase in protein qualities in QPM was similar to the observations made by Idikut *et al.* [2009]. This also indicated that there is considerable genotypic variation in grain carbohydrate and protein quality contents in both QPM and normal cultivars. Duvick [1997] reported a linear increase in grain starch percent and a linear decrease in grain protein percent as grain yield increased over the years in evaluated normal hybrids trials released from 1934 to 1991 in the USA. In the present study, amino acid contents (lysine and tryptophan) and QI were significantly higher compared to normal cultivars, and the threshold set for QPM [Vivek *et al.* 2008] was attained at the various N rates. The superiority of QPM over normal cultivars were shown for grain protein qualities across locations under non-limiting soil fertility conditions in Serbia [Ignjatovic-Micic *et al.* 2013], Zimbabwe and Kenya [Mosisa *et al.* 2007]. Mosisa *et al.* [2008] also found that zein increased more under high-N conditions, whereas grain yields are also higher, compared to other protein fractions (albumins, globulins and glutamins) which contain tryptophan and lysine. Vasal [2001] confirmed a positive relationship between the zein contents and grain yield, and insinuated that zein synthesis could be manipulated by N fertilization through genetic process. It appears that zein was less enhanced in QPM compared to normal cultivars across N conditions, because there was a significant difference between QI values of QPM and normal cultivars. The crude protein content was lowered under low-N compared to optimal-N conditions, and the percentage protein was similar for QPM and normal cultivars under optimal-N in this study. However, increase in crude protein content that was observed when N fertilizer was applied probably increased protein granule size, since the principle increase could be in zein protein fraction [Wu *et al.* 2010]. In this study, QPM hybrids had crude protein comparable to non-QPM versions indicating that there is considerable genotypic variation in grain protein content in both QPM and normal counterparts. This is in consonance with Pixley and Bjarnason [2002] and Zaidi *et al.* [2008]. QPM were also found to accumulate twice the normal concentration of tryptophan. Tryptophan and lysine were lower under low-N compared to optimal-N in both QPM and normal cultivars, although these amino acids level of QPM cultivars under both low-N and optimal-N was higher than the normal maize counterparts. Similarly, it was shown that tryptophan level of QPM under stress and optimum conditions was higher than the tryptophan level of normal maize under optimum conditions [CIMMYT 2003, Bhatnagar *et al.* 2003, Rasheed *et al.* 2004, Mosisa 2005, Worku *et al.* 2007, Ngaboyisonga *et al.* 2012, Ignjatovic-Micic *et al.* 2013]. Prasanna *et al.* [2001] and Gupta *et al.* [2009] also suggested that this might be due to higher N uptake, which significantly enhances these attribute contents in grain. This could also be attributed to the drastic reduction in storage protein (zein) accumulation and the increase accumulation of non-zein endosperm protein which is high in lysine and tryptophan [Tengan 2010].

These results that showed variations in protein qualities in grain of normal endosperm and their QPM hybrids at varying N environments have been described to be under additive and maternal effects of gene action for protein content and non-additive effects for the amino acids [Ngaboyi-songa *et al.* 2008, 2009], which can be exploited for developing QPM genotypes with high levels of these protein and amino acids. Multigenic effects however have been reported to control amino acid contents (Wang *et al.* 2001, Wu *et al.* 2002]. Ultimately, it becomes obvious that the simple genetic nature of *o2* maize has been converted to a classic polygenic character in reference to QPM, and must be manipulated as such in breeding programmes. Krivanek *et al.* [2007] earlier suggested that if tryptophan or lysine levels are not frequently examined during the course of breeding process, the additional gains in protein quality may be lost even though the *o2o2* genotype is maintained.

Maize grain yield is also a multifaceted trait resulting from inter-relationship of its yield characters. Knowledge of correlation coefficients among various plant parameters including grain qualities could assist in ascertaining the degree to which these traits associated with economic productivity. This is practically expressed in this research.

Negative association between grain yield and grain quality parameters is undesirable, but very important in QPM breeding programme as breeders attempt simultaneous selection and improvement of these traits. Across N environments, the grain quality characters, such as protein, tryptophan, and lysine contents in grain, showed a significant negative relationship with grain yields, which were relatively much stronger under low-N stress. This could be explained based on the well-established direct relationship between N and protein contents. Reduced grain N content under low-N might have resulted in reduced quality index, crude protein and amino acids contents in grain under low-N stress. This was eventually reflected as a strong negative relationship of these N-demanding components with grain yield [Zaidi *et al.* 2008]. Carbohydrate (starch) however is the highest component and primary energy source in maize grain. Negative relationship between carbohydrate and crude protein content showed that that crude protein content decrease with increasing starch content of maize grain similar to the report of Idikut *et al.* [2009]. Crude protein content in the grain also decreased as grain yield increased. Mosisa *et al.* [2008] reported a negative relationship between grain yield and grain protein content. Endosperm lysine and tryptophan contents that were highly correlated (r = 0.91**) across N rates was earlier reported by [Idikut *et al.* 2009, Ignjatovic-Micic *et al.* 2013]. Pixley and Bjarnason [2002], Scott *et al.* [2004] and Betran *et al.* [2006] found also a positive and significant correlation between protein content and tryptophan. The authors suggested that any reduction in synthesis of proteins might reduce tryptophan accumulation. Ngaboyisonga *et al.* [2012] and Mosisa *et al.* [2008] also reported that endosperm lysine and tryptophan contents were highly correlated across N environments as observed in this study.

Badu *et al.* [2010] reported that at the initiation of the QPM conversion, normal endosperm extra-early and early maturing populations and elite cultivars were selected for partial conversion to QPM based on high yield potential, resistance to Striga, and tolerance to drought stress, but no conscious effort was made to select for N tolerance. It is therefore glaring that some products of the conversion program were found superior or comparable to the normal versions not only in terms of grain yield but also for low N tolerance. The occurrences of IITA QPM germplasm are competitive with some other cultivars in grain yield; although lowered than that of the best available normal cultivars in some genetic back-grounds, the QPM could bring in the added benefit of enhanced nutritive value because of higher lysine and tryptophan content. In this context, cultivation of QPM in this region will play a pivotal role in eliminating protein-calorie malnutrition. Therefore, this encouraged the need to examine the cause and bring at par or even above with the best normal endosperm cultivars adapted to the region. Taking into cognizance the stability of protein quality and tryptophan content in QPM, this experiment was performed only at one location in two years with the goal to identify QPM hybrids with high values for these two quality components. Considering that yield significantly depends on environmental conditions and stress environments produce high genotype by environment interaction [Banziger and Diallo 2004], and that quality traits also depend on environment [Ignjatovic-Micic *et al.* 2013], field trials on different locations and years are to be performed for the cultivars to test their stability in the agro-ecology.

CONCLUSIONS

The drought tolerant QPM genotypes analyzed in this study have comparable grain yield and maintained their superiority of protein qualities over their normal cultivars at all N rates. 99TY, TYEEC4, 99TYQ and 20SYNEEWQ have viable traits that could be explored for the development of maize varieties with high grain yield and better protein qualities to improve protein availability in maize based diets and feeds for large population of man and livestock.

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