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Quality Protein Maize under Low N and Drought Environments: Endosperm Modification, Protein and Tryptophan Concentrations in Grain

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Abstract: Maize (*Zea mays* L.) is worldwide used as food and feed, supplying carbohydrates and proteins. However, it is deficient in two essentials amino acids namely; lysine and tryptophan. Quality Protein Maize (QPM) has about twice the amount of lysine and tryptophan of normal maize and can be used to correct this deficiency in protein quality. It was developed by combining the genetic systems of the mutant opaque-2 (O2) gene and O2-endosperm genetic modifiers. Current efforts are to expand QPM cultivation in regions experiencing malnutrition. In those regions, maize is produced under stresses among which low N and drought are the most prevalent. However, the effects of those two stresses on QPM characteristics are not known. To study how N and drought affect major characteristics of QPM, 14 QPM inbred lines were received from CIMMYT-Kenya and used to produce 41 Single Crosse Hybrids (SCHs). The 41 SCHs and one normal check were evaluated at Kiboko in Kenya in 2005 and 2006 under optimum, low nitrogen and drought environments and at Rubona in Rwanda in 2005 under optimum and low N environments. Observations were performed on endosperm modification, protein and tryptophan concentrations in grain. The results showed that low N partially reduced the action of O2-endosperm modifiers making QPM endosperm partially soft and opaque. Drought suppressed or reduced significantly the action of O2-endosperm modifiers making QPM endosperm chalky, opaque and soft. Low N and drought reduced significantly protein concentration in grain of genotypes including the non-QPM check whereas they increased the levels of tryptophan except for the non-QPM check. It appeared therefore that nitrogen particularly water played vital roles in modification of O2-maize endosperm. Moreover, QPM genotypes did not lose their nutritional advantages in stressed environments. The adverse effects of low N particularly drought on endosperm modification may have negative impact on adoption of QPM in areas prone to the two stresses and where maize is the major source of food because harvested grain will be inappropriate for human consumption. However because of important genetic variability among genotypes, it is possible to select genotypes less susceptible to low N and drought by using optimum and stressed environments.

Key words: Drought, endosperm modification, low N, optimum, protein, tryptophan concentrations, grain, QPM

INTRODUCTION

Maize (*Zea mays* L.) is an important source of calories and protein in human lives in many countries of the developing world. It is the main staple food in Eastern Africa particularly in Kenya and Tanzania where it supplies >33% of calories and protein (Krivanek *et al*., 2007). Nutritionally, maize is deficient in two essential amino acids: lysine and tryptophan. Therefore, there are concerns about the supply of the two essential amino acids in the regions where it constitutes the daily food. The Quality Protein Maize (QPM) has about twice the levels of lysine and tryptophan compared to normal maize. It was developed by combining the genetic systems of the gene mutant opaque-2 (*O2*) and the genetic of O2-endosperm modifiers (Sofi *et al*., 2009; Krivanek *et al*., 2007; Prasanna *et al*., 2001; Vasal, 2001).

The genetic system of *O2* gene is qualitative and O2 increases the levels of lysine and tryptophan in endosperm by suppressing or reducing the synthesis of zein storage proteins and increasing that of glutelin storage proteins. However, the *O2* gene adversely affects several important agronomic traits including kernel characteristics. It reduces the accumulation of dry matter

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resulting in low grain yield, the kernel phenotype changes to a soft, chalky and opaque appearance. Kernels dry slowly following physiological maturity of the grain and have a higher incidence of ear rots (Sofi *et al*., 2009; Krivanek *et al*., 2007).

Modifiers are genes capable of altering the expression of other genes at different loci in the genome. In QPM, the O2-endosprm modifiers alter the undesirable correlated effects of *O2* gene because the modified endosperm becomes vitreous and hard instead of being opaque, chalky and soft. Thus, QPM varieties look like normal maize and have similar grain yields and other agronomic traits (Prasanna *et al*., 2001; Vasal, 2000, 2001). The mechanisms by which the modifier genes convert the soft and opaque endosperm of O2 maize in desirable phenotype are still poorly understood but it appears that they involve the synthesis of γ27 kDA-zein (Or *et al*., 1993) and altered starch structure in O2 modified endosperm (Gibbon *et al*., 2003; Gibbon and Larkins, 2005).

The nutritional superiority of QPM over normal maize lies in the fact that QPM contains in general >55 of tryptophan, >30 of lysine and <38% of leucine compared to normal (non-QPM) maize. Many reports have shown evidence of the superior nutritional quality of QPM and its ability to correct nutritional defects in people, effects that have been particularly positive in infants and children (Ahenkora *et al*., 2000; Barragan-Delgado and Serna-Saldivar, 2000). The results have been repeated and demonstrated not only in laboratory rats but also in domestic animals particularly monogastrics (Burgoon *et al*., 1992; Osei *et al*., 1999). The use of QPM as supplier of lysine to improve milk production in cattle has been explored and QPM lysine was found to be less available for intestinal absorption. However, it was shown that it could be possible to select QPM for which more lysine could be available for this purpose (Dado, 1999).

The effects of low nitrogen and drought on several traits of normal maize have been largely documented and specific germplasm tolerant to these environmental stresses was developed. The key element in succeeding, in developing and releasing stress tolerant varieties was the identification and the development of Managed Stress Environments (MSEs) in Targeted Population Environment (TPE). The TPE was defined as large area where maize was cultivated under a defined stress whereas MSEs were defined as specialized sites used to manage a specific stress (Edmeades *et al*., 2006; Scott *et al*., 2003; Bnziger *et al*., 2000).

Current research effort on QPM focuses towards increasing its cultivation in the regions experiencing problems of malnutrition and where maize is the staple crop, especially in sub-Saharan Africa. In these regions, however maize is frequently produced under environmental stresses among which low soil nitrogen and drought are the most important. Impacts of low nitrogen and drought on grain yield of ordinarily maize have been extensively studied (Hirel *et al*., 2007; Edmeades *et al*., 2006), however the impacts of those stresses on major characteristics of QPM such as endosperm modification (endosperm hardness), lysine and tryptophan content in grain have not been studied at any extent. Thus, the objectives of this study were to describe and to estimate the impacts of low nitrogen and drought conditions on endosperm modification, protein and tryptophan concentrations in grains of QPM.

MATERIALS AND METHODS

The 13 QPM inbred lines were received from CIMMYT-Kenya and used to produce 41 QPM genotypes (Table 1) at KARI-Kiboko station (2°25'S; 37°75'E, 975 masl) in Kenya during October, 2004 to February, 2005 crop season. The 41 QPM genotypes and one normal

Table 1: Pedigrees of genotypes used in evaluation trials

Codes	Pedigree
G01+	[CML202/CML144]F2-1-1-3-B*4/[CML205/CML176]-B-2-1-B*3
G02+	[CML202/CML144]F2-1-1-3-B*4/[CML389/CML176]B-29-2-B*3
G03	[CML202/CML144]F2-1-1-3-B*4/[CML445/CML176]B-22-2-B*3
G04+	[CML202/CML144]F2-1-1-3-B*4/[CML389/GQL5]B-22-1-B*3
G05	[CML202/CML144]F2-1-1-3-B*4/[CML393/GQL5]B-22-1-B*3
G06+	[CML202/CML144]F2-1-1-3-B*4/[CML159/[MSR/POOL9]C1F2-205-1(OSU23i)-5-3-X-X-1-B-B]-B-10-1-B*3
G07	[CML202/CML144]F2-23-3-1-B*4/[CML205/CML176]-B-2-1-B*3
G08	[CML202/CML144]F2-23-3-1-B*4/[CML389/CML176]B-29-2-B*3
G09	[CML202/CML144]F2-23-3-1-B*4/[CML445/CML176]B-22-2-B*3
G10	[CML202/CML144]F2-23-3-1-B*4/[CML389/GQL5]B-22-1-B*3
G11	[CML202/CML144]F2-23-3-1-B*4/[CML393/GQL5]B-22-1-B*3
G12	[CML202/CML144]F2-23-3-1-B*4/[CML159/[MSR/POOL9]C1F2-205-1(OSU23i)-5-3-X-X-1-B-B]-B-10-1-B*3
G13	[CML202/CML144]F2-35-2-4-1-B*3/[CML205/CML176]-B-2-1-B*#
G14	[CML202/CML144]F2-35-2-4-1-B*3/[CML389/CML176]B-29-2-B*3
G15	[CML202/CML144]F2-35-2-4-1-B*3/[CML445/CML176]B-22-2-B*3
G16	[CML202/CML144]F2-35-2-4-1-B*3/[CML389/GQL5]B-22-1-B*3

Table 1: Continue

Codes	Pedigree
G17	[CML202/CML144]F2-35-2-4-1-B*3/[CML393/GQL5]B-22-1-B*3
G18	[CML202/CML144]F2-35-2-4-1-B*3/[CML159/[MSR/POOL9]C1F2-205-1(OSU23i)-5-3-X-X-1-B-B]-B-10-1-B*3
G19+	[CML202/CML144]F2-66-2-3-B*4/[CML205/CML176]-B-2-1-B*3
G20+	[CML202/CML144]F2-66-2-3-B*4/[CML389/CML176]B-29-2-B*3
G21	[CML202/CML144]F2-66-2-3-B*4/[CML445/CML176]B-22-2-B-B
G22+	[CML202/CML144]F2-66-2-3-B*4/[CML389/GQL5]B-22-1-B*3
G23	[CML202/CML144]F2-66-2-3-B*4/[CML393/GQL5]B-22-1-B*3
G24+	[CML202/CML144]F2-66-2-3-B*4/[CML159/[MSR/POOL9]C1F2-205-1(OSU23i)-5-3-X-X-1-B-B]-B-10-1-B*3
G25+	[CML205/CML182]-B-47-1-B*3/[CML205/CML176]-B-2-1-B*3
G26+	[CML205/CML182]-B-47-1-B*3/[CML389/CML176]B-29-2-B*3
G27	[CML205/CML182]-B-47-1-B*3/[CML445/CML176]B-22-2-B*3
G28+	[CML205/CML182]-B-47-1-B*3/[CML389/GQL5]B-22-1-B*3
G29	[CML205/CML182]-B-47-1-B*3/[CML393/GQL5]B-22-1-B*3
G30+	[CML205/CML182]-B-47-1-B*3/[CML159/[MSR/POOL9]C1F2-205-1(OSU23i)-5-3-X-X-1-B-B]-B-10-1-B*3
G31+	[CML389/CML176]B-11-1-B*3/[CML205/CML176]-B-2-1-B*3
G32+	[CML389/CML176]B-11-1-B*3/[CML389/CML176]B-29-2-B*3
G33	[CML389/CML176]B-11-1-B*3/[CML445/CML176]B-22-2-B*3
G34+	[CML389/CML176]B-11-1-B*3/[CML389/GQL5]B-22-1-B*3
G35	[CML389/CML176]B-11-1-B*3/[CML393/GQL5]B-22-1-B*3
G36+	[CML389/CML176]B-11-1-B*3/[CML159/[MSR/POOL9]C1F2-205-1(OSU23i)-5-3-X-X-1-B-B]-B-10-1-B*3
G37	[CML202/CML144]F2-1-1-3-B*/[CML390/GQL5]-B-18-1-B*3
G38	[CML202/CML144]F2-23-3-1-B*4/[CML390/GQL5]-B-18-1-B*3
G39	[CML202/CML144]F2-66-2-3-B*4/[CML390/GQL5]-B-18-1-B*3
G40	[CML205/CML182]-B-47-1-B*3/[CML390/GQL5]-B-18-1-B*3
G41	[CML389/CML176]B-11-1-B*3/[CML390/GQL5]-B-18-1-B*3
G42+	Normal Check: CML265/CML312

+Genotypes used for determination of protein and tryptophan concentrations in grain

(non QPM) check (CML265/CML312) were evaluated at Kiboko station in Kenya in March-August, 2005 and October, 2005 to February, 2006 crop seasons under optimum, low nitrogen and drought conditions and at ISAR-Rubona station (2°29'S; 29°46'E; 1650 masl) in Rwanda in March-August, 2005 crop season under optimum and low nitrogen environments.

The optimal environments at Kiboko received irrigation throughout the season and fertilizers were applied by supplying 64 kg N ha^{-1} and 46 P ha^{-1} at planting, 46 kg N ha^{-1} 4 weeks after planting and 46 kg ha^{-1} 7 weeks after planting. At Rubona, the optimal environments were achieved by applying 51 kg ha^{-1} N, 51 kg ha^{-1} P_2O_5 and 51 kg ha^{-1} K_2O before planting and 46 kg ha^{-1} N 6 weeks after planting. Water was supplied by rain as Rubona site did not have irrigation facilities to permit managed water supply.

The low nitrogen environments were achieved at Kiboko by not top-dressing nitrogen fertilizers during the season because soils were poor in nitrogen (Table 2). However, a starter nitrogen of 18 kg ha^{-1} was applied at planting to allow for uniform germination, emergence and early seedling growth. Phosphorus was applied at 46 kg ha^{-1} at planting while irrigation was provided during the cropping season. The field was thoroughly cleaned during plowing and all plant residues removed to reduce the effect of organic nitrogen. At Rubona because soils were relatively rich in nitrogen (Table 2), low nitrogen conditions were achieved by depleting nitrogen in the field following the procedures described by Bnzier *et al.* (2000). However, during planting, little nitrogen at a rate of 9 kg ha^{-1} was supplied. Water was supplied by rainfall.

Table 2: Some geographical characteristics of Kiboko and Rubona sites

Characteristics	Kiboko	Rubona
Altitude (masl)	975	1650
Latitude	2°25S	2°29S
Longitude	37°75'E	29°46'E
Soil pH	8.73	6.52
Annual rainfall (mm $year^{-1}$)	400	1020
Average annual temperature (°C)	23.9	19.8
C (%)	0.67	0.82
N (%)	0.05	0.16
C/N	15.35	5.14
P total (ppm)	131.30	144.4
Sand	71.50	72.00
Silt (%)	5.50	8.00
Loam (%)	23.00	20.00
Soil type	Sand-Clay-Loam	Sand-Clay-Loam

Drought environments were achieved at Kiboko by stopping irrigation 1 week before flowering. The field received 64 kg N ha^{-1} and 46 P ha^{-1} at planting, 46 kg N ha^{-1} 4 weeks after planting and 46 kg ha^{-1} 7 weeks after planting similar to the optimum environment. Drought environments were not used at Rubona as the site did not have irrigation facilities and rainfall was high and could make data under drought biased.

At Kiboko before planting, Furadan® 5G (composition: 5% w/w carbonfuran, 10% inert) was applied in rows and covered with little soil to control soil, germination and seedling pests. Additionally, an insecticide called buldock was applied 2 times: 3 weeks

after planting and 6 weeks after planting to control stem borers that are the major biological constraint to maize production at Kiboko.

The experimental design was an incomplete block design (alpha-lattice) with three replications. The plot was made of two rows of 5 m length with the distance between rows and hills measuring 0.75 and 0.25 m, respectively. Planting was performed by putting 2 seeds hill^{-1} and a thinning 3 weeks after planting to a reduced stand of 1 plant hill^{-1}. Thus, a planting density of 53,000 plants ha^{-1} was achieved. Weeding at both sites was performed as required.

The traits measured were Endosperm Modification (EM), Protein (PCG) and Tryptophan (TCG) concentrations in grain. EM scores were recorded on all genotypes and in all environments following the methodology of Ngaboyisonga *et al.* (2009), Vivek (2008) and Pixley and Bjarnason (2002). Ten best ears in each plot were identified and 10 kernels were taken in the middle of the ear to make 100 kernels. Kernels were weighted to obtain the Weight of Hundred Kernels (WHK). The 100 kernels were thereafter sorted and classified into 5 classes of endosperm modification (hardness) using a light table as described by Vivek (2008). The scores were based on appearance of kernel endosperm on the light table. The class 1 was made of 100% modified kernels and looked like those of normal maize. Classes 2-4 were defined as class 2: 75% hard and translucent and 25% soft and opaque; class 3: 50% hard and translucent and 50% soft and opaque; class 4: 25% hard and translucent and 75% soft and opaque. The class 5 comprised kernels that were 100% soft and opaque. Taking A as the number of kernels in class 1, B in class 2, C in class 3, D in class 4 and E in class 5, the endosperm hardness score of a plot was obtained by the equation:

$$EM=\frac{(A\times1)+(B\times2)+(C\times3)+(D\times4)+(E\times5)}{A+B+C+D+E}$$

PCG and TCG were determined on kernel samples of selected 16 QPM genotypes and the non-QPM check from trials of Kiboko environments. Ears harvested in each plot were dried at constant weight and five best ears were chosen. Approximately, 6 kernels from each selected ear having regular size were taken from the middle of the cob and formed a bulk of 30 kernels. The 30 kernels were sent to CIMMYT Cereal Quality Laboratory in Mexico for quality protein analysis. The determination of protein content and quality followed the procedures described by Vivek (2008) and Villegas (1984). The grain samples were finely grounded, the resulting flour was defatted and concentration of nitrogen and tryptophan (%) in grain were calorimetrically determined. The PCG (%) was obtained by multiplying the nitrogen concentration with a factor of 6.35. The estimation and description of effects of low nitrogen and drought were conducted through the combined analysis of variance using GenStat computer package program, Discovery, 3rd Edition (Buysse *et al.*, 2004). Pearson correlation coefficients between endosperm hardness, protein and tryptophan concentrations in grain and 6 agronomic traits were determined using GenStat computer package program, Discovery, 3rd Edition (Buysse *et al.*, 2004). The significance of the coefficients of correlation was obtained by determining an F-statistics by the equeston:

$$F=\frac{(n-2)r^2}{1-r^2}$$

Where:

r = The coefficient of correlation
n = The number of observations used to compute the coefficient of correlation
1 = The degree of freedom of the numerator
n-2 = The denominator using Genstat statistical computer package, Discovery, 3rd Edition (Buysse *et al.*, 2004)

The six agronomic traits included silking, Anthesis Silking Interval (ASI), height, stalk lodging, grain yield and Weight of Hundred Kernels (WHK). Silking was recorded in days by considering the days from planting to when 50% of plants in the plot showed silks. The ASI was obtained by the difference between silking and anthesis. Hence, anthesis was also recorded by considering the days from planting to when 50% of the plants in the plot shed pollen. The height was measured in m on ten plants in the plot from the soil up the 1st branch of the panicle. Grain yield was obtained by weighing the total ears harvested (Fresh Weight in kg, FW) and sampling kernels to obtain Grain Moisture (GM in %) using a portable moisture meter. All ears harvested in a plot were dried and weighted (Dry Weight in kg, DW) then shelled to obtain the Grain Weight (GW in kg). Taking A as the distance (m) between rows and B the distance (m) between hills at planting, C the length (m) of harvested rows and D the number of rows harvested, Grain Yield (GY) in ton ha^{-1} at 15% of grain moisture was obtained as:

$$GY=\frac{FW\times10}{A(B+C)D}\times\frac{100-GM}{100-15}\times\frac{GW}{DW}$$

RESULTS

Endosperm modification: The combined analysis of variance showed highly significant differences between environments ($p<0.01$) and genotypes except under drought where differences between environments were

Table 3: Combined analysis of variance across optimum, low nitrogen, drought for endosperm modification (1-5), protein concentration in grain (%) and tryptophan concentration in grain (%)

		Endosperm modification			Protein concentration in grains		Tryptophan concentration in grains	
Sources of variation	df	MS	F	df	MS	F	MS	F
Environments (E)	7	33.90	53.00***	5	50.123	2882.69***	0.005	229.33***
Optimum	2	3.26	25.10***	1	0.002	0.13NS	1.91×10^{-5}	1.52
Low N	2	4.59	20.00***	1	0.014	0.56NS	1.3×10^{-7}	0.01NS
Drought	1	0.60	0.30NS	1	0.002	0.15NS	2.6×10^{-5}	0.71NS
Crosses (C)	41	2.99	26.27***	16	2.815	161.88***	0.002	80.4***
Under optimum	41	0.85	9.92***	16	2.027	134.25***	8.6×10^{-4}	68.16***
Under low N	41	1.02	9.43***	16	2.336	91.2***	3.9×10^{-4}	23.6***
Under drought	41	1.69	10.33***	16	1.480	129.28***	9.7×10^{-4}	26.62***
E×C	287	0.25	2.19***	80	0.610	35.11***	9.6×10^{-5}	4.41***
Under optimum	82	0.21	2.41***	16	0.018	1.18NS	7.3×10^{-6}	0.58NS
Under low N	82	0.20	1.85***	16	0.005	0.18NS	4.1×10^{-6}	0.25NS
Under drought	41	0.36	2.17***	16	0.001	0.07NS	$\times10^{-5}$	0.27NS
Error	656	0.11	-	96	0.017	-	2.2×10^{-5}	-
Optimum	246	0.09	-	32	0.015	-	1.3×10^{-5}	-
Low N	246	0.11	-	32	0.026	-	1.7×10^{-5}	-
Drought	164	0.16	-	32	0.011	-	3.6×10^{-5}	-

***, **, *Significance at p<0.001, p<0.01, p<0.05, respectively; NSNon Significance (p>0.05)

not significant (p>0.05). Furthermore, the interaction environments x genotypes was highly significant (p<0.01) under all types of environments (Table 3). EM scores varied from 1.73-2.74 under optimum environments with a mean of 2.17 and a coefficient of variation of 13.5%. Under low nitrogen environments, the scores varied from 2.05-2.99 with an average of 2.60 and a coefficient of variation of 12.7 %. Under drought conditions, they varied from 2.62-4.00 with an average of 3.38 and a coefficient of variation of 11.98%. The scores of EM in low nitrogen environments were slightly higher than those from optimum conditions whereas those in drought environments were much higher. The coefficients of variation did not change much from one type of environments to another (Table 4).

The comparison of EM scores means from optimum environments with those from low N and drought conditions showed that the means of genotypes increased between 0 and 50% under low N and between 30 and 100% under drought environments. Furthermore, it showed that drought conditions increased considerably the scores of EM because 68% of genotypes experienced a score increase superior to 50% under drought whereas the increase of scores under low N did not go beyond 35% (Fig. 1).

The distribution of kernels in five classes of endosperm modification showed that the curve of distribution of kernels, characteristic of optimum environments was a decreasing exponential curve from class 1 (Fig. 2). The expected frequencies of classes obtained from the curve were class 1: 49.4%, class 2: 31.5%, class 3: 20.1%, class 4: 12.8% and classes 5: 8.2%. Class 1 and 2 together had a frequency >80%. The $\chi^2_{(df=4)} = 6.33^{NS}$ showed that observed and expected frequencies

Table 4: Endosperm modification (1-5) means under optimum, low nitrogen and drought conditions

Genotypes	Optimum	Low N	Drought	Mean
G01	2.30	2.69	3.58	2.86
G02	1.79	2.22	3.22	2.41
G03	2.03	2.56	3.21	2.60
G04	2.06	2.58	3.31	2.65
G05	1.73	2.21	3.45	2.46
G06	1.92	2.44	3.20	2.52
G07	2.31	2.76	3.75	2.94
G08	2.21	2.54	3.39	2.72
G09	2.23	2.81	3.92	2.99
G10	2.55	2.93	3.63	3.04
G11	2.27	2.66	3.32	2.75
G12	2.13	2.57	3.60	2.77
G13	1.90	2.05	2.62	2.19
G14	1.82	2.20	2.74	2.25
G15	1.94	2.57	2.94	2.48
G16	1.89	2.33	2.94	2.39
G17	2.03	2.15	2.77	2.32
G18	1.77	2.19	2.62	2.19
G19	2.14	2.64	2.89	2.56
G20	1.79	2.53	2.99	2.43
G21	1.87	2.71	3.32	2.63
G22	2.02	2.47	2.98	2.49
G23	1.90	2.31	3.38	2.53
G24	1.98	2.45	3.09	2.51
G25	2.25	2.66	3.77	2.89
G26	1.88	2.46	3.48	2.61
G27	2.16	2.65	3.70	2.84
G28	2.21	2.41	4.00	2.88
G29	2.34	2.64	3.25	2.74
G30	1.80	2.47	3.61	2.63
G31	2.44	2.85	3.60	2.96
G32	2.18	2.53	3.30	2.67
G33	2.43	2.80	3.94	3.06
G34	2.74	2.75	3.62	3.02
G35	2.48	2.83	3.70	3.00
G36	2.62	2.99	3.82	3.14
G37	2.04	2.40	3.56	2.67
G38	2.18	2.67	3.58	2.81
G39	2.08	2.48	3.24	2.60
G40	2.09	2.61	3.25	2.65
G41	2.27	2.87	3.19	2.78
G42	1.00	1.00	1.00	1.00
Mean	2.17	2.60	3.38	2.63
CV (%)	13.5	12.7	12.0	12.8
F	***	***	***	***

***Significant at p<0.001

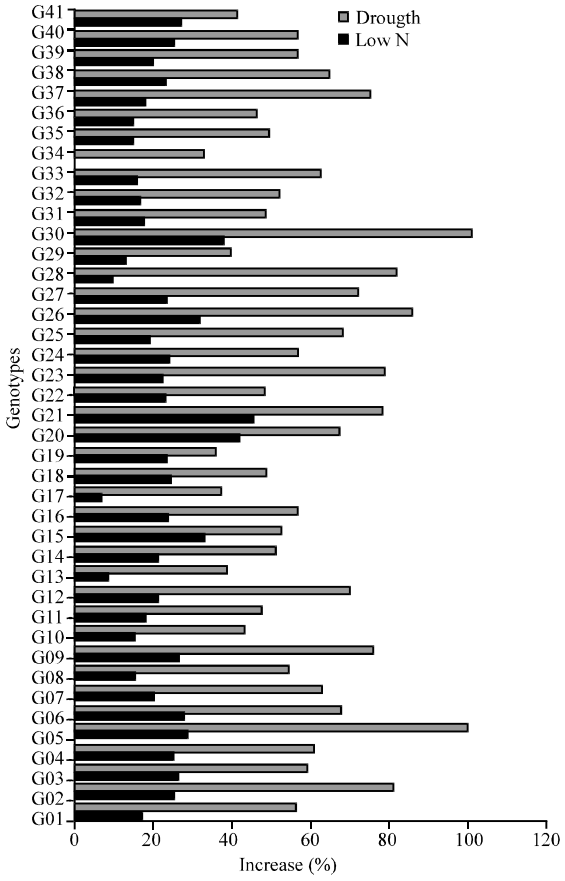

Fig. 1: Endosperm modification score increase (%) under low N and drought relative to optimum conditions

of classes were not significantly different (Fig. 1). The most frequent class was class 1 with approximately 50% of kernels.

The curve of the distribution of kernels in five classes under low nitrogen conditions was a quadratic curve of second order with decreasing concavity from a maximum in class 3 (Fig. 2). The expected frequencies of classes calculated from the curve of distribution were class 1: 20.9%, class 2: 31.2%, class 3: 32.1%, class 4: 23.7% and class 5: 5.8%. Class 2-4 had together a frequency above 80%. The $\chi^2_{(df=4)} = 3^{NS}$ showed that expected and observed frequencies were not different (Fig. 2). The most frequent class was class 3 with 32.1% of kernels.

The distribution of kernels in 5 classes under drought environments was a quadratic curve of 2nd order with decreasing concavity from class 1-3 and then increasing up to class 5 (Fig. 2). The expected frequencies of classes obtained from the curve of distribution were 15.6 for class 1, 14.7 for class 2, 17.0 for class 3, 22.4 for class 4 and 31.1 for class 5. Classes 3-5 occurred together at a frequency >70%. The $\chi^2_{(df=4)} = 3.39^{NS}$ showed that expected and

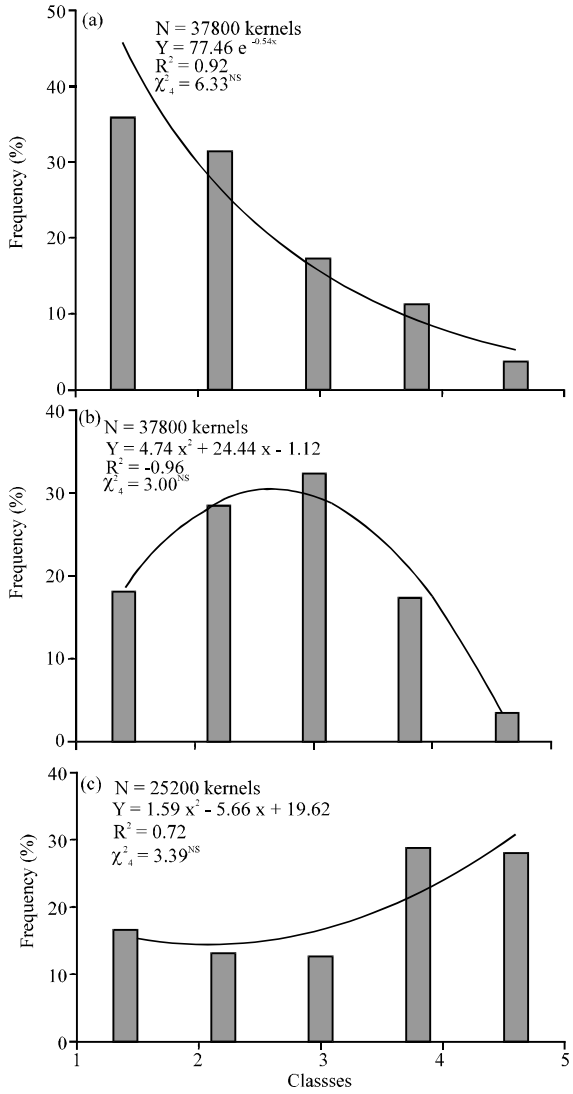

Fig. 2: Distribution of endosperm modification classes: a) Optimum environments; b) Low nitrogen environments; c) Drought environments. Similarity of the three frequency distributions: $\chi^2_8 = 67.9^{***}$

observed frequencies were not significantly different (Fig. 2). Class 5 was the most frequent with 31.1% of kernels.

Furthermore, the $\chi^2_8 = 67.9^{***}$ showed that the three frequency distributions of classes of endosperm modification were high significantly different (Fig. 2).

Protein and tryptophan concentrations in grain: The combined analysis of variance showed highly significant differences ($p<0.001$) between genotypes and between

environments for both PCG and TCG. The interaction environments x crosses was highly significant ($p<0.001$) (Table 3). The PCG varied from 8.19-11.00 under optimum environments with an average of 9.65 while under low nitrogen conditions, it changed from 6.67-9.94 with an average 7.75%. But under drought environment, it changed from 9.39-11.15 with an average of 10.41%. The data clearly showed that mean of genotypes under low N environments were inferior to those under optimum conditions whereas the mean of drought environments were superior to those of optimum conditions (Table 5). The TCG varied from 0.077-0.117 with an average 0.092 under optimum environments and from 0.063-0.089 with an average of 0.074 under low N while it varied from 0.088-0.119 with an average of 100% under drought conditions.

It was clear that means of genotypes in low N environments were inferior to these of optimum conditions whereas the means of genotypes in drought environments were superior to those of low N and optimum conditions. Besides in the 3 types of environments, the non-QPM check had a TCG of approximately 0.51% which was approximately a half of the TCG of QPM genotypes (Table 6).

The comparison of PCG means from optimum environments with those from low N and drought conditions showed that means of genotypes decreased under low N while they increased under drought (Fig. 3). Similarly, the comparison TCG means showed that means of genotypes decreased under low N and increased under drought environments (Fig. 4). Therefore, low N conditions decreased both PCG and TCG whereas drought conditions increased both of them (Fig. 3 and 4).

Correlation of endosperm hardness, protein and tryptophan concentrations in grain with six selected agronomic traits: The coefficient of correlation between EM scores and PCG was significant ($p<0.01$) and positive under optimum (0.33) negative under low N (-0.51) but not significant ($p>0.05$) under drought conditions. Furthermore, the coefficient of correlation between EM scores and TCG was not significant ($p>0.05$) in all environments implying no relationship between EM and TCG.

The correlation between EM scores and six selected agronomic traits was significant ($p<0.001$) and negative for grain yield and WHK (-0.33 and -0.45, respectively) under optimum environments, significant ($p<0.001$) and negative for WHK only in other environments (-0.34 under low N and -0.35 under drought) and not significant ($p>0.005$) for other traits (Table 7). The correlation coefficient between PCG and TCG was significant ($p<0.001$) and positive under optimum (0.54) and low

Table 5: Protein concentration in grain (%) means under optimum, low nitrogen and drought conditions

Genotypes	Optimum	Low N	Drought	Mean
G01	10.88	7.29	11.11	9.76
G02	11.00	7.69	11.14	9.94
G04	9.44	7.45	9.44	8.78
G06	8.19	7.88	10.51	8.86
G19	9.69	9.94	9.94	9.85
G20	9.87	7.71	11.06	9.55
G22	10.31	7.93	10.43	9.56
G24	8.94	7.13	9.39	8.48
G25	9.69	6.57	10.68	8.98
G26	9.44	7.72	10.37	9.18
G28	9.01	8.16	10.59	9.25
G30	9.20	6.89	9.81	8.63
G31	9.38	8.13	11.05	9.52
G32	9.77	8.44	11.15	9.79
G34	10.38	7.63	10.93	9.64
G36	9.08	6.97	9.69	8.58
G42	9.81	8.27	9.70	9.26
Mean	9.65	7.75	10.41	9.27
CV (%)	7.52	9.96	5.68	7.72
F	***	***	***	***

Table 6: Tryptophan concentration in grain (%) means under optimum, low nitrogen and drought conditions

Genotypes	Optimum	Low N	Drought	Mean
G01	0.100	0.089	0.109	0.099
G02	0.117	0.085	0.119	0.107
G04	0.077	0.074	0.111	0.087
G06	0.100	0.081	0.109	0.097
G19	0.095	0.080	0.109	0.094
G20	0.086	0.085	0.096	0.089
G22	0.105	0.074	0.105	0.095
G24	0.088	0.063	0.088	0.080
G25	0.092	0.064	0.119	0.091
G26	0.099	0.073	0.101	0.091
G28	0.092	0.071	0.097	0.087
G30	0.085	0.067	0.098	0.083
G31	0.093	0.078	0.104	0.091
G32	0.089	0.076	0.089	0.085
G34	0.111	0.081	0.111	0.101
G36	0.092	0.066	0.092	0.083
G42	0.050	0.049	0.052	0.051
Mean	0.092	0.113	0.100	0.102
CV (%)	10.83	11.21	11.16	11.07
F	***	***	***	***

***Significance at $p<0.001$

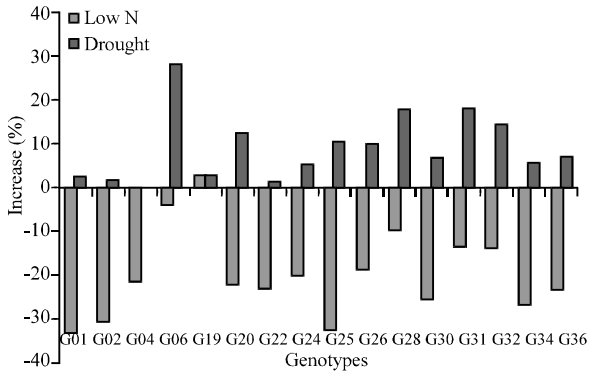

Fig. 3: Protein concentration in grain increase (%) under low N and drought relative to optimum conditions

Table 7: Pearson correlation coefficients between endosperm modification, protein and tryptophan concentrations and six other important agronomic traits of QPM

	Optimum			Low N			Drought		
Traits	EM	PCG	TCG	EM	PCG	TCG	EM	PCG	TCG
EM	1.00	0.33**	-0.01^{NS}	1.00	-0.51***	-0.06^{NS}	1.00	0.05^{NS}	-0.13^{NS}
PCG	0.33**	1.00	0.54***	-0.51***	1.00	0.39***	0.05^{NS}	1.00	0.04^{NS}
TCG	-0.01^{NS}	0.54***	1.00	-0.06^{NS}	0.39***	1.00	-0.13^{NS}	0.04^{NS}	1.00
SIL	-0.05	0.04	-0.33**	-0.06	0.00^{NS}	-0.08^{NS}	0.04^{NS}	0.05^{NS}	0.44***
ASI	0.09^{NS}	-0.13	-0.05^{NS}	0.08^{NS}	0.18^{NS}	-0.50***	0.05^{NS}	-0.07^{NS}	0.27**
HT	-0.16^{NS}	0.23*	-0.06^{NS}	0.04^{NS}	-0.34**	0.08^{NS}	-0.03^{NS}	0.10^{NS}	-0.15^{NS}
STL	0.01^{NS}	0.12^{NS}	-0.18^{NS}	0.02^{NS}	-0.16^{NS}	-0.24*	0.09^{NS}	0.32**	0.35**
GD	-0.33***	-0.10^{NS}	0.16^{NS}	-0.14^{NS}	-0.29**	-0.29**	-0.12^{NS}	-0.04^{NS}	-0.14^{NS}
WHK	-0.45***	-0.14^{NS}	-0.22^{NS}	-0.34**	0.03^{NS}	0.33**	-0.35**	0.31**	0.18^{NS}

EM: Endosperm Modification (1-5); PCG: Protein Concentration in Grain (%); TCG: Tryptophan concentration in grain (%); SIL: Days from planting to 50% Silking (days); ASI: Antheisis-Silking Interval (days); HT: Height (m); STL: Stalk Lodging (1-5); GD: Grain yield (ton ha^{-1} at 15% H_2O); WHK: Wight of 100 Kernels (g); ***,**,*Significance at p<0.001, p<0.05, p<0.05; ^{NS}No Significance (p>0.05)

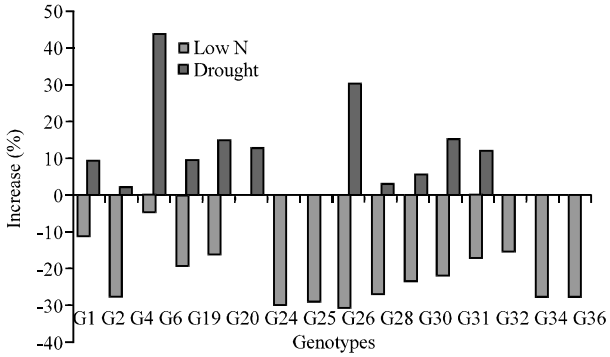

Fig. 4: Tryptophan concentration in grain increase (%) under low N and drought relative to optimum conditions

N (0.39) conditions but not significant under drought (p>0.05). The correlation of EM scores with grain yield and WHK was significant (p<0.001) and negative (-0.33, -0.45) under optimum conditions while it was significant and negative for WHK under low N (-0.34) and drought (-0.35). PCG and TCG were significantly (p<0.01) and negatively correlated (-0.29) with grain yield in low N environments and were not correlated in optimum and drought environments (p>0.05). Moreover, PCG was significantly (p<0.01) and positively correlated (0.31) with WHK under drought whereas TCG was significantly (p<0.01) and positively correlated with WHK (0.33) under low N, silking (0.44), ASI (0.27) and stalk lodging (0.35) under drought (Table 7).

DISCUSSION

Endosperm hardness: The EM scores of genotypes varied from 1.73-2.74 with an average of 2.17 in optimum environments. Hard and translucent kernels (classes 1-2) occurred at >80% implying that genetic modifiers worked and changed the soft, chalky and opaque endosperm of O2 maize into hard and translucent endosperm of QPM as reported by Sofi *et al.* (2009), Krivanek *et al.* (2007), Prasanna *et al.* (2001), Vasal (2001) and Vasal (2000). Under low nitrogen conditions, the EM scores of genotypes varied from 2.05-2.99 with an average of 2.60 and were slightly higher than those under optimum conditions. Partially modified kernels (classes, 2-4) occurred at >80%. Therefore, low N conditions (N deficits) partially suppressed the action of O2-genetic modifiers so that endosperm of kernels became partially hard, opaque and translucent. The EM scores of genotypes varied 2.62-4.00 with an average of 3.38 in drought environments and were much higher than those from optimum and low N conditions. Soft and opaque kernels (classes 3-5) occurred at frequency >70% indicating without doubt that drought suppressed or reduced significantly the action of O2-genetic modifiers so that endosperm of kernels became soft, chalky and opaque. It appeared therefore that N availability and much more water availability plays a vital role in endosperm modification of O2-maize and QPM. The biochemical mechanisms underlying the effects of low nitrogen and drought on endosperm hardness of QPM have not been investigated at any extent and therefore are not well understood. Perhaps, the effect of low nitrogen may be linked to the reduction in synthesis of γ27 kDA-zeins which may consequently soften the endosperm because γ27 kDA-zein families were found to be involved in endosperm modification (Pereira *et al.*, 2008; Moro *et al.*, 1995; Or *et al.*, 1993). Probably when there is a shortage of nitrogen, zein families with low molecular weight such as α19 kDa-zeins, γ16 kDa-zeins, δ10 kDA-zein and β14kDA-zein (Pereira *et al.*, 2008; Moro *et al.*, 1995; Or *et al.*, 1993) are preferentially synthesized instead of the families with high molecular weight like γ27 kDA-zeins with the consequence of softening the endosperm.

The explanation of biochemical effects of drought environments on endosperm modification may be found in altered endosperm starch structures of QPM or O2-modified endosperm (Gibbon and Larkins, 2005;

Gibbon *et al*., 2003). Gibbon *et al*. (2003) found that changes in starch structures in QPM were associated with increased swelling in water and the formation of tight contacts between starch granules in mature endosperm. Consequently, connections between altered starch granules after swelling in water were responsible of restoring the hardness and the translucence of endosperm of O2-modified endosperm. In the absence of water, swelling in water and formation of connections between altered starch granules did not happen and hence, the endosperm of QPM kernels in these circumstances was opaque, chalky and soft. Despite, these probable explanations of effects of low nitrogen and drought conditions there is a need to understand the biochemical processes underlying those phenomena to help selecting QPM genotypes less susceptible to N and water deficits. In fact, the effects of nitrogen and particularly of water deficit may have negative impacts on adoption of QPM varieties in stress prone areas where QPM is destined for direct human consumption. The grain harvested in fields with lower nitrogen levels and particularly if the crop has experienced drought during grain filling is of bad quality so that it is inappropriate to human consumption and therefore, no farmer would like to plant such maize variety. This is particularly important in sub-Saharan Africa where it is estimated that 77 of maize is used for human consumption, 12 serves as feed and 11% serves for other uses (Smale *et al*., 2011) where most of farmers do not afford fertilizers and where drought on maize at grain filling period is frequent. However, high variability between crosses showed that it is possible to develop QPM genotypes less susceptible to N and water deficits in low nitrogen and drought stress prone areas by testing and selecting under optimum environments and stressed environments (Ngaboyisonga *et al*., 2009).

Protein and tryptophan concentrations in grain: Results showed that differences in protein and tryptophan concentrations in grain among crosses existed in each environment. This variation which has been described to be under additive and maternal effects of gene action for PCG and non-additive effects of gene action for TCG (Ngaboyisonga *et al*., 2008) can be exploited for developing QPM genotypes with high levels of PCG and TCG. The results showed clearly that low nitrogen and drought environments reduced PCG but increased TCG. Furthermore, the normal check has levels of PCG comparable with those of QPM genotypes while it had TCG levels approximately a half of those of QPM genotypes in all environments as it has been reported by Sofi *et al*. (2009) and Krivanek *et al*. (2007). Hence, QPM genotypes remained nutritionally superior to normal maize under low N and drought conditions.

The changes caused by low nitrogen and drought environments on PCG in grain by reducing its quantity in kernels showed that nitrogen and water are greatly required in synthesis of amino acids and hence of proteins in maize in general and in QPM in particular (Hirel *et al*., 2007; Roberts *et al*., 2002; Mohammadkhani and Heidari, 2008, Virlouvet *et al*., 2011, Uchida, 2000). Thus if N and water are deficient in soil, less quantity of amino-acids and therefore of proteins are produced compared to non-deficient environments.

Low N and particularly drought environments surprisingly increased TCG. This phenomenon may be linked to the process of O2-endosperm modification and effects of low N and drought conditions on endosperm modification explained in this study. The modification of O2-endosperm from soft, chalky and opaque aspects to hard and vitreous endosperm is accompanied by a slight decrease in lysine and tryptophan levels (Sofi *et al*., 2009; Krivanek *et al*., 2007). This is why development of QPM involved laboratory analysis to monitor lysine and tryptophan in modified kernels and to select those with high levels (Parasanna *et al*., 2001; Vasal, 2000, 2001). Low N particular drought conditions suppress or reduce significantly the action of O2-endosperm modifiers making QPM to become partially or totally O2-maize as explained in this study. Hence tryptophan, lost because of O2-endosperm modification, may be liberated and consequently increase in grain under low N and drought conditions. Consequently, levels of tryptophan and hence of lysine in QPM genotypes under low N nitrogen and drought conditions become much higher than in optimum environments. Moreover, it was shown in this study that QPM does not lose its nutritional superiority over normal maize under low N and drought conditions; instead it gains more nutritional quality as the levels of tryptophan in grain increase. However, this nutritional advantage of QPM in stressed environments is lost with the grain yield reduction and the reduction of kernel quality due to the appearance of chalky, opaque and soft phenotypes.

Correlation of endosperm hardness, protein and tryptophan concentrations in grain with six selected agronomic traits: Results on correlation of EM scores with PCG showed that they were positively correlated under optimum conditions, negatively correlated in low N environments and not correlated in drought environments. The positive correlation in optimum environments implied that factors responsible of increasing endosperm EM scores of QPM increased PCG. Also, Pixley and Bjarnason (2002) found a positive but non-significant correlation between EM scores and PCG. On the contrary in low N environments factors responsible of increasing EM scores of QPM genotypes

reduced PCG. These factors may be linked to the reduction in synthesis of high molecular weight γ27 kDA-zeins accompanied with increase of EM scores and an increase in synthesis of low molecular weight such as α19, γ16, δ10 and β14 kDA-zein and consequently reduction of N accumulated as shown in this study. The correlation between EM scores and PCG under drought environments was not significant indicating that factors responsible of EM scores increase had little or no effect on PCG. The correlation between EM scores and TCG was not significant in all environments indicating weak or no relation between the two traits.

On the contrary, Betran *et al.* (2006) and Scott *et al.* (2004) found in their studies, significant and negative correlations between EM scores and TCG implying that factors responsible of increasing EM scores were responsible of reducing TCG. The coefficient of correlation between PCG and TCG was positive and significant under optimum and low N environments but not significant in drought environments indicating that factors underlying the increase of PCG elevated TCG in those environments as well. Pixley and Bjarnason (2002), Scott *et al.* (2004) and Betran *et al.* (2006) found also a positive and significant correlation between PCG and TCG, hence agreed with this study. In fact, any reduction in synthesis of proteins reduces tryptophan accumulation and any increase in protein elevates tryptophan as well making protein levels in grain to be positively correlated with tryptophan content in grain. Moreover, there was no association between PCG and TCG under drought conditions indicating that factors underlying the two traits under those conditions were different.

The strong and negative correlation coefficient between EM scores, grain yield and WHK indicated that factors underlying dry matter accumulation in grain under optimal environments were responsible of reducing EM scores of QPM genotypes. Interesting are the strong and positive correlations of TCG with silking, ASI and stalk lodging under drought environments. It has been explained in this study that drought conditions (water deficit) suppress the action of O2-endosprm modifiers making the QPM to revert to O2-maize phenotype, i.e., chalky, opaque and soft characteristics. This action affects several agronomic traits by increasing silking time and ASI and making plants very susceptible to stalk lodging (Krivanek *et al.*, 2007; Parasanna *et al.*, 2001). At the same time, levels of tryptophan in grain become increased through the process explained in this study making TCG positively correlated with silking, ASI and stalk lodging. It appears therefore that drought conditions not only affect endosperm modification by suppressing or reducing significantly the action of modifiers but also increase the flowering times and the susceptibility to stalk lodging.

CONCLUSION

Low N conditions soften endosperm and reduce endosperm modification of QPM but drought conditions considerably soften endosperm of QPM by increasing EM score by >50% by inactivating the action of O2-endosperm modifiers, hence undesirable effects of *O2* gene (chalk, opaque and soft phenotypes) appear again. It appears, therefore that nitrogen particularly water plays a vital role in modification of the O2-endosperm. The absence or the deficit in nitrogen and more importantly in water availability at critical stages of O2-endosperm formation significantly suppresses or reduces the action of modifiers. Low N and drought conditions reduce significantly protein concentration in grains of QPM genotypes including the non-QPM check and this show that nitrogen and water are needed in synthesis of amino acids and in accumulation of proteins in QPM.

Low N particularity drought conditions increase the levels of tryptophan in grains. It appears therefore; low N and drought increase the nutritional advantage of QPM in stressed environments, however this advantage is completely lost because of the grain yield reduction and reduction of kernel quality due to the appearance of chalky, opaque and soft phenotypes. Furthermore, under low N and drought conditions, levels of tryptophan, consequently of lysine in grains remain high so that the nutritional advantage of QPM genotypes is not reduced. However, drought conditions not only affect endosperm modification by inactivating or reducing significantly the action of O2-endosperm modifiers but also adversary affects other agronomic traits such as silking time, ASI and the susceptibility to stalk lodging.

Conclusively, adverse effects of low N and particularly of drought on endosperm modification raise important concerns about QPM quality for human consumption and adoption of QPM varieties in stress prone areas such as sub-Saharan Africa where maize is importantly used for human consumption where most farmers do not afford fertilizers and where drought on maize is frequent.

By partially or totally suppressing the action of modifiers and therefore making modified endosperm to become partially or totally opaque, chalky and soft with appearance of several other undesirable effects, low N and drought make the grain harvested inappropriate for human consumption. However, there is a high variability among genotypes so that it is possible to develop QPM varieties less susceptible to low N and drought for stress prone areas by testing and selecting under both optimum and stressed environments.

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