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Quality Protein Maize Under Low-Nitrogen and Drought: Genotype by Environment Interaction for Grain and Protein Qualities

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Abstract: Maize (*Zea mays* L.) is used, as food and feed, although it is deficient in 2 essential amino acids, namely; lysine and tryptophan. Quality Protein Maize (QPM) developed by combining 2 genetic systems: Mutant *opaque-2* (*o2*) gene and o2-endosperm modifiers has about twice the amount of lysine and tryptophan of normal maize. It can be used to correct the deficiency of maize in protein quality. QPM cropping is expanding in the regions experiencing malnutrition where maize is frequently produced under low-nitrogen and drought environments. The interactions of these stresses with QPM genotypes are not well understood. Therefore, this study was undertaken to determine how low-N and drought interact with QPM genotypes in terms of grain and protein qualities. About 36 QPM genotypes were evaluated at Kiboko in Kenya in 2005 and 2006 under optimum environments, low-nitrogen and drought and at Rubona in Rwanda in 2005 under optimum and low-N environments. The AMMI (Additive Main effects and Multiplicative Interaction) models for endosperm modification, protein and tryptophan concentrations in grain were used to analyze data. Results showed that low-N, particularly drought interacted with QPM genotypes by reducing endosperm modification and making QPM partially or totally soft and opaque. Low-N interacted with QPM by reducing the amount of protein and tryptophan in grain while drought interacted with them by increasing the amount of protein and tryptophan in grain. Despite strong interactive forces of environments, the QPM genotypes G02 ([CML202/CML144] F2-1-1-3-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)andG22([CML202/CML144]F2-66-2-3-B*4/[CML389/GQL5]B-22-1-B*3)were stable in all traits across environments. The genotypes G02 and 20 had the same male parent whereas G20 and 22 had the same female parent. Hence, it is possible to identify QPM lines that can provide stable QPM varieties for EM, PCG and TCG under drought and low N environments. The interaction of drought with QPM genotypes for endosperm modification may have negative impact on adoption of QPM varieties in stress prone areas where QPM is destined for human consumption. The grain harvested in fields that have experienced drought would be of bad kernel quality so that it is inappropriate to human consumption. Therefore, no farmer would like to grow such maize variety. However, the existence of stable genotypes that exhibit low interaction effects shows that it is possible to develop QPM genotypes that may be easily acceptable in those drought prone areas.

Key words: Drought, endosperm modification, genotypes×environments interaction, low-N, QPM, grow, protein concentration in grain, tryptophan concentration in grain, quality protein maize

INTRODUCTION

Maize (*Zea mays* L.) is used as staple food crop for millions of people in sub-Saharan Africa providing daily intake of carbohydrates, proteins and lipids (Nuss and Tanumihardjo, 2011). 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 2 essential amino acids: Lysine and tryptophan (Sofi *et al*., 2009; Krivanek *et al*., 2007). The Quality Protein Maize (QPM) has about twice the levels of lysine and tryptophan in grain compared to normal maize (Vivek *et al*., 2008). Hence, it can correct the deficiency of normal maize in lysine and tryptophan. It was developed by combining the genetic systems of the gene mutant *opaque-2* (*o2*) and o2-endosperm modifiers. In fact, *o2*-gene creates several adversary effects such as low grain yield, soft, chalk and opaque kernel phenotypes and high incidence of ear rots. The o2-endosprm modifiers alter these undesirable effects of *o2* gene and the modified endosperm of QPM becomes vitreous and hard. Thus, QPM varieties look like normal maize and have similar grain yields and other agronomic traits (Sofi *et al*., 2009; Krivanek *et al*., 2007).

Current research effort on QPM focuses towards increasing its cultivation in the regions experiencing problems of malnutrition and where maize is the staple

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crop, especially in sub-Saharan Africa (Kirivanek *et al*., 2007). In these regions, however maize is frequently produced under low soil nitrogen and drought (Mhike *et al*., 2012; Worku *et al*., 2007) and maize production will be significantly constrained by climate changes (Cairns *et al*., 2013).

The interactions of low nitrogen and drought with genotypes of normal maize have been largely documented and specific maize cultivars relatively adapted to these stresses were identified, released and utilized in prone areas including sub-Saharan Africa (Anley *et al*., 2013; Hirel *et al*., 2007; Edmeades *et al*., 2006; Campos *et al*., 2004). Also, QPM varieties are being and will be cultivated in low-N and especially drought prone environments where it is not well known how these stresses interact with QPM grain quality especially endosperm modification and protein quality especially protein, tryptophan and lysine content in grain. Therefore, this investigation had the objective of knowing how low-N and drought interact with QPM genotypes for endosperm modification (grain quality), protein, lysine and tryptophan content in grain (protein quality).

MATERIALS AND METHODS

About 12 QPM inbred lines (Table 1) were received from CIMMYT-Kenya and used to produce 36 QPM genotypes (Table 2) at KARI-Kibiko (Table 3) in Kenya during October, 2004 to February, 2005 crop season. The QPM genotypes were thereafter evaluated at KARI-Kiboko station (Table 3) in March to August, 2005 and October, 2005 to February, 2006 crop seasons under optimum, low-N and drought. Moreover, they were evaluated at ISAR-Rubona station (Table 3) in Rwanda in March to August, 2005 crop season under optimum and low-N. Drought environments were not used at Rubona because the amount of annual rain in this site was relatively high (Table 3) and relatively evenly distributed in all months hence this could make data under drought biased.

Table 1: The 12 QPM inbred used to generate the genotypes

Parent	Pedigree	Protein quality
FP1	[CML202/CML144] F2-1-1-3-B*4^{+}	High
FP2	[CML202/CML144] F2-23-3-1-B*4	High
FP3	[CML202/CML144] F2-35-2-4-1-B*3	Medium
FP4	[CML202/CML144] F2-66-2-3-B*4^{+}	Low
FP5	[CML205/CML182]-B-47-1-B*3^{+}	Low
FP6	[CML389/CML176] B-11-1-B*3^{+}	High
MP1	[CML205/CML176]-B-2-1-B*3^{+}	High
MP2	[CML389/CML176] B-29-2-B*3^{+}	High
MP3	[CML445/CML176] B-22-2-B*3	High
MP4	[CML389/GQL5]B-22-1-B*3^{+}	Low
MP5	[CML393/GQL5] B-22-1-B*3	High
MP6	[CML159/[MSR/POOL9] C1F2-205-1 (OSU23i)-5-3-X-X-1-B-B]-B-10-1-B*3^{+}	Low

$^{+}$Parent inbred lines for which protein quality was determined in crosses; FP = Female Parent; MP = Male Parent

Table 2: Pedigrees of 36 QPM genotypes used in evaluation trials

Names	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
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

Table 2: Continue

Names	Pedigree
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

+Genotypes of which protein quality was determined; G = Genotype

Table 3: Geographical characteristics of Kiboko and Rubona sites

Characteristics	Kiboko	Rubona
Altitude (masl)	975	1650
Latitude	2°25S	2°29S
Longitude	37°75E	29°46E
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

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 this site did not have irrigation facilities to permit managed water supply.

The low-N environments were achieved at Kiboko by not top-dressing nitrogen fertilizers during the season because soils were poor in nitrogen (Table 3). 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. At Rubona, because soils were relatively rich in nitrogen (Table 3), low-N conditions were achieved by depleting nitrogen in the field following the procedures described by Banziger *et al.* (2000). However during planting, little nitrogen at a rate of 9 kg ha^{-1} was supplied. Water was supplied by rainfall. 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.

The experimental design was an incomplete block design (alpha-lattice) with 3 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 per hill and a thinning 3 weeks after planting reduced the stand of 1 plant per hill to achieve a planting density of 53.000 plants ha^{-1}.

The traits measured were Endosperm Modification (EM), Protein (PCG) and Tryptophan (TCG) concentrations in grain. Lysine concentration in grain was not determined the correlation between lysine and tryptophan content in grain of QPM genotypes was reported to be 0.99 (Vivek *et al.*, 2008). Therefore, any conclusion to tryptophan would apply to lysine as well.

EM scores were recorded on all genotypes and in all 8 environments following the methodology of Ngaboyisonga *et al.* (2009), Vivek *et al.* (2008) and Pixley and Bjarnason (2002). About 100 kernels from each plot were sorted and classified into 5 classes of endosperm modification using a light table, as described by Vivek *et al.* (2008). The class 1 was made of 100% modified kernels and looked like those of normal maize. Classes 2-4 were defined as follows: 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 EM score of a plot was obtained by the formula:

$$EM = \frac{(A\times 1)+(B\times 2)+(C\times 3)+(D\times 4)+(E\times 5)}{A+B+C+D+E}$$

PCG and TCG were determined on kernel samples of selected 16 QPM genotypes (Table 2) in Kiboko environments (6 environments). Approximately 6 kernels were taken from five selected ear in each plot 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 *et al.* (2008) and Villegas *et al.* (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 AMMI (Additive Main effects and Multiplicative Interactions) was used to analyze the Genotype×Environments Interaction (GEI) (Gauch, 1992). The means and IPCA1 (Interaction Principal Component Axis) scores were used to form the AMM1 biplots while IPCA1 and 2 scores were used to form the AMM2 biplots. The AMMI analysis of variance was performed using genstat statistical computer package, Discovery, 4rd edition (Buysse *et al*., 2007) while biplots were constructed using excell spreadsheet.

RESULTS

The AMMI analysis of variance for EM of 36 QPM genotypes across eight environments showed that the variation due to genotypes, environments and GEI were highly significant ($p<0.01$) The genotype effects accounted for 10.7% of the treatment Sums Squares (SS), environment effects 68.4 and GEI 20.9%. Moreover, this analysis revealed that the 1st 4 IPCAs (Interaction Principal Component Axis) were high significant ($p<0.005$) and captured 80.4% of GEI (Table 4). IPCA1 axis captured 41.7% of GEI, IPCA2 16.4% while both IPCA1 and 2 captured 58.8% of the GEI.

The results of AMMI analysis of variance for PCG and TCG of 16 QPM genotypes across 6 environments revealed that variations due to genotypes, environments and GEI were highly significant ($p<0.01$). The genotype effects explained 13.3% of treatment SS, environment effects 73.2% and GEI effects 13.5% for PCG whereas they captured 10.8, 80.9 and 8.3%, respectively for TCG (Table 5). IPCA1 explained 72.9% of GEI for PCG and 53.0 for TCG, IPCA2 captured 26.7% of GEI for PCG and 39.2 for TCG. IPCA1 and 2 together captured 99.6% for PCG and 92.2% for TCG.

The AMMI1 biplot for EM (Fig. 1) showed that the means of optimum environments (E1, 4 and 6) lied between abscissas 2.0 and 2.5, the means of low-N environments (E2, 4 and 7) between 2.5 and 3.0 while the means of drought environments (E3 and 8) were superior to 3.3. environments E7 and 8 were situated between ordinates -0.2 and +0.2 whereas other environments were either below ordinate -0.2 (E4, 5 and 6) or above ordinate +0.2 (E1 and 2). Furthermore, it revealed three distinct clusters of genotypes. The first cluster comprising the genotypes: G02, G06, G017, G21, G22 and G27 was made by genotypes having means inferior to 2.5 and IPCA1 scores between -0.2 and +0.2. The second comprised the genotypes: G03, G18, G20, G26, G29, G34 and G36 with means inferior to overall mean (2.64) and IPCA1 scores between -0.3 and 0.3. The third had either genotypes with means superior to the overall mean, IPCA1 scores inferior to -0.3 or superior to +0.3.

The AMM1 biplot for PCG (Fig. 2) showed that the means of optimum environments (E1 and 4) were approximately 9.6%, the means of low-N (E2 and 5) environments were approximately 7.7% whereas the means of drought environments (E3 and 8) were approximately 10.4%. Furthermore, it showed that the means of genotypes G20, 22 and 26 were superior to the overall mean and had IPCA1 scores between -0.3 and +0.3 and hence, they were close to ordinate zero. Other genotypes had either means inferior to overall means or IPCA1 scores inferior to -0.3 or superior to +0.3 and therefore, they were far from the ordinate zero.

The AMM1 biplot for TCG (Fig. 3) showed that the means of optimum environments (E1 and E4) were around

Table 4: AMMI analysis of variance for endosperm modification

Sources of variation	DF	SS	MS	F
Total	863	396.37	0.46	
Treatments	287	316.91	1.10	8.92***
Genotypes	35	33.95	0.97	7.83***
Environments	7	216.60	30.94	48.99***
REP/ENV	16	10.11	0.63	5.10***
GEN×ENV	245	66.32	0.27	2.19***
IPCA1	41	27.66	0.68	5.45***
IPCA2	39	10.85	0.28	2.25***
IPCA3	37	8.05	0.22	1.76**
IPCA4	35	6.75	0.19	1.56*
IPCA5	33	5.24	0.16	1.28NS
Residuals	60	7.78	0.13	1.05NS
Error	560	69.36	0.13	

Table 5: AMMI analysis of variance for protein and tryptophan concentrations in grain

Sources of variations	DF	SS	MS	F	SS	MS	F
Total	191	340.50	1.783		0.0513	2.7×10^{4}	
Treatments	95	338.70	3.565	195.60***	0.0506	5.3×10^{4}	80.46***
Genotypes	15	45.01	3.001	164.70***	0.0054	3.6×10^{4}	54.81***
Environments	5	248.09	49.619	1485.10***	0.0409	0.0089	562.60***
ENV/REP	6	0.20	0.033	1.83 NS	0.0001	1.5×10^{5}	2.20NS
GEN×ENV	75	45.59	0.608	33.35***	0.0042	5.6×10^{5}	8.47***
IPCA1	19	33.25	1.750	96.01***	0.0022	1.2×10^{4}	17.71***
IPCA2	17	12.18	0.717	39.32***	0.0017	9.7×10^{5}	14.62***
IPCA3	15	0.10	0.006	0.35 NS	0.0002	1.3×10^{5}	2.01*
Residuals	24	0.07	0.003	0.15 NS	0.0001	5.6×10^{6}	0.85NS
Error	90	1.64	0.018		0.0006	6.6×10^{6}	

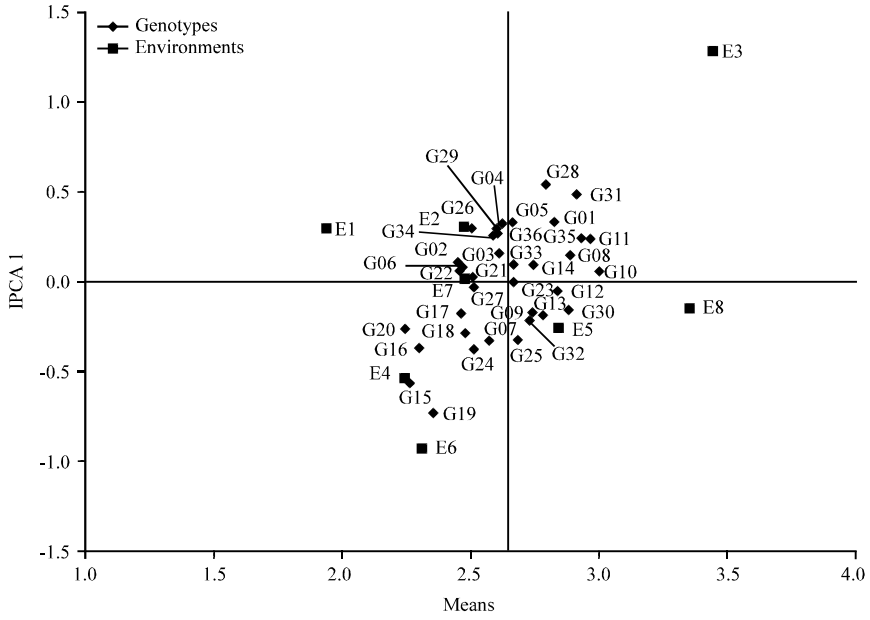

Fig. 1: Biplot of endosperm modification obtained by plotting the means against IPCA1 for 36 crosses evaluated in 8 environments; E1 = Kiboko-optimum-2005; E2 = Kiboko-low N-2005; E3 = Kiboko-drought-2005; E4 = Rubona-optimum-2005; E5 = Rubona-low N-2005; E6 = Kiboko-optimum-2006; E7 = Kiboko-low N-2006; E8 = Kiboko-drought-2006

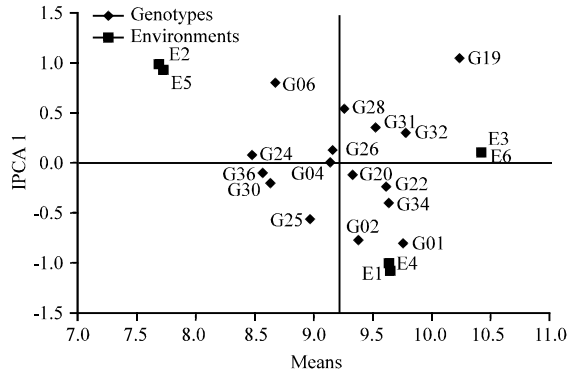

Fig. 2: Biplot of protein concentration in grain obtained by plotting the means against IPCA1 for 16 crosses evaluated in 6 environments; E1 = Kiboko-optimum-2005; E2 = Kiboko-low-2005; E3 = Kiboko-drought-2005; E4 = Kiboko-optimum-2006; E5 = Kiboko-low N-2006; E6 = Kiboko-drought-2006

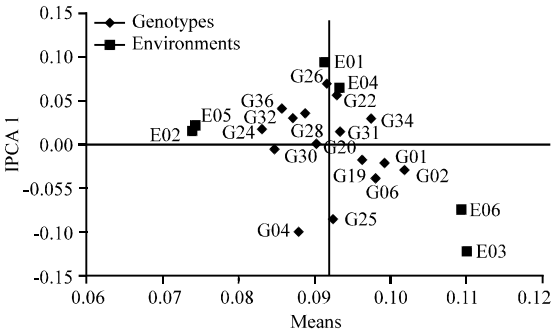

Fig. 3: Biplot of tryptophan concentration in grain obtained by plotting the means against IPCA1 for 16 crosses evaluated in 6 environments; E1 = Kiboko-optimum-2005; E2 = Kiboko-low N-2005; E3 = Kiboko-drought-2005; E4 = Kiboko-optimum-2006; E5 = Kiboko-low N-2006; E6 = Kiboko-drought-2006

the overall means (0.091%) while those of low-N (E2 and 5) were approximately 0.074% and those of drought (E3 and 6) were approximately 0.11%. The genotypes G01, 02, 19, 31 and 34 had means superior or equal to the overall mean (0.091) and IPCA1 scores between -0.3 and +0.3, therefore they were close to abscissa zero while other genotypes were far from this abscissa or had means superior to overall mean.

The AMMI2 biplot (Fig. 4) for EM showed that all environments were situated far from the origin. Environments E1, 2, 5 and 7 had intermediate spokes while E3, 6 and 8 had very long spokes. Angles between the vectors of E4 and 5, E2 and 1 and E7 and 8 were inferior to 15°. About 5 genotypes: G02, 08, 09, 12, 22 and 27, having IPCA1 scores between -0.2 and +0.2 and IPCA2 scores between -0.2 and +0.2 formed a

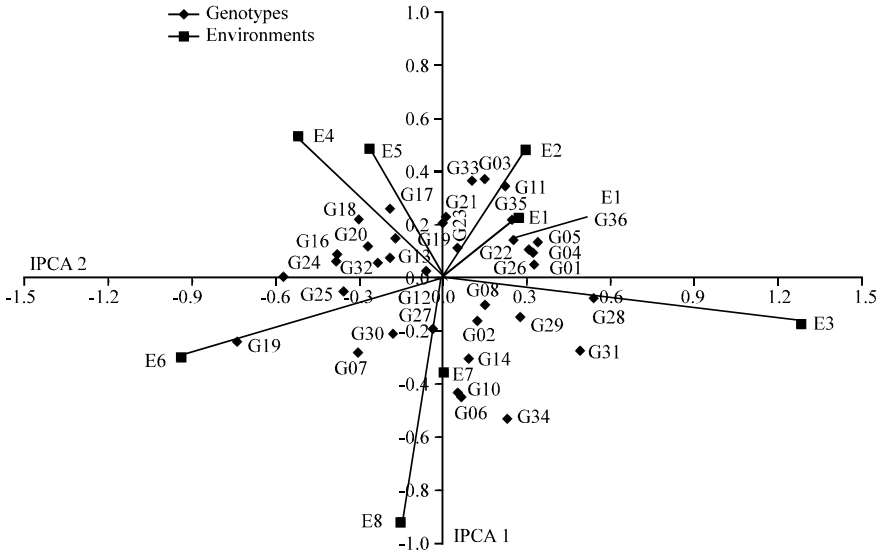

Fig. 4: Biplot of endosperm modification obtained by plotting IPCA1 against IPCA2 for 36 crosses evaluated in 8 environments; E1 = Kiboko-optimum-2005; E2 = Kiboko-low N-2005; E3 = Kiboko-drought-2005; E4 = Rubona-optimum-2005; E5 = Rubona-low N-2005; E6 = Kiboko-optimum-2006; E7 = Kiboko-low N-2006; E8 = Kiboko-drought-2006

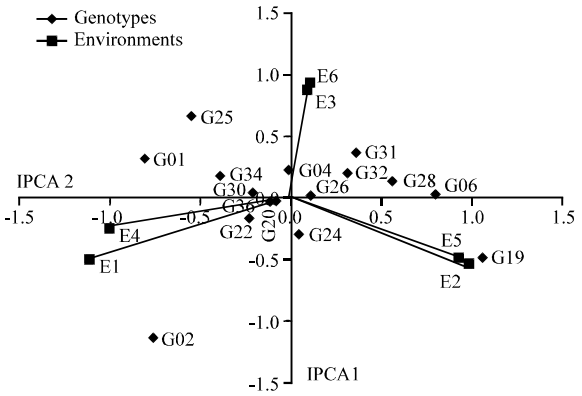

Fig. 5: Biplot of protein concentration in grain obtained by plotting IPCA1 against IPCA2 for 16 crosses evaluated in 6 environments; E1 = Kiboko-optimum-2005; E2 = Kiboko-low N-2005; E3 = Kiboko-drought-2005; E4 = Kiboko-optimum-2006; E5 = Kiboko-low N-2006; E6 = Kiboko-drought-2006

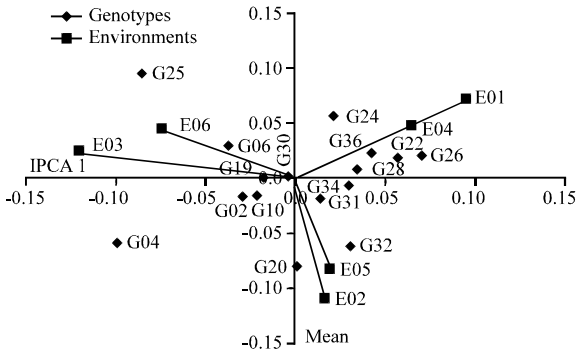

Fig. 6: Biplot of tryptophan concentration in grain obtained by plotting IPCA1 against IPCA2 for 16 crosses evaluated in 6 environments; E1 = Kiboko-optimum-2005; E2 = Kiboko-low N-2005; E3 = Kiboko-drought-2005; E4 = Kiboko-optimum-2006; E5 = Kiboko-low N-2006; E6 = Kiboko-drought-2006

cluster of genotypes very close to the origin. About 6 genotypes: G06, 12, 21, 22, 23 and 27 with IPCA1 scores between -0.1 and +0.1 were very close to IPCA1 axis (IPCA1 zero).

The AMMI2 biplot for PCG (Fig. 5) and TCG (Fig. 6) showed that all environments had long spokes. The vectors of environments of the same type made angles <30° whereas the vectors of environments of different types made angles between 90 and 270°. About 7 genotypes for PCG: G04, 20, 22, 24, 26, 30 and 36 and with IPCA scores between -0.3 and +0.3 were close to the origin while 6 genotypes: G01, 02, 19, 30, 31 and 34 with IPCA scores between -0.3 and +0.3 were considered close to the origin.

DISCUSSION

In AMMI analysis of variance, the treatment variation is subdivided into 3 types of variations:

Variation due to genotypes, variation due to environments and variation due to GEI effects. These 3 sources of variation present different problems and opportunities: The genotypes variation pertains to broad adaptations, the GEI variation pertains to narrow adaptations whereas genotypes and GEI variations jointly determine mega-environments (Gauch, 2006). In the present study, the variation due to environments was far important (>65% of treatments SS) than that of genotypes jointly with GEI effects for the traits under study implying that localized environments effects were more important than mega-environments and genotypes adaptations. There are several studies where environments variation is far important than the 2 other components (Beyene *et al*., 2011; Tonk *et al*., 2011; Mwololo *et al*., 2009; Sadeghi *et al*., 2011) including other crops than maize (Riaz *et al*., 2013; Thiyagu *et al*., 2012). Cases where either genotypes variation or GEI variation were predominant over environments variation have been also reported (Arulselvi and Selvi, 2010; Anandan *et al*., 2009). However, Yan and Tinker (2005) estimated that cases where environment variation was dominant were more frequent.

The AMMI analysis of variance showed that the 2 first IPCA axes captured at least 58% of GEI for the 3 traits under study, hence they were helpful in predicting and analyzing the complexity of the GEI through AMM1 and 2 biplots. This is because AMMI selectively recovers pattern in the first IPCAs whereas it recovers noise in the last IPCA axes (Gauch, 1992).

In AMMI 1 biplot, the usual interpretation of a biplot is that displacements along the abscissa indicate differences in main (additive) effects whereas displacements along the ordinate indicate differences in interaction effects. Genotypes that group together have similar interactions with environments while environments which group together interact with the genotypes in the same way. When a genotype and an environment have the same signs on the IPCA axis, their interaction is positive and if the signs are different, their interaction is negative. If a genotype has high mean (mean>overall mean) and an IPCA1 score closer to zero (near the abscissa), it has small interaction effects and it is considered, as stable across environments (Gauch, 1992).

The AMMI1, biplot for EM clearly showed that low-N, particularly drought environments interacted with QPM genotypes by increasing EM scores, hence reducing endosperm modification so that under low-N, QPM kernels appeared partially soft and opaque while under drought they appeared almost or totally soft and opaque. In fact, Ngaboyisonga *et al*. (2012) showed that nitrogen deficit reduced the action of o2-endosperm modifiers whereas water deficit suppressed them making QPM kernels appearing partially or totally soft and opaque. The genotypes: G02, 06, 017, 21, 22 and 27 were stable across environments for EM because they had means inferior to 2.5 and IPCA1 scores between -0.2 and +0.2. It is important to mention that for EM, lowest scores indicate a high degree of modification with endosperm more vitreous and hard.

The AMM1 biplots for PCG and TCG clearly showed that low-N environments interact with QPM genotypes by significantly reducing the amount of protein and tryptophan in grain (up to 0.074% for TCG) so that QPM loses its protein quality. In fact, modified o2-endosperms are considered to have QPM qualities if the amount of tryptophan in grain is more than 0.075% (Vivek *et al*., 2008). On the contrary, the AMMI1 biplots showed that drought environments interact with QPM genotypes by increasing the amount of proteins and tryptophan in grain. This increase is probably caused by the fact that under water deficits most of QPM kernels become o2 kernels (Ngaboyisonga *et al*., 2012). Additionally, they showed that genotypes G20, 22 and 26 for PCG and genotypes G01, 02, 19, 31 and 34 for TCG were relatively stable across environments because they had means superior to overall mean and IPCA1 scores between -0.3 and +0.3.

The AMMI 2 biplot presents the spatial pattern of the first 2 IPCA axes and helps in visual interpretation of the GEI patterns and identify varieties or locations that exhibit low, medium or high levels of interaction effects. Genotypes near the origin are non-sensitive to environmental interactive forces and those distant from the origin are sensitive and have large interactions. Cultivar and site vectors are defined as vectors from the origin (0, 0) to the end points determined by their marks. Genotypes that appear close together exhibit similar behavior whereas those that are far apart exhibit dissimilar behavior. An angle inferior to 90° or superior to 270° between a cultivar vector and an environment vector indicates a positive response of the genotype in the particular environment. A negative cultivar response is indicated by angle between 90 and 270° between a genotype vector and an environment vector (Van Eeuwijk, 2006; Crossa *et al*., 2002).

The AMMI2 biplot for EM showed that all environments had long spokes and therefore, exhibited strong interactive forces on genotypes. About 5 genotypes: G02, 08, 09, 12, 22 and 27 were closer to the origin (0, 0) and hence, they were not sensitive to environments and exhibited weak interactive forces on environments. About 6 genotypes: G06, 12, 21, 22, 23 and 27 very close to IPCA1 axis had low interactive effects because most of GEI effects are captured in IPCA1 axis (Gauch, 2006).

The AMMI2 biplots for PCG and TCG clearly showed that all environments strongly interacted with genotypes and environments of the same type had similar interaction behavior with genotypes whereas environments of different types have different interaction behavior with genotypes. They showed also that genotypes G04, 20, 22, 24, 26, 30 and 36 for PCG and G01, 02, 06, 19, 30, 31 and 34 for TCG were not very sensitive to the change of environments and hence exerted weak interaction forces on environments.

Despite strong interactive forces of environments, the QPM genotypes: G02 ([CML202/CML144] F2-1-1-3-B*4/[CML389/CML176]B-29-2-B*3), G20([CML202/CML144] F2-66-2-3-B*4/[CML389/CML176] B-29-2-B*3) and G22 ([CML202/CML144] F2-66-2-3-B*4/[CML389/GQL5] B-22-1-B*3 were not sensitive to the change of environments for all traits and therefore, they were stable across environments. The genotypes G02 and G20 had the same male parent: [CML389/CML176] B-29-2-B*3 while G20 and G22 had the same female parent: [CML202/CML144] F2-66-2-3-B*4. Therefore, this implies that it is possible to identify parent QPM lines that can provide stable QPM varieties for EM, PCG and TCG under drought and low N environments.

CONCLUSION

Low-N, particularly drought environments interact with QPM genotypes by increasing their EM scores, therefore reducing endosperm modification and making QPM partially or totally soft and opaque. Low-N environments interact with QPM by significantly reducing the amount of protein and tryptophan in grain so that QPM loses its protein quality while drought environments interact with them by significantly increasing the amount of protein and tryptophan in grain. Despite strong interactive forces of environments, the QPM genotypes G02 ([CML202/CML144] F2-1-1-3-B*4/[CML389/CML176] B-29-2-B*3), G20([CML202/CML144] F2-66-2-3-B*4/[CML389/CML176] B-29-2-B*3) and G22 ([CML202/CML144] F2-66-2-3-B*4/[CML389/GQL5] B-22-1-B*3 were not sensitive to the change of environments for all traits and therefore, they were stable across environments. The genotypes G02 and 20 had the same male parent whereas G20 and 22 had the same female parent. Thus, this indicates that it is possible to identify parent QPM lines that can provide stable QPM varieties for EM, PCG and TCG under drought and low N environments.

The interaction of low-N and particularly of drought with QPM genotypes may have negative impact on adoption of QPM varieties in stress prone areas where QPM is destined for direct human consumption. The grain harvested in fields with nitrogen deficiency and particularly under drought is of bad kernel quality so that it is inappropriate to human consumption. Therefore, no farmer would like to plant such maize variety. However, the existence of stable genotypes that exhibit low interaction effects shows that it is possible to develop QPM genotypes that may be easily acceptable in those drought prone areas.

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