Maydica 53 (2008): 249-260

STABILITY IN PERFORMANCE OF QUALITY PROTEIN MAIZE UNDER ABIOTIC STRESS

P.H. Zaidi[1,*], S.K. Vasal, P. Maniselvan[3], G.C. Jha[4], Mehrajjudin[1], R.P. Singh[3]

[1] *Asian Regional Maize Program, CIMMYT, ICRISAT Campus, Hyderabad, India*
[2] *CIMMYT, Apartado Postal 6-641, 06600 Mexico, D.F., Mexico*
[3] *Directorate of Maize Research, Pusa Campus, New Delhi-12, India*
[4] *Division of Agricultural Economics, IARI, New Delhi-12, India*

Received January 30, 2009

ABSTRACT - Quality protein maize (QPM) has emerged an affordable and viable option to alleviate protein malnutrition and reduce animal feed costs, given that its grain protein contains more than double the lysine and tryptophan concentrations of normal endosperm maize. For commercial success, QPM cultivars must be competitive with normal maize in productivity and should show stable performance across environments, especially with respect to yield and protein quality traits. In the tropics, drought and low-nitrogen (N) fertility are major constraints to maize productivity. In this study, we analyze the stability of performance of CIMMYT tropical and subtropical elite QPM hybrids across stressed (drought and low N) and unstressed environments. In general, stress significantly affected all agronomic traits except male flowering. The effect was comparatively large under drought stress. Among the quality traits, grain protein, tryptophan, and lysine contents showed significant variation across environments. There was an increase in grain protein (12.7%) and in lysine (10.3%) and tryptophan contents (8.1%) under drought stress, while levels of these grain quality traits were reduced under low N by 17.0, 12.5, and 15.6%, respectively. However, the effect of stressed environments was comparatively small on protein quality traits, including tryptophan and lysine content in protein. The variation in protein quality across environments was statistically significant but largely due to genotypic variability. Variation due to environment and genotype by environment (G x E) interaction was statistically non-significant for protein quality traits, except in the case of lysine content in protein, where G x E was significant. Our results suggest that grain yield and grain protein content are the most unstable traits, whereas tryptophan followed by lysine content are the most stable, across stressed and unstressed environments.

KEY WORDS: Quality protein maize; Drought; Low nitrogen; Maize; Quality traits.

* For correspondence (fax: +91-40-30713780; e.mail: phzaidi @cgiar.org).

INTRODUCTION

The nutritional value of cereal protein is a critical constraint in its use for animal and human consumption. Cereal proteins, including protein in maize endosperm, are mostly deficient in essential amino acids, particularly lysine, tryptophan, and methionine (SEGAL *et al.*, 2003). This nutritional deficiency is a global concern, particularly for the countries where maize is a staple food and often used as a major source of protein. Normal maize protein has a biological nutritional value of 40% of that of milk (BRESSANI, 1991), and therefore needs to be used with complementary protein sources such as legumes or animal products. In maize, several approaches have been undertaken to correct this problem, however, a major breakthrough has been the isolation of the *opaque-2* mutant (*o2*), with superior nutritional properties, albeit undesirable agronomic traits (MERTZ *et al.*, 1964). Enthusiasm over the direct use of the *o2* mutation in breeding programs soon subsided after the discovery of serious negative secondary (pleiotropic) effects of this mutation, especially the associated yield penalties and poor grain texture. The soft endosperm of *o2* genotypes initially caused up to 25% yield loss due to lower density of the *opaque* grains, as well as increased susceptibility to fungal ear rots and storage pests (VASAL, 2000). Fortunately, during the process of converting normal maize populations to *o2* versions many researchers including breeders at CIMMYT, Mexico, observed partially hard endosperm (i.e. vitreous) kernels. PAEZ *et al.* (1969) first reported the importance of such grain modification in reducing the negative pleiotropic effects of the *o2* mutation. Selection for hard endosperm modification was rapidly incorporated into *o2* breeding schemes of QPM development. QPM breeding began with the objective of improving the nutritional

value of maize grain protein without the negative pleiotropic effects of the *o2* mutation.

QPM contains the *o2* mutation that alters protein composition of the maize endosperm, resulting in increased concentrations of lysine and tryptophan in protein. *o2*-induced alteration in protein composition results in a 60-to-100% increase in the concentration of these two essential amino acids and about 50% decrease in lysine-deficient prolamine fraction, which eventually increases protein digestibility and nitrogen uptake relative to normal-endosperm maize (VASAL, 2000). Its biological value is about 80%, whereas that of normal maize it is only 40 to 57% (BRESSANI, 1992). QPM is the result of two decades of breeding work to overcome low yield, slow dry-down, ear rot, and various other agronomic deficiencies along with opaque endosperm phenotype associated with the original *opaque-2* maize (BJARNASON and VASAL, 1992). Scientists at CIMMYT used backcross and recurrent selection techniques to convert several maize populations to *opaque-2* and subsequently improved the undesirable traits associated with the mutation (BJARNASON and VASAL, 1992; VILLEGAS *et al*., 1992). The number of genes involved in modifying the *opaque-2* endosperm to translucent and similar to that of normal maize is not known, but most reports indicate that inheritance is complex (BJARNASON and VASAL, 1992; LOPES and LARKINS, 1996) and several cycles of improvement are required to achieve satisfactory modification for most *opaque-2* germplasm (VASAL *et al*., 1980).

Research results have clearly demonstrated the competitiveness for grain yield of QPM with the best normal maize cultivars in numerous tropical environments (CORDOVA and PANDEY, 1999; VERGARA *et al*., 2000; VASAL *et al*., 2008). However, little information is available on the stability of quality traits of QPM cultivars across environment in tropics. PIXLEY and BJARNASON (2002) studied the stability of grain yield, endosperm modification, and protein quality in different type of QPM cultivars under optimal conditions across locations. They concluded that tryptophan concentration in protein was the most stable trait, followed by protein concentration in grain and endosperm modification score. Since most of the modern high yielding cultivars are usually developed under favorable environments and optimal input conditions, it may not be surprising to observe their poor performance under marginal and less favorable environment. In the tropics, much maize is grown in marginal, rainfed environments, and is often affected by multiple biotic and abiotic stresses. Drought and low-N fertility are the major productivity constraints for maize in the lowland tropics (EDMEADES, 2003; ZAIDI *et al*., 2008a). The objective of this study was to assess the stability in performance of new generation QPM hybrids in terms of quality traits across stressed and unstressed environments in tropics.

MATERIALS AND METHODS

Germplasm

The maize hybrids were derived from genetically broad-based, CIMMYT tropical and sub-tropical QPM pools and populations including tropical yellow flint pool 25 and dent pool 26 QPM; sub-tropical yellow flint pool 33 and dent pool 34; tropical white flint population 62 and dent population 63; sub-tropical white flint population 67; white semi-dent population 68; and yellow flint population 69. Tropical QPM pool 25 includes components of Amarillo Cristalino-1 QPM and *Phyllacora*-resistant families from tropical yellow flint QPM pool. Pool 26 includes components of Amarillo Dentado QPM and *Phyllacora*-resistant families from tropical yellow flint QPM pool. Sub-tropical QPM pool 33 includes families selected for flint kernel texture from temperate x tropical QPM populations, Amarillo Bajio QPM, and pool 34 QPM that includes families selected for dent kernel texture from populations Temperate x Tropical QPM, pool 33 QPM, Amarillo Subtropical QPM, and ETO x Illinois QPM. Tropical QPM population 62 was derived originally from population 40 through full-sib selection; population 63 was derived mainly from Tuxpeño-1 QPM, with introgressions from La Posta QPM and pool 24 QPM. Sub-tropical QPM population 67 was derived from pool 31 QPM, population 68 composed of 30-40% temperate and 60-70% tropical germplasm, and population 69 was originally population 41, composed of 30 to 40% temperate and 60 to 70% tropical germplasm. Further details about these QPM pools and populations can be found in CIMMYT's report on maize germplasm (CIMMYT, 1998).

Hybrids involved in this study were elite hybrids selected from various advanced-stage QPM trials of CIMMYT tropical and sub-tropical maize programs, which were conducted across locations under optimal agronomic management conditions. Inbred lines (Table 2) involved in the development of 30 single-cross hybrids were at advance generation of inbreeding, and were derived from QPM pools and populations improved for various agronomic traits, biotic stresses, combining ability, and yield potential under optimal input conditions, but had never been selected and improved for drought or low Nitrogen (N) stress. The 30 experimental hybrids were random combinations among the 21 elite QPM lines. In general, each line was involved in developing only one hybrid, except for CML-142, CML-144, CML-147, CML-161, CML-176, CML-186, and CML-193, which were involved in more than one hybrid.

Experimental site, cultural practices, and stress management

The 30 experimental hybrids were evaluated under normal conditions and low-N stress during the rainy season of 2002 and winter season of 2002-03 at Tlaltizapan, Morelos, Mexico (18°N,

TABLE 1 - *Trial sites and seasons.*

Location	Crop seasons	Soil type	Environment	Nitrogen (N) fertilization (kg ha^{-1})
Tlaltizapan, Mexico	Rainy season, 2002	Clay	Normal	150
Tlaltizapan, Mexico	Dry season, 2002-03	Clay	Normal	150
Tlaltizapan, Mexico	Rainy season, 2002	Clay	Low-N*	-
Tlaltizapan, Mexico	Dry season, 2002-03	Clay	Low-N	-
Tlaltizapan, Mexico	Dry season, 2002-03	Clay	Drought	150
Hyderabad, India	Dry season, 2003-04	Clay loam	Drought	150

* Available soil N through mineralization in top 30.0 cm soil ranges from 8.7-12.3 kg ha^{-1}.

TABLE 2 - *Description of parental lines used in developing single-cross hybrids for the stability trials across stress and non-stress environments.*

No.	Parental lines	Pedigree	Adaptation
1	CML 142	P62C5F93-5-6-S_9	Tropical
2	CML 144	P62C5F182-2-1-S_7	Tropical
3	CML 146	AC8563H35-3-1-S_9	Tropical
4	CML 147	P63C2F53-1-1-S_7	Tropical
5	CML 150	G24QH169-2-1-S_{10}	Tropical
6	CML 159	P63C2F5-1-3-S_7	Tropical
7	CML 161	G25QC18H520-1-1S_{13}	Tropical
8	CML 165	G26QH31-2-2-S_9	Tropical
9	CML 168	G26QSINT-31-1-S_6	Tropical
10	CML 169	G26QC22H7-1-1-S_5	Tropical
11	CML 170	G26QC22H9-3-1-S_6	Tropical
12	CML 171	G25QS4B-H13-5-S_9	Tropical
13	CML 172	G25QS4B-H35-2-B-S_9	Tropical
14	CML 175	P68C0F77-2-3-S_8	Sub-tropical
15	CML 176	(P63-12-2-1/P67-5-1-1)-1-2-S_5	Tropical
16	CML 186	P67C2F26-1-2-S_5	Sub-tropical
17	CML 193	CYO162-B-1-1-S_6	Tropical
18	CML 491	(6207QB/6207QA)-1-4-S_5	Tropical
19	CML 492	P62C3F163-3-3-S_8	Tropical
20	G33Qc25MH103-3-1-S_5	G33Qc25MH103-3-1-S_5	Sub-tropical
21	G34Qc1HC519-2-S_9	G34Qc1HC519-2-S_9	Sub-tropical
22	G34Qc22MH135-4-2-S_8	G34Qc22MH135-4-2-S_8	Sub-tropical
23	P69Qc6HC13-1-S_6	P69Qc6HC13-1-S_6	Sub-tropical

P, population, C3, cycle 3; F, full-sib; H, half-sib; G, germplasm pool; AC8563, synthetic formed in 1985 using families from population 63 that were superior in across location evaluation; S_n, level of inbreeding of the line, e.g. S_9 (pedigree are abbreviated).

940 masl) and under flowering stage drought stress during the winter season of 2002-03 at Tlaltizapan and 2003-04 at Hyderabad, India (17°N, 78°E, 530 masl) (Table 1). The winter cycles at both Tlaltizapan and Hyderabad were similar in terms of being rain-free and free from cloud cover. Vegetative development was slow due to cool temperatures, however, around flowering temperatures rose later in the season so that the interval from flowering to maturity occupied about 40% of the total crop duration. In the trials under drought and normal conditions 75 kg N ha^{-1} as $(NH_4)_2SO_4$ and 22 kg P ha^{-1} as triple super phosphate were applied prior to planting; a second dose of 75 kg N ha^{-1} was side-dressed six weeks after planting. In low-N trials, the same dose of phosphorous was applied, but no N fertilizer was applied in those experiments.

Experiments under normal and drought stress were conducted in same block, but different fields. Experiments under optimal conditions were managed under well-watered and fertilized conditions to provide an ideal growing environment for the crop. In drought experiments, genotypes were exposed to severe drought stress during flowering by withdrawing irrigation about two weeks before 50% male flowering. One additional irrigation was applied about two weeks after 50% anthesis based on population means for anthesis-silking interval (BANZIGER *et al.,* 2000). Low-N experiments were planted in a field with depleted soil nitrogen at Tlaltizapan, Mexico. The field was especially prepared by continuously growing maize for two cycles per year and since 1998, no organic or N fertilizer were applied. As well, crop biomass was removed at harvest to avoid any incorporation of crop residues into the soil. Soil analysis of the field revealed that available soil N in the top 30.0 cm soil ranged from 8.7-12.3 kg ha^{-1}. Under this low-N, grain yield of normal genotypes that were not improved for drought or low-N stress, averaged between 20-30% of the well-fertilized field (ZAIDI *et al*., unpublished data). All the entries in each experiment were over sown and later on thinned to one plant per hill to give a population density of 55,000 plants ha^{-1}. Seeds were planted with 0.25 m spacing within rows and 0.75 m between rows. Experiments were kept free from weeds, insect-pests, and diseases using recommended chemical measures.

Field observations

Plant height in each trial and plot was recorded after completion of male flowering, as the distance between the ground surface and node bearing flag-leaf, using five plants from each row and averaged. Leaf senescence was scored using a 1-10 scale (1 = 10% and 10 = 100% dead leaf area), three times at one-week intervals starting from two weeks after 50% female flowering. *In vivo* chlorophyll concentration in the ear leaf was determined using a Minolta SPAD-502 chlorophyll meter in each plot, two times at two-week intervals starting from 50% male flowering, on 10 plants per plot and averaged. Days from planting to anthesis and silking, indicated when 50% of plants had extruded anther or produced silk, were recorded by daily visual observations during the flowering period. Anthesis-silking interval (ASI) was calculated as the difference between the number of days to 50% silking and 50% anthesis. At maturity, ears were harvested, excluding two plants close to alley from both end of the rows; ear number per plot was determined, and ears per plant (EPP) was calculated. Ear rot was scored in the field after harvest as the percentage of cob area with visibly rotten kernels. Ears were oven dried to a 15% moisture level, and grain yield was recorded on shelled grain basis.

Biochemical analysis

Protein content and quality were determined at CIMMYT's Cereal Quality Laboratory following procedures described by VILLEGAS (1975) and VILLEGAS *et al.* (1984). In brief, whole-grain samples were finely ground, the resulting flour was defatted, and concentration of tryptophan was colorimetrically determined for duplicate sub-samples. Lysine concentration was measured using the procedure described by TSAI *et al*. (1972). Grinding of whole grain rather than endosperm tissue for analyses may have caused some bias due to pericarp pigments affecting colorimetric determinations (VILLEGAS, 1975). This procedure has been used effectively to improve QPM germplasm at CIMMYT and seems justifiable in the light of cost savings relative to separation and analysis of endosperm tissue. Another advantage of analyzing whole-grain samples is that the variability of embryo size can significantly affect protein quantity and quality in the grain (BJARNASON and POLLMER, 1972), and may be exploited.

Experimental design and statistical analysis

Trials were grown with two common QPM check entries in all the experiments using an alpha (0, 1) lattice design (PATTERSON *et al.*, 1978) with three replications, wherein each pair of treatments appeared together in a block once or not at all. All the entries were planted in two row plots (5.0 m x 0.75 m). Data from individual trials under drought, low-N, and normal conditions, were used to analyze the variation due to genotype, environment, and genotype x environment interaction across two seasons/locations. Across environment analyses used raw and lattice-adjusted mean data according to which analysis resulted in the greatest efficacy for each environment. Simple linear regression analyses were used to estimate EBERHART and RUSSEL (1966) parameters for stability of the traits of QPM hybrids across stresses and normal environments. Pearson phenotypic correlation coefficients were calculated between grain yield and different agronomic and quality traits to elicit associations that might help explain environmental fluctuations (lack of stability) for these traits. The additive main effects and multiplicative interactions (AMMI) model, which combines standard analysis of variance with principal component analysis (ZOBEL *et al.*, 1988), was used to study the agronomic nature of genotype x environment interactions. Plots were prepared as described by ZOBEL *et al.* (1988).

RESULTS AND DISCUSSION

Means and variance across environments

Data analysis across two seasons/locations for each environment, i.e. normal, low-N, and drought, showed significant variation for most of the growth and yield traits (Table 3). In general, the effect of season or location was relatively large on grain yield under normal conditions in comparison to low-N and drought stress. This might be because of the fact that under managed stress condition yield losses were almost to the same extent in both seasons/locations. The effect of season was also highly significant on flowering under both normal and low-N stress, which was largely due to environment and genotype x environment interaction. In the case of other traits, variation across season or location was comparatively larger under low-N stress followed by drought. For example, senescence showed no variation due to season under normal conditions, while the variation was significant under low-N and drought stress. This is simply because genotypic variability for these traits is usually low under normal conditions, while under abiotic stresses, the variability increases significantly and genotype x environment interaction plays a significant role (ZAIDI *et al.,* 2004). In addition, the effects of

TABLE 3 - *Means of morpho-physiological traits of quality protein maize hybrids across seasons or locations under normal, low-N, and drought stress.*

Traits	Normal	LSD G	LSD E	LSD GxE	Low-N	LSD G	LSD E	LSD GxE	Drought	LSD G	LSD E	LSD GxE
Plant height (cm)	219.9	*	*	ns	151.7	*	*	*	170.4	*	ns	ns
Chlorophyll (SPAD unit)	51.7	*	ns	ns	25.9	*	*	ns	38.1	*	ns	ns
Senescence score	1.2	ns	ns	ns	5.7	*	**	ns	4.1	*	ns	ns
Days to 50% anthesis	84.5	ns	**	**	87.5	ns	**	**	87.2	*	ns	ns
Days to 50% silking	86.1	ns	**	*	93.4	*	**	*	98.2	*	ns	ns
Anthesis-silking interval (d)	1.6	ns	*	ns	5.9	*	*	ns	11.0	*	ns	ns
Ears per plant	1.1	*	ns	ns	0.9	*	*	ns	0.5	*	ns	ns
Ear rot (%)	10.8	ns	*	ns	30.6	*	**	ns	18.2	*	*	ns
Grain yield (t/ha)	6.8	*	**	**	2.85	*	*	ns	1.59	*	ns	ns
					Quality traits							
Protein (% in grain)	9.97	*	*	ns	8.28	*	*	*	11.20	*	ns	ns
Tryptophan (% in grain)	0.09	*	ns	ns	0.08	*	*	ns	0.10	*	ns	ns
Lysine (% in grain)	0.47	*	ns	ns	0.41	*	*	ns	0.51	*	ns	ns
Tryptophan (% in protein)	0.88	*	ns	ns	0.94	*	ns	ns	0.87	*	ns	ns
Lysine (% in protein)	4.53	*	ns	ns	4.96	*	*	ns	4.59	*	ns	ns

*, ** Significant at P <0.05 and 0.01, respectively. ns indicates non-significant difference.
LSD = Least significant differences, G = genotype, E = environment (season or location).

TABLE 4 - *Analysis of variance for important morpho- physiological and quality traits of QPM hybrids under normal, low-N and drought stress.*

Source	DF	Anthesis-silking interval MSS	% of total SS	Senescence score MSS	% of total SS	Ears per plant MSS	% of total SS	Ear rot MSS	% of total SS	Grain yield MSS	% of total SS
Environment	2	1988.89**	26.08	346.46**	42.28	8.43**	34.84	0.818**	2.55	734.75**	40.76
Error	6	30.38	0.40	1.837	0.22	0.102	0.42	0.231	0.72	10.739	0.60
Genotype	29	39.71**	0.52	0.716**	0.09	0.085**	0.35	0.209**	0.65	3.14**	0.17
G x E	58	26.29**	0.34	0.846**	0.10	0.041**	0.17	0.117 ns	0.37	1.17**	0.06
Error	174	4.53	0.06	0.262	0.03	0.011	0.05	0.093	0.29	0.63	0.03
CV (%)		34.60		15.7		12.78		21.34		22.9	
					Quality traits						
		Protein in grain		Tryptophan in grain		Lysine in grain		Tryptophan in protein		Lysine in protein	
Environment	2	133.7**	32.70	0.007*	28.00	0.17**	26.60	0.074 ns	8.20	3.312 ns	11.62
Error	3	6.733	1.65	0.000	0.00	0.006	0.94	0.014	1.55	0.583	2.05
Genotype	29	1.584**	0.39	0.000**	0.00	0.005**	0.78	0.013**	1.44	0.27**	0.95
G x E	58	0.696*	0.17	0.000 ns	0.00	0.001 ns	0.16	0.003 ns	0.33	0.109*	0.38
Error	87	0.402	0.10	0.000	0.00	0.001	0.16	0.002	0.22	0.069	0.24
CV (%)		6.39		5.75		6.03		4.76		5.58	

*, ** indicate statistical significance at P <0.05 and 0.01, respectively.

low-N stress were more apparent during the rainy season than the dry season, which might be because of comparatively more soil N losses during the rainy season due to frequent heavy rains followed by run-off and leaching. Under low-N stress, all the growth and yield traits showed significant variation across two seasons. The season effect was comparatively large on flowering traits followed by ear rots. Data analysis across two locations under drought stress also showed significant variation for all the traits, but it was mainly governed by genotypic variation, except in the case of ear rot where both genotype and environment contributed to the variation across location. However, across season or location variations were relatively small on protein and grain quality traits. The available variation across seasons under normal conditions was largely due to genotype, except in the case of total protein in grain, where it was due to both genotype and environment. Under low-N stress, the effect of season on grain quality traits was significant on all the traits, in which both genotype and environment have made significant contributions. It might relate to the observed general variation in the low-N stress intensity between the rainy and winter seasons. However, the effect was relatively small on protein quality traits and the observed variation was largely due to genotypic variability for tryptophan. However, the effect of both genotype and environment was significant ($P<0.05$) on lysine contents in protein. Under drought stress, the location effect was relatively small on all the quality traits, including both grain and protein quality traits. The observed variation was due to significant genotype variability that contributed to variation across location.

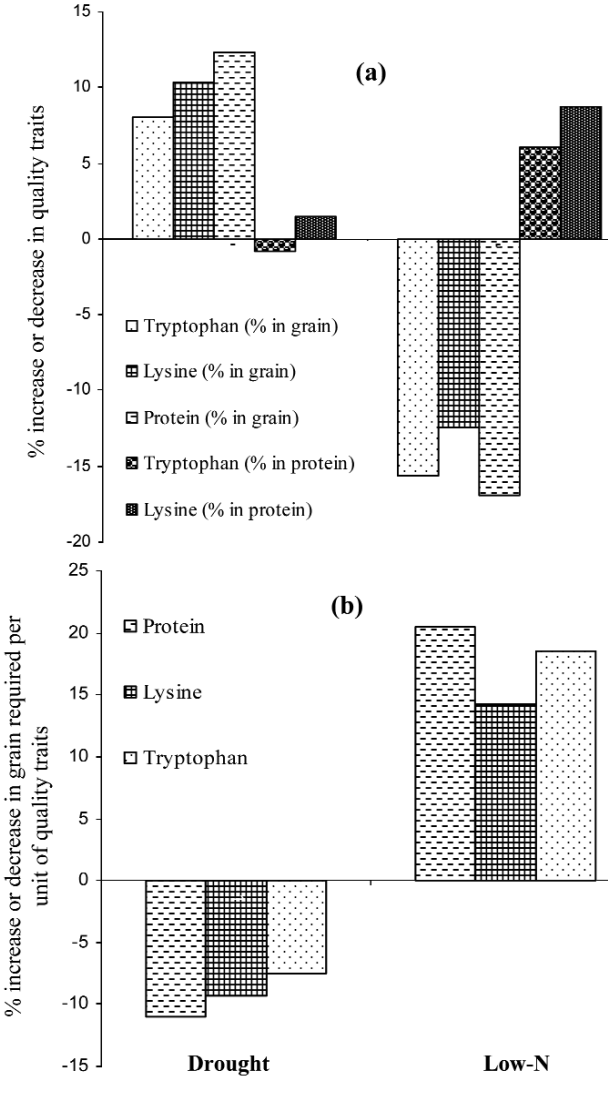

FIGURE 1 - Effect of drought and low nitrogen stresses on protein and grain quality traits.

The mean of the traits across stresses and unstressed environments showed that stress conditions significantly affected all the growth, yield, and quality traits (Tables 3 and 4). Reduction in plant height and leaf chlorophyll content was relatively higher under low-N stress than under drought. Low-N stress caused relatively faster plant senescence and increased ear rots. However, in the case of other traits the inhibitory effect of a stressed environment was comparatively stronger under drought than under low-N. Drought stress severely affected silk emergence, which resulted in prolonged ASI and reduced ears per plant by less than half in comparison to normal. The overall impact of the stresses on various growth and yield attributes eventually resulted in severe yield losses to the extent of 76.4% under drought and 57.8% under low-N stress.

The effect of abiotic stresses was quite large on grain quality traits including protein, lysine, and tryptophan content in grain (Table 3 and Fig. 1a). However, the effect was relatively small on tryptophan and lysine content in total protein. Under drought stress there was a significant increase in protein, lysine, and tryptophan content in grain. Lysine in protein also showed a nominal increase but tryptophan concentration decreased slightly under drought stress (Fig. 1a). However, under low-N stress the trend was opposite, i.e. concentration of protein, lysine, and tryptophan in grain decreased significantly, while both lysine and tryptophan in

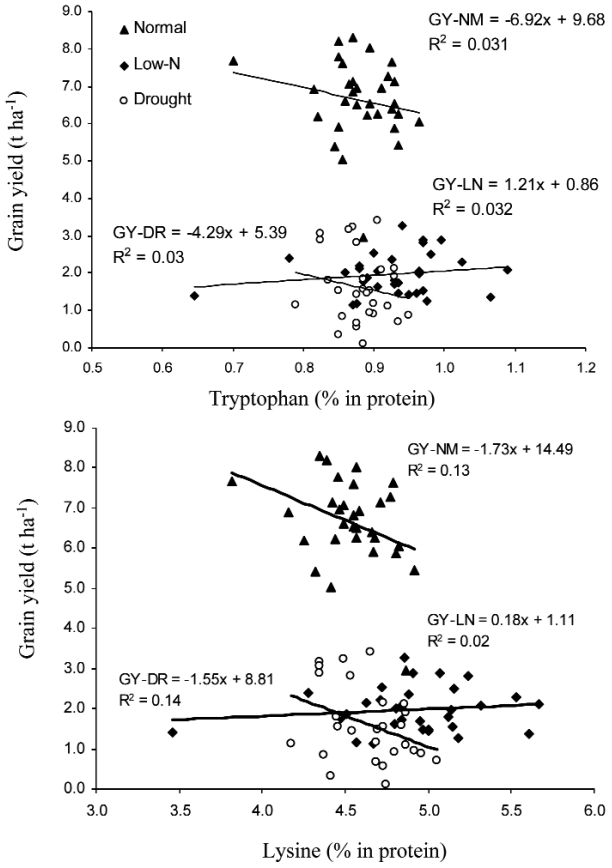

FIGURE 2 - Relationship of grain yield with protein quality traits under normal, drought and low nitrogen stress.

grain showed a significant increase. Data on changes in concentration of quality traits in grain suggested that in the case of low-N stress more quantity of grain would be required to achieve the same quantity of the quality traits, while the requirement of grain would decrease under drought stress (Fig. 2b). For example, in the case of low-N stress, the same quantity of grain protein was recovered with 20.4% more grain, while 10.9% less grain was required to recover the same quantity of protein under drought stress. This might be related to the fact that those quality traits which are directly related to grain N content (such as protein) might be affected more under low-N stress, while under drought stress other grain traits were more affected than quality traits, which resulted in a relative increase of these N-related quality traits under drought.

Analysis of variance across environments showed that variance due to environment, genotype, and genotype x environment was significant (P<0.01) for all the growth and yield traits, except genotype x environment interaction for ear rots (Table 4). However, contribution of environment was highest in overall variation across environments. Next to environment, genotypic variability for the traits significantly contributed in overall variance across environments, except in the case of senescence score where contribution of genotype x environment interaction was slightly higher than genotypic variability. The magnitude of the mean square was highest for ASI followed by grain yield and senescence score, and least for ear rots. Environment variance played a key role in overall variation in the quality traits as well. The variation was significant for all the quality traits, except tryptophan concentration in protein. The magnitude of variation was relatively higher in the case of protein and lysine concentration in grain. There was significant genotypic variability for all the quality traits, which significantly contributed to overall variation across environments (Tables 3 and 4). However, variance due to genotype x environment was significant (P<0.05) only for protein content in grain, and lysine in protein. In general, the coefficient of variation was low in the case of quality traits in comparison to growth and yield traits. It may be due to the relatively small genotypic variability for these traits among QPM hybrids.

Relationship between grain yield and quality traits

Negative association between grain yield and grain quality traits is undesirable and very important for the QPM breeding program because, in general,

TABLE 5 - *Pearson phenotypic correlation coefficients between grain yield and quality and agronomic traits for 30 maize hybrids evaluated under normal, low-N and drought stress.*

Traits	Normal	Low-N	Drought
Protein in grain	-0.27*	-0.43**	-0.38*
Tryptophan in grain	-0.31*	-0.49*	-0.45*
Lysine in grain	-0.35*	-0.57**	-0.52**
Tryptophan in protein	-0.11	0.18	-0.07
Lysine in protein	-0.13	0.13	0.09
Anthesis-silking interval	-0.19	-0.52**	-0.76**
Leaf senescence	-0.14	-0.41*	-0.33*
Ears per plant	0.44*	0.54**	0.84**
Ear rots	-0.21	-0.39*	-0.29*

* and ** indicate statistical significance at P=<0.05 and 0.01, respectively.

TABLE 6 - *Mean and standard deviation, mean linear regression coefficient* (b), *mean coefficient of determination for linear regression model* (r^2), *mean sum of square of deviations from regression* (S^2d), *and mean probability of significance for* S^2d *for grain yield, protein and tryptophan content in grain, and tryptophan and lysine content in protein of 30 QPM single cross hybrids across stresses and unstressed environments.*

Traits	Mean	*b*	r^2	S^2d	*P-value*
Grain yield (t ha^{-1})	3.74±2.61	-3.36±0.77	0.62	19.38	2.2×10^{-5}
Protein in grain (%)	9.82±0.77	-2.88±0.42	0.69	189.6	1.2×10^{-3}
Tryptophan in grain (%)	0.09±0.005	-0.89±0.24	0.82	1.24	0.008
Lysine in grain (%)	0.46±0.023	-1.43±0.21	0.75	1.66	0.006
Tryptophan in protein (%)	0.89±0.012	-0.084±0.08	0.91	0.93	0.082
Lysine in protein (%)	4.69±0.061	-0.112±0.11	0.89	1.35	0.053

± values indicate standard deviation from mean across environment.

breeders attempt simultaneous selection and improvement for these traits. Across environments, the grain quality traits, such as protein, tryptophan, and lysine content in grain, showed a significant negative relationship with grain yields, which were relatively much stronger under low-N stress followed by drought (Table 5). This could be explained based on the well-established direct relationship between nitrogen and protein content. Reduced grain N content under low N (ZAIDI *et al.*, unpublished data) might have resulted in reduced amino acids and protein content in grain under low-N stress (Table 3). This was eventually reflected as a strong negative relationship of these N-demanding traits with grain yield (Table 5). A significant negative relationship of grain yield with grain quality traits was also observed under drought stress. This might be because under drought stress plants face not only the problem of water deficit but also poor availability of nutrients, including nitrogen, i.e. *nutritional drought*, due to slow-nutrient mineralization and mobilization in dry and compact soils (SHEPHERD, 1984; PATTERSON *et al.,* 1993). Accelerated senescence and reduced chlorophyll concentrations under drought stress (Table 3), in spite of well-fertilized conditions, suggest low-N availability in plant tissues under drought. Probably therefore, the relationship between the quality traits with grain yield under drought was similar to low-N stress. These results reflected the well-known negative association of grain yield with grain quality traits in QPM cultivars (KEVIN and BJARNASON, 2002). However, the

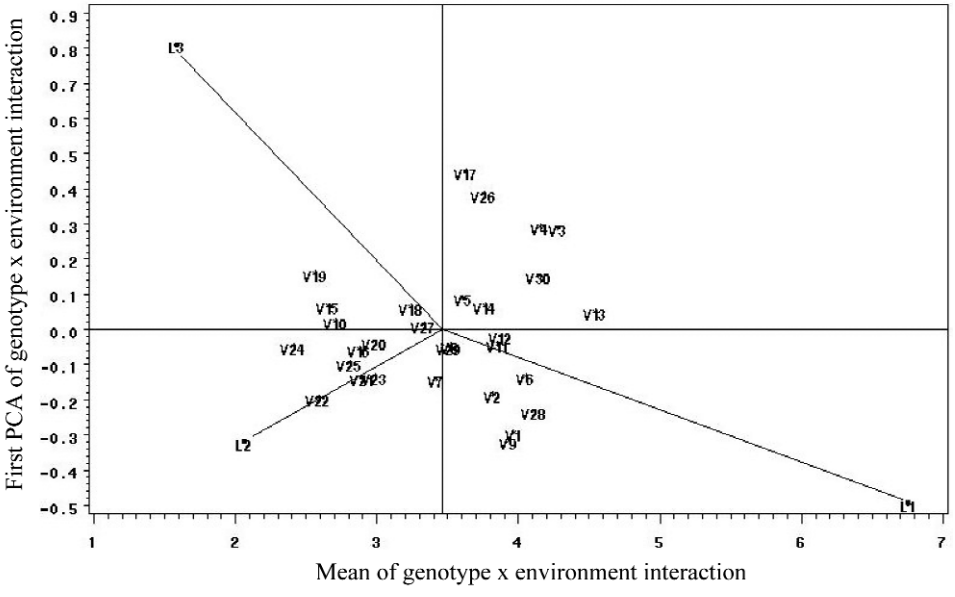

FIGURE 3 - AMMI (additive main effects and multiplicative interactions model) plot for grain yield of QPM hybrids evaluated under low nitrogen and drought stress, and normal conditions across season/locations (L1 = Normal, L2 = Low- nitrogen stress site and L3 = drought stress site).

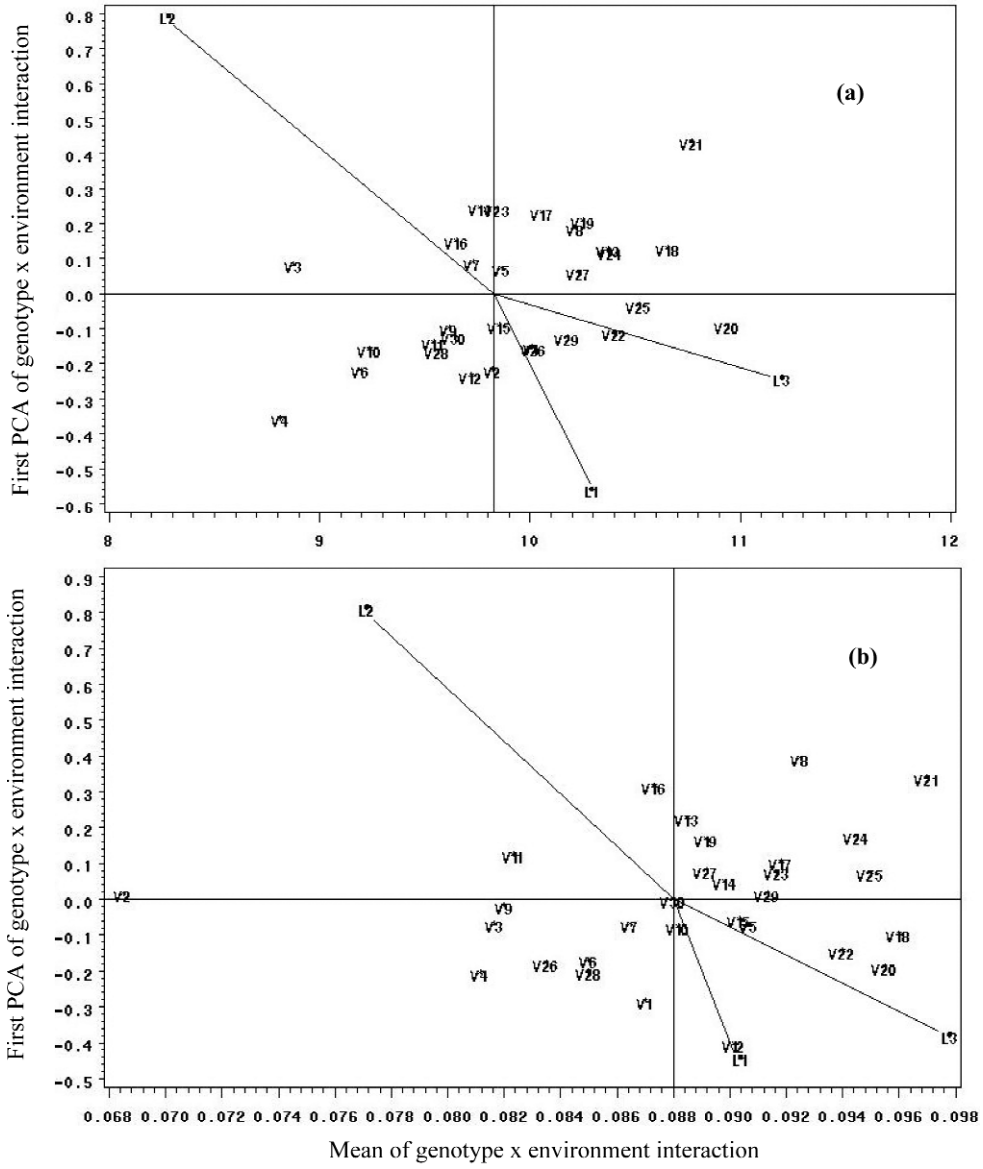

FIGURE 4 - AMMI (additive main effects and multiplicative interactions model) plot for (a) protein and (b) tryptophan content in grains of QPM hybrids evaluated under low nitrogen and drought stress, and normal conditions across season/locations (L1 = Normal, L2 = Low-nitrogen stress site and L3 = drought stress site).

weak associations between grain yield and protein quality traits under normal conditions indicate that the negative linkage between high quality protein and grain yield of QPM is significantly weak in the new generation QPM cultivars (Table 5 and Fig. 2), which should encourage QPM cultivar development efforts. It is interesting to note that concentration of protein quality traits increased under low N (Fig. 1a) and therefore, showed positive (though non-significant) association with grain yield under these stresses (Table 5 and Fig. 2). Since a large proportion of tropical maize is grown under rain-fed marginal environments and is prone to be exposed to these abiotic stresses (GARPACIO and PINGALI, 2007), the positive relationships between yield and protein quality traits should encourage QPM cultivar development for stress-prone marginal environments in the tropics. Apart from the quality traits, the association of various agronomic traits with grain yield under normal and stress-prone environments mostly reflected the well-known relationships (Table 5). The importance of those agronomic traits such as ASI, ears per plant, and senescence increased under stressed environments, as their relationship with grain yield became stronger under stresses, as observed in the present study (Table 5) and also in

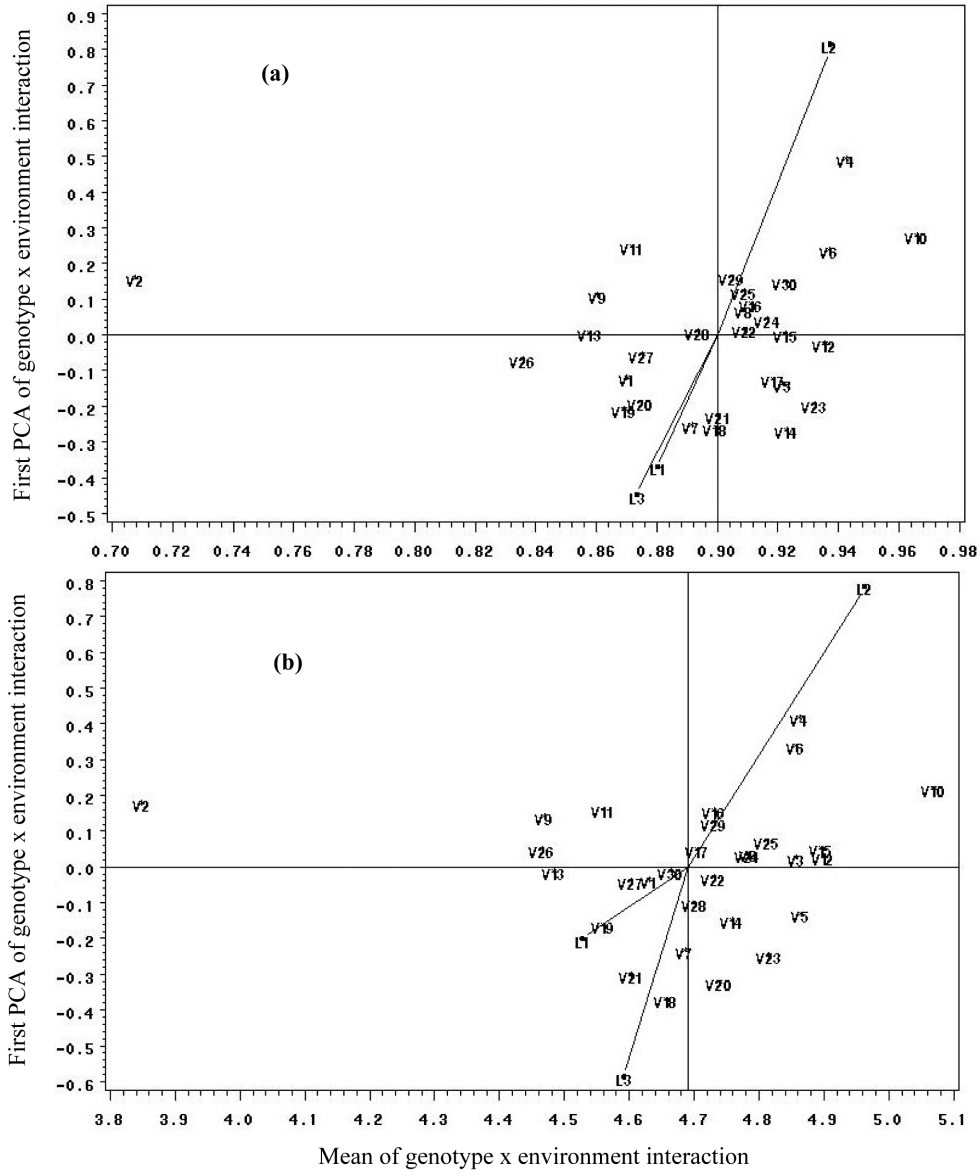

FIGURE 5 - AMMI (additive main effects and multiplicative interactions model) plot for (a) tryptophan and (b) lysine content in grain protein of QPM hybrids evaluated under low nitrogen and drought stress, and normal conditions across season/locations (L1 = Normal, L2 = Low-nitrogen stress site and L3 = drought stress site).

our previous studies on maize under abiotic stresses (ZAIDI *et al.*, 2004, 2008b).

Stability analysis

Grain yield followed by protein content showed a large deviation from environmental mean, whereas the deviation was quite small in the case of protein quality traits (Table 6). The slope of linear regression (*b*) suggested that tryptophan and lysine content in protein were comparatively less responsive to the extreme variation in environment. This agrees with the results from analysis of variance, where the least significant difference across location/year (Table 3) were statistically non-significant for these traits except in the case of lysine under low N stress ($P<0.05$) and variance due environment (Table 4). The average probability of deviations from regression (S^2d) was also highly significant for grain yield and protein content in grain, and for tryptophan and lysine content in grain (Table 6). However, the mean sum of the square of S^2d for protein quality traits did not qualify the statistical significance (EBERHART and RUSSEL, 1966). The results of linear regression parameters for stability agreed with the results of analysis of variance across environments (Table 4) and suggest that protein quality

traits, both tryptophan and lysine content in protein, were comparatively the most stable traits across stresses and un-stressed environments. Pearson phenotypic correlation coefficient analysis (Table 5) also agreed with these stability analysis results, where protein quality traits showed a statistically non-significant relationship with grain yield (the trait with least stability). There is some theoretical criticism that these stability parameters merely indicate appropriateness of the linear regression model for describing data (LIN *et al.*, 1986). However, the small sum of squares for S^2d and relatively large coefficient of determination (r^2) have been widely used to estimate stability of cultivars across environments (EBERHART and RUSSEL, 1966, 1969; JENSEN and SMITH, 1988; KEVIN and BJARNASON, 2002).

AMMI analysis

The AMMI biplot analysis between the mean and the first PCA of G x E interactions indicate the distinct behavior of the environment in segregating QPM hybrids with respect to yield and quality traits (Figs. 3-5). The genotypes close to the X-axis (zero value of the first PCA of G x E interaction) were the most stable genotypes across environments for that particular trait. Among all, the hybrid V13 was the most stable high-yielding hybrid across environments (Fig. 3). The negative PCA score for grain yield under low N and normal conditions and hybrids with negative PCA score indicated a positive G X E interaction effect among them. In general, most of the high-yielding hybrids showed high G X E interaction (deviation from zero value of 1st PCA of G x E interactions) for grain yield, except V11, V12, V13, and V14, which had common genetic backgrounds (parents from pool 33 and 34 QPM). The plot for protein and tryptophan content in grain shows that both the traits had similar trends in terms of discrimination towards high-yielding environments for these traits (Fig. 4). L3 (i.e. drought) was the highest-yielding site, followed by normal; low N was the low-yielding environment for these traits, which agreed with the finding in Table 3. Most of the genotypes, irrespective of their protein content, showed a high level of instability for grain protein content, except V25 and V27 that showed some degree of stability. However, in the case of tryptophan quite a few numbers of genotypes showed good levels of stability across environments. In contrast to the protein and tryptophan content in grain, the trend for tryptophan and lysine content in protein shows that normal and drought go in same direction of relatively low-yielding environments for these traits, whereas low-N tended to be a high-yielding environment (Fig. 5). This might simply be because of the effect of low N availability in grain under low N stress was comparatively large on total protein content (Fig. 1). Also, in terms of stability (deviation from zero PCA1 value) in the genotypes, such as V13, V30, V22, and V15 showed high stability, and most of the other genotypes also showed quite high levels of stability for tryptophan content in protein (Fig. 5a). The result for lysine also showed a similar trend of quite stable performance of a number of genotypes, which had close to zero value for PCA1 of genotype x environment interaction (Fig. 5b).

Our study suggested that drought and low-N stresses significantly affected all the agronomic traits and grain quality traits; including protein, lysine, and tryptophan content in grain (Table 4). Average protein, tryptophan, and lysine content in grain increased under drought stress, while under low-N all these traits showed significant decreases, while the protein quality traits showed comparatively less variation across diverse type of environments (Fig. 1). Both tryptophan and lysine content of protein showed slight increases under low-N, but change was comparatively much smaller under drought stress. Analysis of variances and linear regression analysis both suggested that among the traits studied grain yield was the most unstable trait across stresses and un-stressed environments, followed by protein content in grain. However, in the case of tryptophan and lysine content in protein (the QPM traits), there was variation under stress conditions and particularly under low-N. Nonetheless, the traits showed significant levels of stability and were within the prescribed range for QPM, in spite of quite diverse types of environmental conditions.

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