

Phenotypic Characterization of Quality Protein Maize Endosperm Modification and Amino Acid Contents in a Segregating Recombinant Inbred Population

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ABSTRACT

The protein quality of maize (*Zea mays* L.) can be improved by replacing normal *Opaque2* alleles with nonfunctional recessive alleles (*o2*). Homozygous *o2* kernels have increased levels of lysine and tryptophan. Unfortunately, the associated soft texture of the *o2* kernels causes poor yield and susceptibility to diseases and insects. Breeding has resulted in the development of *o2* genotypes with improved endosperm hardness; such genotypes are referred to as quality protein maize (QPM). Quality protein maize germplasm is utilized in breeding programs worldwide and has been competitive in yield trials. To understand the genetics of endosperm modification, a population of 146 recombinant inbred lines (S_5 to S_7) derived from a cross between the *o2* inbred B73*o2* and the QPM inbred CML161 was evaluated in two Texas locations from 2004 to 2006. The endosperm traits texture, opacity, and vitreousness were highly affected by inbred line genotype, were highly correlated with each other, and exhibited high broad-sense heritability. Relative content of the essential amino acids lysine, tryptophan, and methionine were also highly affected by the inbred line genotype, and exhibited high broad-sense heritability. Negative correlation was observed between endosperm texture traits and amino acid contents. Favorable responses to selection can be expected for both endosperm texture modification and relative content of the essential amino acids if they are efficiently monitored.

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Abbreviations: BLUP, best linear unbiased predictor; CIMMYT, International Maize and Wheat Improvement Center; CS, College Station, TX; GEI, genotype \times environment interaction; Lys, lysine; Met, methionine; QPM, quality protein maize; REML, multivariate restricted maximum likelihood; RIL, recombinant inbred line; Trp, tryptophan; WE, Weslaco, TX.

THE STRUCTURE AND CONTENT of the maize (*Zea mays* L.) endosperm influences the expression of traits targeted for genetic improvement such as grain yield (Salamini et al., 1970; Vyn and Tollenaar, 1998), grain quality (Mazur et al., 1999), suitability for industrial processing (Paulsen and Hill, 1985; Chandrashekar and Mazhar, 1999), ruminal digestibility (Corona et al., 2006), and tolerance to mycotoxin accumulation (Bhatnagar et al., 2003). The protein fraction constitutes only 8 to 9% of the endosperm (Lawton and Wilson, 1987), while starch accounts for about 71% of the kernel (Prasanna et al., 2001). In normal maize, 50 to 70% of the endosperm proteins are of the prolamin type (zeins). The zein proteins are particularly deficient in the essential amino acids lysine (Lys) and tryptophan (Trp). The high proportion of zeins in the endosperm is the primary reason for the poor protein quality of maize (Vasal, 2000).

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Several mutations, both spontaneous and induced, have been identified affecting the composition of the maize endosperm. Among them, those of the *Opaque2* (*O2*) gene are the most extensively studied (Lazzari et al., 2002; Henry et al., 2005). The *O2* gene encodes a leucine-zipper class transcription factor (Schmidt et al., 1990) that induces the transcription of a group of α -zeins and influences expression of other genes such as *b-32* (a Type I ribosome inactivating protein) and *CyPdk1* (a cytosolic pyruvate orthophosphate dikinase) (Bass et al., 1992; Schmidt et al., 1992; Maddaloni et al., 1996). In the 1960s, *o2* received considerable attention as it was associated with a significant increase in the proportion of Lys and Trp in the grain (Mertz et al., 1964). Plant breeders transferred the *o2* allele into different germplasm, aiming to improve nutritional quality, but undesirable traits were associated with the presence of the *o2* allele. In particular, the kernel became soft and more prone to mechanical damage, the yield decreased by 8 to 15%, and the plants were more susceptible to fungi and insects (Lambert et al., 1969; Salamini et al., 1970).

Fortunately, the soft endosperm of *o2* maize can be altered to resemble normal endosperm by the activity of modifier genes (Paez et al., 1969; Vasal, 1971). Modified *o2* genotypes developed at the International Maize and Wheat Improvement Center (CIMMYT) are called quality protein maize (QPM) (Vasal, 2000). The general strategy for the development of QPM initially included backcrossing to develop *o2* varieties and hybrids from normal genotypes, and later, recurrent selection to develop specific genetic backgrounds that restored the vitreous portion of the endosperm. Quality protein maize genetic pools, populations, and inbred lines have resulted from these breeding efforts. Protein quality testing during the breeding process was used in an attempt to avoid excessive losses of Lys and Trp. In general, QPM genotypes retain higher levels of Lys and Trp than normal maize materials (Ortega and Bates, 1983). Yield trials have shown that some QPM germplasm can be competitive with local checks (Pixley and Bjarnason, 1993). The QPM genotypes have been introduced to production systems in several tropical and subtropical countries, where their nutritional advantages have been acknowledged (National Research Council, 1998; Vietmeyer, 2000).

Nevertheless, there are major challenges to develop and use QPM germplasm because of the unknown number of modifier genes required to restore the desired hard-to-soft endosperm ratio, the need to evaluate the grain quality during the breeding process, and the effects specific to the genetic background (Belousov, 1987; Ciceri et al., 2000; Huang et al., 2004; Gibbon and Larkins, 2005).

The objectives of this study were to utilize a population of recombinant inbred lines (RILs) derived from an *o2* line and a QPM line to estimate variance components, phenotypic and genotypic correlations, and heritabilities

and to identify selection strategies for endosperm texture modification and relative content of the essential amino acids Lys, Trp, and methionine (Met).

MATERIALS AND METHODS

Plant Material

A population of RILs was derived from crossing an *o2* temperate line, B73*o2*, with a QPM tropical line, CML161. Inbred CML161 is an exotic tropical-lowland inbred classified as QPM and released by CIMMYT. It is a high-yielding QPM line commonly used in tropical and semitropical breeding programs where it has been used in multiple hybrid combinations (Prasanna et al., 2001; Xingming et al., 2001; Bhatnagar et al., 2003). B73*o2* is an *opaque2* conversion of B73, an Iowa Stiff Stalk inbred. Although both parents are *o2*, CML161 kernels are flinty, whereas B73*o2* kernels are floury. Besides, B73*o2* has showed higher relative content of the essential amino acids Trp, Met, and Lys than CML161 (Scott et al., 2004). A group of 146 RILs were used for field trials (S_5 , S_6 , or S_7 generations) and genotypic analysis (S_6 generation).

Field Design

The RIL population was evaluated in two Texas locations during the years 2004, 2005, and 2006. The College Station (CS) location (latitude 30°37', elevation 96 m above sea level) at the Texas Agricultural Experiment Station in Burleson County has a humid subtropical climate and 99.3 cm of annual precipitation on average, while the Weslaco (WE) location (latitude 26°09', elevation 22.5 m above sea level) has a semiarid subtropical climate and 58.4 cm of annual precipitation on average (Griffiths and Bryan, 1987). The combination of location and years produced the environments WE04 (Weslaco in 2004), WE05 (Weslaco in 2005), CS05 (College Station in 2005), CS06A (College Station in 2006 first planting), and CS06B (College Station in 2006 second planting). A randomized complete block design was used in all environments: WE04 (one replication), WE05 (three replications), CS05 (two replications), CS06A (two replications), and CS06B (two replications). Each plot consisted of one row 5.2 m in length, with 0.76 (CS) or 1 m (WE) of distance between rows. Trials received common management practices according to each research station. At least 10 plants per plot were self-pollinated. All plots were manually harvested.

Endosperm Texture Modification

Endosperm texture was evaluated with four different measurements (TEXT-F, TEXT-L, OPAC, and VITR) associated to the extent of modification of the endosperm in the RIL population. Endosperm texture (TEXT) was a visual rating from 1 (modified = flint-type round crown kernel and vitreous appearance) to 5 (opaque = dent-type kernels with very high proportion of floury endosperm) with increments of 0.5. A value of TEXT was assigned to all the ears self-pollinated and harvested from each plot. Data were taken independently by one observer in the field during harvesting (TEXT-F), and two additional observers in the laboratory before shelling (TEXT-L). Opacity (OPAC) was scored in a light box using a scale of 1 (modified = light passes through the whole kernel) to 5 (opaque = no light transmission due to completely opaque kernels)

(Bjarnason and Vasal, 1992). For the trait vitreousness (VITR), an image analysis-based method was adapted (Leyva-Ovalle et al., 2002). Briefly, eight kernels from bulked ears per plot were arranged embryo down in a metallic grid and sanded with a 114 mm orbital sander (Hitachi Koki, Tokyo, Japan), until approximately one-third of the width of the kernel was removed. An 8-bit black and white image was obtained by scanning the kernels in a tabletop scanner (Hewlett-Packard ScanJet 3970, Palo Alto, CA) using a dark blue background. The negative of the image was used to estimate the area of soft (black) and hard (white) endosperm by the pixel counting option of the UTH-SCSA Image Tool 3.0 software (Wilcox et al., 2002). VITR was defined as the percentage of the area of hard endosperm to the total endosperm area.

Amino Acid Composition

Trp, Met, and Lys were quantified using a microbiological method based on *E. coli* strains auxotrophic for Trp, Met, or Lys as described by Scott et al. (2004). Kernels from bulked ears per plot were ground and measured in triplicate. The concentration of Met, Trp, or Lys in each analysis was calculated using linear regression onto a line fitted to the standards.

The units of the amino acid measurement are arbitrary and are intended to provide relative values within this experiment and not for making comparisons to other methods. The values provided here are intended to rank the samples using a high-throughput method that can be used in breeding programs. This analytical method has been proven highly repeatable and valid to measure the nutritional quality of the grain (Scott et al., 2004).

Statistical Analysis

Analysis of variance was conducted within and across environments for each trait separately, considering all effects in the statistical model as random, in PROC GLM of SAS 9.1 (SAS Institute, 2003). Genotype least-squares means were estimated per environment for each trait. Homogeneity of variances between environments was tested by the Levene's test (Glass, 1966). The best linear unbiased prediction (BLUP) procedure was used to predict the effects of each RIL across environments using the solution statement in PROC MIXED of SAS 9.1 (SAS Institute, 2003). Heritabilities across environments for each trait and their corresponding standard errors were calculated on a plot and entry mean basis using univariate mixed model analysis (all effects random) in PROC MIXED of SAS 9.1 (Holland et al., 2003; SAS Institute, 2003) as follows:

$$H_{pb} = \frac{\hat{\sigma}_g^2}{\hat{\sigma}_g^2 + \hat{\sigma}_{ge}^2 + \hat{\sigma}_e^2}$$

$$H_{mb} = \frac{\hat{\sigma}_g^2}{\hat{\sigma}_g^2 + \frac{\hat{\sigma}_{ge}^2}{e} + \frac{\hat{\sigma}_e^2}{r}}$$

where H_{pb} is heritability on plot basis, H_{mb} is heritability on entry mean basis, $\hat{\sigma}_g^2$ is the total genetic variance, $\hat{\sigma}_{ge}^2$ is the genotype \times environment variance, $\hat{\sigma}_e^2$ is the error variance, e is the number of environments, and r is the number of replicates. VITR, Lys, Trp, and Met measurements were taken on a single replication ($r = 1$) in two or four locations ($e = 2$ or 4, respectively).

The phenotypic (PC) and genotypic correlations (GC) among pair of traits and their corresponding standard errors were computed across environments using multivariate restricted maximum likelihood (REML) estimation in PROC MIXED (SAS Institute, 2003; Holland, 2006). The genotypic correlation between traits i and j is estimated as

$$GC_{ij} = \frac{\hat{\sigma}_{Gij}}{\hat{\sigma}_{Gi}\hat{\sigma}_{Gj}}$$

where $\hat{\sigma}_{Gij}$ is the estimated genotypic covariance between traits i and j and $\hat{\sigma}_{Gi}$ is the estimated genotypic standard deviation for trait i (Holland, 2006).

Direct response to selection (R) using both plots and entry means as selection units was calculated according to Falconer and Mackay (1996):

$$R_x = ih_x\sigma_{AX}$$

where i is the intensity of selection for trait X (1.4 was used corresponding to a selection differential $S = 20\%$), h_x is the square root of the heritability of trait X, and σ_{AX} is the square root of the additive genetic variance. The additive variance was calculated as half the variance among the RILs (Bernardo, 2002).

The correlated response (CR) of trait Y when selecting for trait X using entry means as selection units was calculated according to Falconer and Mackay (1996):

$$CR_Y = ih_xh_yr\sigma_{PY}$$

where i is the intensity of selection for trait X, h_x is the square root of the heritability of trait X, h_y is the square root of the heritability of trait Y, r is the genetic correlation between traits X and Y, and σ_{PY} is the square root of the phenotypic variance of Y.

RESULTS AND DISCUSSION

Trait Means and Variation

Significant ($p < 0.001$) main effects of the RILs were observed in the ANOVA for all traits (Table 1). Significant ($p < 0.001$) main effects of the environments were observed for TEXT-L, OPAC, VITR, Lys, Trp, and Met. In addition, significant genotype \times environment interaction (GEI) ($p < 0.001$) was observed for the traits TEXT-F, TEXT-L, and OPAC. Significant GEIs have been reported for other QPM materials for endosperm modification and other traits such as grain yield, protein in grain, Trp in grain, and protein (Pixley and Bjarnason, 2002; Lou et al., 2005). In normal maize it has been shown that environmental factors such as availability of nutrients in the soil, water stress and heat stress influence the process of kernel filling and the accumulation of storage proteins (Hamilton et al., 1951; Hadi, 2004; Monjardino et al., 2005).

The relative content of the amino acids Lys, Trp, and Met were measured. The parental line B73o2 showed consistently higher values of TEXT-F, TEXT-L, OPAC, Met, Trp, and Lys; whereas CML161 had higher values of VITR (Fig. 1 and 2). As expected, B73o2 had consistent and significantly softer endosperm than CML161.

The mean relative Lys content was 0.100 for B73o2 and 0.087 for CML161. The mean relative Trp content was 0.200 for B73o2 and 0.136 for CML161. The mean relative

Met content was 0.129 for B73o2 and 0.101 for CML161. Inbred B73o2 had consistently higher relative content of these amino acids than inbred CML161. These results are consistent with previous estimates in which B73o2 had high values of Lys, Trp, and Met (Scott et al., 2004).

The frequency distribution of each trait in the population of RILs approached a normal distribution for all traits except for TEXT-F, which showed bimodal distribution with peaks toward the semimodified and opaque categories, and for OPAC, whose intermediate categories had fewer individuals (Fig. 2). Transgressive segregation was not observed for any trait. Both parental inbreds were among the groups with most extreme expression of these traits.

The Levene's test suggested homogeneity of variances for all traits between the different environments except for Trp, in which the two CS locations showed a shift in the distribution toward a higher mean value. Both TEXT-F and TEXT-L correspond to visual estimates of the endosperm texture of whole ears measured under different conditions by different observers. However, the test for homogeneity of variance suggested heterogeneous variances between TEXT-F and TEXT-L, and therefore they were treated as different traits.

Given the contrasting differences between these two inbreds, CML 161 and B73o2, the amount of variation observed was high for all the traits measured. Hence, this RIL population was used as mapping population for a parallel study (Gutierrez-Rojas et al., 2006).

Phenotypic Correlations and Genotypic Correlations

The phenotypic correlations between all the four texture-related traits ranged between 0.66 ± 0.038 and 0.78 ± 0.023 . The genotypic correlations ranged between 0.81 ± 0.032 and 1.00 ± 0.013 . The phenotypic correlation between TEXT-F and TEXT-L was 0.70 ± 0.027 and the genetic correlation was 0.95 ± 0.018 . Due to the scale used in the measurements, VITR showed negative correlation with TEXT-F, TEXT-L, and OPAC (Table 2). The phenotypic correlation between Lys and Met was 0.41 ± 0.038 , whereas the genotypic correlation was 0.63 ± 0.047 . The phenotypic correlation between Lys and Trp was 0.62 ± 0.022 , and the genetic correlation was 0.71 ± 0.025 . The phenotypic correlation between Trp and Met was 0.54 ± 0.014 , and the genetic correlation was 0.68 ± 0.016 . Scott et al. (2004) analyzed a set of opaque and QPM lines and hybrids and observed a general positive correlation between Lys and Trp, but a negative correlation with Met. The positive correlation among amino acids content in this work could

relate to the observation that the o2 parent contained higher relative amounts of Lys, Trp, and Met, while the modified parent CML161 showed lower content of these

Table 1. Combined analysis of variance for endosperm texture in the field (TEXT-F), endosperm texture in the laboratory (TEXT-L), opacity (OPAC), vitreousness (VITR), lysine content (Lys), tryptophan content (Trp), and methionine content (Met) in an o2 × QPM recombinant inbred line population evaluated in Texas between 2004 and 2006.

Trait	Sources of variation†	df	Mean square	p > F	Variance component
TEXT-F (score 1–5)	Env	2	0.09	0.7328	–
	Rep/Env	3	0.40	0.2547	–
	Lines	142	6.30	<0.0001	1.150
	Env × Lines	278	0.47	<0.0001	0.101
TEXT-L (score 1–5)	Env	3	3.21	<0.0001	0.009
	Rep / Env	5	0.55	0.2038	–
	Lines	142	4.63	<0.0001	0.535
	Env × Lines	419	0.53	0.0001	0.072
OPAC (score 1–5)	Env	3	27.80	<0.0001	0.121
	Rep / Env	4	0.53	0.088	–
	Lines	142	4.79	<0.0001	0.719
	Env × Lines	421	0.44	<0.0001	0.104
VITR (% hard/total area)	Env	1	1223.94	<0.0001	9.123
	Lines	142	264.04	<0.0001	114.091
	Residual	128	47.10		
Lys (rel. units)	Env	3	4.15	<0.0001	0.027
	Lines	142	1.35	<0.0001	0.256
	Residual	420	0.33		
Trp (rel. units)	Env	3	179.71	<0.0001	1.260
	Lines	142	10.48	<0.0001	2.121
	Residual	420	2.09		
Met (rel. units)	Env	3	53.60	<0.0001	0.374
	Lines	142	2.75	<0.0001	0.491
	Residual	420	0.81		

†Env, environment; Rep, replication.

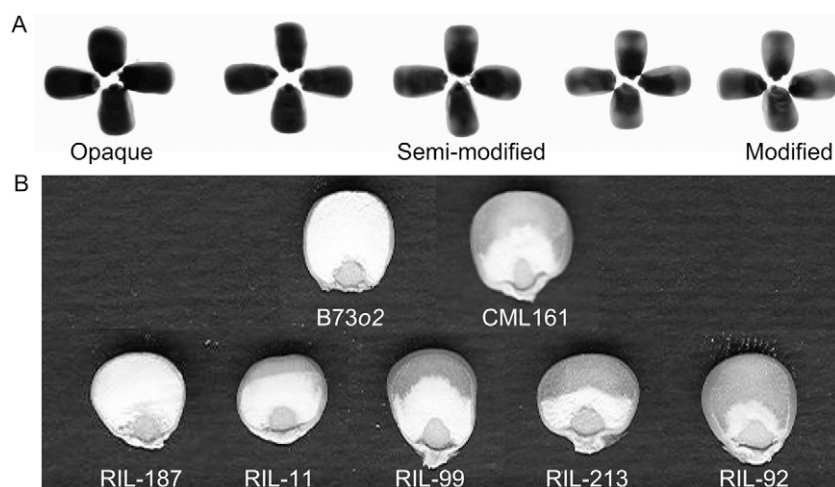


Figure 1. Endosperm texture modification. A. Photograph of backlit kernels showing segregation of the trait opacity (OPAC) in the F₂ of B73o2 × CML161. **B.** Kernel sections (8-bit images) of B73o2, CML161, and five recombinant inbred lines used for measuring the trait vitreousness (VITR).

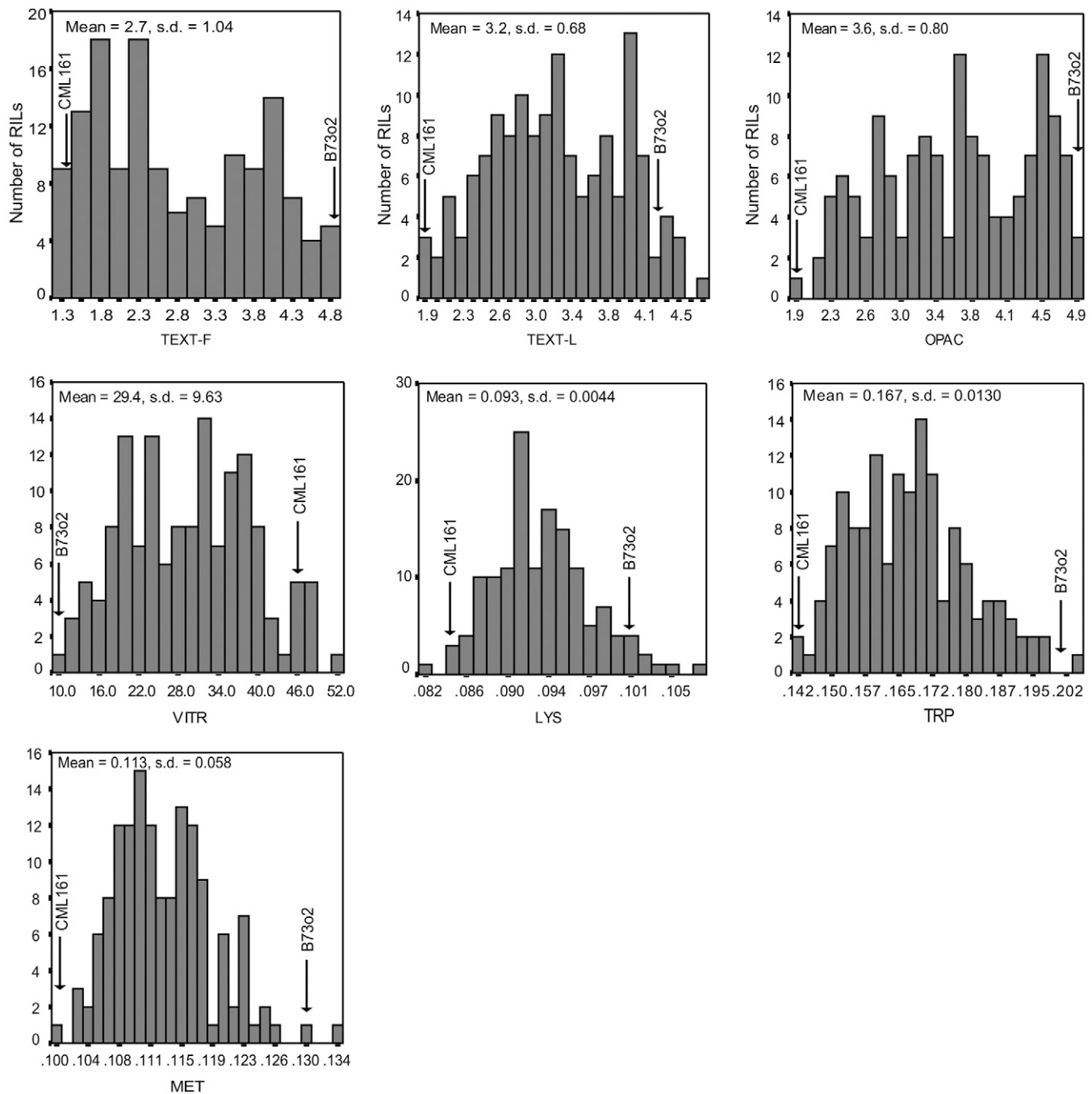


Figure 2. Frequency distributions of endosperm texture modification (TEXT-F, TEXT-L, OPAC, and VITR), and amino acid contents (Lys, Trp, and Met) in an $o2 \times$ QPM recombinant inbred line (RIL) population evaluated in Texas between 2004 and 2006. Mean values of the parents B73o2 and CML161 are showed by arrows.

amino acids. In addition, the nature of diversity displayed by the RIL population used in this study and that displayed among the lines with different origin used by Scott et al. (2004) is different.

In the population of RILs derived from B73o2 \times CML161, there was a positive correlation between endosperm opacity and relative content of Lys, Trp, and Met. The relative values of these amino acids decreased as the inbred lines tended from opaque toward flint/dent modified kernels (Fig. 3). However, there were RILs with high content of amino acids and degree of

modification suggesting the possibility to select for both simultaneously. Contradictory reports have described both neutral (Pixley and Bjarnason, 2002; Bantte and Prasanna, 2004) and negative (Paez et al., 1969; Robutti et al., 1974; Wessel-Beaver et al., 1985) correlations for endosperm modification and amino acid content. Here, grain yield analysis was not included, although there is evidence for lack of phenotypic correlations between yield and endosperm modification, but negative correlation between yield and amino acid content (Pixley and Bjarnason 2002).

Table 2. Phenotypic (PC) and genotypic correlation (GC) estimates for endosperm texture in the field (TEXT-F), endosperm texture in the laboratory (TEXT-L), opacity (OPAC), vitreousness (VITR), lysine content (Lys), tryptophan content (Trp), and methionine content (Met) in an $\alpha 2 \times$ QPM recombinant inbred lines population evaluated in Texas between 2004 and 2006.

	TEXT-F		TEXT-L		OPAC		VITR		Lys		Trp	
	PC	GC	PC	GC	PC	GC	PC	GC	PC	GC	PC	GC
TEXT-F	-	-										
SE												
TEXT-L	0.70	0.95	-	-								
SE	0.027	0.018										
OPAC	0.78	0.94	0.67	1.00	-	-						
SE	0.023	0.017	0.028	0.013								
VITR	-0.68	-0.81	-0.66	-0.95	-0.75	-0.94	-	-				
SE	0.022	0.032	0.038	0.032	0.030	0.057						
Lys	0.32	0.46	0.24	0.43	0.32	0.50	-0.18	-0.30	-	-		
SE	0.057	0.083	0.053	0.088	0.050	0.080	0.014	0.045				
Trp	0.39	0.55	0.31	0.58	0.41	0.63	-0.34	-0.33	0.62	0.71	-	-
SE	0.054	0.073	0.051	0.073	0.048	0.065	0.004	0.007	0.022	0.025		
Met	0.36	0.54	0.24	0.52	0.27	0.51	-0.21	-0.31	0.41	0.63	0.54	0.68
SE	0.055	0.081	0.054	0.085	0.093	0.146	0.008	0.024	0.038	0.047	0.014	0.016

Broad-Sense Heritability and Gain from Selection

The heritabilities (entry mean basis) calculated across environments for TEXT-F, TEXT-L, and OPAC were 0.94 ± 0.010 , 0.90 ± 0.014 , and 0.92 ± 0.107 , respectively. The heritabilities (plot basis) calculated for TEXT-F, TEXT-L,

and OPAC were 0.75 ± 0.027 , 0.54 ± 0.036 , and 0.66 ± 0.032 , respectively (Table 3).

The estimates of heritability for VITR were 0.83 ± 0.030 (entry mean basis) and 0.71 ± 0.043 (plot basis). Heritabilities on entry mean basis calculated across environments for Lys, Trp, and Met were 0.76 ± 0.034 , 0.80 ± 0.027 , and 0.71 ± 0.040 , respectively. Heritabilities on plot

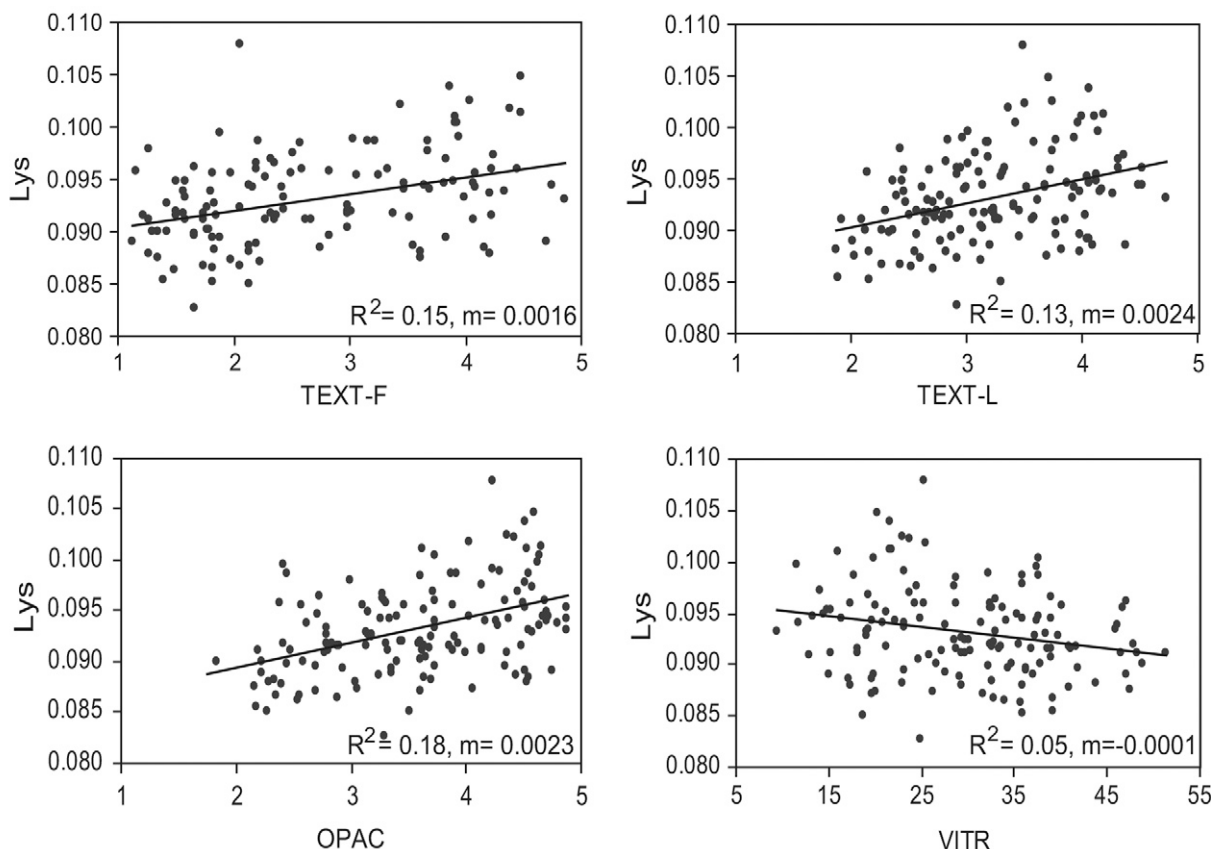


Figure 3. Phenotypic correlation between lysine content (Lys) and endosperm modification (TEXT, OPAC, and VITR) in an $\alpha 2 \times$ QPM recombinant inbred line population.

Table 3. Heritability estimates for endosperm texture in the field (TEXT-F), endosperm texture in the laboratory (TEXT-L), opacity (OPAC), vitreousness (VITR), lysine content (Lys), tryptophan content (Trp), and methionine content (Met) in an $\alpha 2 \times$ QPM recombinant inbred line population evaluated in Texas between 2004 and 2006.

Trait (environments, repetitions)	Plot basis	Entry mean basis
TEXT-F ($e = 3, r = 6$)	0.75	0.94
SE	0.027	0.010
TEXT-L ($e = 4, r = 9$)	0.54	0.90
SE	0.036	0.014
OPAC ($e = 4, r = 8$)	0.66	0.92
SE	0.032	0.107
VITR ($e = 2, r = 2$)	0.71	0.83
SE	0.043	0.030
Lys ($e = 4, r = 4$)	0.44	0.76
SE	0.045	0.034
Trp ($e = 4, r = 4$)	0.50	0.80
SE	0.043	0.027
Met ($e = 4, r = 4$)	0.38	0.71
SE	0.046	0.040

basis calculated across environments for Lys, Trp, and Met were 0.44 ± 0.045 , 0.50 ± 0.043 , and 0.38 ± 0.046 , respectively (Table 3). When inbred lines are developed from an F_2 population, the variance among inbred lines is equal to twice the original additive variance in the starting F_2 population (Bernardo, 2002). Therefore, the heritability/repeatability estimates calculated from inbred lines could be higher than estimates made using other mating designs.

With the estimates of heritability and the additive variance it is possible to predict the response to selection (R) (Falconer and Mackay, 1996). The direct selection response was calculated using a selection intensity of 20%. As expected, the results suggested that a substantial gain can be expected when selection is applied to endosperm texture traits. Gains were higher when entry means were used as selection units. The maximum predicted gain was about 40%, for the trait TEXT-F with entry means selection (Table 4). For amino acid content, the gain was much

lower, with a maximum of 7.7% of gain for Trp. Calculated gain in Lys was 4.7%. As for endosperm texture, selection based on entry means produced higher gain in amino acid content (Table 4).

As mentioned before, amino acid content and endosperm modification traits were negatively correlated in the population of RILs. The correlated response to selection (CR) can be predicted to anticipate the change in amino acid content when selecting for endosperm modification (Table 5). Selection for any of the four texture-related traits produced a decrease in the content of the three amino acids. For each unit increase in endosperm texture rating, Lys content was reduced by approximately 3%. (Table 5). Hence, if the correlated loss in amino acid content caused by selection for endosperm modification is found to be significant, then evaluation for both traits must be considered when developing QPM genotypes (Wessel-Beaver et al., 1985). However, selection for amino acid content based on direct measurements of each amino acid is not a feasible alternative for most breeding programs (Prasanna et al., 2001). Alternatives such as specific calibrations of near-infrared reflectance spectroscopy for grain quality traits have shown that thousands of samples can be processed in a relatively short period of time at reasonable cost (Fontaine et al., 2002; Montes et al., 2006).

CONCLUSIONS

Genetic variation for endosperm and amino acids content was observed in this study. This variation supported intermediate to high heritabilities and the existence of RILs with desirable combinations of high amino acids content and endosperm vitreousness. The development of segregating populations between opaque with high amino acids content and QPM material could be a breeding approach to enhance further the protein quality of QPM lines (e.g., CML161). The lack of significant transgressive segregation may be the consequence of using parents with very extreme expression of these traits. Parents with intermediate expression should provide segregating populations showing transgressive segregation.

Table 4. Predicted response to selection for endosperm texture in the field (TEXT-F), endosperm texture in the laboratory (TEXT-L), opacity (OPAC), vitreousness (VITR), lysine content (Lys), tryptophan content (Trp), and methionine content (Met) with both plot basis and entry means-based selection units.

Trait	Selection response		Selection response as percentage of the mean	
	Plot basis	Entry mean basis	Plot basis	Entry mean basis
%				
TEXT-F (score 1–5)	0.92	1.03	35.49	39.68
TEXT-L (score 1–5)	0.53	0.69	16.51	21.36
OPAC (score 1–5)	0.68	0.81	18.90	22.39
VITR (%hard/total area)	8.89	9.63	30.05	32.52
Lys (rel. units)	0.33	0.44	3.55	4.67
Trp (rel. units)	1.02	1.29	6.12	7.72
Met (rel. units)	0.43	0.58	3.78	5.18

Table 5. Correlated response of lysine content (Lys) when selecting for endosperm texture in the field (TEXT-F), endosperm texture in the laboratory (TEXT-L), opacity (OPAC), vitreousness (VITR), lysine content (Lys), tryptophan content (Trp), and methionine content (Met) with entry means-based selection units.

Selecting for	GC _{x-Lys} [†]	Entry mean basis	Change in Lys
			%
TEXT-F (score 1–5)	0.455	0.31	3.35
TEXT-L (score 1–5)	0.429	0.29	3.09
OPAC (score 1–5)	0.503	0.34	3.68
VITR (% hard/total area)	-0.297	-0.19	-2.06

[†]GC, genetic correlation.

High heritabilities and genetic correlations suggest high direct and indirect genetic gain possible by selection for single traits. Nevertheless, negative correlation between desirable endosperm traits and amino acids contents may indicate the need of monitoring both types of traits and the use of selection indices during the inbreeding and selection process.

The quantification of endosperm vitreousness by image analysis was conducted successfully and can be used in breeding programs. It was highly correlated with visual endosperm ratings, but some differences were observed among RIL rankings.

Based on the results presented here, it may be possible to further enhance the protein and nutritional value of QPM by measuring endosperm characteristics and levels of Lys, Trp, and Met during breeding and selection of recombinant lines (i.e., conventional or double haploids) and by selecting those having vitreous endosperm and high levels of these amino acids.

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