Maydica 49 (2004): 303-311

TRYPTOPHAN AND METHIONINE LEVELS IN QUALITY PROTEIN MAIZE BREEDING GERMPLASM

M.P. Scott[1], S. Bhatnagar[2], J. Betrán[2]*

[1] *USDA-ARS, Corn Insects and Crop Genetics Research Unit, Ames, IA 50014, USA*
[2] *Texas A&M University, Department of Soil and Crop Sciences, College Station, TX 77843, USA*

Received July 14, 2004

ABSTRACT - Because maize (*Zea mays* L.) is often used either as food for humans or as feed for monogastric animals, essential amino acid levels are important. Maize kernels containing the *opaque-2* (*o2*) mutation have improved amino acid balance and poor agronomic qualities including opaque kernels that are soft and susceptible to mechanical and biological damage. Quality Protein Maize (QPM) developed through plant breeding has improved amino acid balance conferred by the *opaque-2* (*o2*) mutation, but lacks the agronomic deficiencies normally associated with this mutation. To characterize the amino acid balance in QPM breeding germplasm, we determined the levels of nutritionally limiting amino acids tryptophan and methionine. Tryptophan levels were negatively correlated with endosperm translucence, a measure of kernel hardness suggesting the process of selection for hard-kernels reduces tryptophan levels. On average, germplasm containing the *o2/o2* mutation had lower methionine levels than *O2/O2* germplasm regardless of kernel hardness, suggesting methionine levels could be reduced by the *o2/o2* mutation. A series of inbred lines was test-crossed to the *o2/o2* soft endosperm inbred line Tx804. The predictive value of the characteristics of the inbred line for the characteristics of the hybrids was examined. The amino acid levels of the inbred lines were significantly correlated with those of the hybrids, although the predictive value was low (R^2 = 0.13 and 0.27 for methionine and tryptophan, respectively). The reduction in tryptophan during conversion to the hard-kernel phenotype and the reduction in methionine in *o2* germplasm both reduce the nutritional value of QPM. It may be possible to correct these deficiencies by breeding and selection for levels of tryptophan and methionine.

KEY WORDS: Tryptophan; Methionine; Quality protein maize.

* For correspondence (fax: +1 979 862 1931, e-mail: javierbetran@tamu.edu)

INTRODUCTION

The majority of the maize grown worldwide is used in food or feed. One of the main nutritional limitations of maize grain is its essential amino acid content does not match the nutritional requirements of monogastric animals, including humans. Lysine and tryptophan levels in maize normally do not meet the minimum requirements established for human growth and development (FAO/WHO/UNU, 1985) or for growth and development of monogastric animals. In legumes, the sulfur amino acids, cysteine and methionine, are limiting. Because maize is frequently complemented with legume protein in diets, the methionine level can be limiting in some diets. Thus, increasing the levels of these nutritionally limiting amino acids is an important objective of plant breeding programs. Lysine levels have been shown to be correlated with tryptophan levels, so rapid chemical methods to measure tryptophan are used to assess amino acid balance in plant breeding programs (HERNANDEZ and BATES, 1969).

Plant breeding was first used to alter the composition of maize grain more than a century ago (HOPKINS, 1899) and has been used with success since that time. With the discovery that maize carrying the *o2* mutation has an improved amino acid balance (MERTZ *et al.*, 1964), breeders in programs directed toward improving amino acid balance began to work with *o2* germplasm. The *o2* mutation is characterized by soft, opaque kernels with low density and high susceptibility to mechanical and biological damage. One of the main objectives of breeding programs working with the *o2* mutation, therefore, was to overcome these adverse pleiotropic effects of the *o2* mutation. By selection for harder kernels in *o2* germplasm, breeders have developed maize designated Quality Protein Maize (QPM) (VASAL, 2001), which contains the *o2* mutation and the ele-

vated lysine levels accompanying it, but has high-density, translucent endosperm.

While it was noted that methionine levels are reduced in an early characterization of *o2* strains (MERTZ et al., 1964), methionine levels in *o2* breeding programs are seldom monitored. Studies have shown that *o2* germplasm has lower methionine levels than normal germplasm (ROBUTTI *et al.*, 1971; EGGUM *et al.*, 1979). Similarly, a QPM variety was shown to have significantly lower methionine levels than two out of three normal endosperm hybrids and no significant difference from a third normal hybrid (ZARKADAS *et al.*, 1995) and a set of 15 QPM varieties was found to have a highly significant ($p<.001$) lower methionine level than two normal check hybrids (ZARKADAS *et al.*, 2000). While methionine levels were reported in all of these references, only one of them commented on the difference in methionine levels in the text (MERTZ *et al.*, 1964).

The Texas A&M University maize breeding program has developed high lysine inbreds with different levels of endosperm hardness that are adapted to temperate Southern U.S. growing conditions. These lines were developed from CIMMYT (The International Wheat and Maize Improvement Center) QPM populations, conversions of elite normal inbreds, or temperate soft endosperm breeding populations. These lines, which represent a wide range of genetic backgrounds and kernel characteristics, and their hybrids, constitute suitable breeding germplasm to estimate levels of essential amino acids and their relationships with other traits.

Several studies have examined amino acid concentrations in *o2* maize germplasm. A study of 93 inbred lines, including some *o2* lines, revealed lysine content is correlated with the level of non-zein proteins (MORO *et al.*, 1996). A study of several traits, including tryptophan levels and endosperm opacity in QPM hybrids and open pollinated cultivars concluded tryptophan levels and level of endosperm opacity are not correlated, and tryptophan levels are very stable across environments (PIXLEY and BJARNASON, 2002). In a study of tryptophan content in *o2*, modified endosperm *o2* and wild-type versions of inbred lines, the level of tryptophan was found to be reduced on average in the modified endosperm *o2* lines relative to the unmodified *o2* lines (GENTINETTA *et al.*, 1975). The relationship between lysine and endosperm opacity has been studied more thoroughly, and it has been determined that lysine levels in hard endosperm *o2* breeding germplasm were intermediate between *o2* and normal germplasm (ORTEGA and BATES, 1983; ROBUTTI *et al.*, 1974) and lysine levels are negatively correlated with endosperm hardness (WESSEL-BEAVER *et al.*, 1985).

This research has three objectives related to developing maize with improved nutritional value: First, to determine if tryptophan levels are altered by conversion from the soft-kernel to the hard-kernel phenotype in our breeding germplasm. This objective is similar to that of an earlier study, (PIXLEY and BJARNASON, 2002) but we used germplasm with a wider range of endosperm translucence so trends may be easier to detect. Because methionine is potentially nutritionally limiting and normally not monitored in QPM breeding programs, the second objective is to characterize the variation in methionine content in QPM breeding germplasm. The relationship between amino acid levels of inbred lines and their hybrids, and the level of transmission to hybrids of the amino acid contents from parental inbreds are important issues in hybrid development. Therefore, the third objective is to estimate the relationship between inbred lines and their testcrosses for tryptophan and methionine levels. The information gained in this study is useful for designing breeding strategies for the development of QPM with improved amino acid balance.

MATERIALS AND METHODS

Germplasm

Evaluations were conducted on three sets of germplasm with each set containing about 80 accessions: (1) QPM lines developed from CIMMYT QPM populations 65 (yellow flint), 66 (yellow dent), 69 (temperate yellow flint), 70 (temperate yellow dent), Temperate x Tropical High-Oil, Pools 26 (tropical late yellow dent), 33 (subtropical intermediate yellow flint), and 34 (subtropical intermediate yellow dent). This set is referred to as "inbreds 1"; (2) Crosses of lines in set 1 with soft endosperm *o2/o2* inbred Tx804. This set is referred to as "testcrosses"; and (3) experimental lines ranging from *o2/o2* soft endosperm lines developed from breeding crosses provided by Crow's Seed Company and classified as Iowa Stiff Stalk Synthetic or non Stiff Stalk heterotic groups, to translucent QPM lines. This set is referred to as "inbreds 2". Both QPM and opaque lines were advanced and selected in Texas maize nurseries (summer nursery at College Station, TX, and fall-winter nursery at Weslaco, TX) based on endosperm opacity, maturity, grain color, grain yield, lodging, lysine content and plant traits. High lysine inbreds (Tx802, CML161, Do940y, B73 *o2/o2*, and Tx804), and normal inbreds (Tx772, NC300, FRB73, FR2128, B104, and Tx601y) were included as checks in the inbred line evaluations. The commercial *o2/o2* hybrid Crow's SL53, and the normal endosperm hybrids Pioneer Brand 31B13 and 32R25, Dekalb DK668 and DK687, and Asgrow RX897 were included as checks in the evaluations of hybrids.

Because our objective was to identify trends across a broad range of germplasm rather than to obtain values for any specific genotype, we used a single replication of the experiment. This allowed us to survey many samples, providing a good illustration of general trends in a breeding program where lines are advanced and selected in single replications, although precision in the individual measurements was sacrificed. Grain of these three sets of germplasm was produced in single plots at Texas A&M University during the summers of 2001 and 2002 at College Station. A subset of the grain produced in 2001 was used to examine the correlation between lysine and tryptophan (Fig. 1) and for the study of tryptophan and methionine content in different classes of germplasm (Table 1). The 2002 grain was used for all other studies. Plots were irrigated and fertilized with 350 kg ha^{-1} of 32-0-0 and 6 units of zinc before planting and 180 kg ha^{-1} of 32-0-0 at V6 stage. All ears in a line or testcross used in this study were self pollinated by hand, harvested, bulked within genotypes, and the grain dried to approximately 12% moisture. Endosperm opacity was measured using 50 kernels per genotype that were individually classified in three classes: opaque, semi-vitreous, and vitreous by visual observation on a light box. A weighted average per genotype was calculated using a 1 to 5 scale (Figs. 2 and 4; opaque = 1, semi-vitreous = 3, and vitreous translucent = 5). Genotypes with *o2/o2* grain with a score greater than 3 were defined as QPM.

Quantification of amino acids

Lysine was quantified using the AOAC standard method for determination of lysine levels in grain (AOAC, 1990). Separation and analysis of amino acids were done with a Beckman 6300 Amino Acid Analyzer (Elk Grove, GA) equipped with a high performance cation-exchange resin column, and amino acid detection was done with a post-column ninhydrin derivation. Norleucine was used as the internal standard.

Tryptophan and methionine were quantified using a microbiological method suited to the high-throughput needs of plant breeding programs. Kernels from bulked ears were ground using a coffee grinder so that greater than 70% of the ground material passed through an 80 micron mesh screen. The ground grain was mixed and 10 mg of the resulting powder was weighed into a randomly assigned well of a 96-well plate. Ten wells were not filled to accommodate standards. In order to extract and hydrolyze protein in the ground grain, one-hundred microliters of 50 mM KCl adjusted to pH 2.0 with HCl containing 0.2 mg of pepsin were added to each well and the plate was shaken 16 hours at 37°C. This time was used because the level of tryptophan released by the hydrolysis reaches a constant level at this point (data not shown). The plate was then centrifuged at 3000 x g for 20 minutes, and 4 µl of the supernatant for methionine analysis or 7 µl of the supernatant for tryptophan analysis was transferred to the corresponding well of a second plate for analysis. Five microliters of standards consisting of commercially obtained (Sigma, St. Louis, MO) methionine or tryptophan in the concentrations of 0.1 to 0.8 mM and 0.1 to 0.6 mM, respectively, were added to the empty wells of the plate. This plate was inoculated with a bacterial strain auxotrophic for either tryptophan (CAG18455, (SINGER *et al.*, 1989) or methionine (P4X, (JACOB and WOLLMAN, 1961) in 100 µl M9 minimal media. This plate was incubated with shaking at 37°C for 20 h for tryptophan analysis or 16 hours for methionine analysis. Following incubation, the 595 nm light scattered by the culture was measured in a microplate reader to give a value reflecting the level of amino acid per mass of tissue. The level of each amino acid is expressed as a fraction of the maximum value in a given comparison.

TABLE 1 - *Average tryptophan (Trp) and methionine (Met) contents in entries selected from the three groups of germplasm described in the materials and methods.*

		grouping[a]					mean[b]	S.E.	Observations
Trp[c]	Yellow *o2/o2* Inbreds	A					0.784	0.019	13
	Yellow *o2/o2* Advanced Inbreds		B				0.730	0.013	25
	White *o2/o2* Advanced Inbreds		B	C			0.683	0.019	12
	White *o2/o2* Inbreds		B	C			0.682	0.025	7
	White & Yellow *o2/o2* Hybrids			C	D		0.652	0.025	7
	Yellow Normal Inbreds				D	E	0.602	0.024	8
	White Normal Inbreds					E	0.534	0.047	2
Met	Yellow Normal Inbreds	A					0.833	0.024	8
	White Normal Inbreds		B				0.647	0.048	2
	Yellow *o2/o2* Inbreds		B				0.602	0.019	13
	Yellow *o2/o2* Advanced Inbreds			C			0.493	0.014	25
	White & Yellow *o2/o2* Hybrids			C			0.481	0.026	7
	White *o2/o2* Advanced Inbreds			C			0.473	0.020	12
	White *o2/o2* Inbreds			C			0.470	0.026	7

[a] Grouping based on comparison of means by Fisher's protected LSD at p=0.05. Groups with the same letter are not significantly different from each other.

[b] Mean amino acid content per mass of tissue for the group indicated, relative to the highest individual value which was set to 1.

[c] Trp = Tryptophan; Met = Methionine.

Statistical methods

Each ground sample was measured in triplicate, with each measurement made on an independently randomized 96-well plate. This is a randomized complete block design with three plates each representing a block, and each sample evaluated once in each block. The methionine and tryptophan concentration in each analysis was calculated using linear regression onto a line fitted to the standards. The predicted value of each sample was calculated from the three individual measurements using a linear ANOVA model with the plate considered a fixed effect. In each comparison of means, statistical differences within and among groups were characterized using a one-way ANOVA with a probability threshold of $p>0.05$. When the F tests were significant, groupings were assigned using pairwise Students T tests with $p>0.05$ as the significance threshold (Fisher's protected LSD). To estimate the relationship between amino acid levels on inbreds and their testcrosses, simple regression of testcross means on means for parental inbreds was computed using linear regression with the least squares procedure.

RESULTS

Tryptophan level is negatively correlated with endosperm opacity

It has been suggested that concentrations of lysine and tryptophan are correlated in maize grain (HERNANDEZ and BATES, 1969). We measured these amino acids in an array of breeding germplasm and found a similar significant relationship (Fig. 1). Given that tryptophan is one of the most nutritionally limiting amino acids and tryptophan levels are correlated with lysine levels, we conclude that in this germplasm measurement of tryptophan is an efficient way to evaluate the amino acid quality of grain.

By determining correlations between tryptophan levels and endosperm opacity scores, statistically

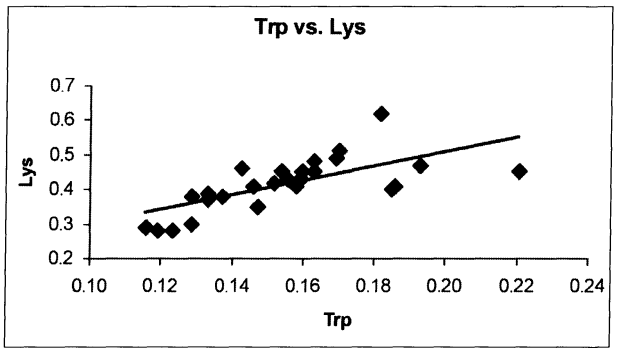

FIGURE 1 - Relationship between tryptophan (Trp, relative values) and lysine (Lys, mg/100 mg sample) for 28 maize genotypes (5 QPM hybrids, 6 QPM white inbreds, 10 QPM yellow inbreds, 4 normal inbreds and 1 normal hybrid). This germplasm is a subset of the three germplasm sets described in the materials and methods.

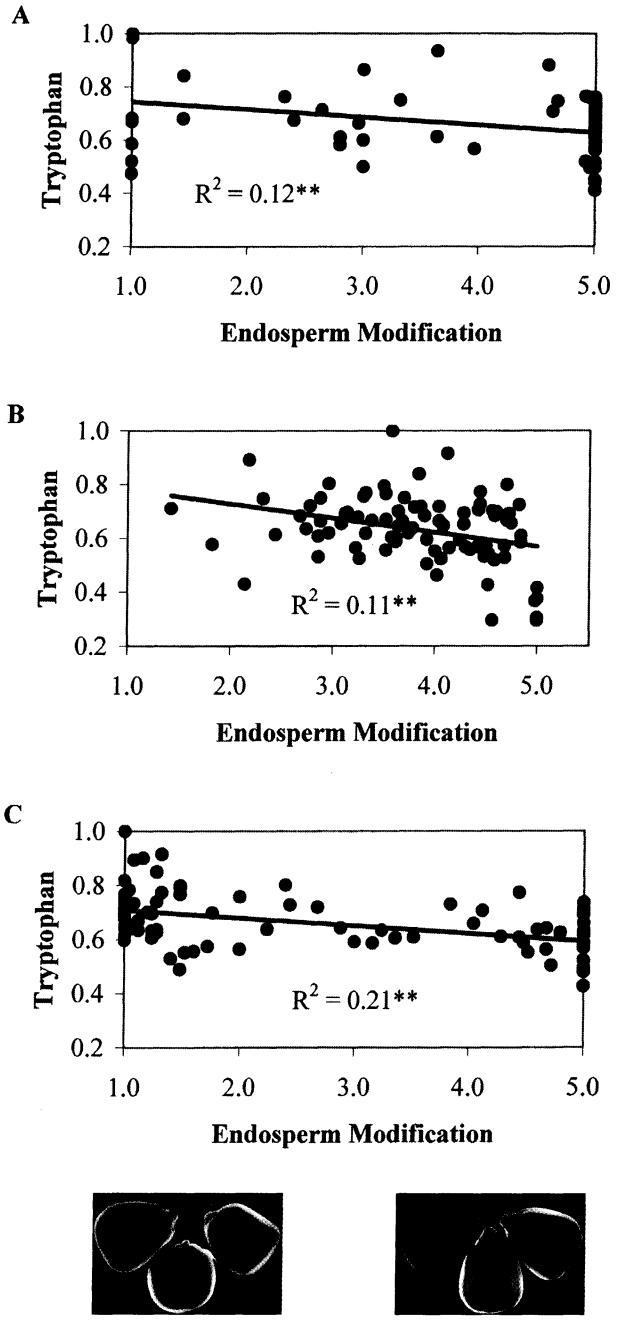

FIGURE 2 - Relationships between tryptophan and maize endosperm opacity (1 = opaque, 5 = translucent). A. Kernels resulting from self-pollination of five *O2/O2* inbred lines (circled) and 81 high lysine lines (inbreds 1). B. F2 kernels from self pollination of F1 plants from crosses of each inbred line in A with the *o2/o2* inbred Tx804 (testcrosses). C. Kernels from self-pollination of 85 *o2/o2* inbred lines and one *O2/O2* inbred line (inbreds 2). Pictures at the bottom illustrate typical back-lit kernel phenotypes for endosperm opacity score 1 (opaque) and 5 (translucent).

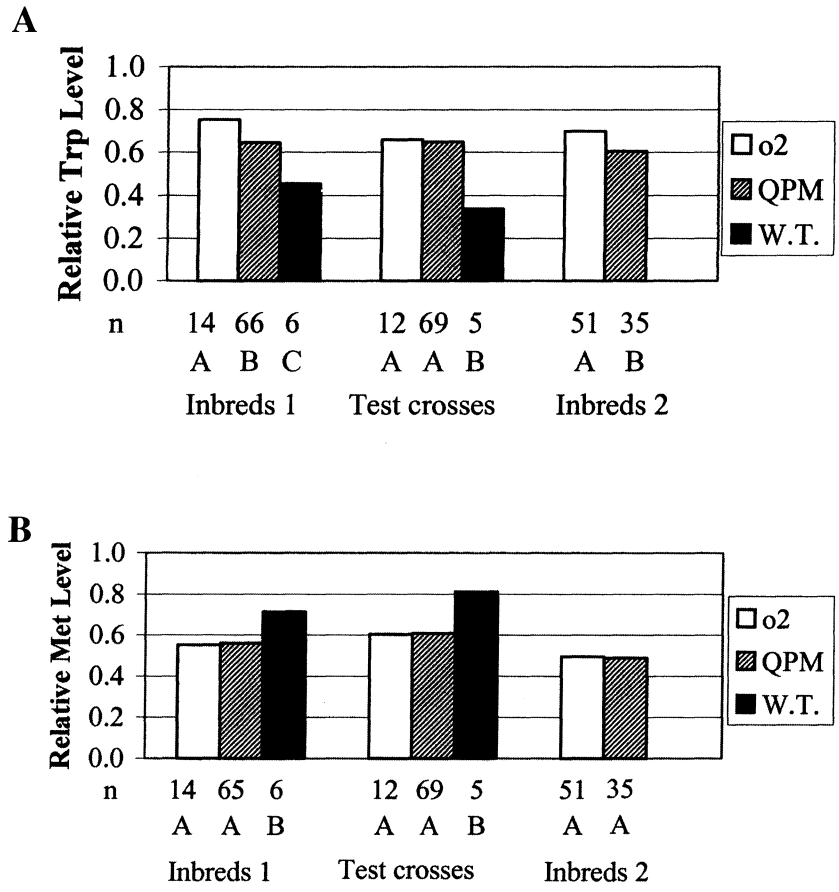

FIGURE 3 - A. Mean tryptophan (trp) content of maize germplasm sorted by phenotype. The number of entries in each group is given in the top line of the x-axis label. Groupings resulting from mean comparison within each of the three groups (Inbreds 1, Testcrosses and Inbreds 2) with Fisher's protected LSD test at $p<0.05$ are given on the middle line of the x-axis label. B. As in panel A, but for mean methionine (met) content.

significant ($p<0.01$) negative relationships between tryptophan and endosperm opacity were found in all three sets of germplasm examined (Fig. 2). This figure also illustrates that the distribution of endosperm modification scores is skewed toward the extremes in germplasm sets 1 and 3 (Fig. 2A and 2B, respectively) because these sets contain relatively large numbers of completely opaque or completely transluscent samples. Germplasm set 2, the testcrosses, are more normally distributed (Fig. 2B).

When the accessions within each set were grouped according to their level of translucence as *o2* (endosperm translucence <3) or QPM (endosperm translucence >3), the mean tryptophan levels of the *o2* group was significantly ($p>0.05$) greater than the QPM group in two of the three sets (Fig. 3).

Methionine is reduced in high lysine germplasm

Methionine is the third limiting amino acid in maize used in non-ruminant diets after lysine and tryptophan, and it is the first limiting amino acid in the legumes. When considering a complete diet often consists of maize and a legume, methionine levels can be nutritionally limiting. One of our objectives was to characterize methionine levels in QPM breeding germplasm. First, to determine if methionine levels were different in high lysine (*o2/o2*) and normal germplasm, we examined methionine levels in an array of high lysine (*o2/o2*) and normal inbreds and hybrids. This germplasm consisted of selections from the three sets of germplasm described earlier and produced in 2001. While the average tryptophan content of the "normal inbreds" was in the two lowest groupings, the average methionine content of the "normal inbreds" group was in the two highest groupings (Table 1). To examine this trend further, we determined the methionine content in each of the three germplasm sets described in the Materials and Methods. Thus, we examined methionine levels in inbreds grouped as *O2/O2* (w.t.), *o2/o2* (o2) or QPM (inbreds 1 and inbreds 2, Fig. 3). In addition, we made the same comparison

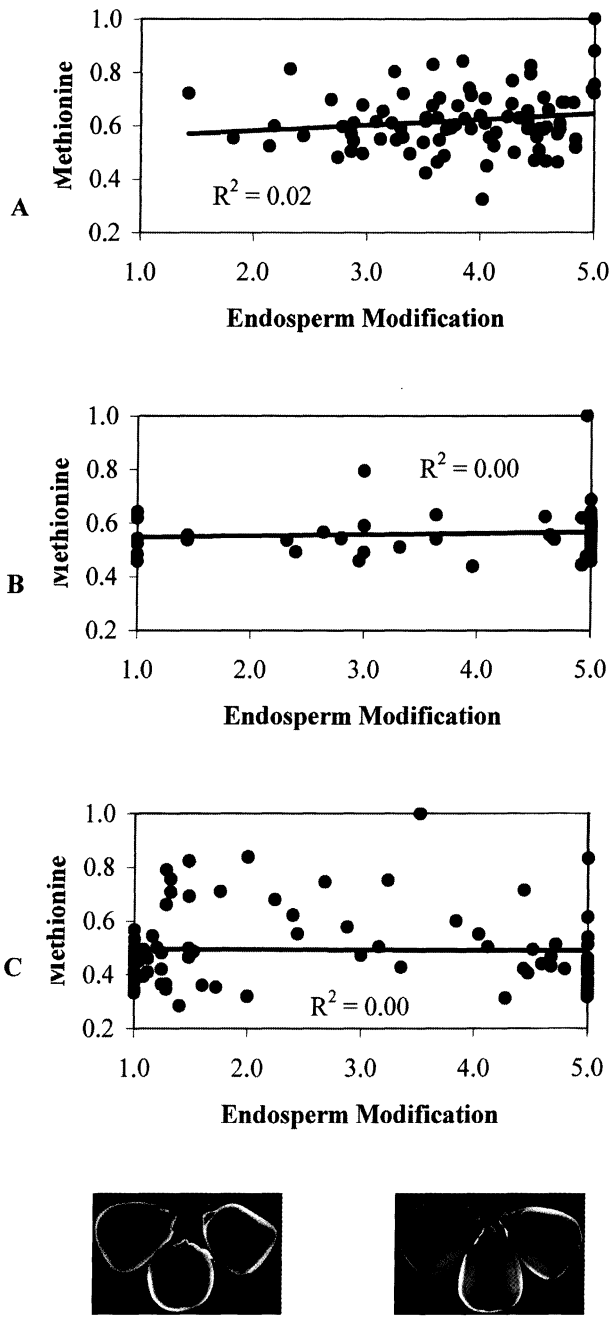

FIGURE 4 - Relationships between methionine and maize endosperm opacity (1 = opaque, 5 = translucent). A. Kernels from self-pollination of five *O2/O2* inbred lines and 81 high lysine lines (inbreds 1). B. F2 kernels from selfing in crosses of each inbred line in A with the *o2/o2* inbred Tx804 (testcrosses). C. Kernels from self-pollination of 85 *o2/o2* inbred lines and one *O2/O2* inbred line (inbreds 2). Pictures at the bottom illustrate typical back-lit kernel phenotypes for endosperm opacity score 1 (opaque) and 5 (translucent).

for the F2 seed produced by selfing the F1 crosses of each of these inbred lines with the *o2/o2* tester, Tx804 (Testcrosses, Fig. 3). In contrast to the average tryptophan level, which was higher in the *o2/o2* germplasm, the average methionine levels of the *o2* and QPM genotypes were not significantly different, while the *O2/O2* genotypes were significantly higher. Taken together, these data indicate methionine levels are lower in *o2/o2* germplasm. It seems that the process of selecting for translucent kernels does not substantially effect methionine levels, because methionine levels were very similar in *o2/o2* and QPM germplasm in all three sets of germplasm evaluated (Fig. 3B). In addition, inbreds classified as Iowa Stiff Stalk Synthetic type had higher methionine levels than Non-Stiff Stalk lines (data not shown). This is in agreement with OLSEN et al. (2003), who reported higher methionine levels in B73 and BSSS53 (Iowa Stiff Stalk Synthetic type) than in Mo17 (Non-Stiff Stalk type). Finally, there were no statistically significant correlations between methionine levels and endosperm translucence (Fig. 4).

Relationship between tryptophan and methionine levels in parental inbreds and their testcrosses

We examined the predictive value of tryptophan and methionine levels of inbred lines for tryptophan and methionine levels of testcross hybrids of each inbred with a common tester, Tx804, a Non-Stiff Stalk *o2/o2* tester with high lysine levels. Regressions of tryptophan and methionine levels in 80 hybrids on *o2/o2* parental inbred lines with a range of endosperm opacity were highly significant ($p > 0.01$) with R^2 values of 0.13 for methionine and 0.27 for tryptophan (Fig. 5). In the methionine data, two inbred lines have unusually high values and therefore strongly influence the correlation. The R^2 for this correlation is 0.16 when these two points are not considered in the analysis. This indicates the amino acid levels of parental inbreds have a low value for predictive amino acid levels in their hybrids. Most of the inbred parents (germplasm set 1) used in the regressions had vitreous endosperms (Fig. 2A and 4A). The tester inbred, *o2/o2* Tx804 has soft endosperm. The testcrosses (germplasm set 2) have a greater segregation of endosperm opacity (F2 seeds derived from QPM x soft crosses) than their parents (Fig. 2B and 4B). Sixty-eight percent of the inbreds examined had fully vitreous kernels, but the F2 kernels derived from crosses of these inbreds with

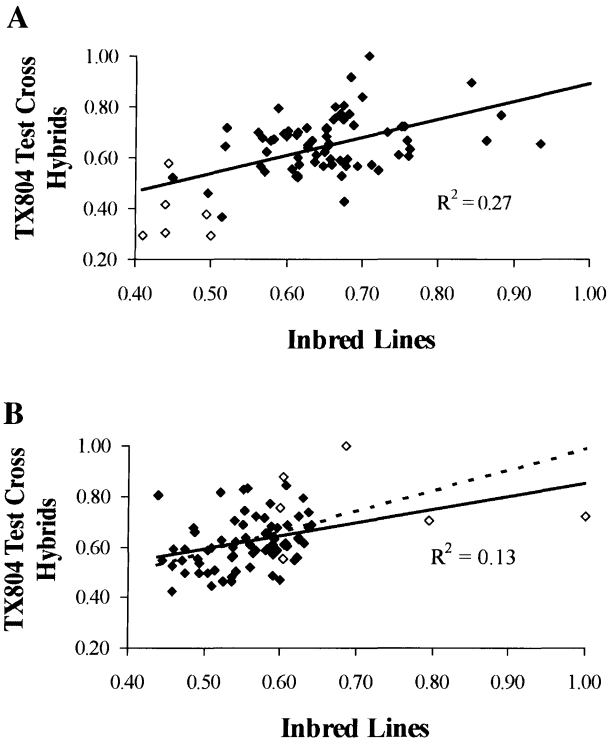

FIGURE 5 - Correlation of amino acid content between maize kernels produced on self-pollinated ears of parental inbreds and corresponding F2 kernels of their crosses with *o2/o2* inbred Tx804. Open points indicate *O2/O2* parental inbreds and filled points are *o2/o2* parental inbreds. A. Tryptophan. B. Methionine. The R^2 shown on the graphs is for the fit of the solid line. The dashed line in panel B is fit to the data excluding the two points with the highest values on the inbred lines axis. The R^2 for this fit is 0.16.

Tx804 gave a wider range of endosperm opacity scores (Fig. 6). There was not a statistically significant correlation between the endosperm opacity scores of the inbred lines with endosperm opacity scores less than 5 and the F2 kernels derived from crosses of these inbreds with Tx804.

DISCUSSION

The development of agronomically adapted varieties of maize with improved amino acid balance benefits livestock producers and people who depend on maize for dietary protein. The use of the *o2* mutation for the development of QPM is an established method of improving the amino acid balance of maize. In this study we addressed several issues that have important implications for plant breeders developing QPM maize varieties.

First, we characterized the relationship between tryptophan levels and endosperm translucence, and found tryptophan content is negatively correlated with endosperm opacity. Opaque phenotypes had more tryptophan than vitreous QPM phenotypes. This observation is consistent with a similar observation made using *o2* inbred lines (GENTINETTA *et. al.*, 1975). This observation is contrary, however, to an earlier report (PIXLEY and BJARNASON, 2002) that examined this relationship in QPM germplasm. Because our study included QPM and opaque non-QPM germplasm, it may be that we had a greater range of endosperm opacity values than the earlier study, and this allowed us to detect the correlation. In light of the correlation between lysine and tryptophan levels, our observations are consistent with observations that lysine levels in hard endosperm *o2* breeding germplasm are lower than soft endosperm *o2* germplasm (ORTEGA and BATES, 1983; ROBUTTI *et al.*, 1974) and lysine levels are negatively correlated with endosperm hardness (WESSEL-BEAVER *et al.*, 1985). A negative correlation between tryptophan or lysine levels and endosperm translucence could mean selection for translucent kernels reduces the beneficial effects of the *o2* mutation. This observation underscores the importance of monitoring amino acid levels throughout the breeding process, as has been suggested (WESSEL-BEAVER *et al.*, 1985). In this way, it may be possible to reduce the loss of tryptophan and lysine during conversion from soft to hard kernels.

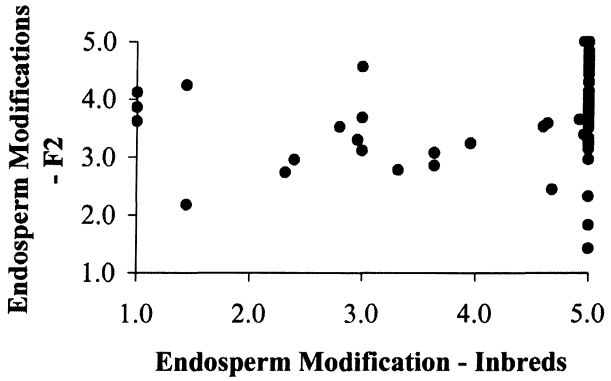

FIGURE 6 - Comparison of endosperm translucence between maize kernels from self-pollinated ears of parental inbreds and corresponding F2 kernels of their crosses with *o2/o2* inbred Tx804.

The second objective of this study was to examine methionine levels in QPM breeding material. We observed methionine levels were lower in *o2* germplasm regardless of endosperm hardness. This observation has important nutritional implications, especially when viewed in light of a complete diet. Complete diets often rely on legumes for protein, and legumes tend to be deficient in methionine. It is possible that QPM, has sufficient levels of lysine and tryptophan but reduced levels of methionine. Methionine levels are not correlated with endosperm translucence and do not exhibit a regular trend across different classes of endosperm opacity but are reduced in *o2/o2* material we examined regardless of endosperm hardness. It is therefore possible the reduced level of methionine in QPM is not a product of the conversion to QPM, but rather is a pleiotropic effect of the *o2* mutation itself. This is a plausible explanation because *o2* is a regulator of zein accumulation (MOTTO *et al.*, 1989), and the delta zeins are particularly rich in methionine (KIRIHARA *et al.*, 1988; PEDERSEN *et al.*, 1986) and have reduced transcript levels in *o2* relative to normal kernels (HUNTER *et al.*, 2002). An attractive strategy to develop nutritionally enhanced maize would therefore be one that elevates levels of both tryptophan and methionine simultaneously. Maize with elevated methionine levels has been developed by manipulation of the content of methionine-rich zeins by genetic enginerring (ANTHONY *et al.*, 1997; LAI and MESSING, 2002), or breeding (PHILLIPS *et al.*, 1981) and perhaps these strategies could be incorporated into a QPM breeding program.

Inbred line performance was a poor predictor of hybrid performance with a common *o2/o2* tester for tryptophan and methionine levels. Evaluating different testers with variable levels of endosperm opacity and amino acid levels it may be possible to develop a better understanding of the gene action controlling these traits.

In QPM breeding programs, the majority of effort is often devoted to altering physical properties of the endosperm, maintaining the *o2* mutation, and improving agronomic traits. Based on results presented here, it may be possible to further increase the nutritional value of QPM by monitoring tryptophan and methionine levels during breeding and selecting genotypes having both vitreous endosperm and high levels of these amino acids.

ACKNOWLEDGEMENTS - The authors wish to thank Erik Mottl and Merinda Struthers, and Texas A&M University personnel for technical assistance, and Dr. Slobodon Trifunovic for valuable discussions. This paper is a joint contribution from the Corn Insects and Crop Genetics Research Unit, USDA-ARS, and project no. 3781 of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA 50011. Names are necessary to report factually on the available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may be suitable.

REFERENCES

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS (AOAC), 1990 Method 15; 982.30 E (a, b, c). Official methods of analysis. 15th Ed., Washington, DC.

ANTHONY J., W. BROWN, D. BUHR, G. RONHOVDE, D. GENOVESI, T. LANE, R. YINGLING, K. AVES, M. ROSATO, P. ANDERSON, 1997 Transgenic maize with elevated 10 KD zein and methionine. pp. 295-297. *In:* W.J. Cram, L.J. De Kok, I. Stulen, C. Brunold, H. Rennenberg (Eds)., Sulphur metabolism in higher plants: molecular, ecophysiological and nutritional aspects. Leiden, Backhuys.

FAO/WHO/UNU, 1985 Energy and protein requirements. Report of a joint FAO/WHO/UNU Expert Consultation WHO, Geneva.

EGGUM B.O., E.M. VILLEGAS, S.K. VASAL, 1979 Progress in Protein quality of Maize. J. Sci. Food Agric. **30:** 1148-1153.

GENTINETTA E., 1975 Protein studies in 46 *Opaque-2* strains with modified endosperm Texture. Maydica **20:**145-164.

HERNANDEZ H., L.S. BATES, 1969 A modified method for rapid tryptophan analysis of maize. pp. 1-7. International maize and wheat improvement center Research bulletin.

HOPKINS C.G., 1899 Improvement in the chemical composition of the corn kernel. Illinois Agric. Experiment. Station Bulletin **55:** 205-240.

HUNTER B.G., M.K. BEATTY, G.W. SINGLETARY, B.R. HAMAKER, B.P. DILKES, B.A. LARKINS, R. JUNG, 2002 Maize opaque endosperm mutations create extensive changes in patterns of gene expression. Plant Cell **14:** 2591-2612.

JACOB F., E. WOLLMAN, 1961 Analyse de groupes de liaison genetique de differentes souches donatrices d'*Escherichia coli.* Comptes rendus de l'Academie des Sciences de Paris **245:** 1840-1843.

KIRIHARA J.A., J.B. PETRI, J. MESSING, 1988 Isolation and sequence of a gene encoding a methionine-rich 10-kDa zein protein from maize. Gene **71:** 359-370.

LAI J., J. MESSING, 2002 Increasing maize seed methionine by mRNA stability. Plant J. **30:** 395-402.

MERTZ E., L. BATES, O.E. NELSON JR, 1964 Mutant gene that changes protein composition and increases lysine content of maize endosperm. Science **16:** 279-280.

MOTTO M., N. DI FONZO, H. HARTINGS, M. MADDALONI, F. SALAMINI, C. SOAVE, R.D. THOMPSON, 1989 Regulatory genes affecting maize storage protien synthesis. Oxf. Surv. Plant Mol. Cell Biol. **6:** 87-114.

MORO G.L., J.R. HABBEN, B.R. HAMAKER, B.A. LARKINS, 1996 Characterization of the variability in lysine content for normal and *opaque2* maize endosperm. Crop Sci. **36:** 1651-1659.

OLSEN M.S., T.L. KRONE, R.L. PHILLIPS, 2003 BSSS53 as a donor source for increased whole-kernel methionine in maize: selection and evaluation of high-methionine inbreds and hybrids. Crop Sci. **43:** 1634-1642.

ORTEGA E.I., L.S BATES, 1983 Biochemical and agronomic studies of two modified hard-endosperm *Opaque-2* maize (*Zea mays* L.) populations. Cereal Chem. **60:** 107-111.

PEDERSEN K., P. ARGOS, S. NARAVANA, B. LARKINS, 1986 Sequence analysis and characterization of a maize gene encoding a high- sulfur zein protein of Mr 15,000. J. Biol. Chem. **261:** 6279-6284.

PHILLIPS R.L., P.R. MORRIS, F. WOLD, B.G. GENGENBACH, 1981 Seedling screening for lysine-plus-threonine feedback resistant maize. Crop Sci. **21:** 601-607.

PIXLEY K.V., M.S. BJARNASON, 2002 Stability of grain yield, endosperm modification, and protein quality of hybrid and open-pollinated quality protein maize (QPM) cultivars. Crop Sci. **42:** 1882-1890.

ROBUTTI J., R.C. HOSENEY, C.W. DEYOE, 1974 Modified Opaque-2 corn endosperms. I. Protein Distribution and Amino Acid Composition. Cereal Chem. **51:** 163-172.

SINGER M., T.A. BAKER, G. SCHNITZLER, S.M. DEISCHEL, M. GOEL, W. DOVE, K.J. JAACKS, A.D. GROSSMAN, J.W. ERICKSON, C.A. GROSS, 1989 A collection of strains containing genetically linked alternating antibiotic resistance elements for genetic mapping of *Escherichia coli*. Microbiol. Rev. **53:** 1-24.

VASAL S.K., 2001 High Quality Protein Corn. pp. 85-129. *In:* A.R. Hallauer (Ed.), Specialty Corns. Second Edition. CRC Press LLC, Boca Raton, Fl.

WESSEL-BEAVER L., R.J. LAMBERT, J.W. DUDLEY, 1985 Genetic Variability and Correlations in a Modified Endosperm Texture *Opaque-2* Maize Population. Crop Sci. **25:** 129-132.

ZARKARDAS C.G., Y. ZIRAN, R.I. HAMILTON, P.L. PATTISON, N.G.W. ROSE, 1995 Comparison Between the Protein Quality of Northern Adapted Cultivars of Common Maize and Quality Protein Maize. J. Agric. Food Chem. **43:** 84-93.

ZARKADAS C.G., R.I. HAMILTON, Z.R. YU, V.K. CHOI, S. KHANIZADEH, N.G.W. ROSE, P.L. PATTISON, 2000 Assessment of the protein quality of 15 new northern adapted cultivars of quality protein maize using amino acid analysis. J. Agric. Food Chem. **48:** 5351-5361.