Assessment of the Protein Quality of 15 New Northern Adapted Cultivars of Quality Protein Maize Using Amino Acid Analysis

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Amino acid determinations were carried out on 15 new northern adapted cultivars of quality protein maize (QPM) containing *opaque-2* modifier genes to ascertain whether their amino acid scoring patterns could be used to select high-lysine QPM genotypes and to assess their protein quality. Total protein in these cultivars ranged from 8.0 to 10.2% compared to two commercial maize varieties, Dekalb DK435 (7.9%) and Pioneer 3925 (10.3%). Four of these QPM genotypes, QPM-C26, QPM-C21, QPM-C79, and QPM-C59, contained high levels of lysine (4.43−4.58 g of lysine/100 g of protein), whereas the remaining varied from 3.43 to 4.21 g of lysine/100 g of protein, compared to Dekalb DK435 and Pioneer 3925, which contained 2.9 and 3.1 g of lysine/100 g of protein, respectively. Although lysine is the first limiting amino acid in QPM inbreds, the high-lysine QPM genotypes may supply ∼70.2−72.6% of human protein requirements, compared to 46.2% for Dekalb DK435 and 50.1% for Pioneer 3925, 55−63% for oats, and 59−60.3% for barley. Northern adapted QPM genotypes may have the potential to increase their lysine content even further, either by an increase in specific high-lysine-containing nonzein proteins, such as the synthesis of factor EF-1a, or by a further reduction in the 19 and 22 kDa α-zein in the endosperm or both. This knowledge could assist maize breeders in the selection of new high-performance QPM genotypes with improved protein quality and quantity.

Keywords: *Maize; quality protein maize; QPM; opaque-2 maize; QPM performance; protein quality; amino acid composition; amino acids; essential amino acids; corrected amino acid score*

INTRODUCTION

Maize (*Zea mays* L.) is the third largest cereal crop in the world and is a major source of energy, protein, and other nutrients for both humans and livestock (FAO, 1992). Maize contains 7−13% protein (Moro et al., 1996). Like all cereal grains, maize seeds contain three groups of proteins: the storage proteins, which constitute an amino acid reserve deposited early in seed development; the enzymes involved in metabolism; and the structural proteins, that is, ribosomal, chromosomal, and membrane proteins. The predominant proteins in maize are a family of alcohol-soluble prolamin storage proteins called zeins, which accumulate in the protein bodies of maize endosperm during development (Burr and Burr, 1976; Lee et al., 1976; Lending and Larkins, 1989). The zeins are divided into four structurally distinct types on the basis of their molecular weights determined on SDS−polyacrylamide gels: α-, *β*-, *γ*-, and *δ*-zeins (Petersen et al., 1980; Esen, 1987; Wang and Esen, 1986; Larkins et al., 1989; Wallace et al., 1990; Shewry and Tatham, 1990; Schmidt, 1993; Magnavaca et al., 1993). Typically, the endosperm contains ∼90% of the total protein, with the zeins accounting for ∼60−70% of the total endosperm protein at maturity. All of the prolamins are characterized by a high content of glutamine, leucine, and proline, but they are nearly devoid of lysine and tryptophan (Habben and Larkins, 1995), which account for the presence of between 1.8 and 2.0% and between 0.6 and 0.8% of the total protein, respectively (Zarkadas et al., 1995).

The most important mutant gene that has been shown to improve the amino acid composition and protein quality of maize is the *opaque-2* (*o2*) mutation (Emerson et al., 1935; Mertz et al., 1964; Alexander et al., 1969; Nelson, 1969; Neuffer et al., 1997). The *opaque-2* gene is a mutation in one of the regulatory loci of the short arm of chromosome 7. It is inherited as a simple Mendelian recessive and regulates zein protein gene transcription (Schmidt et al., 1987; Kodrzycki et al., 1989; Lending and Larkins, 1989: Aukerman and Schmidt, 1991; Aukerman et al., 1991), particularly that of the most abundant α-zein (Bjarnason and Vasal, 1992; Hartings et al., 1989). Several studies have demonstrated that the principal changes which occur in storage proteins of *opaque-2* maize genotypes are a 50% reduction of both the 19 and 22 kDa α-zein in the endosperm (Wallace et al., 1990; Geetha et al., 1991), a 2−3-fold increased synthesis of the 27 kDa *γ*-zein, a proline and cysteine-rich protein (Wallace et al., 1990; Paiva et al., 1991), and an increase in nonzein proteins, especially in the content of the elongation factor 1α (EF-1α) (Bressani, 1991, 1992; Damerval and de Vienne,

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1993; Habben et al., 1993; Schmidt, 1993; Durso and Cyr, 1994; Shiina et al., 1994; Morelli et al., 1994; Habben et al., 1995). Aukerman et al. (Aukerman and Schmidt, 1991; Aukerman et al., 1991) have shown that the reduction in zein gene expression in the *opaque-2* mutant is a result of the inability of the mutant transcriptional activator protein (bZIP) to bind the zein promoters (Or et al., 1993; Pysh et al., 1993). The concentration of lysine in the endosperm has recently been shown to be highly correlated with the content of a single nonzein protein called the protein synthesis factor EF-1α (Habben et al., 1993, 1995; Habben and Larkins, 1995; Moro et al., 1996), a lysine rich (11% lysine) abundant protein that mediates the binding of aminoacyl-tRNAs to the ribosome (Merrick, 1992) and performs a number of other cellular functions (Durso and Cyr, 1994; Shiina et al., 1994; Morelli et al., 1994; Moro et al., 1996). Genes encoding catalase-2 (7% lysine) and trypsin inhibitor (1% lysine) are also more actively expressed in *opaque-2* mutants than in normal maize (Habben and Larkins, 1995). Quantitation of the EF-1a factor has been proposed as an index for determining improved protein quality in maize breeding programs (Moro et al., 1996).

Compared to standard flint or dent inbreds, however, most of the early developed *opaque-2* genotypes and hybrids had many negative characteristics including soft, chalky endosperm, lower yields, and increased susceptibility to diseases and mechanical damage (Nelson, 1969). To overcome some of these difficulties, maize breeders at the International Maize Improvement Center in Mexico (CIMMYT, 1981, 1985; Villegas et al., 1992) and at the University of Natal, South Africa, (Gevers and Lake, 1992), through backcrossing and several cycles of recurrent selection (Hallauer, 1992), have combined the high-lysine potential of the *opaque-2* mutation with the two modifier genes. They have successfully developed new cultivars, mainly for tropical and subtropical regions, that are similar in yield and other agronomic properties to normal maize (Bjarnason and Vasal, 1992; Villegas et al., 1980, 1992; Ortega et al., 1991). These new maize inbreds, collectively called quality protein maize (QPM), have nearly normal yields and protein contents and a vitreous endosperm, are resistant to disease and mechanical damage, and have increased lysine and tryptophan levels compared to normal maize (Villegas et al., 1992; Shmidt et al., 1993; Habben and Larkins, 1995).

These new high-lysine QPM genotypes have been extensively used in Brazil (Magnavaga, 1993), and large scale production is becoming of major interest to seed producers and industry in Canada and United States because of their potential advantages in human and animal nutrition (Bockholt and Rooney, 1992). Attempts have been made to introduce QPM lines to the temperate regions of eastern Canada (Spaner et al., 1992a,b), but research is now underway to develop cultivars better adapted to the more northern latitudes (latitude >45° N), which have long daylengths (>16 h) and a short growing season. The amino acid composition of a new QPM inbred (QPM-C13) grown in Ottawa, Canada, indicated that the lysine content of this cultivar was 40 mg/g of total protein, compared to 25−30 mg/g of total protein in normal flint and dent maize (Zarkadas et al., 1995).

The aims of the present study were to quantitatively measure and compare genetic variability of total protein, the lysine content, and the individual amino acids in 15 new QPM cultivars, a high-yielding QPM genotype, QPM-C13, and two commercial maize cultivars, that is, Pioneer 3925 and Dekalb DK435, all developed for northern latitudes; and to assess the protein quality and nutritional adequacy of these new cultivars from digestibility and amino acid compositional data in order to select cultivars with the highest lysine content and nutritive value (FAO/WHO/UNU, 1985; FAO/WHO, 1991).

MATERIALS AND METHODS

Materials. The amino acid standards were obtained as follows: norleucine from Pierce Chemical Co., Rockford, IL; and the standard amino acid calibration mixture from Beckman Instruments, Inc., Palo Alto, CA. Octanoic acid was obtained from Eastman Kodak Co., Rochester, NY, and phenol was a product of J. T. Baker Chemical Co., Phillipsburg, NJ. Hydrochloric acid (Analar), hydrobromic acid (Aristar), formic acid (88.0%), and hydrogen peroxide (30.0%) were purchased from BDH Inc., Poole, U.K. High-purity sodium hydroxide (50.0% w/w), which was used to prepare all buffers and reagents, was a product of Allied Fisher Scientific, Fair Lawn, NJ. All other chemicals and reagents were of the highest purity commercially available and were used without further purification.

Experimental Procedures. *Plant Material and Sample Preparation*. The 15 northern adapted QPM inbred lines selected for this investigation were developed from either Northern Temperate Zone 1 (NTR-1) QPM or Subtropical Temperate Zone (STR) QPM gene pools, which originated from the International Maize and Wheat Improvement Center (CIMMYT, 1981) in Mexico (Villegas et al., 1992). The CIMMYT NTR-1 QPM gene pool consists of material primarily derived from the U.S. germplasm developed through shuttle breeding between Mexico and the northern United States (Spaner, 1992a,b). The STR QPM gene pool consists of tropical material adapted for temperate climates developed through breeding between Mexico and Germany. Both QPM inbred lines are early-maturing inbreds with superior combining ability and agronomic traits, and both inbred lines, which were designed for the 46−52° N and S latitudinal range (CIMMYT, 1981), are well adapted to the more northerly temperate regions of Canada (latitude >45° N), where the minimum average daily temperature in May and June ranges from 10 to 15 °C and the growing season is short. Both NTR-1 and STR QPM synthetics were inbred to the S5 generation by Dr. R. I. Hamilton's maize breeding program at the Central Experimental Farm (CEF), Plant Research Centre, Agriculture and Agri-Food Canada, Ottawa, ON, and resulted in the new QPM genotypes containing the high-lysine *opaque-2* gene and set of modifier genes that favor improved kernel characteristics and increased tryptophan and lysine contents.

All QPM inbreds share one common male parent and should therefore differ in their amino acid composition and protein content only by virtue of genetic variance in the base population and environmental effects. Valid estimates of heritability and genetic variance can be made only on randomly selected genotypes from a population. A complete description of the QPM germplasm and their pedigree are as follows:

The NTR-1 QPM inbred lines were QPM-C2, QPM-C12, QPM-C18, QPM-C21, QPM-C26, QPM-C36, QPM-C39, QPM-C40, QPM-C42, QPM-C74, QPM-C79, and QPM-C94.

The QPM-C59, QPM-C68, and QPM-C72 inbred lines, which originated from the International Maize and Wheat Improvement Center (CIMMYT, 1981) in Mexico, were developed from the STR QPM gene pool.

For purposes of comparison the two well-established commercial high-yielding hybrids used to evaluate these 15 new QPM hybrid lines were high-yielding QPM inbred to evaluate designated QPM-C13, which was also developed from the NTR-1 gene pool, Pioneer 3925, which is a single-cross yellow Dent hybrid (FAO maturity classification: FAO 350), and

Dekalb DK435 (FAO 400). The QPM-C13 genotype contained the high-lysine *opaque-2* gene and modifier genes (Zarkadas et al., 1995).

Representative samples of seeds of the NTR-1 and STR QPM cultivars were obtained from Dr. R. I. Hamilton of the Eastern Cereal an Oilseed Research Centre and Drs. D. M. Spaner and D. E. Mather of the Department of Plant Science, McGill University, Macdonald Campus, Ste-Anne-de-Bellevue, Quebec, and were from experimental trials grown at the Central Experimental Farm, Agriculture Agri-Food Canada, Ottawa, ON (latitude 45° 31′ N; FAO 300−400). Agronomic performance and combining ability studies on both NTR-1 and STR QPM inbred lines were carried out by Spaner et al. (1992a,b). The experimental layout was a randomized complete block design with four replications. Each plot consisted of a single row, 5 m long. Plots were overseeded and subsequently thinned to achieve a final density of 48000 plants ha^{-1}. The distance between rows was 75 cm. The two commercial maize hybrids used as testers were detasselled prior to flowering to prevent pollen contamination of experimental gerplasm. Plants were hand harvested and mechanically shelled. Following shelling, a random sample of between 80 and 100 g of kernels per plot was collected from five ears at harvest to determine protein content and amino acid composition.

Representative samples of the whole kernels were freeze-dried and then pulverized in a standard electrically driven end runner mill (Cyclone sample mill, U. D. Corp., Fort Collins, CO), passed through a 1.0-mm mesh sieve, lyophilized, and stored at −20 °C in polypropylene bottles until used.

Preparation of Tissue Hydrolysates. Complete amino acid analyses were carried out in each of the four replicate maize samples. Each of the replicates was divided into two subsamples, A and B, which were then hydrolyzed in duplicate (50.0 mg) in Pyrex (No. 9860) test tubes (18 × 150 mm) under vacuum (below 10 mmHg) with triple-glass-distilled constant-boiling HCl (6.0 M) containing 0.2% (v/v) phenol and 5 μL of octanoic acid at 110 ± 0.5 °C for 24 h. Analyses of individual acid hydrolysates were performed on the clear filtrate in duplicate according to methods described previously (Zarkadas et al., 1986, 1987, 1988a−c, 1990). The data reported in Tables 1 and 2 represent the average values of 32 determinations.

Procedures for Amino Acid Analyses. The most direct and accurate method for determining lysine and other amino acids and total protein in maize is ion-exchange chromatography (Moore and Stein, 1963). Amino acid analyses were carried out on a Beckman Spinco System 6300 fully automated high-perfomance amino acid analyzer using single-column expanded protein hydrolysate methodology (Slocum et al., 1987). The automated instrument was equipped with a Beckman model 406 analog interface module, the system Gold (Beckman Instrument, Inc., Altex Division, San Ramon, CA) chromatographic data reduction system, and an IBM (486 series) compatible personal computer.

Amino acid analyses of the QPM genotypes were carried out on each of the eight replicate maize samples (50.0 mg) per cultivar according to the standard procedures described previously (Zarkadas et al., 1986, 1987, 1988a−c). Methionine was also determined from the 24 h acid hydrolysates. The data presented in Tables 1 and 2 represent the average of 32 determinations per cultivar. The values for cyst(e)ine and tryptophan were taken from data reported previously on QPM-C13 (Zarkadas et al., 1995).

When a large number of seed samples must be screened, however, an economical way to determine lysine is by the following accelerated method, which enabled 45 lysine analyses to be carried out per day compared to 19 for the complete amino acid analysis of maize samples.

Accelerated separation of lysine from the other basic amino acids in maize hydrolysates was also carried out on a high-performance cation-exchange 12 × 0.4 cm Beckman column using a two-buffer system (buffer F, pH 4.25, and buffer D, pH 6.4). The second buffer D was introduced at 5.0 min and 6450 kN/m^2 (1250 psi). The buffer flow rate was 14.0 mL/h. The initial temperature was 70 °C and changed to 77 °C at 5.0 min. The internal standards selected to test the accuracy of this method were either norleucine, eluting between leucine and tyrosine, or l-2-amino-3-guanidinopropionic acid (AGPA), which elutes before ammonia. For standardization of the instrument, 1000 pmol/0.05 mL calibration standard was applied to the microcolumn. For analytical work, 5−10 μg/0.05 mL of maize hydrolysate samples, which corresponds to 0.5−1.5 μg of protein and yields 100−500 pmol of basic amino acids, was applied to the high-performance column. Excellent separation of lysine from all other amino acids was obtained with this system in 19.3 min.

Protein Determination. Recoveries of amino acids were calculated on the basis of the protein content of individual hydrolysates determined according to the method of Horstmann (1979) as described previously (Zarkadas et al., 1988a−c, 1994). According to this method, a mean residue weight, WE (in micrograms per nanomole), was calculated for the amino acids constituting the proteins in maize as

$$\mathrm{WE} = \sum_{i=1}^{18}(a_i b_i) \tag{1}$$

where a is the mole fraction of an amino acid i found in the analyzed aliquot and b is the molecular weight of amino acid residue i (in micrograms).

A conversion factor CF, which represents the apparent average residue molecular weight (in micrograms per nanomole) of the proteins in maize, but in the absence of tryptophan, methionine, and cyst(e)ine, and protein concentration of each hydrolysate were then calculated as described previously (Zarkadas et al., 1988a,b, 1995; Khanizadeh et al., 1989, 1992).

The protein content of each sample was calculated by multiplying CF by the nanomoles of total amino acids (χ_i) in each acid hydrolysate as

$$P = \mathrm{CF}\sum_{i=1}^{15}\chi_i \tag{2}$$

where χ_i is the nanomoles of each amino acid i found in the analyzed aliquot. The values reported in Table 1 for the content of total protein in each of the 15 QPM genotypes and commercial maize samples investigated are the averages of 32 determinations.

Assessment of Protein Quality of Maize. The method for calculating the protein digestibility corrected amino acid score (PDCAAS) of maize can be defined according to Young and Pellett (1994) as follows:

$$\mathrm{PDCAAS} = \frac{\text{AA content (mg/g of protein) of food protein} \times \text{digestibility}}{\text{AA content of FAO/WHO/UNU (1985) pattern for a 2−5-year-old child}} \tag{3}$$

These authors have defined the amino acid score as the concentration of the limiting amino acid in the food protein, which is expressed as a proportion of the concentration of the same amino acid in a standard or reference amino acid pattern such as for a 2−5-year-old child.

Digestibility is included in this amino acid scoring procedure (eq 3) to allow for differences in the digestibility between plant and animal sources. The true protein digestibility values for maize quoted in this study (Table 4) were taken from the U.S. *Federal Register*'s Appendix B, pp 2193−2195 (U.S. Food and Drug Administration, 1993).

Statistical Analysis. Data processing of the results was carried out by an EXCEL version 7 for Windows spread-sheet computer program developed for this purpose. Analysis of variance, conducted on the amino acid data, for a completely randomized block design (factorial) was done by the general linear model procedure using SAS under the Windows operat-

Table 1. Comparison of the Amino Acid (AA) Composition and Protein Contents (Grams of AA per Kilogram of Total Protein; Mean ± SEM) of 15 New Northern Adapted Cultivars of QPM with Commercial Hybrid Maize, Pioneer 3925, and Dekalb DK435

	CIMMYT maize cultivars[a] (QPM; *opaque-2*)									QPM tester *opaque-2*
AA	QPMC2	QPMC12	QPMC18	QPMC21	QPMC26	QPMC36	QPMC39	QPMC40	QPMC42	QPMC13
aspartic acid	80.19 ± 2.47	86.03 ± 1.71	84.21 ± 0.86	83.86 ± 1.75	86.15 ± 1.37	79.91 ± 1.28	76.05 ± 1.35	73.95 ± 1.08	73.28 ± 0.8	85.92 ± 1.66
threonine	34.76 ± 0.48	35.36 ± 0.77	37.29 ± 0.57	37.07 ± 0.63	36.58 ± 0.62	35.67 ± 0.58	34.82 ± 0.34	33.41 ± 0.79	34.68 ± 0.6	38.86 ± 0.62
serine	44.74 ± 0.91	42.51 ± 0.48	41.76 ± 0.32	43.37 ± 0.73	43.09 ± 1.95	43.31 ± 0.78	44.12 ± 0.54	43.57 ± 1.67	42.37 ± 1.1	45.99 ± 1.47
glutamic acid	176.98 ± 1.9	161.60 ± 1.4	169.88 ± 1.4	168.18 ± 1.4	159.93 ± 1.5	171.57 ± 1.4	167.06 ± 1.4	171.73 ± 1.9	172.9 ± 1.5	139.82 ± 8.0
proline	89.99 ± 1.28	86.91± 1.51	94.17 ± 0.79	90.33 ± 1.91	94.68 ± 0.97	87.67 ± 0.58	85.13 ± 0.82	85.54 ± 1.00	97.41 ± 1.2	85.71 ± 4.13
glycine	32.34 ± 0.85	38.55 ± 0.58	38.31 ± 0.44	39.51 ± 0.63	41.18 ± 0.54	35.12 ± 0.98	35.21 ± 0.39	34.52 ± 0.27	35.29 ± 0.5	45.23 ± 3.01
alanine	64.13 ± 0.64	58.69 ± 084	57.99 ± 0.29	58.37 ± 0.31	54.86 ± 0.39	62.23 ± 0.82	63.92 ± 0.62	62.46 ± 0.81	62.96 ± 0.6	59.84 ± 0.52
cysteine[b]	49.13 ± 1.89	53.59 ± 1.62	50.32 ± 1.04	44.48 ± 2.03	42.45 ± 1.38	44.27 ± 1.85	49.47 ± 1.02	53.42 ± 0.87	48.68 ± 0.3	48.18 ± 2.43
valine	45.59 ± 0.64	54.54 ± 0.65	50.29 ± 0.83	50.71 ± 0.34	53.22 ± 0.28	48.59 ± 0.39	52.74 ± 0.74	53.04 ± 0.63	50.18 ± 1.7	57.39 ± 0.46
methionine	19.58 ± 2.61	21.09 ± 0.74	21.93 ± 1.68	17.83 ± 0.86	18.19 ± 0.52	15.95 ± 0.61	24.11 ± 1.41	22.17 ± 0.73	19.42 ± 0.3	23.10 ± 1.09
isoleucine	36.08 ± 3.62	31.05 ± 0.23	32.83 ± 0.32	33.37 ± 0.18	32.08 ± 0.16	33.18 ± 0.24	30.87 ± 0.39	33.45 ± 0.44	33.29 ± 0.4	30.92 ± 1.09
leucine	111.58 ± 4.9	94.82 ± 1.88	93.30 ± 2.47	96.85 ± 1.07	87.59 ± 1.34	108.44 ± 1.2	105.9 ± 1.58	110.5 ± 1.59	110.2 ± 1.6	93.14 ± 2.54
tyrosine	42.64 ± 0.94	39.32 ± 0.41	38.39 ± 0.57	38.98 ± 0.27	39.02 ± 0.41	41.57 ± 1.13	41.61 ± 0.47	41.85 ± 0.77	40.55 ± 1.3	38.22 ± 1.01
phenylalanine	48.29 ± 1.11	43.24 ± 0.45	43.05 ± 0.54	43.83 ± 0.27	41.45 ± 0.29	44.52 ± 1.71	45.52 ± 0.45	46.96 ± 0.42	44.52 ± 1.4	43.68 ± 0.43
histidine	30.53 ± 0.78	33.84 ± 1.14	34.93 ± 0.31	36.47 ± 0.50	38.23 ± 0.32	32.45 ± 0.44	29.86 ± 0.28	30.18 ± 0.35	33.42 ± 0.5	36.35 ± 0.34
lysine	34.32 ± 1.58	39.99 ± 1.83	40.86 ± 1.13	45.29 ± 1.06	45.79 ± 0.88	39.70 ± 1.34	35.07 ± 1.86	35.51 ± 0.69	38.06 ± 1.1	41.17 ± 1.03
arginine	48.66 ± 1.52	71.62 ± 2.42	60.77 ± 0.92	66.18 ± 1.88	77.74 ± 1.93	66.49 ± 0.94	65.14 ± 1.52	60.61 ± 2.53	53.65 ± 0.6	70.79 ± 0.78
tryptophan[b]	7.81 ± 0.18	8.39 ± 0.26	8.32 ± 0.41	7.74 ± 0.44	7.52 ± 0.55	7.59 ± 0.37	8.42 ± 0.16	8.49 ± 0.19	8.98 ± 0.3	8.27 ± 0.17
ammonia	17.58 ± 1.14	29.02 ± 3.25	16.88 ± 0.77	30.56 ± 3.58	19.41 ± 0.64	28.65 ± 5.33	37.97 ± 1.36	34.43 ± 2.42	16.81 ± 0.7	30.53 ± 5.01
WE[d]	0.111683	0.111387	0.111145	0.110977	0.111084	0.111261	0.111453	0.112419	0.110927	0.110601
CF[d]	0.113415	0.113611	0.113549	0.112885	0.113093	0.113103	0.114326	0.114082	0.113134	0.113056
total protein[d] g/kg of dry matter	85.57 ± 1.42	79.11 ± 2.42	84.92 ± 1.7	97.41 ± 4.63	101.15 ± 3.5	97.19 ± 3.72	86.18 ± 1.75	79.73 ± 1.33	84.65 ± 2.7	90.01 ± 5.45

	CIMMYT maize cultivars[a] (QPM opaque-2)						commercial maize cultivars[a]		signif levels among cultivars[c] overall			QPM vs not
AA	QPMC59	QPMC68	QPMC72	QPMC74	QPMC79	QPM94	Pioneer 3925	Dekalb DK435	SEM	CV	*F*	*F*
aspartic acid	87.02 ± 1.02	79.09 ± 1.65	83.53 ± 1.66	80.09 ± 1.03	100.21 ± 2.6	83.98 ± 1.71	70.27 ± 1.02	69.73 ± 0.75	4.10.	5.10	17.6***	25.7***
threonine	37.43 ± 0.32	33.79 ± 1.53	38.69 ± 1.27	36.15 ± 0.29	37.25 ± 0.42	36.03 ± 0.22	34.35 ± 0.74	35.21 ± 0.29	1.89	5.29	4.28***	5.75***
serine	42.78 ± 0.39	42.64 ± 2.32	43.09 ± 0.86	41.07 ± 0.75	42.46 ± 0.24	44.90 ± 1.44	43.09 ± 0.83	42.56 ± 0.32	3.28	7.58	1.03ns	1.21ns
glutamic acid	164.33 ± 3.36	174.10 ± 1.9	145.29 ± 5.9	161.76 ± 1.4	160.91 ± 2.8	163.48 ± 1.1	180.37 ± 1.59	173.89 ± 1.6	7.46	4.46	15.5***	21.8***
proline	94.21 ± 0.86	96.09 ± 1.77	94.06 ± 2.69	96.41 ± 1.18	91.36 ± 1.42	94.26 ± 1.39	92.33 ± 0.92	86.36 ± 0.76	4.39	4.82	6.90***	10.2***
glycine	39.95 ± 0.22	34.96 ± 0.67	40.36 ± 0.44	40.54 ± 0.60	40.32 ± 0.54	39.13 ± 0.61	31.41 ± 0.50	32.22 ± 0.49	2.54	6.89	12.8***	18.7***
alanine	57.55 ± 0.36	61.77 ± 0.79	57.13 ± 0.59	55.32 ± 0.39	57.11 ± 0.72	57.55 ± 0.26	66.38 ± 0.59	65.24 ± 1.68	2.02	3.34	20.8***	30.2***
cysteine[b]	41.59 ± 1.32	39.23 ± 1.06	53.68 ± 1.70	46.69 ± 1.45	51.40 ± 2.91	45.21 ± 1.21	44.39 ± 1.57	53.73 ± 1.61	4.79	10.2	9.37***	0.78***
valine	53.62 ± 0.31	50.54 ± 0.39	58.16 ± 2.02	52.79 ± 1.2	51.06 ± 0.64	50.78 ± 0.58	46.79 ± 0.28	49.22 ± 0.32	1.82	3.54	16.2***	23.8***
methionine	15.85 ± 0.63	18.61 ± 0.68	19.88 ± 1.85	18.93 ± 0.42	16.12 ± 0.63	20.50 ± 0.46	22.75 ± 2.37	21.01 ± 0.24	3.01	15.3	3.77***	5.14***
isoleucine	33.21 ± 0.14	33.81 ± 0.37	31.60 ± 0.33	32.22 ± 0.21	31.87 ± 0.37	32.89 ± 0.19	33.68 ± 0.25	35.27 ± 0.42	2.58	7.81	1.93**	2.51**
leucine	89.22 ± 2.39	110.6 ± 2.51	91.32 ± 1.59	93.73 ± 1.54	91.97 ± 2.55	97.87 ± 1.77	122.18 ± 1.65	116.91 ± 3.3	6.38	6.21	19.1***	27.9***
tyrosine	39.42 ± 0.27	41.84 ± 0.46	39.29 ± 0.36	39.82 ± 0.19	34.63 ± 1.66	40.55 ± 0.38	44.02 ± 0.36	41.76 ± 0.49	2.05	5.05	6.65***	9.37***
phenylalanine	43.23 ± 0.19	47.21 ± 0.72	42.53 ± 0.31	41.83 ± 0.37	38.36 ± 1.92	43.72 ± 0.23	48.80 ± 0.29	48.16 ± 0.49	2.25	5.02	9.80***	14.3***
histidine	37.70 ± 0.33	33.88 ± 0.43	37.47 ± 0.27	38.59 ± 0.31	36.99 ± 0.44	36.58 ± 0.28	27.01 ± 0.5	28.66 ± 0.16	1.27	3.76	43.5***	64.1***
lysine	44.25 ± 0.44	35.58 ± 0.88	42.06 ± 1.03	40.93 ± 0.55	45.15 ± 0.93	41.36 ± 1.11	31.29 ± 0.8	29.15 ± 1.22	3.23	8.33	14.6***	21.4***
arginine	72.82 ± 2.73	59.78 ± 1.78	73.54 ± 2.36	74.93 ± 2.52	64.18 ± 1.32	63.42 ± 1.54	57.87 ± 1.41	54.29 ± 1.28	5.16	8.06	14.3***	22.1***
tryptophan[b]	7.74 ± 0.42	6.49 ± 0.32	8.69 ± 0.34	8.15 ± 0.45	8.37 ± 0.58	7.73 ± 0.27	9.71 ± 2.14	9.04 ± 0.33	1.73	21.2	1.09ns	ns
ammonia	20.81 ± 1.05	20.39 ± 2.61	29.68 ± 5.7	17.06 ± 0.73	15.85 ± 0.11	13.86 ± 0.48	20.93 ± 1.05	19.01 ± 1.94	8.58	34.5	3.48***	5.12***
WE[d]	0.111433	0.110907	0.110973	0.111234	0.110732	0.110899	0.111301	0.111716	10^{-4}	0.85	1.21ns	1.68*
CF[d]	0.112676	0.112876	0.113200	0.113355	0.112615	0.113105	0.113423	0.113668	10^{-4}	0.50	4.09***	5.75***
total protein[d] g/kg of dry matter	101.05 ± 3.24	97.78 ± 11.3	79.31 ± 2.27	90.06 ± 2.96	83.59 ± 4.33	93.54 ± 2.43	102.67 ± 4.7	79.24 ± 2.04	11.7	13.1	2.81***	4.03***

[a] Mean values and standard error of measurements (SEM) for four replicates ($N = 4$) and 32 determinations. [b] Taken from Zarkadas et al. (1995). [c] Significance: *F*, values from analysis of variance among cultivars, ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; ns, not significant; CV, coefficient of variation. [d] Computed according to the method of Horstmann (1979) and Zarkadas et al. (1988a,b).

Table 2. Amino Acid (AA) Composition and Nitrogen Contents (Grams of Amino Acids per 16 g of Nitrogen; Mean ± SEM) of 15 New Northern Adapted Cultivar of QPM with Commercial Hybrid Maize, Pioneer 3925, and Dekalb DK435

	CIMMYT maize cultivars (QPM; *opaque-2*)									QPM tester (*opaque-2*)
AA	QPMC2	QPMC12	QPMC18	QPMC21	QPMC26	QPMC36	QPMC39	QPMC40	QPMC42	QPMC13
aspartic acid	7.95 ± 0.24	7.42 ± 0.25	7.99 ± 0.07	7.23 ± 0.24	7.81 ± 0.12	7.17 ± 0.19	6.27 ± 0.28	6.54 ± 0.09	7.05 ± 0.08	7.99 ± 0.07
threonine	3.43 ± 0.05	3.04 ± 0.08	3.54 ± 0.05	3.23 ± 0.08	3.31 ± 0.052	3.19 ± 0.07	2.87 ± 0.11	2.83 ± 0.11	3.34 ± 0.05	3.40 ± 0.10
serine	4.42 ± 0.10	3.66 ± 0.10	3.96 ± 0.03	3.76 ± 0.12	3.91 ± 0.16	3.89 ± 0.13	3.67 ± 0.11	3.86 ± 0.15	4.07 ± 0.09	4.02 ± 0.14
glutamic acid	17.08 ± 0.21	13.96 ± 0.51	16.13 ± 0.18	14.62 ± 0.33	14.51 ± 0.19	15.39 ± 0.32	13.77 ± 0.54	15.19 ± 0.22	16.65 ± 0.1	12.88 ± 0.44
proline	8.72 ± 0.14	7.52 ± 0.32	8.94 ± 0.09	8.03 ± 0.23	8.59 ± 0.11	7.87 ± 0.18	7.01 ± 0.29	7.57 ± 0.11	8.76 ± 0.65	7.83 ± 0.26
glycine	3.23 ± 0.09	3.32 ± 0.11	3.64 ± 0.03	3.41 ± 0.08	3.73 ± 0.03	3.15 ± 0.09	2.89 ± 0.09	3.06 ± 0097	3.65 ± 0.28	3.55 ± 0.06
alanine	6.19 ± 0.05	5.07 ± 019	5.51 ± 0.04	5.07 ± 0.09	4.95 ± 0.03	5.58 ± 0.11	5.27 ± 0.20	4.69 ± 0.07	6.06 ± 0.07	5.23 ± 0.12
cysteine[b]	4.85 ± 0.08	4.74 ± 0.20	4.78 ± 0.09	3.86 ± 0.17	3.85 ± 0.05	3.98 ± 0.20	4.06 ± 0.12	4.73 ± 0.09	4.83 ± 0.18	4.13 ± 0.19
valine	4.46 ± 0.08	4.71 ± 0.16	5.29 ± 0.13	4.43 ± 0.11	4.79 ± 0.04	4.36 ± 0.09	4.32 ± 0.46	4.690.07	4.69 ± 0.03	5.03 ± 0.11
methionine	2.02 ± 0.20	1.76 ± 0.13	2.11 ± 0.14	1.45 ± 0.08	1.63 ± 0.05	1.43 ± 0.05	1.98 ± 0.13	1.960.07	1.86 ± 0.03	1.97 ± 0.14
isoleucine	3.16 ± 0.03	2.68 ± 0.09	3.32 ± 0.22	2.90 ± 0.06	2.89 ± 0.02	2.97 ± 0.06	2.55 ± 0.11	2.86 ± 0.11	3.21 ± 0.05	2.71 ± 0.11
leucine	11.23 ± 0.24	8.19 ± 0.36	8.33 ± 0.68	8.41 ± 0.17	7.92 ± 0.15	9.72 ± 0.19	8.73 ± 0.37	9.78 ± 0.17	10.63 ± 0.1	8.27 ± 0.29
tyrosine	4.12 ± 0.08	3.39 ± 013	4.21 ± 0.57	3.39 ± 0.08	3.59 ± 0.04	3.73 ± 0.11	3.43 ± 0.12	3.70 ± 0.06	4.51 ± 0.67	3.34 ± 0.10
phenylalanine	4.66 ± 0.11	3.74 ± 0.13	4.22 ± 0.57	3.82 ± 0.08	3.76 ± 0.04	4.15 ± 0.09	3.61 ± 0.26	4.16 ± 0.05	4.24 ± 011	3.83 ± 0.09
histidine	3.00 ± 0.07	2.90 ± 0.11	3.32 ± 0.03	3.17 ± 0.08	3.47 ± 0.03	2.91 ± 0.06	2.46 ± 0.09	2.67 ± 0.04	3.22 ± 0.05	2.90 ± 0.11
lysine	3.63 ± 0.14	3.53 ± 0.20	3.87 ± 0.11	4.03 ± 0.12	4.15 ± 0.06	3.67 ± 0.13	2.86 ± 0.11	3.16 ± 0.06	3.66 ± 0.09	3.53 ± 0.20
arginine	4.83 ± 0.11	6.13 ± 0.24	5.77 ± 0.08	5.77 ± 0.18	7.05 ± 0.16	5.98 ± 0.19	5.39 ± 0.23	5.37 ± 0.24	5.16 ± 0.05	6.13 ± 0.24
tryptophan[b]	0.75 ± 0.01	0.740.02	0.79 ± 0.03	0.67 ± 0.04	0.68 ± 0.04	0.68 ± 0.04	0.68 ± 0.02	0.83 ± 0.08	0.86 ± 0.03	0.740.02
ammonia	1.61 ± 0.11	2.98 ± 3.54	1.60 ± 0.07	2.85 ± 0.33	1.76 ± 0.05	2.49 ± 0.37	3.98 ± 0.54	3.03 ± 0.18	1.62 ± 0.06	2.98 ± 3.54
total AAN[d]										
g of AAN/kg of protein	164.79 ± 0.9	180.05 ± 2.7	168.54 ± 0.74	84.77 ± 3.95	176.39 ± 0.7	178.95 ± 4.2	184.09 ± 0.6	180.91 ± 1.6	166.25 ± 0.6	183.39 ± 4.2
g of AAN/kg of dry mass[d]	14.94 ± 0.86	14.76 ± 0.58	14.31 ± 0.29	17.76 ± 0.74	17.49 ± 0.56	17.39 ± 0.92	16.52 ± 0.49	14.43 ± 0.31	14.41 ± 0.34	16.46 ± 0.91
g of AA/16 g of N	96.61 ± 0.42	88.83 ± 1. 2	94.94 ± 0.41	87.93 ± 0.90	90.76 ± 0.36	90.88 ± 1.01	87.37 ± 0.60	88.49 ± 0.77	96.25 ± 0.4	88.81 ± 1.43

	CIMMYT maize cultivars[a] (QPM; *opaque-2*)								signif levels among cultivars[c]			
									overall			QPM vs not
AA	QPMC59	QPMC68	QPMC72	QPMC74	QPMC79	QPM94	Pioneer 3925	Dekalb DK435	SEM	CV	*F*	*F*
aspartic acid	7.89 ± 0.09	7.43 ± 0.18	7.19 ± 0.24	7.41 ± 0.15	9.43 ± 0.23	8.02 ± 0.14	6.13 ± 0.21	7.01 ± 0.23	0.56	7.59	10.5***	15.1***
threonine	3.39 ± 0.03	3.16 ± 0.12	3.33 ± 0.14	3.34 ± 0.04	3.51 ± 0.403	3.44 ± 0.02	2.99 ± 0.10	3.41 ± 0.04	0.25	7.63	4.34***	6.35***
serine	3.88 ± 0.05	3.98 ± 0.18	3.70 ± 0.08	3.79 ± 0.08	3.99 ± 0.03	4.29 ± 0.14	3.87 ± 0.09	4.15 ± 0.08	0.35	6.32	2.02***	2.83***
glutamic acid	14.62 ± 0.15	16.36 ± 0.32	12.46 ± 0.38	14.95 ± 0.22	15.15 ± 0.28	15.63 ± 0.16	16.13 ± 0.41	16.67 ± 0.2	0.94	6.18	10.6***	15.2***
proline	8.55 ± 0.09	9.02 ± 0.14	8.08 ± 0.12	8.91 ± 0.13	8.59 ± 0.12	9.26 ± 1.39	8.01 ± 0.29	7.97 ± 0.57	0.88	10.7	2.75***	3.78***
glycine	3.63 ± 0.02	3.28 ± 0.08	3.47 ± 0.07	3.74 ± 0.04	3.79 ± 0.04	3.01 ± 0.16	2.78 ± 0.07	3.41 ± 0.32	0.38	11.3	4.23***	6.04***
alanine	5.23 ± 0.07	5.81 ± 0.13	4.92 ± 0.12	5.12 ± 0.06	5.37 ± 0.07	3.74 ± 0.05	5.94 ± 0.15	6.28 ± 0.17	0.36	6.47	8.67***	12.7***
cysteine[b]	3.77 ± 0.11	3.71 ± 0.11	4.60 ± 0.09	4.31 ± 0.12	4.84 ± 0.28	4.35 ± 0.17	4.26 ± 0.10	5.18 ± 0.17	0.54	12.7	2.88***	3.77***
valine	4.87 ± 0.02	4.75 ± 0.09	5.01 ± 0.25	4.88 ± 0.04	4.84 ± 0.04	4.85 ± 0.04	4.26 ± 0.10	4.73 ± 0.06	0.29	6.38	3.81***	5.59***
methionine	1.44 ± 0.06	1.46 ± 0.08	1.72 ± 0.17	1.75 ± 0.04	1.55 ± 0.08	1.96 ± 0.05	1.71 ± 0.12	2.02 ± 0.02	0.31	17.5	3.09***	4.29***
isoleucine	3.02 ± 0.02	3.17 ± 0.06	2.72 ± 0.07	2.98 ± 0.03	2.99 ± 0.03	3.14 ± 0.02	3.01 ± 0.07	3.39 ± 0.05	0.26	8.66	4.58***	6.54***
leucine	8.21 ± 0.23	10.39 ± 0.31	7.82 ± 0.17	8.66 ± 0.17	8.66 ± 0.25	9.34 ± 0.19	11.03 ± 0.27	11.26 ± 0.35	0.89	9.51	11.2***	16.6***
tyrosine	3.58 ± 0.04	3.93 ± 0.08	3.37 ± 0.5	3.68 ± 0.04	3.26 ± 0.16	3.87 ± 0.04	3.924 ± 0.10	4.02 ± 0.07	0.50	13.5	2.03**	2.79**
phenylalanine	3.93 ± 0.03	4.46 ± 0.11	4.17 ± 0.5	3.88 ± 0.05	3.63 ± 0.18	4.17 ± 0.03	4.41 ± 0.10	4.64 ± 0.07	0.32	7.86	6.64***	9.73***
histidine	3.42 ± 0.02	3.18 ± 0.06	3.22 ± 0.07	3.57 ± 0.03	3.48 ± 0.03	3.49 ± 0.02	2.51 ± 0.08	2.76 ± 0.03	0.19	6.46	18.4***	27.7***
lysine	4.02 ± 0.03	3.34 ± 0.09	3.62 ± 0.13	3.78 ± 0.05	4.25 ± 0.07	3.97 ± 0.08	2.67 ± 0.11	3.282 ± 1.58	0.35	9.63	10.2***	15.1***
arginine	6.63 ± 0.23	5.62 ± 0.18	6.34 ± 0.30	6.91 ± 0.19	6.04 ± 0.11	6.06 ± 0.12	4.94 ± 0.17	5.23 ± 0.13	0.54	9.22	9.03***	3.77***
tryptophan[b]	0.70 ± 0.03	0.61 ± 0.02	0.75 ± 0.02	0.75 ± 0.04	0.78 ± 0.05	6.06 ± 0.12	0.62 ± 0.02	0.87 ± 0.04	0.12	16.2	2.88***	3.77***
ammonia	1.88 ± 0.08	1.92 ± 0.21	2.82 ± 0.38	1.57 ± 0.05	1.55 ± 0.08	1.32 ± 0.04	3.25 ± 0.5	1.81 ± 0.17	0.92	40.0	4.15***	6.04***
total AAN[d]												
g of AAN/kg of protein	176.29 ± 1.0	170.51 ± 2.2	186.4 ± 3.68	173.14 ± 1.14	169.98 ± 0.55	167.39 ± 0.7	183.65 ± 5.8	166.33 ± 1.6	9.33	5.31	3.74***	5.43***
g of AAN/kg of dry mass	17.79 ± 0.50	18.81 ± 0.46	14.76 ± 0.34	15.59 ± 0.46	14.72 ± 0.60	15.67 ± 0.44	17.63 ± 0.34	13.19 ± 0.37	1.69	10.5	5.52***	8.15***
g of AA/16 g of N	90.78 ± 0.52	93.94 ± 1.22	87.67 ± 1.16	92.44 ± 0.603	94.13 ± 0.31	95.59 ± 0.41	88.09 ± 2.53	96.28 ± 0.98	3.75	4.11	4.82***	7.08***

[a] Mean values and standard error of measurements (SEM) for four replicates ($N = 4$) and 32 determinations. [b] Taken from Zarkadas et al. (1995). [c] Significance: *F*, values from analysis of variance among cultivars; ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; ns, not significant; CV, coefficient of variation. [d] Total amino acid nitrogen (AAN) was determined according to the methods of Heidelbaugh et al. (1975), Horstmann (1979), and Zarkadas et al. (1988a,b).

Table 3. Comparison of Performance of 15 New Northern Adapted Cultivars of QPM with Commercial Hybrid Maize, Pioneer 3925, Pioneer 3953, Dekalb DK435, Dent (C0251), and Flint (C0255)

maize cultivar	lysine,[a] g/100 g of protein	protein,[a] g/100 g of dry matter	N, g of AA N/100 g of dry mass	PDCAAS,[b] %
QPMC26	4.58[a]	10.15[a]	1.75	72.6
QPMC21	4.53[ab]	9.74[abc]	1.78	71.8
QPMC79	4.52[ab]	8.36[cd]	1.47	71.6
QPMC59	4.43[abc]	10.11[a]	1.78	70.2
QPMC72	4.21[abd]	7.93[d]	1.48	66.7
QPMC94	4.14[cde]	9.36[abc]	1.57	65.6
QPMC13	4.17[cde]	9.01[abcd]	1.65	65.3
QPMC74	4.10[cd]	9.07[abcd]	1.56	64.9
QPMC18	4.09[cde]	8.49[bcd]	1.43	64.9
QPMC12	3.99[de]	7.91[d]	1.48	63.4
QPMC36	3.97[de]	9.72[abc]	1.74	62.9
QPMC42	3.81[ef]	8.46[bcd]	1.44	60.3
QPMC68	3.56[fg]	9.78[ab]	1.88	56.4
QPMC40	3.55[fg]	7.97[d]	1.44	56.3
QPMC39	3.51[fg]	8.62[bcd]	1.65	55.6
QPMC2	3.43[gh]	8.56[bcd]	1.49	54.4
Pioneer 3925	3.16[hi]	10.27[a]	1.76	50.1
Pioneer 3953[c]	2.89[c]	8.43[c]	1.28[c]	45.9
Dekalb DK435	2.92[i]	7.99[d]	1.32	46.2
Dent (CO251)	1.99[c]	10.16[c]	1.65[c]	33.4[c]
Flint (CO255)	1.77[c]	10.47[c]	1.79[c]	30.5[c]

[a] Means along a horizontal row with different superscripts are significantly different at the $P < 0.001$ level by Duncan's new multiple-range test (Duncan, 1955). [b] True protein digestibility values were taken from the U.S. Food and Drug Administration (1993; *Federal Register*, Appendix B). [c] Data taken from Zarkadas et al. (1995). [k] Calculation of protein ratings of the 15 new northern adapted QPM cultivars was carried out by comparison of the amino acid composition of hen's whole egg with that of the reference pattern established by FAO/WHO/UNU (1985) for a preschool child (2−5 years old).

ing system, release 6.2 (SAS, 1991), and represents the average values from four replicates (32 determinations) per genotype.

RESULTS AND DISCUSSION

Fifteen new northern adapted QPM inbred cultivars, which contain the *opaque-2* and modifier genes quoting for high lysine in maize, were evaluated in the present study. They included 12 from CIMMYT's (1981) northern temperate zone QPM gene pool, namely, QPM-C2, QPM-C12, QPM-C18, QPM-C21, QPM-C26, QPM-C36, QPM-C39, QPM-C40, QPM-C42, QPM-C74, QPM-C79, and QPM-C94, and 3 additional inbred cultivars, designated QPM-C59, QPM-C68, and QPM-C72, which originated from CIMMYT's (1981) subtropical temperature zone QPM gene pool. Precise determinations of total protein and amino acids were carried out by single ion-exchange amino acid chromatographic methods (Moore and Stein, 1963; Slocum et al., 1987; Zarkadas et al., 1986, 1987, 1990).

The overall amino acid composition of the 15 QPM cultivars and levels of statistical significance obtained from analysis of variance, which represent the average values of 32 analyses per cultivar, are shown in Table 1. These values show deviations of <3.0% from the average values obtained between replicates within the same cultivar. Expressing the amino acid data as grams of amino acids per kilogram of anhydrous fat- and ash-free protein (Table 1) allowed comparisons to be made of the protein and amino acid contents of the various cultivars with the recommended FAO/WHO (1991) reference amino acid patterns for humans (Tristram and Smith, 1963; Zarkadas et al., 1988a−c, 1990, 1995). The mean residue weight (WE, micrograms per nanomole) and conversion factor (CF, micrograms per nanomole), given in Table 1, can be used in all subsequent quantitations of the same maize tissue following standard procedures as described by Horstmann (1979), Zarkadas et al. (1988a−c), and Zarkadas (1997).

Analyses of maize for both protein and lysine contents have been previously reported (Aquirre et al., 1953; Bressani et al., 1962; Tello et al., 1965; Paez et al., 1969; Zuber et al., 1975; Paiva et al., 1991; Kniep and Mason, 1991; Zarkadas et al., 1995; Moro et al., 1996; Zarkadas, 1997). These studies revealed a broad range of variability in both protein and lysine contents. The data presented in Table 1 indicate that the protein content among these maize cultivars investigated differed significantly ($P < 0.001$). For example, the QPMC26 and QPMC59 inbred lines were consistently higher in total protein (10.1%) than any of the other 13 QPM cultivars, which varied from 9.7% (QPMC21, QPMC36, and QPMC68) to 7.9% (QPMC12 and QPMC40). The QPMC13 genotype contained 9.0% protein (Zarkadas et al., 1995). These results are in accord with those of Ortega et al. (1991), Kniep and Mason (1991), and Glover (1992), who reported values ranging from 8.3 to 9.7% for their normal and hard endosperm *opaque-2* maize cultivars. It should be noted that the total protein of the new QPM cultivars is equal to or higher than that of the most common commercial maize varieties (Table 3).

These results, however, were considerably lower than those reported by Bjarnnason and Vasal (1992) and Vasal et al. (1993a,b), who determined total protein according to the Kjeldahl method. Moro et al. (1996) reported variable protein contents by the microKjeldahl method, in a diverse set of 93 maize inbred lines investigated, which ranged from 6.7−13.5% for normal maize to 3.4−9.4% for *opaque-2* genotypes. Similar high maize protein values have been obtained using the Kjeldahl method for the maize population GR-OP-319 (Bletsos and Goulas, 1999) and for the Illinois high protein maize strain, which after 90 generations of selection is reported to be 32.0% protein (Dudley and Lambert, 1992). The accuracy of the Kjeldahl method, however, varies depending upon the amount of nonprotein nitrogen present in the sample (Khanizadeh et al., 1992). To correct for nonprotein nitrogen the U.S. Department of Health Services, Food and Drug Administration (U.S. FDA., 1993) changed the U.S. *Federal Register* methodology 101.9(c)(7)(ii) for assessing the protein content of foods. It states that specific conversion factors are to be used when converting nitrogen into protein content and for assessing the protein digestibility-corrected amino acid score (PDCAAS). In the present study the best estimate of the protein and nitrogen contents of these new QPM cultivars was made by the summation of the weights of the amino acid residues or amino acid nitrogen, of which each of these cultivars is composed, as described by Tristram and Smith (1963), Heidelbaugh et al. (1975), and Horstmann (1979). The results, summarized in Tables 1−3, show that this method yields accurate estimates of the absolute amount of protein by simple summation of the amino acids and nitrogen present among the cultivars evaluated.

For purposes of comparison, the amino acid data and nitrogen contents from this study have been calculated as recommended by Block and Mitchell (1946), Heidel-

Table 4. Comparison of the Essential Amino Acid (EAA) Scores of 15 New Northern Adapted Cultivars of QPM with Commercial Hybrid Maize, Pioneer 3925, and Dekalb DK435, Hen's Whole Egg, and the EAA Requirements of a 2−5-Year-Old Child

EAA	EAA[a] requirements for a preschool child (2−5-year-old)	CIMMYT maize cultivars (QPM; *opaque-2*)								
		QPMC2	QPMC12	QPMC18	QPMC21	QPMC26	QPMC36	QPMC39	QPMC40	QPMC42
		Milligrams of Amino Acid per Gram of Total Protein[b]								
histidine	19	30	34	35	36	38	32	30	30	33
isoleucine	28	36	31	33	33	32	33	31	33	33
leucine	66	111	95	93	97	86	108	106	110	110
lysine	58	34	40	41	45	46	40	35	36	38
methionine + cyst(e)ine[b]	25	68	74	72	62	61	60	74	76	68
phenylalanine + tyrosine	63	79	77	78	80	80	77	75	91	78
threonine	34	35	35	37	37	36	36	35	33	35
tryptophan[b]	11	8	8	8	7	8	8	9	9	8
valine	35	46	55	50	51	53	49	53	53	50
% total protein										
EAA_9c	33.9	44.7	44.9	44.7	44.8	44.0	45.5	44.8	47.1	45.3
		Percent True Protein Digestibility[d] in Man								
		92	92	92	92	92	92	92	92	92
		Percent Amino Acid Score[d]								
		58.6	68.9	70.6	77.6	79.3	68.9	60.3	62	65.5
		Protein Digestibility Corrected Amino Acid Score[d]								
		53.9	63.4	64.9	71.8	73.0	62.9	55.5	56.3	60.3

EAA	EAA[a] requirements for a preschool child (2−5-year-old)	CIMMYT maize cultivars quality protein maize (QPM; *opaque-2*)							commercial maize cultivars		animal product
		QPMC59	QPMC68	QPMC72	QPMC74	QPMC79	QPMC94	QPMC13 testers	Pioneer 3925	Dekalb DK435	egg
		Milligrams of Amino Acid per Gram of Total Protein[c]									
histidine	19	38	34	37	39	37	37	36	27	29	22
isoleucine	28	33	34	32	32	32	33	31	34	35	54
leucine	66	89	110	91	94	92	98	93	122	117	86
lysine	58	44	36	42	41	45	42	41	31	29	70
methionine + cyst(e)ine[b]	25	57	58	74	66	68	66	71	67	75	57
phenylalanine + tyrosine	63	81	89	82	82	73	84	82	91	92	93
threonine	37	37	34	38	36	37	36	38	34	35	47
tryptophan[b]	11	8	7	9	8	8	8	8	9	9	17
valine	54	51	58	53	51	51	57	57	47	49	66
% total protein											
EAA_9c	33.9	43.8	46.0	45.8	44.9	44.3	46.1	45.7	46.2	47.0	50.9
		Percent True Protein Digestibility[d] in Man									
		92	92	92	92	92	92	92	84	84	97
		Percent Amino Acid Score[d]									
		75.8	62.1	72.4	70.7	77.6	72.4	68.9	54.5	50.0	
		Protein Digestibility Corrected Amino Acid Score[d]									
		69.8	57.1	66.6	65.0	71.4	66.6	65.3	50.1	46.2	97

[a] Data from FAO/WHO/UNU (1985) and FAO/WHO (1991). [b] Taken from Zarkadas et al. (1995). [c] Calculation of protein ratings of the 15 new northern adapted QPM cultivars was carried out by comparison of the amino acid composition of hen's whole egg with that of the reference pattern established by FAO/WHO/UNU (1985) for a preschool child (2−5 years old). [d] True protein digestibility values were taken from the U.S. Food and Drug Administration (1993; *Federal Register*, Appendix B).

baugh et al. (1975), FAO/WHO/UNU (1985), and FAO/WHO (1991) and are expressed as grams of amino acids per 16 g of total nitrogen (Table 2). The total amino acid nitrogen of these QPM maize inbred samples ranged from 1.43 to 1.88%. By comparison, Dekalb DK435 and Pioneer 3925 commercial maize contained 1.32 and 1.76% amino acid nitrogen, respectively.

A comparison of the amino acid profiles of the maize cultivars, as presented in Tables 1 and 2, shows that the two commercial cultivars, Dekalb DK435 and Pioneer 3925 cultivars, were very similar in amino acid composition. Both were high in several amino acids, including glutamic acid (17.4−18.0% of the total amino acids), leucine (11.6−12.2%), and alanine (6.5−6.6%). However, lysine accounted for only 2.92−3.14% of the total. Thus, the total basic amino acids, which include lysine, arginine, and histidine, constituted 11.2−11.9% of the total amino acids. These values are considerably lower than the acidic amino acids, which represent 24.4−25.1% of the total amino acid residues. These results were close to those reported by Nelson et al. (1965), Zarkadas et al. (1995), and Zarkadas (1997).

By contrast, the 15 new QPM cultivars had an overall amino acid composition very different from those of the two commercial maize cultivars investigated in this study. The variation of amino acids noted among the QPM cultivars was also found to be highly significant ($P < 0.01$ to $P < 0.001$) for each amino acid analyzed, except for serine. Of particular interest were the significantly increased levels ($P < 0.001$) of lysine in QPM maize genotypes compared to normal maize cultivars. A summary of the performance of the QPM cultivars showed that four of these QPM inbred lines, QPM-C26, QPM-C21, QPM-C79, and QPM-C59, contained the highest levels of lysine, ranging from 4.43 to 4.58 g of lysine/100 g of protein along with high levels of total protein (Table 3). These results are in accord with the lysine values found by Kniep and Mason (1991), who reported a range from 4.1 to 4.3% lysine for short- and long-season *opaque-2* maize, respectively. The lysine

values found among the other 11 QPM inbreds ranged from 3.43 to 4.21 g/100 g of protein compared to 2.92 and 3.14 g of lysine/100 g of protein in Dekalb DK435 and Pioneer 3925 commecial maize, respectively. Habben et al. (1995) and Moro et al. (1996) reported a wide variation of lysine content among the *opaque-2* inbred lines investigated, which ranged from 2.77 to 4.46% lysine. Their data, however, were based upon a single lysine analysis per cultivar.

From the amino acid composition of the new QPM cultivars it became apparent that considerable differences exist in lysine content among the QPM genotypes studied, depending upon their genetic background. In addition to an almost 2-fold increase in lysine, the contents of aspartic acid, proline, alanine, and histidine increased in QPM inbreds, whereas the levels of glutamic aicd, leucine, tyrosine, and phenylalanine decreased sharply, compared to the commercial varieties. Several studies have demonstrated that the principal changes which occur in storage protein among the *opaque-2* inbred lines investigated include 50% reduction of both the 19 and 22 kDa α-zein subunits (Wallace et al., 1990; Geetha et al., 1991), an increase in γ-zein, a proline- and cysteine-rich protein (Wallace et al., 1990; Paiva et al., 1991), and an increase in nonzein proteins, especially in the content of the elongation factor 1α (EF-1α) (Durso and Cyr, 1994; Shiina et al., 1994; Morelli et al., 1994; Habben et al., 1995; Moro et al., 1996). Wallace et al. (1990) presented data showing that QPM contained 2−4 times more γ-zein than normal maize varieties or *opaque-2* and *floury-2* genotypes. Paiva et al. (1991) and Lobes and Larkins (1991) indicated that both soft and hard regions of QPM endosperm are enriched in γ-zein, which influences kernel hardness in the QPM genotypes. The increased synthesis of the 27 kDa γ-zein in QPM genotypes leads to a significant increase in aspartic acid, proline, alanine, histidine, and arginine (Table 1). Sequence studies have shown that γ-zein contained 7% histidine (Shewry and Tatham, 1990), which might explain the results in the present study.

The data in Table 1 also indicate a highly significant increase ($P < 0.001$) in basic, totally charged, and hydrophilic amino acids, compared to Pioneer 3925 and Dekalb DK435. These results suggest because zeins contain no lysine (Shewry and Tatham, 1990), the increase in lysine and a decrease in hydrophobicity in QPM reflect a higher proportion of nonzein proteins, which have markedly higher lysine contents. Habben et al. (1995) and Moro et al. (1996) have shown that the elongation factor EF-1α is overexpressed in QPM endosperm compared to normal maize and that a high correlation exists between lysine and nonzein proteins primarily between lysine and EF-1α contents. Although the biological basis for the correlation between the high lysine content of the QPM inbred cultivars and EF-1α remains to be elucidated, these authors suggested that genetic selection for genotypes with a high EF-1α content can significantly improve the nutritional quality of maize.

The essential amino acid (EAA) profiles and the protein digestibility corrected amino acid score (PDCAAS) protein ratings of the 15 QPM inbred cultivars, 2 typical commercial cultivars Pioneer 3925 and Dekalb DK435, and QPM-C13 are compared with those of the reference pattern (FAO/WHO/UNU, 1985; FAO/WHO, 1991) for a 2−5-year-old child, and the results are summarized in Tables 3 and 4. The data indicate that four of these QPM inbred lines, QPM-C26, QPM-C21, QPM-C79, and QPM-C59, contained the highest amino acid score, ranging from 70.2 to 72.6%, reflecting the high levels of lysine present. It should be noted that although lysine averaged 45.2 mg/g of QPM proteins, which is considerably higher than normal maize, it is still below the recommended FAO/WHO (1991) reference lysine standard value of 58 mg/g of dietary protein for the 2−5-year-old child (Table 4). The amino acid scores found among the remaining 11 QPM cultivars ranged from 54.4 to 66.7% compared to 30.5, 33.4, 46.2, 45.9, and 50.1% values found for Flint CO255, Dent Co251, Dekalb DK435, and Pioneer 3953 and 3925, respectively (Tables 3 and 4).

The large difference in the corrected amino acid scores between common commercial maize cultivars and QPM inbreds is attributed to their low lysine values, which ranged from 1.7 to 3.16 g of amino acid/100 g of total protein. Thus, in common maize the primary essential amino acid deficiency is lysine. However, early nutritional studies with rats by Benton et al. (1955) showed that the other limiting amino acid in common maize is isoleucine. These authors have indicated that although common maize is not deficient in isoleucine or threonine, the presence of large amounts of leucine in diets of zein or maize has caused both amino acid imbalances in rats and interference of isoleucine absorption (Harper et al., 1955; Benton et al., 1956). Similar concerns exist for humans fed primarily maize, whose niacin requirements are increased; excess leucine could be partly responsible for the development of pellagra in humans fed primarily maize (FAO, 1992). The ratio of leucine/isoleucine found among the QPM inbreds ranged from 2.8 to 3.1 compared to 3.3 and 3.7 in Dekalb DK435 and Pioneer 3925, respectively, suggesting that the QPM proteins provide an even better EAA balance than is indicated from the calculated amino acid profile.

Although lysine is the first limiting amino acid in QPM inbreds for humans, the overall balance of their EAA for humans is superior to those of normal maize and other cereals. The results presented in Table 4 indicate that high-lysine QPM maize may supply ∼70.2−72.6% of human protein requirements, compared to 28−50% for common maize, 55−63% for oats, and 59−60.3% for barley.

This study also indicates that northern adapted QPM genotypes containing the *opaque-2* modifier genes may have the potential to increase their lysine and protein contents even higher, either by an increase in specific high-lysine-containing nonzein proteins, such as the synthesis of factor EF-1a, or by a further reduction in the 19 and 22 kDa α-zein in the endosperm or both. This knowledge could assist maize breeders in the selection of new high-performance QPM genotypes with improved protein quality and quantity.

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LITERATURE CITED

Aguire, F.; Robles, C. E.; Serimsha, N. S. The nutritive value of Central American corn. II. Lysine and methionine content of twenty-three varieties in Guatemala. *Food Res.* **1953**, *18*, *368*−*372*.

Alexander, D. E.; Lambert, R. J.; Dudley, J. W. Breeding problems and potentials of modified protein maize. In *New Approaches to Breeding for Improved Plant Protein*; Panel Proceedings Series; International Atomic Energy Agency: Vienna, Austria, 1969; pp 55−65.

Aukerman, M. J.; Schmidt, R. J. Regulation of α-zein gene expression during maize endosperm development. In *Results and Problems in Cell Differentiation*; Nover, L., Dennis, E., Eds.; Springer-Verlag: Berlin, Germany, 1991; Vol. 20, pp 209−233.

Aukerman, M. J.; Schmidt, R. J.; Burr, B.; Burr, F. A. An arginine to lysine substitution in the bZIP domain of an *opaque-2* mutant in maize abolishes specific DNA binding. *Genes Dev.* **1991**, *5*, 310−320.

Benton, D. A.; Harper, A. E.; Elvehjem, C. A. Effect of isoleucine supplementation on the growth of rats fed zein or corn diets. *Arch. Biochem. Biophys.* **1955**, *57*, 13−19.

Benton, D. A.; Harper, A. E.; Spivey, H. E.; Elvehjem, C. A. Leucine, isoleucine and valine relationships in the rat. *Arch. Biochem. Biophys.* **1956**, *60*, 147−155.

Bjarnason, M.; Vasal, S. K. *Breeding of Quality Protein Maize* (*QPM*); Janick, J., Ed.; Plant Breeding Rev. 9; Wiley: New York, 1992; pp 181−216.

Bletsos, E. A.; Goulas, C. K. Mass selection for improvement of grain yield and protein in a maize population. *Crop Sci.* **1999**, *39*, 1302−1305.

Block, R. J.; Mitchell, H. H. The correlation of the amino acid composition of proteins with their nutritive value. *Nutr. Abstr.* **1946**, *16*, 249−278.

Bockholt, A. J.; Rooney, L. W. QPM hybrids for the United States. In *Quality Protein Maize*; Mertz, E. T., Ed.; American Association of Cereal Chemists: St. Paul, MN, 1992; pp 111−121.

Bressani, R. Protein quality of high-lysine maize for humans. *Cereal Foods World* **1991**, *36*, 806−811.

Bressani, R. Nutritional value of high-lysine maize in humans. In *Quality Protein Maize*; Mertz, E. T., Ed.; American Association of Cereal Chemists: St. Paul, MN, 1992; pp 205−224.

Bressani, R.; Elias, L. G.; Schrimshaw, N. S.; Guzman, M. A. Nutritive value of Central American corn. VI. Varietal and environmental influences on the nitrogen, essential amino acid, and fat content of ten varieties. *Cereal Chem.* **1962**, *39*, 59−67.

Burr, B.; Burr, F. A. Zein synthesis in maize endosperm by polyribosomes attached to protein bodies. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 515−519.

CIMMYT (Centro Internacional de Mejoramiento de Maiz y Trigo). *CIMMYT Report on Maize Improvement*; CIMMYT: Mexico City, 1981.

CIMMYT. *Boosting Protein Quality in Maize*; CIMMYT Research Highlights; CIMMYT: Mexico City, 1985.

Damerval, C.; de Vienne, D. Quantification of dominance for proteins pleiotropically affected by *opaque-2* in maize. *Heredity* **1993**, *70*, 38−51.

Dudley, J. W.; Lambert, R. J. Ninety generations of selection for oil and protein in maize. *Maydica* **1992**, *37*, 81−87.

Duncan, D. B. Multiple range and multiple F Test. *Biometrics* **1955**, *11*, 1−42.

Durso, N. A.; Cyr, R. J. A calmodulin-sensitive interaction between microtubules and a higher plant homolog of elongation factor 1α. *Plant Cell* **1994**, *6*, 839−905.

Emerson, R. A.; Beadle, G. W.; Fraser, A. C. A summary of linkage groups in maize. *Cornell Univ. Agric. Exp. Sta. Mem.* **1935**, *No. 180*.

Esen, A. A proposed nomenclature for the alcohol-soluble proteins (Zeins) of maize (*Zea mays* **L.**). *J. Cereal Sci.* **1987**, *5*, 117−128.

FAO. *Maize in Human Nutrition*; Report Series 25; Food and Agriculture Organization: Rome, Italy, 1992.

FAO/WHO Expert Consultation. *Protein Quality Evaluation*; FAO/WHO Nutrition Meetings, Report Series 51; Food and Agriculture Organization/World Health Organiztion: Rome, Italy, 1991.

FAO/WHO/UNU Expert Committee. *Energy and Protein Requirements*; FAO/WHO Nutrition Meetings, Report Series 724; Food and Agriculture Organization/World Health Organization: Geneva, Switzerland, 1985.

Geetha, K. B.; Lending, C. R.; Lopes, M. A.; Wallace, J. C.; Larkins, B. A. *Opaque-2* modifiers increase γ-zein synthesis and alter its spatial distribution in maize endosperm. *Plant Cell* **1991**, *3*, 1207−1219.

Gevers, H. O.; Lake, J. K. Development of modified *opaque-2* maize in South Africa. In *Quality Protein Maize*; Mertz, E. T., Ed.; American Association of Cereal Chemists: St. Paul, MN, 1992; pp 49−78.

Glover, D. V. Corn Protein-Genetics, Breeding, and Value in Foods and Feeds. In *Quality Protein Maize*; Mertz, E. T., Ed.; American Association of Cereal Chemists: St. Paul, MN, 1992; pp 9−26.

Habben, J. E.; Larkins, B. A. Improving Protein Quality in Seeds. In *Seed Development and Germination*; Kigel, J., Galili, G., Eds.; Dekker: New York, 1995; Chapter 29, pp 791−810.

Habben, J. E.; Kirleis, A. W.; Larkins, B. A. The origin of lysine-containing proteins in *opaque-2* maize endosperm. *Plant Mol. Biol.* **1993**, *23*, 825−838.

Habben, J. E.; Moro, G. L.; Hamaker, B. R.; Larkins, B. A. Elongation factor 1α concentration is highly correlated with the lysine content of maize endosperm. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 8640−8644.

Hallauer, A. R. *Recurrent Selection in Maize*; Janick, J., Ed.; Plant Breeding Rev. 9; Wiley: New York, 1999; pp 115−179.

Harper, A. E.; Benton, D. A.; Elvehjem, C. A. L-Leucine, an isoleucine antagonist in the rat. *Arch. Biochem. Biophys.* **1955**, *57*, 1−11.

Hartings, H.; Madaloni, M.; Lazzroni, N.; Fonzo, N.; Motto, M.; Salamini, F.; Thomson, R. The *o2* gene which regulates zein deposition in maize enosperm encodes protein with structural homologies to transcriptional activators. *EMBO J.* **1989**, *8*, 2795−2801.

Heidelbaugh, N. D.; Huber, C. S.; Bernarczyk, J. R.; Smith, M. D.; Rambart, P. C.; Sheller, H. O. Comparison of three methods for calculating protein content of foods. *J. Agric. Food. Chem.* **1975**, *23*, 611−613.

Horstmann, J. H. A Precise method for the quantitation of proteins taking into account their amino acid composition. *Anal. Biochem.* **1979**, *96*, 130−138.

Khanizadeh, S.; Buszard, D.; Zarkadas, C. G. Seasonal variation of proteins and amino acids in apple flower buds (*Malus pumilla* Mill., cv McIntoch/M7). *J. Agric. Food Chem.* **1989**, *37*, 1246−1252.

Khanizadeh, S.; Buszard, D.; Zarkadas, C. G. Comparison of three methods for calculating protein content in developing apple flower buds. *J. Assoc. Off. Anal. Chem.* **1992**, *75*, 734−737.

Kniep, K. R.; Mason, S. C. Lysine and protein content of normal and *opaque-2* maize grain as influenced by irrigation and nitrogen. *Crop Sci.* **1991**, *31*, 177−181.

Kodrzycki, R.; Boston, R. S.; Larkins, B. A. The *opaque-2* mutation of maize differentially reduces zein gene transcription. *Plant Cell* **1989**, *1*, 105−114.

Larkins, B. A.; Wallace, J. C.; Galili, G.; Lending, C. R.; Kawata, E. E. Structural analysis and modification of maize storage proteins. *Dev. Ind. Microbiol.* **1989**, *30*, 203−209.

Lee, K. H.; Jones, R. A.; Dalby, A., Tsai, C. Y. Genetic regulation of storage protein content in maize endosperm. *Biochem. Genet.* **1976**, *14*, *641*−*650*.

Lending, C. R.; Larkins, B. A. Changes in the zein composition of protein bodies during maize endosperm development. *Plant Cell* **1989**, *1*, 1011−1023.

Lopes, M. A.; Larkins, B. A. γ-Zein content is related to endosperm modification in quality protein maize. *Crop Sci*. **1991**, *31*, 1655−1662.

Magnavaca, R.; Larkins, B. A.; Schaffert, R. E.; Lopes, M. A. Improving Protein Quality of Maize and Sorghum. In *International Crop Science I*; Buxton, D. R., Shibles, R., Forsberg, R. A., Blad, B. L., Asay, K. H., Paulsen, G. M., Wilson, R. F., Eds.; Crop Science Society of America: Madison, WI, 1993; Chapter 85.

Merrick, W. C. Mechanism and regulation of eukaryotic protein synthesis. *Microbiol. Rev*. **1992**, *56*, 291−315.

Mertz, E. T.; Bates, L. S.; Nelson, O. E. Mutant gene that changes protein composition and increases lysine content of maize endosperm. *Science* **1964**, *45*, 279−280.

Moore, S.; Stein, W. H. Chromatographic determination of amino acids by the use of automatic recording equipment. *Methods Enzymol*. **1963**, *6*, 819−831.

Morelli, J. K.; Shewmaker, K.; Vayda, M. E. Biphasic stimulation activity correlates with induction of translation factor 1 subunit α. *Plant Physiol*. **1994**, *106*, *897−903*.

Moro, G. L.; Habben, J. E.; Hamaker, B. R.; Larkins, B. A. Characterization of the variability in lysine content for normal and opaque-2 maize endosperm. *Crop Sci*. **1996**, *36*, 1651−1659.

Nelson, O. E. The Modification by Mutation of Protein Quality in Maize. In *New Approaches to Breeding for Improved Plant Protein*; Panel Proceedings Series; International Atomic Energy Agency: Vienna, Austria, 1969; pp 41−54.

Nelson, O. E.; Mertz, E. T.; Bates, L. S. Second mutant gene affecting the amino acid pattern of maize endosperm proteins. *Science* **1965**, *150*, 1469−1470.

Neuffer, M. G.; Coe, E. H.; Wessler, S. R. The *o2* Gene. In *Mutants of Maize*; Cold Spring Harbor Laboratory Press: New York, 1997; Chapter 5, p 361.

Or, E.; Boyer, S. K.; Larkins, B. A. *Opaque-2* modifiers act post-transcriptionally and in a polar manner on γ-zein gene expression in maize endosperm. *Plant Cell* **1993**, *5*, 1599−1609.

Ortega, E. I.; Villegas, E.; Bjarnason, M.; Short, K. Changes in dry matter and protein fractions during kernel development of quality protein maize. *Cereal Chem*. **1991**, *68*, 482−486.

Paez, A. V.; Ussary, J. P.; Helm, J. L.; Zuber, M. S. Survey of maize strains for lysine content. *Agron. J*. **1969**, *61*, 896−899.

Paiva, E.; Kriz, A. L.; Peixoto, M. J. V. V. D.; Wallace, J. C.; Larkins, B. A. Quantitation and distribution of γ-zein in the endosperm of maize kernels. *Cereal Chem*. **1991**, *68*, 276−279.

Pedersen, K.; Bloom, K. W.; Andersen, J. N.; Glover, D. V.; Larkins, B. A. Analysis of the complexity and frequency of zein genes in the maize genome. *Biochemistry* **1980**, *19*, 1644−1650.

Pysh, L. D.; Aukerman, M. J.; Schmidt, R. J. OHP1: A maize basic domain/leucine zipper protein that interacts with *opaque-2*. *Plant Cell* **1993**, *5*, 227−236.

SAS (Statistical Analysis System). *User's Guide: Basics*; SAS Institute: Cary, NC, 1991.

Schmidt, R. J. *Opaque-2* and zein gene expression. In *Control of Plant Gene Expression*; Verma, D. P. S., Ed.; CRC Press: Boca Raton, FL, 1993; Chapter 21, pp 337−355.

Schmidt, R. J.; Burr, F. A.; Burr, B. B. Transposon tagging and molecular analysis of the maize regulatory locus *opaque-2*. *Science* **1987**, *238*, 960−963.

Shewry, P. R.; Tatham, A. S. The prolamin storage proteins of cereal seeds: Structure and evolution. *Biochem. J*. **1990**, *267*, 1−12.

Shiina, N.; Gotoh, Y.; Kubomura, N.; Iwamatsu, A.; Nishimura, E. Microtubule severing by elongation factor 1α. *Science* **1994**, *266*, 282−285.

Slocum, R.; Lee, P.; Arrizon-Lopez, V. Accelerated protein hydrolyzate analysis. Beckman Bulletin System 6300/7300, A6300-AN-001A, 1987; pp 1−5.

Spaner, D.; Mather, D. E.; Hamilton, R. I. Genetic and agronomic evaluation of short-season Quality Protein Maize. *Can. J. Plant Sci*. **1992a**, 1171−1183.

Spaner, D.; Mather, D. E.; Hamilton, R. I. Agronomic potential of northern temperate zone Quality Protein Maize germplasm in eastern Canada. *Plant Varieties Seeds* **1992b**, *5*, 113−121.

Tello, F.; Alvarez-Tostado, M. A.; Alvarado, G. A study on the improvement of the essential amino acid balance of corn protein. I. Correlation between racial and varietal characteristics and lysine levels of corn. *Cereal Chem*. **1965**, *42*, 368−384.

Tristram, G. R.; Smith, R. H. In *The Amino Acid Composition of Some Purified Proteins*; Anfinsen, C. B., Anson, M. L., Edsall, J. T., Eds.; Advances in Protein Chemistry 18; Academic Press:, New York, 1963; pp 276−279.

U.S. Food and Drug Administration (FDA), Department of Health and Human Services (HHS), 21 CFR Parts 1 and 101. Food Labeling: Mandatory Status of Nutrition Labeling and Nutrient Content Revision, Format for Nutrition Label. *Fed. Regist*. **1993**, *58*, *2079−2178*.

Vasal, S. K.; Srinivasan, G.; Pandey, S.; Gonzalez, C.; Crossa, J.; Beck, D. L. Heterosis and combining ability of CIMMYT's quality protein maize germplasm: I. Lowland tropical. *Crop Sci*. **1993a**, *33*, 46−51.

Vasal, S. K.; Srinivasan, G.; Gonzalez, C.; Crossa, J.; Beck, D. L.; Crossa, J. Heterosis and combining ability of CIMMYT's quality protein maize germplasm: II. Subtropical. *Crop Sci*. **1993b**, 33, 51−57.

Villegas, E. M.; Eggum, B. O.; Vasal, S. K.; Kohli, M. M. Progress in nutritional improvements of maize and triticale. *Food Nutr. Bull*. **1980**, *2*, 17−24.

Villegas, E. M.; Vasal, S. K.; Bjarnason, M. Quality protein maize-what is it and how was it developed. In *Quality Protein Maize*; Mertz E. T., Ed.; American Association of Cereal Chemists: St. Paul, MN, 1992; pp 27−48.

Wallace, L. C.; Lopes, M. A.; Paiva, E.; Larkins, B. A. New methods for extraction and quantitation of zeins reveal a high content of γ-zein in modified *opaque-2* maize. *Plant Physiol*. **1990**, *92*, 191−196.

Wang, S. Z.; Esen, A. Primary structure of a proline-rich zein and its cDNA. *Plant Physiol*. **1986**, *81*, 70−74.

Young, V. R.; Pellett, P. L. Plant proteins in relation to human protein and amino acid nutrition. *Am. J. Clin. Nutr*. **1994**, *59*, 1203S−1212S.

Zarkadas, C. G. Assessment of the protein quality of native white floury maize, designated IPO-13, by amino acid analysis. *J. Agric. Food Chem*. **1997**, *45*, *1062−1069*.

Zarkadas, C. G.; Zarkadas, G. C.; Karatzas, C. N.; Khalili, A. D.; Nguyen, Q. Rapid methods for determining desmosine, isodesmosine, 5-hydroxylysine, tryptophan, lysinoalalanine and the amino sugars in proteins and tissues. *J. Chromatogr*. **1986**, *378*, 67−76.

Zarkadas, C. G.; Rochemont, J. A.; Zarkadas, G. C.; Karatzas, C. N.; Khalili, A. D. Determination of methylated basic, 5-hydroxylysine, elastin cross-links, other amino acids, and the amino sugars in protein and tissues. *Anal. Biochem*. **1987**, *160*, 251−266.

Zarkadas, C. G.; Meighen, E. A.; Zarkadas, G. C.; Karatzas, C. N.; Khalili, A. D.; Rochemont, J. A.; Berthelet, M. The determination of the myofibrillar and connective tissue proteins in the bovine diaphragm. *J. Agric. Food Chem*. **1988a**, *36*, 1095−1109.

Zarkadas, C. G.; Drouliscos, N.; Karatzas, C. J. Comparison of the total protein, nitrogen and amino acid composition of selected additives and ingredients used in composite meat products. *J. Agric. Food Chem*. **1988b**, *36*, 1121−1131.

Zarkadas, C. G.; Karatzas, C. N.; Khalili, A.; Khanizadeh, S.; Morin, G. Quantitative determination of the myofibrillar proteins and connective tissue content in selected porcine skeletal muscle. *J. Agric. Food Chem*. **1988c**, *36*, 1131−1146.

Zarkadas, C. G.; Meighen, E. A.; Rochemont, J. A.; Zarkadas, G. C.; Khalili, A. D.; Nguyen, Q. Determination of methylated basic amino acids, 5-hydroxylysine, and elastin cross-

links in proteins and tissues. In *Amino Acids*: *Chemistry, Biology and Medicine*; Lubec, C., Rosenthal, G. A., Eds.; ESCOM Science Publishers: Leiden, The Netherlands, 1990; pp 201−216.

Zarkadas, C. G.; Yu, Z.; Voldeng, H. D.; Hope, H. J.; Minero-Amador, A.; Rochemont, J. A. Comparison of the protein-bound and free amino acid contents of two northern adapted soybean cultivars. *J. Agric. Food Chem*. **1994**, *42*, 21−33.

Zarkadas, C. G.; Yu, Z.; Hamilton, R. I.; Pattison, P. L.; Rose, N. G. W. Comparison between the protein quality of northern adapted cultivars of common maize and quality protein maize. *J. Agric. Food Chem*. **1995**, *43*, *84*−*93*.

Zuber, M. S.; Skrdla, W. H.; Choe, B. Survey of maize selections for endosperm lysine content. *Crop Sci*. **1975**, *15*, 93−94.

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