Phenotypic Effects of *opaque2* Modifier Genes in Normal Maize Endosperm

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ABSTRACT

The *opaque2* modifier genes convert the soft, chalky endosperm, as found in maize *opaque2* mutants, to a hard, vitreous phenotype. Modified *opaque2* genotypes, also known as Quality Protein Maize (QPM), have increased levels of the essential amino acid lysine and a normal appearing kernel. The development of vitreous endosperm in QPM is associated with a two- to threefold increase in the γ-zein storage protein. To determine whether an increased concentration of γ-zein can influence hardness and the formation of vitreous endosperm in normal genotypes, we derived 13 modified normal inbreds from a cross of the normal line W64A with Pool 34 QPM. Analysis of protein content, kernel density and hardness revealed variation among the 13 inbreds. The modified normal inbreds show an increased content of γ-zein in the endosperm, similar to that of QPM. But the high level of γ-zein did not increase hardness and density above a threshold level. The modified normal inbreds did not have an increased lysine content, suggesting modifier genes, per se, do not improve the nutritional quality of maize endosperm.

Discovery that the maize *opaque2* (*o2*) mutation dramatically increases the lysine content of the grain (Mertz et al 1964) led to the development of high lysine corn (reviewed in Mertz 1992). However, the soft, starchy endosperm of this mutant, which causes the kernel to be susceptible to pests and mechanical damage (Ortega and Bates 1983), prevented significant utilization of the mutation. After the initial characterization of *o2*, genes were identified that alter the mutant phenotype, giving it a normal appearance. These genes, designated *o2* modifiers (Paez et al 1969), were subsequently used by plant breeders at the International Maize and Wheat Improvement Center (CIMMYT) (Villegas et al 1992) and Pietermaritzburg, South Africa (Geevers and Lake 1992) to develop *o2* varieties with normal kernel hardness and protein content, as well as an enhanced percentage of lysine. These modified *o2* mutants are called Quality Protein Maize (QPM) (Vasal et al 1980).

The mechanism by which *o2* modifiers convert the starchy endosperm of *o2* mutants to a hard, vitreous phenotype is not understood. The major biochemical difference between QPM and standard *o2* mutants is a two- to threefold increase in γ-zein protein and mRNA (Wallace et al 1990, Geetha et al 1991, Or et al 1993). It has been demonstrated that the degree of modification (vitreousness) and the increased accumulation of γ-zein are highly correlated and dependent on the dosage of modifier genes (Lopes and Larkins 1991). Thus, it appears the γ-zein protein is involved in some way with the formation of vitreous endosperm, which is generally related to hardness (Watson 1987).

Although there have been many reports describing the effect of *o2* modifiers in *o2* mutant backgrounds, we are not aware of any descriptions of the effect of these genes in normal endosperm genotypes. Consequently, we do not know the extent to which increased concentrations of γ-zein can increase hardness, density, and vitreousness in wild type backgrounds, nor do we know the extent to which *o2* modifiers affect the nonzein fraction, the source of lysine-containing proteins (Habben et al 1993).

To address these questions, we developed 13 inbreds homozygous for the wild type *O2* gene and containing *o2* modifiers. We have designated these genotypes as "modified normals" or QPM+. In this article, we describe the physical (hardness and density) and biochemical characteristics of kernels from these inbreds. We also describe an enzyme-linked immunosorbent assay (ELISA) procedure to estimate the content of nonzein proteins using a complex antiserum. This technique could provide an alternative, sensitive method to estimate the content of lysine-containing protein in maize endosperm.

MATERIALS AND METHODS

Development of Modified Normal Inbreds

The W64A line was used as the source of the normal *Opaque2* allele and Pool 34 QPM was used as the source of modifier genes (recurrent parent). Although Pool 34 is an open-pollinated population, its kernels have a uniformly modified endosperm, indicating that the modifier genes are fixed. W64A × Pool 34 F1 plants were selfed, and F2 seeds were visually selected for normal, vitreous phenotype (dominant allele in the *opaque2* locus). F2 plants were backcrossed (BC) to the Pool 34 QPM parent, and the resultant seeds were again visually selected for normal phenotype and used in the next cycle of backcross. The BC5 seeds had a uniformly normal phenotype.

Selected BC5 plants went through three generations of self-pollination and visual selection for normal phenotype to eliminate the *opaque2* allele. BC5-S3 plants were tested for the presence of a mutant *opaque2* allele by two criteria. Because *O2* regulates expression of a gene encoding a ribosome-inactivating protein (RIP) (Lohmer et al 1991, Bass et al 1992), the presence of RIP verifies the expression of the wild type *O2* allele. Southern blot analysis with an *O2* cDNA clone was used to confirm the presence of the wild type allele. The 13 BC5-S3 families homozygous for the *O2* allele were selfed, and their seeds (BC5-S4) were used for biochemical characterization.

Sample Preparation

For the Stenvert hardness test, micro Kjeldahl determinations (total protein, total zeins, nonzeins), and amino acid analysis, seed samples were conditioned for at least two weeks at 67% rh, 27°C, to a moisture content of 12.5%–13%, and stored in sealed containers until used. For ELISA measurements (α-zein, γ-zein, and nonzeins) and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), seeds were allowed to dry at room temperature. Whole-kernel samples were used for the Stenvert hardness test. For the other analyses, kernels were degermed using a variable speed dental-type drill (Dremel, Racine, WI) and pulverized to flour using a ball mill.

Kernel Hardness and Density

The Stenvert hardness test was performed using a modification of the method of Pomeranz et al (1985). Due to small sample

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size, 15 g of seed was used instead of the normal 20 g. Preliminary tests showed comparable reproducibility to the standard method ($r = 0.895$, $P < 0.01$). Samples were ground in a microhammer mill (Glen Creston type 4, Glen Mills, Maywood, NJ), using a 2.0-mm aperture screen and a hammer speed of 3,600 rpm. Grain hardness was measured as the time in seconds to grind a volume of 17 ml.

Kernel densities were measured using an air pycnometer (fabricated by the Department of Agricultural Engineering, Purdue University) corrected to actual volumes using a standard curve.

Total Protein, Zein, and Nonzein Determination

Micro Kjeldahl determinations were made of protein fractions (total protein, zein, and nonzein) prepared by an adaptation of the method of Wallace et al (1990). Flour samples (300 mg) were extracted for 1 hr on a shaker at room temperature with 0.0125*M* sodium borate (pH 10), 1% SDS, 2% 2-ME at a ratio of 1:10 (flour-solvent). The suspension was centrifuged at 5,000 × *g* for 20 min and the supernatant was saved. This was repeated twice and the supernatants were pooled. For the zein-nonzein determination, ethanol was added to the total protein extract to a final concentration of 70%. The mixture was allowed to stand for 2 hr with occasional stirring, and then centrifuged. The supernatant fraction contained the zein fraction and the pellet contained the nonzein fraction. Total flour protein, and zein, nonzein, and residual protein (unextracted N) were analyzed for N by the micro Kjeldahl method (method 46-13, AACC 1983). N values were multiplied by 5.70 to estimate the protein content (Mossé 1990). Previous testing showed that the presence of SDS did not interfere with N determination.

SDS-PAGE with a gradient of 7.5–18% acrylamide was performed for both zein and nonzein fractions according to the procedure of Fling and Gregerson (1986).

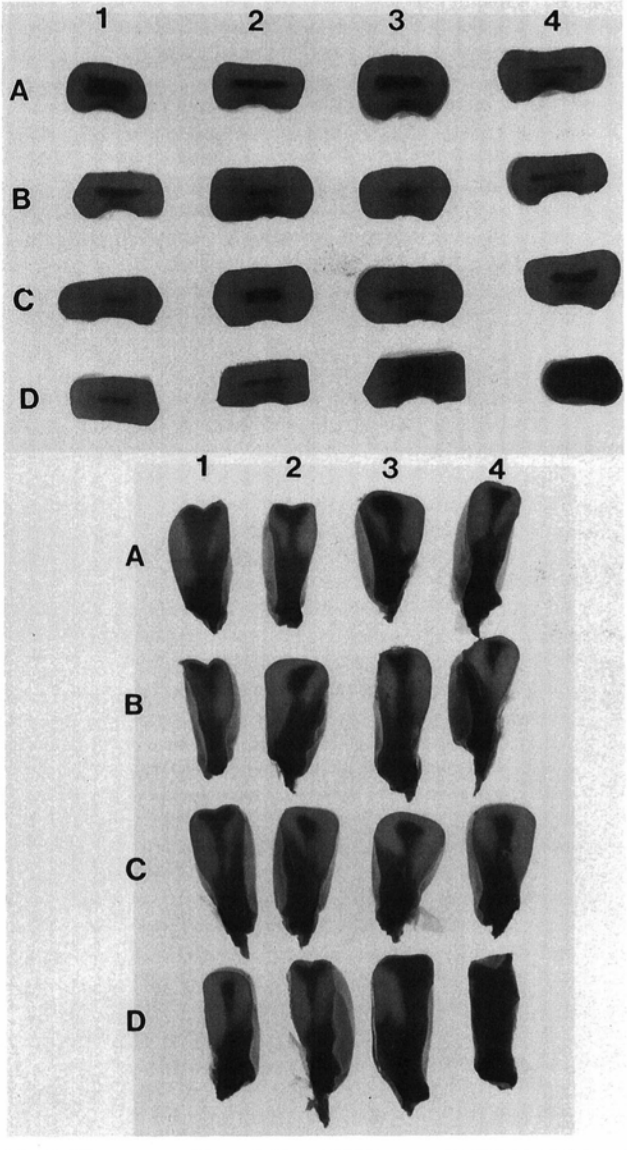

Fig. 1. Phenotypes of kernels from W64A normal (A1), QPM+ (modified normal) 1–13 (A2–A4, B1–B4, C1–C4, and D1–D2), QPM (modified *opaque2)* Pool 34 (D3), and W64A *opaque2* maize genotypes. Kernels are shown in cross-section (top) and longitudinal section (bottom).

ELISA Measurements

Protein extraction and zein-nonzein fractionation were performed as described by Wallace et al (1990). The alcohol-soluble protein fraction was used to quantify γ- and α-zein. For ELISA of γ-zein, the alcohol fraction (equivalent to an extract from 15 mg of flour) was diluted 10,000-fold in carbonate-coating buffer (CCB) (Clark et al 1986); 100 μL of this dilution (equivalent to an extract from 150 ng of flour) was mixed with 100 μL of CCB in a well of an ELISA plate (Immulon 2, Dynatech). Using a multichannel pipette, the samples were homogenized and four twofold dilutions were made into adjacent wells of a different plate (to ensure uniformity in loading of different samples) containing CCB. The antigen was allowed to bind to the plate overnight at 4°C. Subsequently, the antigen was removed by aspiration, the wells were washed twice using TTBS (25 mM Tris-HCl, pH 7.5; 0.9% NaCl, 0.05% Tween 20) and 100 μL of the rabbit anti-γ-zein serum diluted 1:1,000 in TTBS was added and allowed to react for 4 hr. The primary antibody was removed, the wells were washed twice with TTBS and the secondary antibody, goat antirabbit IgG alkaline conjugate (Sigma) diluted 1:4,000 in TTBS, was added and allowed to bind for 2 hr. After the secondary antibody was removed, the wells were washed twice with TTBS and 200 μL of the substrate for alkaline phosphatase (Sigma) diluted in diethanolamine substrate buffer (Clark et al 1986) was added. The color was allowed to develop for 1 hr and the absorbance was read at 410 nm on a Dynatech MR700 ELISA plate-reader.

The ELISA for α-zein was performed the same way as for γ-zein with some modifications. The dilution and antigen binding steps were done using a 40% ethanol, 10% acetic acid solution (Wallace et al 1990) and the primary antibody (rabbit anti-α-zein) dilution was 1:2,000.

For ELISA of total nonzeins, a freshly isolated nonzein pellet was resuspendend in 1 ml of 0.1*N* NaOH, 1% SDS with vortexing and boiling for 5 min. This nonzein protein solution was diluted 1,000-fold in CCB; 100 μL of this dilution (equivalent to an extract from 1.5 μg of flour) was mixed with 100 μL of CCB in the well of an ELISA plate (Immulon2, Dynatech). After all samples were loaded, a multichannel pipette was used to make seven twofold dilutions into the adjacent wells containing CCB. From this point, the procedure is similar to that described for γ-zein, except the incubation time for the alkaline phosphatase substrate was 30 min. The primary antibodies used in this assay were from a rabbit complex antiserum produced against the nonzein endosperm proteins. The antiserum recognized most of the major nonzein proteins (Habben et al 1993).

The range of protein concentrations chosen for all ELISA analyses was such that the relationship of absorbance versus relative antigen content was approximately linear; a regression analysis was performed. As the slope of this regression is proportional to the antigen content, it was used to measure the relative protein content. The results were normalized to the values of the W64A inbred. Results reported are the average of two independent experiments in which three measurements were taken.

Amino Acid Analysis

Amino acid analysis was performed at the University of Missouri Experiment Station Chemical Laboratories. The complete amino acid profile was obtained by sample hydrolysis, performic acid oxidation followed by acid hydrolysis for cysteine,

and alkaline hydrolysis for tryptophan, according to the standard method (AOAC 1990). Separation and analysis of amino acids was done on an amino acid analyzer (Beckman 6300, Elk Grove, CA) equipped with a high-performance cation-exchange resin column with amino acid detection accomplished with postcolumn ninhydrin derivation. Norleucine was used as the internal standard.

Statistical Analysis

All statistical analyses were performed using an analysis of variance (ANOVA) computer software package. For ELISA, the regression equations used for estimation of relative protein content were always significant (F test, $P < 0.01$) and had an r^2 greater than 0.90. The correlations were compared using a standard T test (significance levels are shown).

RESULTS

The 13 inbreds homozygous for the wild type *O2* gene and containing *o2* modifier genes were developed by crossing the W64A inbred with Pool 34 QPM using Pool 34 QPM as the recurrent parent. The identity of the *O2* locus in the modified normal inbreds (QPM+) was confirmed by SDS-PAGE of the α-zein and RIP proteins, and by restriction fragment length polymorphism (RFLP) analysis using an *O2* cDNA probe. The phenotype of the parental and progeny kernels is illustrated in Figure 1. The effect of *o2* modifier genes on a homozygous *o2* background is observed by comparing the endosperms of W64A*o2* and Pool 34 QPM. The completely soft, starchy endosperm of W64A*o2* contrasts with the vitreous phenotype of the modified *o2* mutant, which resembles the wild type W64A. Seeds with the wild type *O2* locus and *o2* modifiers have normal phenotypes, except they are generally more translucent and have a smaller channel of starchy endosperm in the central region of the endosperm.

Coincident with a vitreous phenotype, modified *o2* mutants typically show a two- to threefold increase in γ-zein protein relative to their unmodified *o2* counterparts (Geetha et al 1991, Or et al 1993). Figure 2A shows SDS-PAGE separation of the zein fraction from Pool 34 QPM and W64A, as well as the 13 QPM+ inbreds. The concentration of γ-zein was estimated using an ELISA with polyclonal antibodies against the γ-zein protein (Fig. 3). Relative to W64A, which has a high γ-zein content (Or et al 1993), Pool 34 QPM contains 1.80 times more γ-zein. Eight out of the 13 QPM+ inbreds contain 1.5 times more γ-zein than W64A; all of them have significantly more γ-zein than W64A.

The presence of a wild type *O2* gene in QPM+ inbreds allows expression of a number of genes encoding α-zeins, especially the 22 kDa α-zeins (Kodrzycki et al 1989, Schmidt et al 1992) (Fig. 2A). Most of the 13 QPM+ inbreds resemble W64A in having high concentrations of 22 and 19 kDa α-zeins (Fig. 3). With the exception of QPM+3, which has nearly the same α-zein content as W64A, most of the QPM+ lines contain ~12–25% less α-

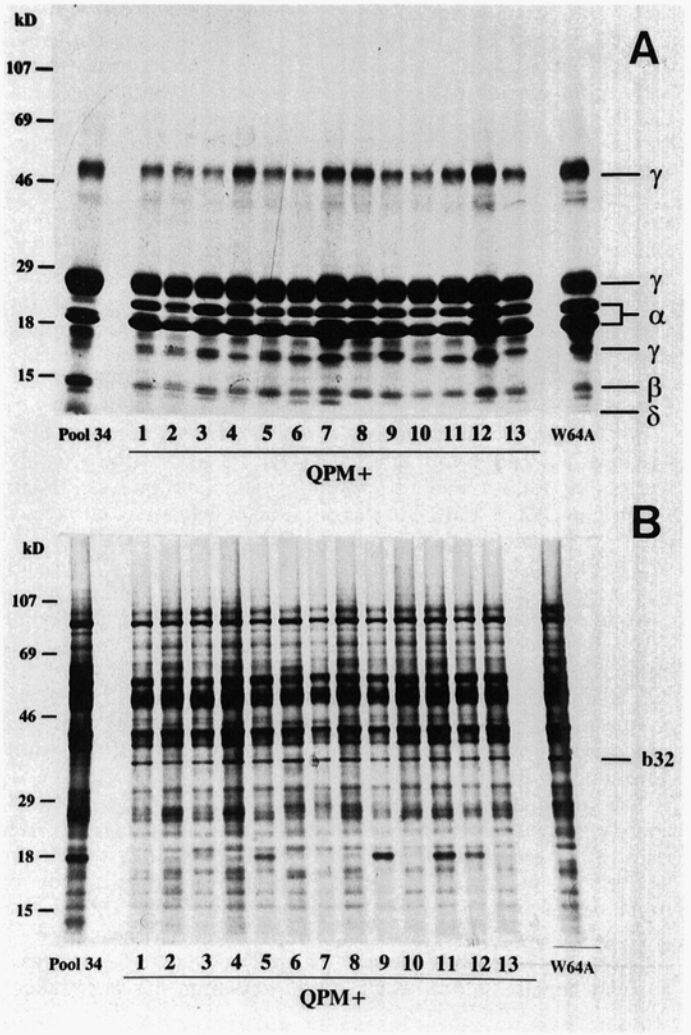

Fig. 2. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of total zeins (A) and nonzeins (B) extracted from endosperm of W64A, Pool 34, and modified normal QPM+ families (1–13). The protein loaded onto each lane was from 1.5 mg of endosperm flour. Molecular weight standards (kDa) are indicated on the left side of each panel.

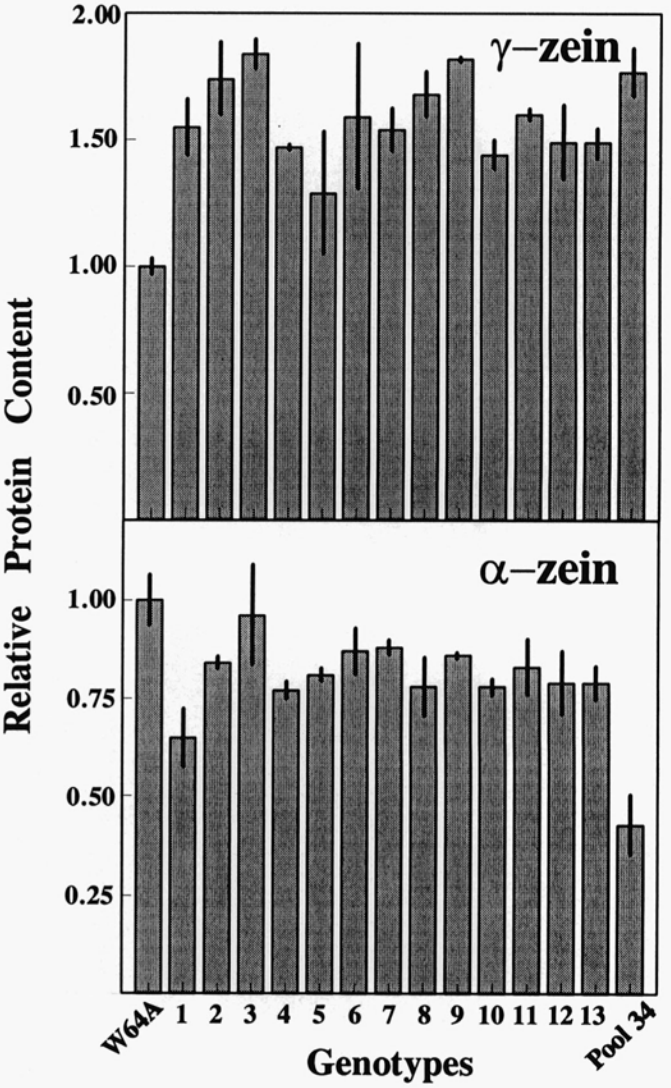

Fig. 3. Content of γ- and α-zeins in W64A, Pool 34, and modified normal QPM+ families (1–13), measured by enzyme-linked immunosorbent assay. The results indicated by the bars were normalized to the inbred W64A. Lines on the top of the bars represent the standard deviations.

zein protein than W64A. But while the total quantity of α-zeins in the QPM+ lines is somewhat lower than W64A, it is nearly double the Pool 34 QPM. A doubling of the α-zein content is consistent with restoration of the *o2* mutation to wild type (Kodrzycki et al 1989).

SDS-PAGE separation of the nonzein fraction from the 13 QPM+ lines revealed a few qualitative differences in protein composition (Fig. 2B). In addition to α-zein gene transcription, *O2* regulates expression of a gene encoding an RIP protein (b-32) (Lohmer et al 1991, Bass et al 1992). This protein is not detected in the nonzein fraction of Pool 34 QPM, but it is found in W64A and all 13 QPM+ lines (Fig. 2B). Another qualitative difference between the parental genotypes is an 18 kDa polypeptide, which is found only in the Pool 34 QPM parent. This protein, whose function is unknown, is also present in some of the QPM+ progeny (inbreds 5, 9, 11, and 12).

To estimate quantitative variation in nonzein proteins in the parents and progeny, we developed an ELISA assay using a polyclonal antiserum against the nonzein fraction (Habben et al 1993). As this antiserum reacts with the major nonzein proteins in W64A, and most of these are common with Pool 34 QPM, we thought this procedure might provide a rapid and sensitive estimate of the nonzein fraction. Based on this assay, using W64A as standard for comparison, Pool 34 QPM contains ~75% more nonzein protein per unit of flour (Fig. 4). As illustrated for representative inbreds, there is some variation in the nonzein content among the QPM+ progeny, but these inbreds tend to resemble W64A.

As an additional basis for comparing the protein content of the parents and progeny, micro Kjeldahl assays measured total protein and zein and nonzein fractions. Data presented in Table I show W64A contains more protein per unit of flour than does Pool 34 QPM. This is mainly the result of a lower α-zein content in Pool 34 QPM, resulting from the *o2* mutation (Fig. 3). Most of the QPM+ inbreds have a total protein content intermediate between W64A and Pool 34 QPM. The only exception is QPM+3, which has a protein content similar to that of W64A. The micro Kjeldahl analysis revealed that the nonzein fraction of most QPM+ inbreds is lower than either parent, although inbreds 2 and 6 are exceptions.

The QPM+ kernels were not necessarily denser or harder than W64A kernels. Kernel densities for W64A (1.418 g/cm^2), Pool 34 QPM (1.429 g/cm^2) and the 13 QPM+ inbreds (from 1.245 g/cm^2 to 1.404 g/cm^2) were not statistically different (data not shown). In contrast, kernel hardness, as measured with a Stenvert test, showed a broad range of variation (Table I). Perhaps surprisingly, kernels of Pool 34 QPM are harder than W64A. Among the QPM+ inbreds, three statistically distinct groups differing in hardness were identified. The hardest group, with values comparable to that of Pool 34 QPM, includes inbreds 3, 5, 6, 7, 9, 11, and 12. A second group includes inbreds 8 and 13, which have hardness values comparable to that of W64A. The third group, inbreds 1, 4, and 10, are somewhat softer than W64A. Seven of the 12 QPM+ inbreds analyzed by the Stenvert test were significantly harder than W64A; however, all inbreds tested would be considered to have a hard phenotype. Stenvert values well above 20 represent very hard endosperm kernel types. For comparison, a recent study of 42 yellow food maize hybrids grown in West Lafayette, IN, some of which were developed for hard kernel characteristics, showed a range of Stenvert values from 11.0 to 19.1 (Darrah et al 1993).

The variation in zein content, especially α- and γ-zeins, and the difference in nonzein content affect the amino acid composition of the parents and QPM+ progeny. Because of its high nonzein content, Pool 34 QPM has the highest percentage of lysine (Table II). With the exception of the inbreds QPM+ 2 and 6, the lysine content of all the other QPM+ inbreds is lower than that of W64A. The concentrations of most other amino acids in the QPM+ progeny are intermediate to the parental types. The reduction in glutamic acid, proline, and alanine, when compared to W64A, reflects the reduction in α-zein protein in QPM+ inbreds. The tryptophan content shows little variation between the parents and progeny.

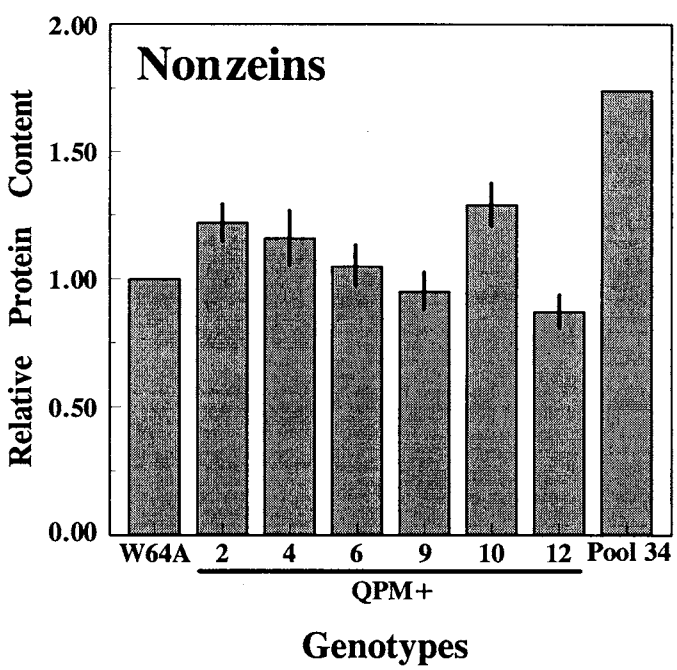

Fig. 4. Content of nonzeins in W64A, Pool 34, and six families of modified normal QPM+, measured by enzyme-linked immunosorbent assay. The results indicated by the bars were normalized to the inbred W64A. Lines on the top of the bars represent the standard deviations.

DISCUSSION

There is variability in total protein, the content of specific storage proteins, and hardness among the progeny inbreds we developed of the W64A × Pool 34 QPM cross. This heterogeneity reflects the fact that the recurrent parent, Pool 34 QPM, represents an open-pollinated population and consequently is genetically diverse. Nevertheless, the traits we measured were uniform for each QPM+ inbred line.

The identification and evaluation of *o2* modifiers is traditionally done by crossing a specific genotype to an *o2* mutant, selfing,

TABLE I
Stenvert Hardness and Total Protein, Total Zein and Non Zein Contents of QPM+ Families

Genotypes	Hardness[a] (sec)	Total Protein[b] (% of flour)	Zeins[b] (% of flour)	Non Zeins[b] (% of flour)	Res.[c]
W64A +	24.3 B[c]	12.9 A	7.9 B	1.8 B	0.67
QPM + 1	19.5 C	9.2 D	5.7 D	1.6 C	0.48
QPM + 2	*[d]	10.0 C	7.1 B	1.9 B	0.43
QPM + 3	28.4 A	12.9 A	8.7 A	1.7 B	0.46
QPM + 4	20.2 C	9.7 D	6.1 D	1.7 B	0.39
QPM + 5	25.9 A	9.4 D	5.8 D	1.5 C	0.46
QPM + 6	28.9 A	10.9 B	7.7 B	2.1 A	0.46
QPM + 7	26.3 A	10.7 B	7.3 B	1.6 C	0.48
QPM + 8	23.5 B	10.2 C	6.7 C	1.6 C	0.57
QPM + 9	27.0 A	9.9 C	6.7 C	1.4 C	0.30
QPM + 10	22.3 C	9.6 D	5.7 D	1.3 C	0.98
QPM + 11	26.1 A	10.0 C	6.1 D	1.6 C	0.43
QPM + 12	25.5 A	9.6 D	6.1 D	1.4 C	0.55
QPM + 13	23.8 B	10.1 C	6.3 D	1.5 C	0.70
Pool 34	27.1 A	8.5 E	4.2 E	2.1 A	0.55

[a] Stenvert time (sec). Average of two replicates. Higher values represent harder kernels.
[b] Estimated by micro-Kjeldahl. Average of two replications.
[c] Means in the same column not followed by the same capital letter (A–E) are significantly different by the test of Scott-Knott ($P < 0.05$).
[d] Value not determined.
[e] Residue nitrogen.

and assessing for the degree of vitreousness among the F2 progeny. The biochemical parameters we describe here allow one to monitor accumulation of modifier genes in wild type genotypes without the need for systematic crossing with an *o2* mutant. The approach is based on the linkage between modifier gene activity and enhanced synthesis of γ-zein. In addition, by assaying the 32 kDa RIP and 22 kDa α-zein proteins, we can assess *O2* gene expression. Specific *O2* alleles can be determined by RFLP analysis. Geevers and Lake (1992) used a somewhat similar approach to evaluate modified *o2* lines.

In *o2* mutants, the high concentration of γ-zein endowed by *o2* modifier genes is directly related to an increase in vitreousness, density, and hardness (Lopes and Larkins 1991, Paulis et al 1993). However, in normal *(O2)* genotypes, this effect of the protein is not obvious. High concentrations of γ-zein in the QPM+ inbreds did not increase kernel density, at least when compared to W64A. Furthermore, hardness was only weakly correlated to γ-zein content ($r = 0.40$, $P = 0.098$), suggesting other factors affecting hardness segregate in these progeny lines. However, it is also possible there is a threshold of density and hardness in normal endosperm above which it is no longer possible to detect an effect of γ-zein content.

Hardness of the QPM+ inbreds is highly correlated with α-zein content ($r = 0.86$, $P < 0.0001$). However, this too is not a simple relationship. All the QPM+ inbreds contain less α-zein than W64A, and yet one third are harder. Furthermore, Pool 34 QPM has one half the α-zein content of W64A and the QPM+ inbreds, but it has the highest Stenvert value. The relationship between α-zein content and hardness, like that between γ-zein and hardness, is not simply quantitative. It has been reported that hardness is a function of the ratio of vitreous and starchy endosperm, and the starchy endosperm contains much less α-zein (Dombrink-Kurtzman and Bietz 1993, Paulis et al 1993). However, it is also possible α-zein content relates to hardness as a function of size and number of protein bodies, which would also be expected to reflect the spatial pattern of α-zein distribution.

Hardness among the QPM+ genotypes is also correlated with cysteine content ($r = 0.67$, $P = 0.0082$). Since γ-zein contains 7% cysteine (Prat et al 1985) and is a major protein component of the endosperm, it is somewhat surprising there is such a weak relationship between hardness and γ-zein content ($r = 0.40$, $P = 0.098$). The correlation between γ-zein and cysteine content is $r = 0.57$ ($P = 0.0199$), suggesting there are other major sources of cysteine-containing proteins. Nevertheless, the correlation between cysteine and hardness lends support to the hypothesis that cross-linkage between cysteine-containing proteins plays a role in kernel hardness (Lopes and Larkins 1991).

It appears that α-zein and γ-zein synthesis are to some degree regulated independently. This is supported by the magnitude of the correlation between α- and γ-zein ($r = 0.49$, $P = 0.0432$). Although there is variation in α-zein content among the QPM+ inbreds, three of them, QPM+ 2, 3, and 9 have high levels of both α-zein and γ-zein. We do not have a non-*o2* version of Pool 34 QPM with which to compare the α-zein content of the QPM+ inbreds; however, if we assume it would be approximately double that of Pool 34 QPM, and nearly the same as that of W64A, then three of the inbreds have near maximal amounts of both α- and γ-zein.

Most of the QPM+ inbreds contain reduced amounts of nonzein protein compared to Pool 34 QPM. Since these proteins are the primary source of lysine ($r = 0.82$, $P < 0.0001$), the smaller content of nonzeins results in a depression of the lysine percentage. Nevertheless, it does appear there is genetic variability in nonzein content, and this segregates independently of both α- and γ-zein. This demonstrates selection for a vitreous phenotype, or γ-zein content alone, will not ensure a high lysine content in QPM materials.

Our results suggest that an ELISA for nonzeins using antibodies against these proteins (Habben et al 1993) may be suitable for genetic selection to increase lysine content. Lysine estimates based on the ELISA show high correlation with measurements obtained by amino acid analysis ($r = 0.75$, $P = 0.0148$). But while the complex antiserum recognized most of the abundant nonzeins (Habben et al 1993), the correlation between the ELISA estimates and the micro Kjeldahl measurements of nonzeins is weak ($r = 0.50$, $P = 0.1045$). Perhaps the conditions used for this assay did not allow equivalent binding of all nonzein proteins to the ELISA plate. This would not be surprising, since the nonzeins are a highly complex group of proteins. Considering the correlation with absolute lysine content, it is nevertheless clear that a considerable portion of the protein that contributes to the lysine content is recognized. We are expanding the genetic base of this analysis to evaluate the efficiency of this method.

In summary, the expression of *o2* modifier genes in normal backgrounds leads to enhanced synthesis of γ-zein proteins. To an extent, this increases the proportion of vitreous endosperm and affects hardness. However, the modifier genes, per se, do not increase the nutritional quality of wild type endosperm.

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TABLE II
Amino Acid Profile (w/w % flour basis) of QPM+ Families

Amino Acid	W64A+	QPM+ 1	2	3	4	5	6	7	8	9	10	11	12	13	Pool 34
Asp	0.82	0.58	0.66	0.70	0.61	0.58	0.69	0.64	0.63	0.60	0.56	0.53	0.57	0.57	0.59
Thr	0.45	0.33	0.37	0.43	0.34	0.34	0.39	0.39	0.37	0.36	0.32	0.30	0.34	0.34	0.33
Ser	0.60	0.41	0.47	0.57	0.44	0.45	0.54	0.51	0.48	0.46	0.42	0.40	0.45	0.44	0.36
Glu	2.96	2.07	2.26	2.81	2.15	2.12	2.55	2.46	2.29	2.28	1.99	1.91	2.13	2.13	1.53
Pro	1.30	1.05	1.16	1.49	1.10	1.12	1.31	1.27	1.20	1.20	1.05	1.00	1.12	1.13	0.98
Gly	0.35	0.29	0.34	0.37	0.30	0.30	0.38	0.33	0.33	0.33	0.29	0.30	0.30	0.30	0.37
Ala	1.16	0.79	0.88	1.07	0.82	0.82	1.02	0.93	0.87	0.87	0.77	0.77	0.80	0.82	0.54
Cys	0.25	0.23	0.25	0.33	0.24	0.25	0.31	0.28	0.27	0.28	0.23	0.23	0.25	0.24	0.30
Val	0.64	0.49	0.55	0.63	0.51	0.48	0.58	0.53	0.53	0.51	0.47	0.45	0.48	0.50	0.46
Met	0.23	0.17	0.21	0.30	0.17	0.21	0.26	0.20	0.20	0.24	0.18	0.19	0.20	0.17	0.16
Ile	0.53	0.39	0.42	0.49	0.39	0.38	0.48	0.44	0.41	0.41	0.36	0.38	0.37	0.39	0.30
Leu	2.23	1.51	1.64	2.08	1.55	1.59	1.84	1.84	1.65	1.68	1.47	1.47	1.55	1.57	0.97
Tyr	0.52	0.33	0.38	0.47	0.35	0.36	0.43	0.41	0.36	0.40	0.31	0.33	0.36	0.36	0.27
Phe	0.79	0.54	0.59	0.71	0.55	0.55	0.66	0.65	0.58	0.58	0.51	0.51	0.53	0.55	0.39
His	0.35	0.32	0.34	0.41	0.34	0.31	0.37	0.36	0.36	0.33	0.30	0.29	0.32	0.33	0.36
Lys	0.22	0.20	0.23	0.19	0.20	0.18	0.22	0.18	0.21	0.18	0.18	0.18	0.18	0.18	0.27
Arg	0.44	0.35	0.40	0.42	0.37	0.35	0.42	0.39	0.38	0.38	0.33	0.31	0.35	0.35	0.44
Trp	0.06	0.05	0.06	0.06	0.06	0.05	0.06	0.06	0.06	0.06	0.05	0.06	0.05	0.05	0.08

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