

Protein Distribution Pattern in Floury and Vitreous Endosperm of Maize Grain

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ABSTRACT

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Alpha-amino nitrogen compounds of floury and vitreous parts of hand-dissected endosperm from eight maize (*Zea mays* L.) inbred lines, representing a broad range of vitreousness (42–95%), were isolated as nonprotein nitrogen, albumin-globulins, zeins, and true glutelins. The three protein classes averaged, respectively, 13, 48, and 35% of total nitrogen in floury endosperm, and 4, 79, and 15% of that in vitreous endosperm. For six inbreds, floury endosperm was richer in 27 kDa γ -zein than vitreous endosperm; the reverse was found for an Argentine flint inbred (ARGL 256), and only traces of 27 kDa γ -zein occurred in both floury and vitreous endosperm of inbred F113. Results were compared

with protein distribution patterns reported in the literature of whole endosperm of wild-type and mutant genotypes of maize, and with wild relatives of maize, *Tripsacum*, and teosintes. When percentage of salt-soluble nitrogen increased from 2% (*Tripsacum*) to 22% (in double mutant Oh43o2;bt2), zeins decreased from 87 to 22%, and true glutelins increased from 11 to 57%. The pattern of whole endosperm of *Zea perennis* was very similar to that of the vitreous endosperm of line ARGL 256. The mean pattern for whole endosperm of six *o2* inbred lines was identical to that of floury endosperm of eight wild-type lines, consistent with a lack of synthesis of α -zeins due to the mutation in the O2 gene.

Maize kernels have both vitreous (also described as horny, hard, flint, translucent, or glassy) and floury (soft, opaque, or chalky) endosperm in various proportion. These two endosperm types are differentiated by their hardness, which is a comparative measure based on tests involving abrasion, cutting, crushing, or penetration (Rasper 1991).

Few studies have examined protein compositions of the two endosperm types. Hamilton et al (1951) first reported such quantitative data. They dissected 100 grains from each of 40 samples (five hybrids grown under eight culture conditions) and pooled samples to obtain means of hybrids for each culture condition. Soil fertilization and crop rotation increased the proportion of vitreous endosperm, which accounted for 30–60% of total endosperm weight and its protein content of 6.8–10.1%. Floury endosperm contained 6.2–9.1% protein. The concentration of alcohol-soluble nitrogen (corresponding to only free α -zein subunits, using Greek letter nomenclature) in vitreous endosperm dry matter was twice that of floury endosperm. These data agreed with microscopic observations of Duvick (1961) about the size of protein bodies at different locations in the developing endosperm.

Paiva et al (1991) examined vitreous and floury endosperm of six wild, mutant and modified *opaque-2* genotypes, and reported that zein (α -, β -, γ -, and δ subunits) contents, expressed as wt%, were twice as high in vitreous as in floury endosperm. This agreed with data of Hamilton et al (1951). However, zein contents of vitreous and floury endosperm, on the basis of total protein, were identical in wild-type and floury genotypes, suggesting incomplete extraction of zeins from vitreous endosperm.

Using reversed-phase HPLC, Dombrink-Kurtzman and Bietz (1993) examined compositions of alcohol-soluble proteins in vitreous and floury endosperm of nine maize samples. Vitreous endosperm were richer in total zeins, especially α subunits, and poorer in 27 kDa γ -zein, on a weight basis. Based on the model of protein body development proposed by Lending and Larkins (1989), they concluded that floury endosperm contained “immature protein bodies”.

More recently, Robutti et al (1997) ground and sifted grains from 24 maize inbreds, separating them into coarse and fine portions representing vitreous and floury endosperm, respectively. Zein compositional data from chromatography and electrophoresis agreed with data of Dombrink-Kurtzman and Bietz (1993), suggesting that endosperm fractionation can be performed by mechanical means.

The present study examines dry matter, protein content, and protein distribution of vitreous and floury endosperm from eight maize inbreds differing in kernel vitreousness. This information is relevant to protein synthesis in endosperm and affords insight into the contribution of protein composition to texture and into fractionating the endosperm into diverse particles by dry milling.

MATERIALS AND METHODS

Plant Material

Eight maize inbred lines (F113, COEST6, A188, CM105, CO255, LH74, F7, and ARGL256) of different grain types were grown in a glasshouse at Orsay (30 km south of Paris, France) during 2000. Grain samples were harvested at physiological maturity and stored at room temperature.

Sample Preparation

Pericarp, germ, and endosperm were isolated by hand-dissection of duplicate samples of four grains (two for COEST6 and six for F7 and ARGL256) previously soaked in water for 30 min. Fractions were then lyophilized and weighed. Vitreous and floury fractions were isolated from whole endosperm using an adjustable-speed grinding wheel and then weighed. The degree of vitreousness was estimated as the weight ratio of vitreous fraction to whole endosperm expressed as percent.

Single analyses were performed on two independent samples of each genotype rather than duplicate analyses on single samples.

Extraction and Quantitation of Protein and NPN

Duplicate samples (40 mg) of vitreous and floury endosperm flour were first defatted with cold hexane. Sequential protein extraction (E) then involved 1) 0.5M NaCl; 2) water at 4°C (combined saline and water extracts E_{1,2}); 3) 55% 2-propanol + 0.02% dithiothreitol (DTT) (E₃); and 4) 0.5M NaCl buffered at pH 10 + 0.02% DTT (E₄). The remaining residue (E_{5,6}) contained proteins usually extracted by SDS (E₅) plus unextracted proteins (E₆). Furthermore, nonprotein nitrogen (E₀ by extension) was isolated from precipitated proteins by adding trichloroacetic acid (TCA) in E_{1,2} extracts to a final concentration of 10%. No significant differences have been found between E₀ extracted with TCA directly or isolated after protein precipitation (Landry et al 2000).

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RESULTS

Nonprotein nitrogen and protein were quantified by ninhydrin assay of α -NH₂ groups (and ammonia) liberated after sample hydrolysis, using an equimolar mixture of 17 amino acids and ammonium sulfate (Pierce) for calibration and a conversion factor of 1.06 μ g of protein for 10 nmol of amino acids. Nonprotein nitrogen and proteins from E₀, E₁₋₂, and E₄ extracts were hydrolyzed in 3M NaOH for 45 min (Landry and Delhaye 1998). Proteins from flour, E₃ extract (after removing solvent), and residue (E₅₋₆) were hydrolyzed in constant boiling HCl at 115°C for 18 hr.

Under these conditions, nonprotein nitrogen (NPN), albumins plus globulins, zeins, and true glutelins corresponded to α -amino nitrogen contained in E₀, E₁₋₂ – E₀, E₃ + E₄, E₅₋₆, respectively. No duplicate nitrogen content was determined for E₅₋₆ because the second residue was stored for further analyses.

The data in this study was compared with that from literature regarding protein distribution patterns of whole endosperm of diverse maize lines and their wild relatives (Misra et al 1975; Landry and Moureaux 1980; Magoja and Nivio 1982; Paiva et al 1991; Mestres and Matencio 1996; Landry et al 2000).

Electrophoresis

SDS-PAGE was performed according to Laemmli (1970) using precast 4–20% polyacrylamide gradient gels (Novex). Samples applied to the gel contained 20–25 μ g of protein.

Statistical Analysis

One-way analyses of variance were performed on the protein, zein, and albumin plus globulin contents in the vitreous and floury parts of the endosperm, with genotype as the factor.

Sample Characteristics

Table I lists characteristics of eight maize lines with vitreous and floury endosperm subjected to sequential protein extraction and SDS-PAGE of zeins. Grain (k) weight, estimated from sum of weights of lyophilized pericarp (b), germ (g), and endosperm, averages 226 mg. Vitreousness range was 42–95% (average 74%). The mean protein content of vitreous endosperm (18.4%) was nearly twice as high as that of floury endosperm (9.8%). No genotypic effect was found for protein content of floury endosperm, while F113 and COEST6 had significantly higher protein content in their vitreous endosperm than the other six inbred lines. Protein contents of floury and vitreous endosperm appeared to have low correlation ($r = 0.380$) and high correlation ($r = -0.888$) correlated to vitreousness, respectively.

Protein Distribution Pattern of Floury Endosperm

Protein distributions of floury endosperm from eight selected inbreds are listed in Tables II (% of dry matter) and III (% of total protein), ranked according to increasing grain vitreousness (top to bottom). Floury endosperm of inbreds differing the most in vitreousness (COEST6 and ARGL256) showed extreme (maximal or minimal) values for the contents in salt-soluble proteins (A + G), zeins extracted in the presence of alcohol (E₃), total zeins (E₃ + E₄) and total proteins (Table I), whether expressed on the basis of dry matter or nitrogen. This was not the case for true glutelins (E₅₋₆), where maximum and minimum contents were found in inbreds CM105 and CO255, respectively. There was no regular increase or decrease in content of protein fractions with increasing vitreousness,

TABLE I
Features of Maize Grains Subjected to Manual Dissection^{a,b}

Genotype	Type	n	Wk	Wb/Wk	Wg/Wk	We/Wk	Wve/We	Pe	Pve	Pfe
COEST6	Floury	4	213.4	6.0	11.8	82.2	41.5	16.5	25.5a	10.2a
F113	Dent	8	230.0	7.6	11.1	81.4	61.7	18.9	23.8a	11.0a
A188	Dent	8	252.6	9.1	12.3	78.6	68.0	14.5	17.0b	9.3a
CO255	Flint-dent	8	203.0	6.1	9.3	84.6	74.8	14.5	16.1b	9.6a
LH74	Dent	8	236.0	8.5	11.3	80.3	77.6	15.4	17.2b	9.2a
CM105	Dent	8	222.6	7.8	10.3	82.0	83.9	16.7	17.9b	10.7a
F7	Flint	12	186.5	9.1	11.0	80.0	89.7	15.5	16.2b	9.2a
ARGL256	Flint	12	265.3	6.7	13.2	80.2	95.0	13.8	14.0b	9.6a
Mean			226.2	7.6	11.3	81.1	74.0	15.7	18.5	9.9
SD			24.0	1.2	1.1	1.7	16.0	1.5	3.7	0.7
CV%			11	15	10	2	22	10	20	7

^a Grain weight (Wk, mg); pericarp weight (Wb, %); germ weight (Wg, %); endosperm weight (We, %); vitreous endosperm weight (Wve, %). Endosperm protein content (Pe, wt% of dissected part); vitreous endosperm protein content (Pve, %); floury endosperm protein content (Pfe, %).

^b Values followed by the same letter are not significantly different ($P < 0.05$).

TABLE II
Protein Distribution of Floury Endosperm (% of dry matter)

Genotype	NPN ^a	A+G ^b	E ₃	E ₄	Zeins ^c	E ₅₋₆ ^d	Extraction Yield %
COEST6	0.23	0.65cf ^e	6.67	0.19	6.85a	3.48	110
F113	0.80	1.75ab	5.38	0.19	5.56ab	3.97	110
A188	0.40	1.17bc	4.10	0.21	4.31bc	3.80	105
CO255	0.28	0.78c	4.88	0.25	5.13b	3.28	98
LH74	0.37	1.24bc	4.22	0.19	4.41bc	3.47	103
CM105	0.41	1.13bc	5.28	0.30	5.58ab	4.09	105
F7	0.31	1.32a-c	4.47	0.22	4.69ab	3.43	107
ARGL256	0.61	2.21a	3.09	0.31	3.40bc	3.40	100
Mean	0.43	1.28	4.76	0.23	4.99	3.62	105
SD	0.18	0.50	0.99	0.05	0.97	0.28	4
CV%	42	39	21	20	20	8	

^a Nonprotein nitrogen.

^b Albumins + globulins (E₁₋₂ – NPN).

^c E₃ + E₄.

^d Glutelins.

^e Values followed by the same letter are not significantly different ($P < 0.05$).

though zein content tended to decrease with increasing vitreousness ($r^2 = 0.613$). Mean values for the eight samples showed higher amounts of zeins and true glutelins than of salt-soluble proteins. Genetic effect was significant for both salt-soluble proteins and zeins. Coefficients of variation (CV) indicate a variability among genotypes, low for true glutelins, moderate for zeins, and high for salt-soluble proteins and nonprotein nitrogen. The same was true when contents were expressed as percent of total proteins: salt-soluble proteins 5.8–22.9% (average 14.6%); zeins 32.2 and 59.5% (mean 45.9%); and true glutelins 31.1–39.3% (mean 35.2%). Finally, the mean extraction yield, which corresponds to the mean ratio of the sum of nitrogen recovered in fractions to nitrogen assayed in sample, was slightly higher than 100%, suggesting an overestimation of protein fractions due to the probable presence of some ninhydrin-positive carbohydrates.

Protein Distribution Pattern of Vitreous Endosperm

Protein compositions of vitreous endosperm are listed in Table IV (% of dry matter) and Table V (% of total protein). Overall obser-

vations about floury endosperm also apply to vitreous endosperm when data are expressed on a dry matter basis, but some differences exist. Level of E₃ in both floury and vitreous endosperm was highest for COEST6 and lowest for ARGL256. The maximum content of true glutelins was in A188. Zein content decreased with increasing vitreousness ($r^2 = 0.728$), reflecting the close relationship between total protein and vitreousness.

The data expressed as % of total protein (Table V) did not show consistent trends, however, in spite of large variation in protein content (Table I). Salt-soluble nitrogen was no more than ≈5% of total proteins (average 3.8%, showing medium variability; CV 17%). Zeins (E₃ + E₄) predominated, averaging 78.8% with low variability (CV 3%). True glutelins (E_{5,6}) were present at a moderately high level, averaging 15.8%, and showing the same medium variability (CV 16%) as salt-soluble proteins.

Zein Electrophoresis

SDS-PAGE patterns of zeins isolated in E₃ extracts from vitreous and floury endosperm of all the examined lines were depicted

TABLE III
Protein Distribution of Floury Endosperm (% of total nitrogen)^{a,b}

Genotype	NPN	A+G	E ₃	E ₄	Zeins	E _{5,6}
COEST6	2.1	5.8b	59.5	1.7	61.1a	31.1c
F113	6.6	14.5ab	44.5	1.5	46.0bc	32.9bc
A188	4.1	12.1b	42.3	2.2	44.4bc	39.3a
CO255	3.0	8.3b	51.4	2.6	54.1ab	34.7a-c
LH74	3.9	13.0b	44.5	2.0	46.5b	36.6ab
CM105	3.7	10.0b	47.1	2.6	49.8b	36.5ab
F7	3.2	13.5b	45.9	2.2	48.1b	35.2a-c
ARGL256	6.3	22.9a	32.2	3.2	35.3c	35.4a-c
Mean	4.1	12.5	45.9	2.3	48.2	35.2
SD	1.5	4.8	7.3	0.5	7.0	2.3
CV%	37	38	16	23	15	7

^a See abbreviations in Table II.

^b Values followed by the same letter are not significantly different ($P < 0.05$).

TABLE IV
Protein Distribution of Vitreous Endosperm (% of dry matter)^{a,b}

Genotype	NPN	A+G	E ₃	E ₄	Zeins	E _{5,6}	Extraction Yield %
COEST6	0.56	1.17a	18.94	0.42	19.36a	3.16	95
F113	0.48	0.75ab	18.08	0.36	18.44a	2.29	92
A188	0.41	0.77ab	12.97	0.39	13.35b	3.52	107
CO255	0.35	0.64ab	12.93	0.31	13.24b	2.56	104
LH74	0.35	0.52b	12.11	0.37	12.48b	2.60	93
CM105	0.33	0.49b	14.34	0.44	14.77b	3.20	105
F7	0.23	0.60ab	12.21	0.37	12.58b	2.46	98
ARGL256	0.23	0.63ab	11.48	0.38	11.85b	2.49	108
Mean	0.37	0.70	14.13	0.38	14.51	2.79	100
SD	0.11	0.21	2.66	0.04	2.67	0.41	6
CV%	29	31	19	10	18	15	6

^a See abbreviations in Table II.

^b Values followed by the same letter are not significantly different ($P < 0.05$).

TABLE V
Protein Distribution of Vitreous Endosperm (% of total nitrogen)^{a,b}

Genotype	NPN	A+G	E ₃	E ₄	Zeins	E _{5,6}
COEST6	2.3	4.8ns	78.1	1.7	79.8ab	13.0c
F113	2.2	3.4ns	82.4	1.6	84.0a	10.4d
A188	2.3	4.3ns	71.8	2.1	73.9c	19.5a
CO255	2.1	3.8ns	77.1	1.8	78.9bc	15.3bc
LH74	2.2	3.3ns	75.9	2.3	78.2bc	16.3b
CM105	1.8	2.0ns	76.3	2.3	78.6a-c	17.1b
F7	1.4	3.0ns	77.0	2.3	79.3a-c	15.5b
ARGL256	1.5	4.0ns	75.5	2.5	78.0bc	16.4b
Mean	2.0	3.8	76.7	2.1	78.8	15.4
SD	0.3	0.6	2.7	0.3	2.6	2.5
CV%	16	17	4	14	3	16

^a See abbreviations in Table II.

^b Values followed by the same letter are not significantly different ($P < 0.05$); ns, not significant.

in Fig. 1. The 22 kDa α -, 19 kDa α -, 16 kDa γ -, and 14 kDa β -zeins occurred in all samples in variable proportions, according to genotype and endosperm texture. It is noteworthy that protein profiles of zeins isolated in E_4 extracts (data not shown) were characterized by many streaks.

The 27 kDa γ - and 10 kDa δ -zeins were absent in both floury and vitreous endosperm of F113. Furthermore, 27 kDa γ -zeins of COEST6, A188, CM105, CO255, LH74, and F7 were relatively more abundant in floury than in vitreous endosperm, agreeing with observations of Dombrink-Kurtzman and Bietz (1993) performed on two inbreds and seven hybrids. The reverse occurred in ARGL256, however. These unexpected results for F113 and ARGL256 led us to subject E_3 extracts from two independent samples of each line to SDS-PAGE. Patterns were similar (data not shown), indicating that conditions of zein isolation and characterization did not cause these atypical results. Therefore, there is no clear-cut relationship between the occurrence of 27 kDa γ -zein and vitreousness.

DISCUSSION

Data in this study represent a limited number of grains selected at random from all grains harvested for each genotype. To show variability of protein distribution and vitreousness within genotypes, we preferred performing single analyses on two independent samples of each genotype, rather than duplicate analyses on single samples. Vitreousness was determined on the basis of abrasion resistance.

Protein distributions of floury and vitreous endosperm appeared significantly different. Therefore, protein composition of whole endosperm in any genotype reflects that of a proportionate mixture of floury and vitreous endosperm (respectively, poor and rich in proteins). Likewise, it is interesting to compare mean protein distribution patterns of floury and vitreous endosperm with those of whole endosperm displaying various protein contents. Table VI compares our data with that from the literature regarding protein distributions of whole endosperm of diverse maize lines and their wild relatives harvested at maturity. For sake of accuracy, only distributions determined using classical Landry-Moureaux (1970) extraction were considered. Thus, zeins (also used to define alcohol-

soluble *Tripsacum* proteins) were considered as proteins in fractions F_{II} (zein), F_{III} (G_1 -glutelins or zein-like), and F_{IV} (G_2 -glutelins or glutelin-like) (Landry et al 2000). Unextracted proteins were combined with fraction F_V (G_3 -glutelins) under the name "true glutelins". Table VI data constitute a substantial sample of the total body of published results. They do not include protein distributions of Illinois Low and High Protein maizes (Balconi et al 1993) because this study omitted fraction F_{IV} while quantifying zeins. Similarly, results of Gentinetta et al (1975) were not considered because these authors used a modified Landry-Moureaux extraction scheme, leading to an overestimation of salt-soluble proteins (presence of F_{IV} proteins) and a corresponding underestimation of zeins (absence of F_{IV} proteins). Salt-soluble nitrogen in endosperm from some double mutants (e.g., Oh43sh4;o2) can be as high as 43% of total nitrogen in a study reported by Misra et al (1975a). This high content is associated with high NPN (Misra et al 1975b), leading to overestimation of salt-soluble proteins and underestimation of zeins and glutelins. Because of this, protein distributions of these double mutants are not shown in Table VI.

Genotypes in Table VI are listed in order of decreasing zein content. To facilitate comparisons, in addition to listing mean distributions of vitreous and floury endosperm, distributions of some inbreds are also included to indicate maximum and minimum zein contents of vitreous and floury endosperm, respectively. Table VI data show that the salt-soluble nitrogen range was 2–22% of total nitrogen, depending on genotype. As salt-soluble nitrogen increases, zeins decrease from 87 to 21%, and true glutelins increase from 11 to 57%. Endosperm of one maize inbred and of wild maize relatives had very high protein levels. They were also richest in zeins and poorest in glutelins. Lines with a zein content >78% can be considered to have highly or completely vitreous endosperm (flint). Conversely, endosperm with <60% zein would be mainly floury. This would be also true for some mutants (such as *opaque-2*) or double mutants (such as *o2o2*), but not for quality protein maize (QPM). QPM corresponds to *o2* lines genetically modified for hard endosperm (Wessel-Beaver and Lambert 1982). These data thus confirm the absence of a simple relationship between vitreousness and zein content. Endosperm with 61–78% of zeins would contain variable proportions of vitreous region.

Whole endosperm of teosintes (*Z. diploperennis* and *Z. perennis*) had a protein distribution virtually identical to that of vitreous endosperm of F113 and ARGL256, respectively. Similarly, whole endosperm of a hybrid between *Z. perennis* and maize is comparable to that of CR29086 line with high protein content. *Tripsacum* endosperm contains more zeins than those of teosintes and maizes, consistent with the conclusions of Paulis and Wall (1977) that teosintes are more closely related to maize than to *Tripsacum*. Note that popcorns, whose small flint-type grains have a high proportion of vitreous endosperm, could be the most primitive of races of maize (Galinat 1979).

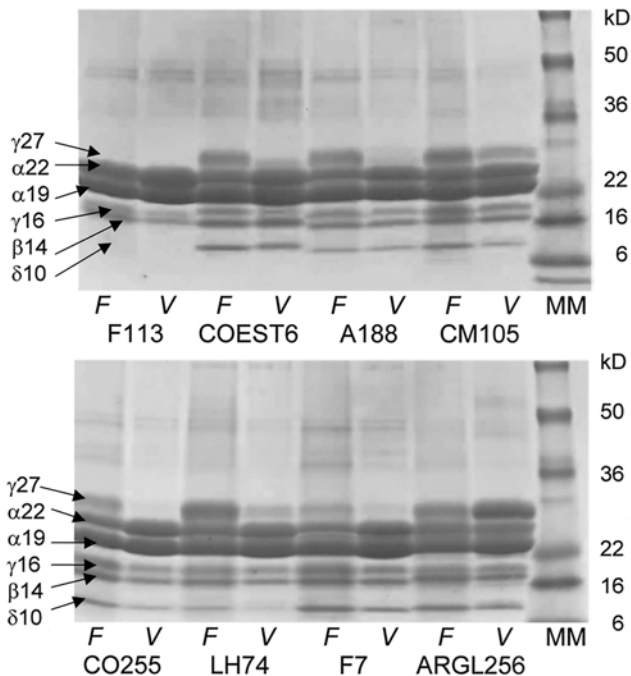


Fig. 1. SDS-PAGE of alcohol extracts (E_3) of floury (F) and vitreous (V) endosperm. MM, molecular masses of standard proteins.

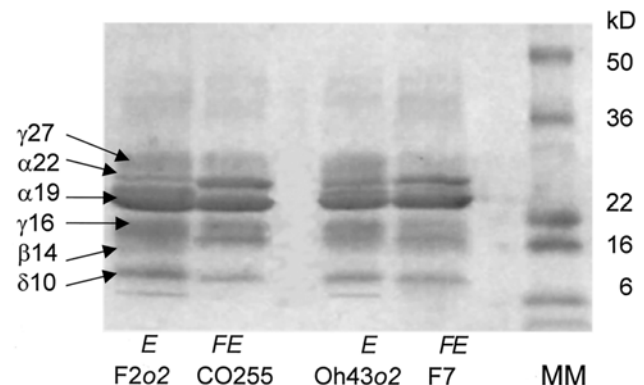


Fig. 2. SDS electrophoretic comparison of zeins (E_3) from whole endosperm (E) of F2o2 and Oh43o2 and from floury endosperm (FE) of CO255 and F7.

Whole endosperm of some wild-type (ecotype E211), mutant (F2o2, Oh43o2), and double-mutant (W22o7;o2) maizes had a protein distribution similar to that of flourey endosperm of some wild-type lines we examined (CO255, F7, LH74). More generally, mean protein distribution in endosperm of six o2 lines paralleled that of flourey endosperm of the eight wild-type lines examined, showing that the general features of whole o2 endosperm are similar to those of flourey endosperm of wild-type maizes. Protein bodies of flourey endosperm, as compared with those of horny endosperm, were fewer, smaller, and developed earlier (Duvick 1961). Furthermore, protein bodies of flourey endosperm contained a higher percentage of γ -zeins, according to the model of protein body development of Lending and Larkins (1989).

This was confirmed by numerous comparisons of wild-type inbreds and their o2 versions. Electrophoretic comparison of alcohol-soluble protein (E₃) from whole F2o2 and Oh43o2 endosperm with those from flourey CO255 and F7 endosperm, (Fig. 2) shows that, for the same amount of protein, o2 endosperm are richer in γ - and δ -zeins and poorer in 22 kDa α -zeins. Zeins from F2o2, however, appeared richer in 19 kDa α -subunits than did Oh43o2 zeins, in keeping with the fact that the nonprotein nitrogen level, due to impaired synthesis of α -zeins, was five times higher in Oh43o2 than in F2o2.

These results are consistent with the main effect of O2 as a transcriptional activator of α -zein synthesis, more precisely 22 kDa

α -subunits in the outlying region of endosperm that promote vitreousness (Schmidt et al 1992). Reduced synthesis of zeins would promote limited increases of albumin + globulins and true glutelins, resulting in a protein distribution similar to that of flourey endosperm in wild-type lines, and similar contents of true glutelins in o2 and wild-type endosperm, consistent with findings of Landry et al (2002).

CONCLUSIONS

The use of a procedure for extracting maize proteins selectively, leading to exhaustive isolation of zeins, coupled with a consistent microassay of α -NH₂ allowed a thorough picture of protein distribution pattern in vitreous and flourey endosperm to be described, and nonprotein nitrogen to be quantitated. We found that vitreous endosperm was richer in zeins and poorer in nonprotein nitrogen than flourey endosperm. The former can be considered as being at a more advanced stage of physiological development. The protein synthesis in flourey endosperm as well as in whole endosperm of some mutants (specifically o2) could be considered arrested at a lower physiological maturity on the basis of their high nonprotein nitrogen content. The present results, together with those taken from literature, enable the influence of phenotype, and mainly genotype, on the protein distribution to be assessed. However, the relationship between vitreousness and the quantity of total zeins or γ -zeins in endosperm is far from strict.

TABLE VI
Protein Distribution Pattern in Endosperm of Various Maize Genotypes and Wild Relatives (% of total protein)

Genotypes ^{a,b}	Proteins (% db)	Salt-Soluble Proteins (%)	Zeins (%)	True Glutelins (%)	Similarity ^c	Reference ^c
1. <i>Tripsacum</i> (<i>Td</i>)	27.4	1.8	87.2	11.0		1
2. Maize (<i>Zm</i>) VE F113	21.9	5.2	84.3	10.5		PS
3. Diploperennial teosinte (<i>Zp</i>)	21.0	3.1	83.8	13.1	VE F113	1
4. Perennial teosinte (<i>Zd</i>)	27.0	1.8	81.1	17.1	VE ARGL 256	1
5. <i>Zm</i> VE 8 lines +	18.5 (3.7) ^d	3.9 (1.9)	80.3 (2.0)	15.8 (2.8)		PS
6. <i>Zp</i> × <i>Zm</i>	21.2	3.8	79.9	16.3	E CR29086	1
7. <i>Zm</i> CR29086	30.7	4.0	79.1	19.5		2
8. <i>Zm</i> 6 inbred lines +	13.3	4.9	75.5	19.7		3
9. <i>Zm</i> VE A188	17.0	6.6	73.9	19.5		PS
10. <i>Zm</i> 10 inbred lines +	9.6	5.7	73.8	20.5		1
11. <i>Zm</i> IRAT 171	11.9	7.4	71.8	20.8		4
12. <i>Zm</i> Oh43sh1	nr	8.2	70.4	21.4		5
13. <i>Zm</i> Inra 260	10.0	8.8	69.5	21.7		6
14. <i>Zm</i> W22	8.5	6.9	68.7	24.4		3
15. <i>Zm</i> 18 cultivars	9.6 (1.6)	8.0 (1.4)	61.8 (4.5)	28.2 (7.1)		4
16. <i>Zm</i> FE COEST6	10.2	8.8	61.1	31.1		PS
17. <i>Zm</i> F2o2	10.3	10.3	54.5	35.2	FE CO255	3
18. <i>Zm</i> E211	6.1	11.6	53.8	34.6	FE CO255	4
19. <i>Zm</i> Oh43sh2	nr	12.3	53.8	33.9	FE CO255	5
20. <i>Zm</i> QPM 3 samples	10.1 (1.1)	12.6 (3.1)	50.9 (0.4)	36.5 (3.6)		7
21. <i>Zm</i> FE 8 lines +	9.9 (0.7)	16.6 (6.2)	48.2 (7.2)	35.2 (2.5)		PS
22. <i>Zm</i> 6 o2 lines	11.1 (1.0)	16.2 (3.5)	47.8 (4.6)	36.1 (2.1)	FE 8 lines+	3
23. <i>Zm</i> Oh43o2	10.4	17.8	47.1	35.2	FE F7	3
24. <i>Zm</i> W22 o7;o2	7.6	17.6	45.1	37.3	FE LH74	5
25. <i>Zm</i> FE ARGL256	9.6	29.3	35.3	35.4		PS
26. <i>Zm</i> Oh43o2;bt2	12.9	22.3	20.6	57.1		5

^a E, whole endosperm; VE, vitreous endosperm; FE, flourey endosperm; nr, not reported.

^b 1) *Tripsacum dactyloides*; 2) maize = *Zea mays*: inbred line, vitreous endosperm has highest zein content; 3) *Z. diploperennis* (teosinte); 4) *Z. perennis* = *Euchlaena perennis* (teosinte); 5) lines under consideration; 6) F1 hybrid *Z. perennis* × *Z. mays*; 7) inbred line; 8) inbred lines W64A, W22, W23, Oh43, F2, and B37; 9) inbred line, vitreous endosperm has lowest zein content; 10) wild-type inbred lines not specified; 11) composite maize from Burkina; 12) *shrunk-1* version of Oh43 inbred; 13) commercial three-way hybrid ([F7 × F2] × W122E); 14) inbred line; 15) set of cultivars collected from seed farms in West African countries and French West Indies with various genetic backgrounds (five are ecotypes, seven composites, one a population, two are varieties, and three are single hybrids); 16) inbred line, flourey endosperm with highest zein content; 17) *opaque2* version of F2 inbred; 18) ecotype from Mali; 19) *shrunk-2* version of Oh43 inbred; 20) three samples of QPM (Quality Protein Maize): Blanco Dentado, Yellow Flint, and Pool 25; 21) see 5; 22) *opaque2* versions of lines indicated in 9; 23) *opaque2* version of Oh43 inbred; 24) *opaque7-opaque2* double mutant of W22 inbred; 25) inbred line, flourey endosperm has lowest zein content; 26) *opaque2-brittle2* double mutant of Oh43 inbred.

^c PS, present study; 1, Magoja and Nivio (1982); 2, unpublished data; 3, Landry et al (2000); 4, Mestres and Matencio (1996); 5, Misra et al (1975); 6, Landry and Moureaux, 1980; 7, Paiva et al (1991).

^d Standard deviation in parentheses.

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