CORN ENDOSPERM: PROTEIN DISTRIBUTION AND AMINO ACID COMPOSITION IN AMYLOMAIZE *vs.* NORMAL DENT HYBRID

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ABSTRACT

Commercial amylomaize hybrids had a significantly higher proportion of saline-soluble protein and glutelins and a lower ethanol-soluble protein content than normal dent hybrid corn. In agreement with this pattern of protein distribution, lysine content of endosperm protein was significantly higher than that of normal dent hybrid corn, varying from 2.3 g/16 g N in class 5 amylomaize (62% amylose) to 2.9 g/16 g N in class 8 amylomaize (81% amylose) as compared with 1.6 g/16 g N in normal dent hybrid (28% amylose). Other essential amino acids were also higher than those of normal dent hybrid corn but the differences were not significant. In contrast to amylomaize hybrids, endosperm proteins of the high-amylose inbred line W64A*ae* (61% amylose) were similar to those of the normal counterpart W64A (32% amylose) with respect to both protein distribution and amino acid composition.

The effect of the amylose extender gene (*ae*) in altering maize endosperm starch by greatly increasing its iodine affinity is well known. Through its effect on enzyme composition, the *ae* gene might also influence the overall protein composition of amylomaize endosperm. Analyses of high-amylose corn selections indicate that the lysine content of this genotype is indeed higher than that of normal maize (1,2). Baudet *et al.* (1) found, in addition, that combination of the *ae* and o_2 genes exerted a cumulative effect on the lysine level of maize proteins which resulted in a double recessive genotype with lysine content higher than that produced by either gene alone. In contrast to these findings, Boundy *et al.* (3) concluded that the *ae* gene had no influence on protein composition or distribution.

Previous studies of high-amylose endosperm proteins were based on whole kernel analyses of inbred lines (1).

We report here the pattern of protein distribution and amino acid composition in isolated endosperm of commercial amylomaize hybrids in comparison with those of normal dent hybrid corn. Analyses of endosperm proteins of amylomaize hybrids are of special interest not only because such hybrids are now commercially available but because of the diversity of their genetic background which may lead to extensive changes in protein composition.

MATERIALS AND METHODS

Samples of corn homozygous for the recessive gene *ae* and its normal counterpart in the inbred line W64A background were obtained from M. S. Zuber (U.S. Department of Agriculture, ARS, Columbia, Mo.).

A series of single cross hybrids from Bear Hybrid Corn Co. (Decatur, Ill.) was used. The series included, as a reference corn, a dent hybrid containing starch

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with an amylose content typical of hybrid corn widely produced in the Corn Belt. This hybrid has a pedigree similar to that of other hybrids grown in the central Corn Belt and has a wide adaptation in that area. For convenience, this control corn is referred to as "normal" dent hybrid or as dent hybrid corn. The three high-amylose hybrids, each with starch of a different amylose level, are designated as classes 5, 7, and 8. Hybrids of classes 5 and 7 are available commercially, but class 8, with the highest amylose content of the series, is not presently in commercial production. The terms high-amylose corn and amylomaize are synonymous.

Chemical Analyses

Kernels were soaked in distilled water for 15 to 30 min and then dissected into pericarp, germ, and endosperm. The three parts were equilibrated in air before weighing to calculate percentage of each part in the kernel.

The endosperm was ground in a Wiley mill (40 mesh) and extracted with hexane. Proteins of the defatted endosperm were partially fractionated by extracting, in order, the saline-soluble and alcohol-soluble proteins. The remaining protein (which we call "residue protein"), mostly glutelins, was not extracted. The detailed procedure follows: 1.1 g of the endosperm was shaken with 25 ml of 0.5 *M* sodium chloride for 5 hr. The mixture was centrifuged (1,100 $\times g$) and the residue was washed once with 0.5 *M* NaCl. The washings were added to the saline extract. The residue was then extracted by shaking with 25 ml of 70% EtOH for 5 hr. This alcoholic extract was combined with an alcohol wash.

Total nitrogen was determined, in duplicate, on the ground, defatted endosperm, on the saline-soluble and alcohol-soluble fractions, and on the final residue by a micro-Kjeldahl procedure (4). Protein content was calculated by multiplying the total nitrogen by 6.25. The standard deviation was $\pm 0.23\%$.

Amino acid analyses were carried out on ground, hexane-extracted endosperm and on endosperm remaining after extraction of saline-soluble and alcohol-soluble proteins as described above. Sample size was adjusted for analysis to contain approximately 1 mg of nitrogen. The samples were refluxed for 24 hr in 6*N* HCl (2 ml of acid per mg of dry sample). Hydrolysates were evaporated to dryness. The residue was dissolved in 10 ml of pH 2.2 citrate buffer for analysis on a Beckman-Spinco amino acid analyzer using the 3-hr procedure of Benson and Patterson (5). Data were computed as described by Cavins and Friedman (6). Amino acid analyses were replicated five times on the inbred varieties and twice on the hybrid corns. All essential amino acids but tryptophan were tabulated. Tryptophan is not stable to acid hydrolysis and therefore was not recovered in the above procedure. For conciseness, the only additional acids tabulated were glycine and proline because they were the only nonessential acids which showed significant differences between amylomaize and normal dent hybrid corn.

Total starch and amylose content of the starch were determined as described previously (7,8).

Analytical data are reported on a moisture-free basis.

RESULTS

Characteristics of normal dent and high-amylose corns are summarized in Table I. The high-amylose level characteristic of amylomaize starch is

TABLE I
Percentage of Component Parts in Kernel; Starch and Amylose Content of Corns

Corn	100-Kernel Weight, g, db	Component Parts of Kernel (db)			Starch[a]	Amylose[b]
		Endosperm %	Germ %	Pericarp %	%	%
W64A	20.30	82.6	10.0	7.4	80	32
W64A*ae*	22.26	78.5	12.5	9.0	78	61
Normal dent	27.10	82.5	10.5	7.0	84	28
Amylomaize class 5	26.82	78.7	12.5	8.8	77	62
Amylomaize class 7	26.77	76.3	14.5	9.2	78	76
Amylomaize class 8	25.18	74.4	14.0	11.6	78	81

[a]Percentage of endosperm (db).
[b]Percentage of starch (db).

TABLE II
Total Protein and Distribution of Protein in Endosperm Fractions

Corn	Total Protein[a] %	Protein in Fraction[b]		
		Saline-Soluble[c] %	Ethanol-Soluble[d] %	Residue[e] %
W64A	15.4	4.2	44.3	49.4
W64A*ae*	18.7	6.8	42.8	51.0
Normal dent	11.1	5.8	39.2	51.6
Amylomaize class 5	12.5	6.9	18.5	69.6
Amylomaize class 7	12.4	7.6	26.0	61.8
Amylomaize class 8	13.7	10.8	27.5	61.3

[a]Percentage of endosperm, db (N × 6.25).
[b]Percentage of total endosperm protein, db.
[c]Least significant difference (LSD) = 1.43.
[d]LSD = 4.76.
[e]LSD = 4.91.

accompanied by significant changes in other kernel characteristics. There is less endosperm and starch in amylomaize than in normal dent corn and, as a consequence, percentage of both germ and pericarp is high in amylomaize. The germ of W64A*ae* is at least 25% larger than that of the normal counterpart W64A, and other high-amylose selections may have 40% more germ tissue than the normal dent hybrid (Table I). Similar kernel changes influenced by the *ae* gene were noted by Zuber *et al.* (9) in maize of other genetic backgrounds. It is reasonable to expect that any enzymatic changes controlled by the single gene *ae* might be accompanied by a change in endosperm proteins. The most obvious variation is an increase in total protein concentration in amylomaize endosperm over that of normal corn endosperm (Table II) amounting to 23% in class 8 amylomaize. This rise in protein concentration is partly a result of the reduced starch content of amylomaize endosperm (Table I). However, despite a reduction in total endosperm of amylomaize kernels (Table I), there is an increase in total endosperm protein per kernel of approximately 2 to 6% in hybrid amylomaize and 26% in the near-isogenic line.

Inbred Line Comparisons

Comparison of the nearly isogenic pair W64A and W64A*ae* shows a small increase in saline-soluble protein in the W64A*ae* endosperm over the normal counterpart but no significant difference in zein or glutelins (Table II). The basic amino acids lysine, histidine, and arginine are slightly higher in W64A*ae* than in W64A (Table III), but the differences are not significant. Likewise, concentrations of other essential amino acids are similar in the two endosperm types. Thus, the *ae* gene in itself, which has a profound effect on starch composition and on kernel structure, appears to have little influence on endosperm protein composition and on the amino acid pattern.

Amylomaize Hybrids

In contrast to the small differences between *ae* and normal endosperm in the inbred line, high-amylose hybrids showed significant differences in both protein distribution and amino acid composition relative to normal dent hybrid. The combination of high saline-soluble protein and glutelins and low alcohol-soluble protein in amylomaize hybrids, compared to the normal dent hybrid (Table II), contributed to their high-lysine content.

The difference in lysine content between amylomaize and normal dent hybrid corn proteins increased as the amylose level increased with the highest lysine concentration occurring in class 8 amylomaize (Table IV). Expressed as total endosperm lysine per kernel, the increase in lysine varied from 53 to 87% in the different classes of amylomaize, exceeding increases which can be calculated from concentration data (Table IV). The other basic amino acids, histidine and arginine, were also higher in amylomaize than in normal dent hybrid, but only the arginine increases were significant. Other essential amino acids, including methionine, tended to be higher in amylomaize than in the normal dent hybrid; but again the differences were not significant. Proline, a nonessential amino acid, was significantly lower in amylomaize than in normal dent hybrid.

DISCUSSION

Similarity in the amino acid patterns of W64A and W64A*ae* (Table III) suggests that the single gene difference, *ae*, has little effect on composition of endosperm proteins. Thus, our results are at variance with those of Baudet *et al.* (1) who found that W64A*ae* had a higher lysine content than the normal counterpart W64A. The relatively high lysine values reported by these workers were obtained by analysis of whole kernel corn in which much of the lysine was contributed by the germ (10); consequently, their data are not directly

TABLE III
Amino Acids of Whole Endosperm and Residue Proteins in W64A and W64A*ae*

Amino Acid[a]	Whole Endosperm Proteins		Residue Proteins	
	W64A (Normal)	W64A*ae*	W64A (Normal)	W64A*ae*
Lysine	1.6	1.8	2.1	2.6
Histidine	2.7	2.8	3.3	3.7
Arginine	3.6	3.8	4.1	4.6
Threonine	3.4	3.4	3.4	3.7
Proline	9.6	9.4	9.7	10.2
Glycine	2.9	3.1	3.5	4.0
Valine	4.9	4.9	5.0	5.4
Methionine	2.4	2.2	3.2	3.0
Isoleucine	3.8	3.8	3.4	3.5
Leucine	15.4	15.0	12.6	12.2
Phenylalanine	5.8	5.6	4.6	4.9

[a]Expressed as g/16 g of nitrogen.

TABLE IV
Amino Acid Content of Whole Endosperm Proteins and of Residue Proteins in Normal Dent Hybrid Corn and Amylomaize

Amino Acid[a]	Whole Endosperm Proteins[b]				Residue Proteins[b]				
	Normal	Amylomaize			Normal	Amylomaize			
	Dent	Class 5	Class 7	Class 8	Dent	Class 5	Class 7	Class 8	LSD
Lysine	1.6	2.3	2.4	2.9	2.2	2.7	3.1	3.6	0.33
Histidine	2.8	3.1	2.9	3.1	4.4	3.9	4.0	3.7	0.37
Arginine	3.5	3.9	3.8	4.6	4.3	4.9	4.9	5.4	0.71
Threonine	3.5	3.8	3.9	3.8	3.7	4.0	4.2	4.0	0.35
Proline	16.0	11.0	7.5	10.4	11.8	11.2	10.5	10.0	3.02
Glycine	3.0	3.7	4.0	3.6	4.2	4.1	4.5	4.5	0.18
Valine	4.8	5.1	5.4	5.5	6.2	5.6	5.9	6.1	0.66
Methionine	1.7	2.0	1.9	2.0	1.9		2.4	2.2	1.19
Isoleucine	3.5	3.9	4.1	3.9	3.1	4.1	3.9	3.8	0.54
Leucine	14.4	14.2	15.0	13.4	15.0	13.1	12.3	12.7	2.10
Phenylalanine	5.4	4.9	4.9	5.0	4.2	5.1	4.9	4.6	0.91

[a]Expressed as g/16 g of nitrogen.
[b]Average of two analyses.

comparable to ours which was obtained by analysis of dissected endosperm.

The high-lysine content of amylomaize hybrids (Table IV), compared with amylomaize inbred corn (Table III), may be due to the greater complexity of the genetic background of the hybrids. Zuber *et al.* (9) emphasize the importance of the gene modifier complex in the buildup of high-amylose starch. The cumulative effect of *ae* and gene modifiers, introduced in the course of the breeding program, may be responsible for the altered protein composition of amylomaize hybrids.

Despite the relative indigestibility of amylomaize starch (11), the performance of rats fed high-amylose corn was significantly superior to that of rats on a normal corn diet (2), presumably due to the higher lysine and tryptophan levels of amylomaize proteins. This experience suggests that by-product feed supplements, produced in the processing of amylomaize for high-amylose starch, may have special value in the feeding of monogastric animals.

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