Comparison of the Protein Compositions of Selected Corns and Their Wild Relatives, Teosinte and *Tripsacum*

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To establish evolutionary and genetic relationships, the proteins of a hybrid field corn, an Illinois high-protein corn, and an Argentine popcorn were compared to those of two wild relatives, teosinte and *Tripsacum*. After sequential extractions of grain meals by a modified Osborne–Mendel scheme, the protein classes obtained were subjected to amino acid analyses and electrophoresis in various systems. *Tripsacum* yielded less zein and more alcohol-soluble reduced glutelin than the other grains. These differences resulted in *Tripsacum* grain having a higher methionine content than the others and deviating from the inverse correlation between lysine content and zein content demonstrated in corn. Most electrophoretic patterns of zeins and alcohol-soluble reduced glutelins of the teosintes were similar to their counterparts in the corns but those of *Tripsacum* showed marked differences. These data support other evidence that teosinte is closely related to corn and may be its direct ancestor whereas *Tripsacum* is less closely related.

Recent crop losses of corn (maize, *Zea mays L.*) due to disease and drought have pointed out the need for further diversification of germ plasm in lines used for production of high-yielding hybrids. Wild ancestors of many cereal grains have served as sources of genetic variation. However, all races of corn are domesticated and dependent on man for propagation. For this reason, considerable interest and importance is currently being attached to the relationship of corn to the self-propagating wild grasses, teosinte [*Zea mexicana* (Schrader) Kuntze] and *Tripsacum* (L. spp.). To investigate the genetic similarities of corn to these wild relatives, we have compared their seed proteins by sensitive biochemical techniques.

In Mexico, extensive interbreeding of corn with teosinte has been attributed by Wilkes (1972) to result in heterotic vigor in corn. Beadle (1939) considers corn to have evolved from teosinte based on similarity of chromosome number and homology and on cross-breeding experiments, despite great differences in appearance of their female spikes and fruits. However, Mangelsdorf (1974) suggests that the progenitor of corn was a wild grass, now extinct or undiscovered. Earlier, Mangelsdorf and Reeves (1939) regarded teosinte as a secondary product of corn–*Tripsacum* hybridization and not on the direct course of evolution of corn. Like corn and teosinte, *Tripsacum* spp. are classified in the subtribe Tripsacinae of the tribe Andropogoneae of the grass family (Clayton, 1973). Diploid as well as tetraploid *Tripsacum* (*T. dactyloides* and *T. floridanum*) can be experimentally crossed with corn despite differences in chromosome number.

Bressani and Mertz (1958) and Rotar et al. (1975) established some similarities among solubility classes of proteins in corn, teosinte, and *Tripsacum*. We have compared in more detail the proteins of a modern hybrid dent corn to those of seeds of two strains of teosinte and one variety of *Tripsacum dactyloides* (gama grass). The proteins were extracted by a modified Osborne–Mendel sequential solubility scheme. Glutelin, after reduction of its disulfide bonds, was further fractionated into alcohol-soluble and alcohol-insoluble fractions (Paulis et al., 1975). Yields, amino acid analyses, electrophoretic behavior in starch gel electrophoresis (SGE) and polyacrylamide gel electrophoresis (PAGE), and molecular weights (mol wt) of subunits by polyacrylamide gel electrophoresis in sodium dodecyl sulfate media were determined on the protein fractions. Because grains of the wild species were smaller in size and contained more protein than the dent hybrid corn, we also compared their proteins to those of a small kernel Argentine popcorn which readily interbreeds with teosinte (Mangelsdorf, 1974) and an Illinois high-protein corn.

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EXPERIMENTAL SECTION

Grain Sample Preparation. Grains of two strains of teosinte, Chalco (Tc) and El Salada (Ts), were obtained from plants growing wild in Mexico. The designations of the teosintes represent areas near their points of collection. The fruit cases were removed from both samples with a Miag Vario roller mill and the kernels separated into various size fractions by a box sifter. Whole kernels in +8 mesh fractions from Ts and in +5 and +6 mesh fractions from Tc were used for these studies.

Spikelets of *Tripsacum dactyloides* (tetraploid) (Trip) were collected near Huey, Ill. (Newell and deWet, 1974). Kernels were released by breaking the fruit cases between two heavy metal weights.

The corn samples consisted of an Argentine popcorn (Pop), an Illinois high protein (H.P.), and a normal (N) PAG commercial hybrid SX52.

Whole kernels (generally 10 g) were ground to 60 mesh with a Udy Cyclone Sample mill. The meal was then partially defatted by intermittent shaking with cold petroleum ether at a 3:1 (v/w) solvent to meal ratio for 1 h. The suspensions were filtered and the solids washed with cold solvent on a Buchner funnel and air-dried overnight.

Fractionation of Protein. Extraction of the protein fractions from the defatted meals followed an earlier procedure (Paulis et al., 1975) summarized in Table I. Globulins were precipitated after dialysis against water of 0.5 M NaCl extracts and separated from albumins by centrifugation. Although the term zein is generally restricted to designate the prolamines of the genus *Zea*, in this paper we also designate as zein protein extracted from *Tripsacum* by 70% ethanol–5% sodium acetate. The zein extracts were dialyzed against only one change of cold 70% ethanol and then against several changes of cold water. The dialysate was centrifuged at room temperature at

Table I. Protein Distribution in Defatted Meals of Corns and Related Grains

Fraction	Proteins	N[a]	Pop[a]	H.P.[a]	Ts[a]	Tc[a]	Trip[a]
		% protein in meal (as is)					
		10.9	13.3	26.2	28.7	17.6	29.3
		% total protein recovered in extracts					
		95.1	90.5	89.5	95.3	96.2	83.6
		% of total recovered protein					
0.5 M NaCl 1 × 5:1 (v/w) +1 × 5:2 (v/w) 1 h 4 °C	Albumins, globulins, and nonprotein nitrogen	19.1	12.4	16.9	4.7	8.8	6.9
70% EtOH-0.5% NaOAc 3 × 10:1 (v/w) 1 h 25 °C	Zeins	41.4	46.4	49.2	61.1	54.1	44.7
70% EtOH-0.5% NaOAc-0.1 M ME 2 × 10:1 (v/w) 0.5 h 25 °C	Alcohol-soluble reduced glutelins	12.6	14.5	12.2	10.0	11.6	22.4
Residue	Alcohol-insoluble reduced glutelins	26.8	26.7	24.0	24.5	25.5	26.0

[a] Abbreviations signify the following grains: N = normal (PAG SX52 Hybrid) corn; Pop = Argentine popcorn; H.P. = Illinois high protein corn; Ts = teosinte El Salada; Tc = teosinte Chalco; Trip = *Tripsacum dactyloides* (tetraploid).

1000*g* for 10 min and the precipitated protein lyophilized to dryness. The alcohol-soluble reduced glutelin (ASG) fractions were dialyzed only against cold water in a Spectropor membrane tubing having mol wt cutoff 6000–8000, and the dialysates were lyophilized to dryness.

Because of the limited amount of Trip seed available, only 5.0 g was extracted. Yields were compared to extract of a 5.0-g sample of H.P.

Analytical Methods. Aliquots of extracts or portions of weighed dried materials were assayed for nitrogen by a semi-micro Kjeldahl method. Crude protein was estimated by multiplying nitrogen content by 6.25 and is given on an "as is" basis.

Samples for amino acid analysis were hydrolyzed by refluxing in 6 N HCl (2 ml per mg of sample) for 24 h. Amino acids of each hydrolysate were quantitatively determined with a Beckman Model 121 automatic analyzer (Benson and Patterson, 1965). The results were integrated on an Infotronics Model CH210 integrator and calculated by computer (Cavins and Friedman, 1968). Methionine was partially oxidized during hydrolysis to methionine sulfoxide and both were calculated from the chromatograms. Tryptophan, cysteine, and cystine were not determined. All amino acids were corrected to 97% recovery of nitrogen for comparison between samples.

Albumins and globulins were analyzed by SGE using the procedure of Paulis and Wall (1969) except that the 16% starch gel contained half the described concentration of aluminum lactate and lactic acid in the 8 M urea-containing buffer. Electrophoresis was at 100 V.

Polyacrylamide gel electrophoresis of zeins and ASG's was carried out for 6 h at 350 V according to Jones and Beckwith (1970) on a 0.4 × 13 × 16.5 cm gel slab prepared from 4.75% acrylamide and 0.25% *N,N*-methylenebisacrylamide. The apparatus had a gel cooling tray with 200-cm^3 buffer wells. Coomassie blue was used to stain the proteins in the gels (Paulis et al., 1975).

All urea-containing buffers were prepared from a 10 M urea solution which was deionized on a 1 × 4 cm column of AG501-X8 (Bio-Rad) to remove cyanates. After addition of all reagents and filtration through an 0.8-μm Millipore filter, the urea was diluted to 8 M with water. For the polyacrylamide gel electrophoresis buffer, the catalyst was added after filtration.

The proteins for polyacrylamide gel electrophoresis and SGE (0.4 to 0.6 mg of nitrogen) were reduced overnight at room temperature in 0.1 ml of 1% (v/v) β-mercaptoethanol (ME) and deionized 8 M urea. The sulfhydryls were alkylated by adding 20 μl of 8.1% (w/v) acrylonitrile–8 M urea, and reacting for 1 h. The pH was then adjusted to 3.1 by addition of 10 μl of 3.2% aluminum lactate–6.2% (w/v) lactic acid–8 M deionized urea. Samples of the above solutions were absorbed on filter paper squares and inserted in gels for electrophoresis of proteins.

Molecular weights of albumins, globulins, and alcohol-insoluble reduced glutelin (AIG) were estimated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis on 5% gels according to a procedure described by Paulis et al. (1975). For zein and ASG, sodium dodecyl sulfate–polyacrylamide gel electrophoresis was carried out on 10% polyacrylamide gel slabs (16.5 × 13 × 0.4 cm). To increase band intensity, gels were stained and destained twice (Bietz et al., 1975).

RESULTS AND DISCUSSION

Meal Protein Composition. Analyses and extractions were conducted on whole meals rather than endosperm because Ts, Tc, and Trip had small germs which were difficult to remove. Protein contents vary considerably among the whole grain samples (Table I). H.P., Trip, and Ts have about twice the protein content of the N or Pop corns.

The amounts of proteins sequentially extracted from the different grains with each solvent in sequence are given in Table I. Yields of salt-soluble proteins from the two teosintes and Trip are much lower than from the three corn varieties. This difference may be due to smaller germs in these grains than in the corns; germ is rich in albumins, globulins, and nonprotein nitrogen which constitute this solubility fraction. The two teosintes have higher zein contents than the corns, but the zein content of Trip is similar to that of the corns. A very notable difference between the grains is the very high content of ASG in Trip, almost twice that of the corns. The yield of ASG in the teosinte meals is slightly less than that of the corns. The quantity of residue protein (AIG) is similar among all the grains.

Total protein recovery was poorest from Trip, H.P., and Pop grains (Table I). These three grains are small and contain much pericarp. Since the floating pericarp was filtered off from the 0.5 M NaCl extractions, it might retain some grain protein.

The amino acid analyses of the defatted whole grains are compared in Table II. In general, increased protein content is accompanied by a decrease in lysine and an elevation of glutamic acid and leucine in the grain protein. This tendency is expressed to a lesser degree in H.P. whose relatively larger germ contributes a high albumin and globulin content (Table I). Trip has a higher methionine content than the other grains.

Salt-Soluble Proteins. Only small differences exist among the amino acid compositions of albumins extracted from the different varieties (Table III). Similarly, var-

Table II. Amino Acid Composition of Defatted Grain Meals (g/100 g of Protein)

Amino acid	N	Pop	H.P.	Ts	Tc	Trip
Lys	2.8	2.5	2.3	1.3	1.8	1.4
His	2.9	2.9	2.5	2.2	2.5	2.5
NH_3	2.8	2.9	3.1	3.5	2.9	3.3
Arg	4.7	4.4	4.1	3.0	3.7	3.2
Asp	6.8	6.2	7.1	6.0	6.0	5.6
Thr	3.7	3.6	3.3	3.4	3.4	3.3
Ser	5.1	5.0	5.1	5.3	5.4	5.0
Glu	20.5	20.7	21.2	23.7	22.9	22.9
Pro	8.7	9.8	7.9	9.2	8.8	8.6
Gly	3.7	3.6	2.9	2.2	2.7	2.4
Ala	8.1	8.3	8.8	9.4	9.1	10.1
Val	4.8	5.2	5.1	4.8	4.8	4.5
Met	2.3	2.2	1.8	1.9	2.8	3.6
Ile	3.7	3.7	3.9	4.0	4.1	3.9
Leu	13.1	13.4	14.8	16.7	16.0	15.5
Tyr	4.7	4.9	5.3	4.7	5.0	5.0
Phe	5.5	5.2	6.4	6.0	6.1	5.4

Table III. Amino Acid Composition of Albumin Fractions (g/100 g of Protein)

Amino acid	N	Pop	H.P.	Ts	Tc	Trip
Lys	7.3	7.0	6.5	6.3	6.6	6.9
His	2.8	2.6	2.7	2.6	2.4	2.4
NH_3	2.2	2.1	1.8	2.0	2.2	2.0
Arg	8.2	8.0	7.7	8.5	8.1	7.9
Asp	9.0	9.4	9.6	9.1	9.8	9.7
Thr	4.8	4.8	5.3	4.5	5.0	5.3
Ser	4.8	4.7	5.4	4.5	5.5	6.0
Glu	13.4	13.3	13.4	13.4	13.5	13.0
Pro	4.4	4.5	4.1	5.4	4.4	4.9
Gly	6.5	6.5	7.1	5.9	6.4	6.6
Ala	7.3	7.8	7.6	8.0	8.2	7.9
Val	5.9	6.2	5.7	6.3	5.7	5.7
Met	1.4	1.6	2.2	2.0	1.4	1.8
Ile	3.7	3.9	3.6	3.8	3.6	3.6
Leu	5.8	5.9	5.5	5.9	5.6	5.7
Tyr	2.9	3.2	3.5	3.0	2.9	3.2
Phe	3.4	3.4	3.2	3.1	2.9	3.0

Table IV. Amino Acid Composition of Globulin Fractions (g/100 g of Protein)

Amino acid	N	Pop	H.P.	Ts	Tc	Trip
Lys	6.4	6.7	6.5	6.0	5.9	6.5
His	3.8	3.5	3.1	3.3	3.2	3.4
NH_3	1.3	1.4	1.4	1.6	1.3	1.5
Arg	11.8	11.2	9.7	11.5	10.2	11.2
Asp	7.5	7.8	8.5	7.5	8.0	7.8
Thr	3.5	3.9	4.5	3.7	4.0	3.9
Ser	5.4	5.5	6.1	5.5	5.6	5.0
Glu	14.7	14.1	15.0	15.0	15.4	14.4
Pro	3.2	3.6	5.0	3.6	7.6	4.6
Gly	5.5	5.7	6.2	5.6	5.8	5.7
Ala	5.9	6.2	6.6	6.0	6.4	6.1
Val	5.2	6.1	6.3	5.8	6.2	6.2
Met	2.0	1.4	1.3	1.7	1.1	1.2
Ile	3.5	3.9	3.6	3.5	3.4	3.6
Leu	6.8	7.3	7.3	7.1	7.0	6.8
Tyr	3.4	3.2	3.4	3.2	3.2	3.1
Phe	4.9	5.1	5.4	5.0	5.1	4.7

iations in amino acid content are small among globulins of the different grains (Table IV). Both protein fractions from the teosintes contain slightly less lysine than those from the other grains. The albumins of each variety have slightly more lysine, aspartic acid, threonine, glycine, and alanine than the globulins but less arginine, serine, leucine, and phenylalanine.

Electrophoresis of albumins and globulin fractions of the different varieties separated their constituent proteins

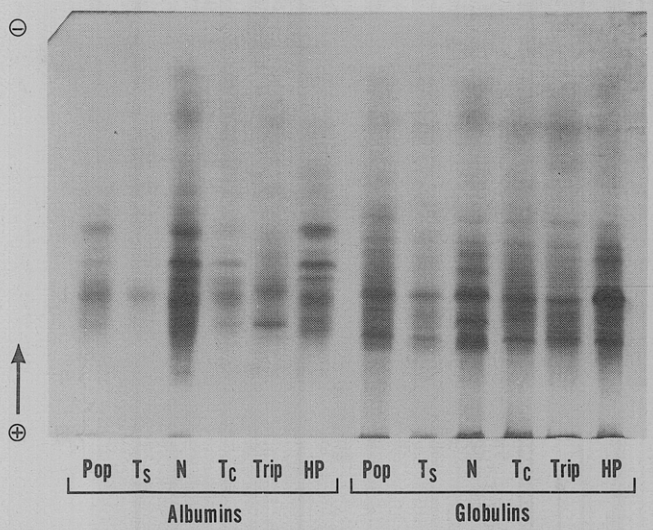

Figure 1. Starch gel electrophoresis of salt-soluble proteins. Origin at bottom. The abbreviations signify the following grains: Pop = Argentine popcorn; Ts = teosinte El Salada; N = normal (PAG SX52 Hybrid) corn; Tc = teosinte Chalco; Trip = *Tripsacum* dactyloides (tetraploid); H.P. = Illinois high-protein corn.

Table V. Amino Acid Composition of Zein Fractions (g/100 g of Protein)

Amino acid	N	Pop	H.P.	Ts	Tc	Trip
Lys	0.1	0.1	0.1	0.1	0.1	0.1
His	1.5	1.5	1.4	1.5	1.4	1.7
NH_3	3.5	3.1	3.6	3.6	3.6	3.4
Arg	2.1	2.0	1.8	1.8	1.8	1.7
Asp	5.5	5.7	6.0	5.8	5.5	5.3
Thr	3.1	3.1	2.9	3.0	3.0	3.1
Ser	5.7	5.9	5.7	5.5	5.7	5.2
Glu	26.0	27.1	27.0	26.4	26.0	26.6
Pro	9.7	10.0	10.0	9.2	10.5	9.7
Gly	1.5	1.5	1.4	1.2	1.4	1.3
Ala	10.3	10.7	10.7	9.9	10.6	12.1
Val	4.0	4.5	4.6	4.3	4.0	4.1
Met	1.3	1.2	1.0	2.0	1.7	2.8
Ile	4.3	4.2	4.3	4.2	4.2	4.2
Leu	20.1	20.2	20.1	20.1	19.6	18.8
Tyr	5.4	5.6	5.1	5.3	5.2	5.5
Phe	7.4	7.3	7.1	7.0	7.3	6.2

based on charge and size differences (Figure 1). Albumins of the corns and Tc have similar SGE patterns; the major bands show quantitative variations. The Ts albumins are not distinct and do not show minor bands. In contrast, most of the major Trip albumins have mobilities that differ significantly from those of the other albumins. The globulins, except Tc, show no consistent major qualitative differences between species, but appreciable quantitative differences in some bands can be discerned, even between the three corn varieties. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of albumins and globulins resolved them according to molecular weights, but the complexity of the patterns precluded assignment of genetic relationships.

Zeins. The amino acid compositions of the zeins from the different grains are very similar (Table V). All are practically devoid of lysine. Trip zein had slightly more methionine and alanine and less arginine and leucine than the others.

Polyacrylamide gel electrophoresis patterns of the reduced and alkylated zeins from the six grains exhibited marked similarities as well as significant differences (Figure 2). All zeins had relatively low mobilities in this system. The pattern of Trip deviated considerably from those of

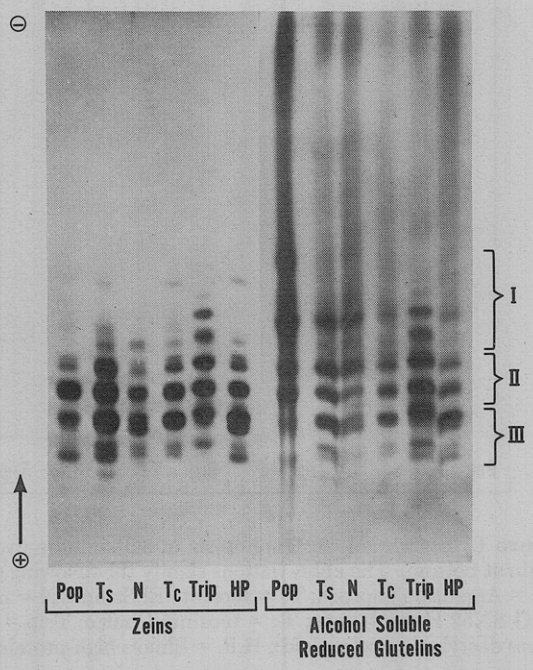

Figure 2. Polyacrylamide electrophoresis of alcohol-soluble proteins. Origin at bottom.

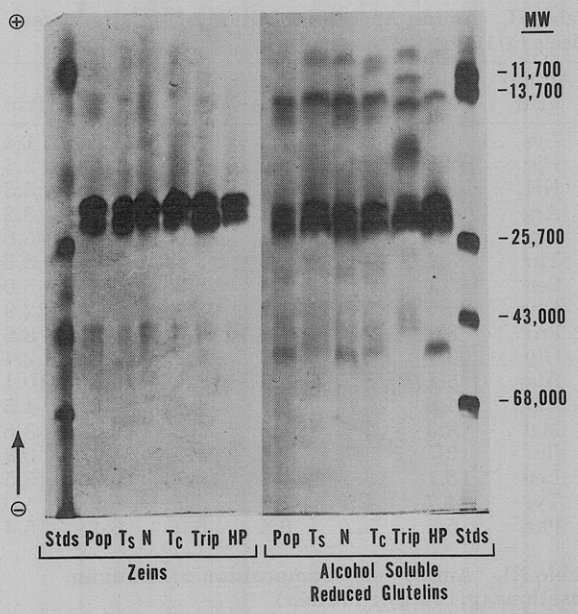

Figure 3. Polyacrylamide gel electrophoresis in sodium dodecyl sulfate of alcohol-soluble proteins. Origin at bottom.

the others in the number and mobilities of the bands. Two prominent Trip prolamine components migrated faster than major zeins from the other grains. The Trip pattern consisted of six or seven well-separated bands while a similar number of components in zein patterns of the corns and teosintes included at least one set of two bands in close proximity (doublet). The patterns of the two teosintes (Ts and Tc) resembled that of N except that a doublet in region III of N is replaced by an intense single band in the teosintes. The H.P. zein pattern resembles the N pattern, but the Pop pattern shows only a single band instead of a doublet for the slowest components. Yamaleyeva and Kissel (1973) observed differences in native zeins from endosperms of corns of different genetic backgrounds.

In our earlier sodium dodecyl sulfate–polyacrylamide gel electrophoresis studies of corn proteins, a 5% polyacrylamide gel was used to separate reduced proteins (Paulis et al., 1975). Zein appeared as a single intense diffuse band. Misra et al. (1976) found that sodium dodecyl sulfate–polyacrylamide gel electrophoresis on 10% gels resolved zein into two bands. Patterns in Figure 3 verify that zeins could be separated on a 10% gel into two components having mol wt of 22 000 and 24 000. The two zein bands are of equal intensity from all grains except Trip in which the mol wt 24 000 band is more intense. As evidenced by a greater number of components in the polyacrylamide gel electrophoresis patterns (Figure 2), each sodium dodecyl sulfate–polyacrylamide gel electrophoresis band must consist of several proteins varying in charge and amino acid content.

Alcohol-Soluble Reduced Glutelin (ASG). There are some small differences among the amino acid compositions of the ASG's from the different grains (Table VI); however, like the zeins, all ASG's are very low in lysine. Again, Trip ASG shows the greatest variation from the others, being higher in methionine, alanine, glutamic acid, and leucine and lower in arginine, proline, and glycine.

The ASG proteins exhibit polyacrylamide gel electrophoresis patterns (Figure 2, right) similar to the corresponding patterns of zein (Figure 2, left) except that the teosintes and corns contain additional fast-moving bands in region I. Also, the Pop sample exhibits a still faster moving component. Most Trip ASG's have slightly faster mobilities than those of related corn or teosinte ASG's indicating nonidentity of the Trip ASG's with those from the other grains. The ASG's of the corns and teosintes are similar in terms of band mobilities and intensities except that the doublet in the upper III region of the corns appears as a single band in the teosintes.

Among the sodium dodecyl sulfate–polyacrylamide gel electrophoresis patterns of the ASG's (Figure 3, right), Trip exhibited the greatest variation. While the other ASG's had bands of equal intensities at 22 000 and 24 000 daltons, the former band was much less intense in the Trip extract, as observed for the zeins. An additional prominent band at 17 000 daltons and one at 12 500 daltons also distinguishes the Trip ASG from the others. The low molecular weight components in ASG contain polypeptides rich in methionine (Paulis and Wall, 1971; Paulis et al., 1975). Large amounts of the fast bands in Trip ASG may contribute to its high methionine content, 5.9%. H.P. has the least amounts of the fast migrating low molecular weight ASG bands and its ASG has the lowest content of methionine (Table VI). A protein of mol wt 54 000 is present in the ASG of three corns and two teosintes (Figure 3), but it is absent or deficient in Trip.

Table VI. Amino Acid Composition of Alcohol-Soluble Glutelins (g/100 g of Protein)

Amino acid	N	Pop	H.P.	Ts	Tc	Trip
Lys	0.3	0.2	0.2	0.2	0.3	0.2
His	4.6	5.2	4.4	3.1	3.4	3.1
NH_3	2.9	2.5	2.9	3.2	3.2	3.2
Arg	3.4	3.7	2.6	2.6	2.6	2.1
Asp	2.5	2.0	3.4	4.0	3.6	4.1
Thr	3.3	3.5	3.4	3.3	3.6	3.6
Ser	4.4	4.1	4.6	4.9	5.2	5.1
Glu	21.9	22.2	23.9	23.9	23.7	24.9
Pro	15.1	15.2	13.9	11.6	13.1	10.4
Gly	4.1	4.4	3.1	3.1	3.2	2.6
Ala	7.1	7.0	8.4	8.7	8.1	9.9
Val	4.3	4.7	5.4	4.5	4.6	4.2
Met	4.9	3.6	2.4	5.1	5.5	5.9
Ile	2.4	2.2	3.1	3.0	3.0	3.6
Leu	12.8	12.1	14.8	15.2	14.7	15.7
Tyr	4.9	5.2	5.2	5.6	5.1	5.4
Phe	3.1	2.6	4.0	4.1	4.2	4.5

Table VII. Amino Acid Composition of Alcohol-Insoluble Glutelins (g/100 g of Protein)

Amino acid	N	Pop	H.P.	Ts	Tc	Trip
Lys	5.2	5.1	4.5	4.0	4.9	3.7
His	4.0	3.6	3.0	3.4	3.7	3.1
NH_3	2.0	1.6	2.6	2.5	2.2	3.0
Arg	6.3	6.3	5.0	5.3	5.7	5.1
Asp	7.7	8.6	8.2	7.8	8.1	7.3
Thr	4.4	4.9	4.3	4.2	4.4	3.8
Ser	5.1	5.2	5.1	5.4	4.9	4.9
Glu	16.4	16.2	18.2	21.8	17.6	18.2
Pro	7.2	8.1	6.6	7.9	7.5	6.7
Gly	5.2	5.6	4.3	3.9	4.8	4.4
Ala	6.7	7.2	7.5	8.6	7.0	7.6
Val	6.2	6.8	6.3	6.1	6.5	5.5
Met	2.3	2.4	2.4	2.5	2.6	3.1
Ile	4.1	4.1	4.3	4.3	4.3	3.9
Leu	9.5	9.8	11.7	13.4	10.4	11.0
Tyr	4.7	4.4	4.4	5.0	4.3	4.6
Phe	5.1	5.0	4.8	5.4	4.6	4.6

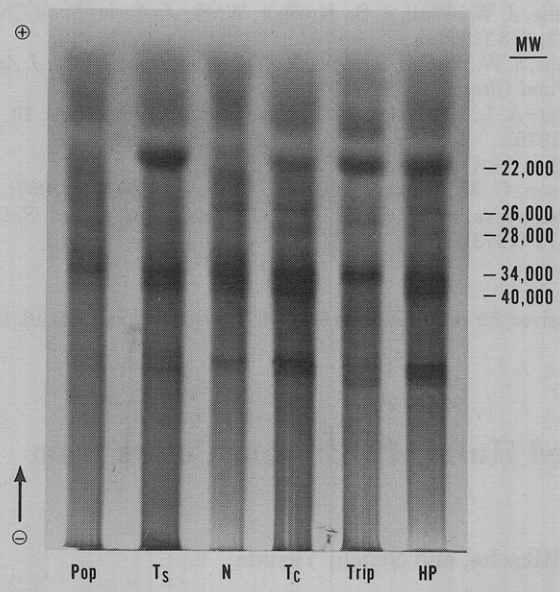

Figure 4. Polyacrylamide gel electrophoresis in sodium dodecyl sulfate of alcohol-insoluble proteins.

Residue Proteins. The residue protein consists mainly of AIG. The amino acid compositions of the AIG's from the different grains differ from each other (Table VII). For example, lysine varies from 3.7% of the AIG protein in Trip to 5.2% in N; glutamic acid ranges from 21.8% in the Ts sample to 16.2% in that from Pop; and leucine is highest in Ts AIG, 13.4%, and lowest in that from N, 9.5%.

Distinct qualitative and quantitative differences are apparent in the sodium dodecyl sulfate–polyacrylamide gel electrophoresis analysis of AIG proteins of the different grains in Figure 4. An intense band appears in the Ts, Trip, and H.P. sample at mobility corresponding to 22 000 daltons. The low amounts of lysine and high levels of leucine in this sample suggest that residual ASG protein was retained in these AIG samples and may be responsible for this band. Other variations in mobility and band intensity indicate that the Trip AIG is most different from the others. Bands are present in the Trip pattern at 26 000 and 40 000 daltons that do not occur in the other AIG's; bands at 28 000 and especially 34 000 daltons are absent in Trip that can be seen in the other patterns.

CONCLUSIONS

The amino acid compositions of the whole cereal grains are determined by both the grain contents of the various

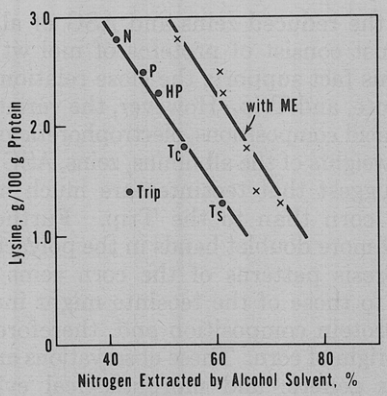

Figure 5. Lysine content of whole grain meals vs. extracted alcohol-soluble nitrogen.

protein classes and the amino acid analyses of those proteins. Paulis et al. (1974a,b) demonstrated that in widely differing genotypes of corn an inverse linear relationship (correlation coefficient, 0.87) exists between lysine and zein content of the grain. When for all six grains in this study, percent lysine in meal protein is plotted against zein (nitrogen extracted by 70% ethanol–0.5% sodium acetate solvent) (Figure 5) the correlation coefficient of the regression plot is only −0.66. Excluding data for Trip from the calculation yields a better correlation, −1.00. Thus, the teosintes appear to follow the relationship established for corns. The ASG protein like zein is also low in lysine (Table VI). When the total alcohol-soluble, low-lysine proteins (zein plus ASG) are plotted against percent lysine in meal protein (Paulis and Wall, 1975), all values including that for Trip lie close to the regression line (Figure 5) to give a correlation coefficient of −0.96. Thus, although the amount of total alcohol-soluble protein correlates well with lysine for all grains tested, in Trip a significantly larger amount exists as part of the glutelin complex rather than as zein.

The large amount of ASG in Trip results in its high content of methionine because ASG proteins are high in methionine (Table VI) (Paulis and Wall, 1971). Of the ASG samples isolated, that from trip has the highest methionine content. The contribution of ASG to the methionine contents of the meals can be calculated; in normal corn 25.5% of the methionine in the recovered fractions was present in ASG, in Pop, 22.2%, but in Trip, 40.1%. Perhaps introduction of Trip genes into corn may increase its ASG fraction at the expense of zein and thereby elevate corn's methionine content.

During evolution, changes in amino acid sequence of seed storage proteins may not be as lethal for plant development as would comparable changes to enzymes. Thus, major changes in storage proteins may frequently occur and be retained in progeny. In the species studied, the number of electrophoretic bands of the prolamines and ASG's are small enough to permit easy comparisons between varieties. Differences were noted in the prolamine polyacrylamide gel electrophoresis patterns of the three corns. In contrast, the numerous globulin proteins must consist of a large number of enzymes which differ greatly in charge and molecular weight. Considerable variability in quantities of enzymes present in such different genotypes of corn (Dalby and Davis, 1967; Wilson and Alexander, 1967) may make difficult interpretations of globulin patterns. Electrophoretic patterns of zeins and glutelins might serve as a better key to the genetic backgrounds of corn races.

It is noteworthy that the sodium dodecyl sulfate–polyacrylamide gel electrophoresis patterns demonstrated

that both the reduced zeins and ASG of all the grains studied must consist of proteins of mol wt 22 000 and 24 000. This fact supports the close relationship among corn, teosinte, and Trip. However, the variations among the amino acid compositions, electrophoretic patterns, and molecular weights of the albumins, zeins, ASG's and AIG's strongly suggest that teosintes are much more closely related to corn than to the Trip. Furthermore, the presence of more doublet bands in the polyacrylamide gel electrophoresis patterns of the corn zeins and ASG's compared to those of the teosinte might indicate more complex protein composition and, therefore, later evolutionary origin of corn. These observations are consistent with other genetic and morphological evidence that modern teosinte is closely related to the ancestor of corn but that *Tripsacum*, although a close relative, lies on a divergent evolutionary branch among the Tripsacinae.

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LITERATURE CITED

Beadle, G. W., *J. Hered.* **30**, 245 (1939).
Benson, J. V., Jr., Patterson, J. A., *Anal. Chem.* **37**, 1108 (1965).
Bietz, J. A., Sheppard, K. W., Wall, J. S., *Cereal Chem.* **52**, 513 (1975).
Bressani, R., Mertz, E. T., *Cereal Chem.* **35**, 227 (1958).
Cavins, J. F., Friedman, M., *Cereal Chem.* **45**, 172 (1968).
Clayton, W. D., *Kew Bull.* **28**, 49 (1973).
Dalby, A., Davis, I., *Science* **155**, 1573 (1967).
Jones, R. W., Beckwith, A. C., *J. Agric. Food Chem.* **18**, 33 (1970).
Mangelsdorf, P. C., "Corn. Its Origin, Evolution, and Improvement", The Belknap Press of Harvard University, Cambridge, Mass., 1974.
Mangelsdorf, P. C., Reeves, R. C., *Tex. Agric. Exp. Stn. Bull.* **574**, 1 (1939).
Misra, P. S., Mertz, E. T., Glover, D. V., *Cereal Chem.* **53**, 765 (1976).
Newell, C. E., deWet, M. J., *J. Am. Bot.* **61**, 45 (1974).
Paulis, J. W., Bietz, J. A., Wall, J. S., *J. Agric. Food Chem.* **23**, 197 (1975).
Paulis, J. W., Wall, J. S., *Cereal Chem.* **46**, 263 (1969).
Paulis, J. W., Wall, J. S., *Biochim. Biophys. Acta* **251**, 57 (1971).
Paulis, J. W., Wall, J. S., "Protein Nutritional Quality of Foods and Feeds", Friedman, M., Ed., Marcel Dekker, New York, N.Y., 1975, p 381.
Paulis, J. W., Wall, J. S., Kwolek, W. F., *J. Agric. Food Chem.* **22**, 313 (1974a).
Paulis, J. W., Wall, J. S., Kwolek, W. F., Donaldson, G. L., *J. Agric. Food Chem.* **22**, 318 (1974b).
Rotar, A. I., Borovskii, M. I., Pashkav, S. I., *Skh. Biol.* **10**, 522 (1975).
Wilkes, H. C., *Science* **177**, 1071 (1972).
Wilson, C. M., Alexander, D. E., *Science* **155**, 1576 (1967).
Yamaleyeva, A. A., Kissel, N. I., *Tr. Prikl. Bot. Genet. Sel.* **52**, 171 (1973).

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Carbohydrates, Polyphenols, and Lignin in Seed Hulls of Different Colors from Turnip Rapeseed

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The carbohydrates, polyphenols, and lignins in seed hulls from turnip rapeseed, *Brassica campestris*, of different colors and in hulls from white mustard, *Sinapis alba*, were studied. Twelve low molecular weight carbohydrates were identified of which eight were quantified. The amount of polysaccharides in the yellow hulls is higher and the amount of Klason lignin is lower than in the dark hulls. The relative carbohydrate compositions of the polysaccharides were rather similar. Oxidative degradation of kraft-cooked (heating with aqueous sodium hydroxide–sodium sulfide) samples (methylated and hexadeuteromethylated) showed that dark hulls have a high polyphenol content and light hulls a low content; lignin contents, however, are about the same.

Rapeseed (*Brassica napus* L.) and turnip rapeseed (*Brassica campestris* L.), the most important oilseed crops grown in Sweden, usually have dark hulls which form about 15–20% of the seeds. Recently, plant breeders have obtained yellow-hulled seeds which have higher oil and protein content and lower crude fiber content compared with dark-hulled seeds. They have thinner hulls and the cells, especially the palisade cells, are smaller and the embryos are heavier (Jönsson and Bengtsson, 1970; Stringam et al., 1974).

The dark-hulled seeds give a darker oil which needs to be decolorized before it is used for food products. Rapeseed meal obtained after oil extraction is today an important fodder product because of its high content of proteins and well-balanced amino acid composition. Rapeseed protein concentrate (Anjou and Fecske, 1974) and rapeseed protein isolate (Gillberg and Törnell, 1977) may be useful for human consumption. These are greyish when prepared from dark-hulled seeds but are lighter and more attractive when prepared from yellow-hulled seeds.

Polysaccharide fractions from rapeseed hulls have been studied (Aspinall, 1974) with paper chromatography (PC) and the structure of the pectin from rapeseed hulls has

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